

To: Dr. Suhair Shallal, EPA Designated Federal Official

From: W. Heiger-Bernays, PhD, T.F. Webster, DSc, J. Schlezinger, PhD – Boston University School of Public Health

Date: Wednesday, December 29, 2021

Re: Comments on “Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water” [PFAS Charge.pdf] and on the “External Peer Review Draft: Draft Framework for Estimating Non-Cancer Health Risks Associated with Mixtures of Per- and poly-fluoroalkyl Substances (PFAS). EPA Document No. 822D-21-003.”

The following comments are provided on the document titled: “Charge Questions for SAB Review of the Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goals for PFOA and PFOS in Drinking Water” [PFAS Charge.pdf]

Noncancer Hazard Identification:

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

Comments: We have reviewed the document titled “Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water” and refer to this document in our comments as, “EPA Proposed Approaches.” While we agree that immune system dysfunction is an important adverse human health endpoint, there also is strong epidemiological evidence of PFAS-induced increases in serum triglycerides and non-high density lipoprotein cholesterol (non-HDL), as well as emerging evidence of associations with aortic disease and cardiovascular disease risk [1]. It is concerning that an important study showing a strong association between PFOA and PFOS with serum non-HDL in the general US population was not included in the EPA analysis [2]. Here, we provide the strengths of the animal model serum lipid data and its consistency with the human epidemiological data. The animal model data support both inclusion of PFAS-induced effects on serum lipids as an alternative endpoint for the derivation of the Reference Dose (RfD) and can provide support for the selection of Cardiovascular Disease Risk Reduction as a Result of Reduced PFOA and PFOS Exposure in Drinking Water.

Serum lipids overall

On Page 188 of the EPA Proposed Approaches document, there is an incorrect over-arching interpretation of study results. *“Overall, studies have generally reported consistent decreases in serum lipids including TC, triglycerides, LDL cholesterol, HDL cholesterol, and/or non-HDL cholesterol in rats {Martin et al., 2007, 758419; Loveless et al., 2008, 988599; Elcombe et al., 2010, 2850034; NTP, 2019, 5400977; NTP, 2020, 7330145} and mice {Loveless et al., 2008, 988599; De Witt et al., 2009, 1937261; Minata et al., 2010, 1937251; Yahia 2010, 1332451 ; Yan, 2014, 2850901; Quist et al., 2015, 6570066; Blake et al., 2020, 6305864}.”* It is true that multiple animal model studies have shown a decrease in serum lipids at high PFOA exposure levels. However, the statement above is not true of studies that include lower, human-relevant PFAS serum levels, as discussed below.

Serum triglycerides

We appreciate that EPA does recognize exposure levels and their relationship to effects of serum triglycerides. On page 189 of the EPA Proposed Approaches document: *“For serum triglyceride levels, significant increases were observed at lower exposure concentrations of PFOA (0.31 and 1.25 mg/kg/day) while significant decreases were seen following exposure to higher PFOA concentrations (5 and 10 mg/kg/day).”* [3]. What is not discussed is that the 1.25 mg/kg/day dose resulted in a PFOA serum concentration of approximately 60 µg/mL, a concentration of PFOA found in highly exposed fluorochemical workers [4]. Thus, PFOA induced decreases in serum triglycerides *only* at serum levels significantly greater than those measured in even occupationally exposed people.

In male mice, studies have reported that perfluorocarboxylic acids dose-dependently induced increases in serum triglycerides and decreases in serum triglycerides [3, 5, 6, 14]. In studies with male rats, perfluorocarboxylic acid exposure also was associated with dose-dependent increases in serum triglycerides and decreases in serum triglycerides [5, 7-12]. Some studies in rodents also have reported no effect [8, 13, 14]. There are two studies that are important to note, in which full dose response assessments were conducted and serum PFOA concentrations were measured [3, 14]. Across these two studies, at lower PFOA body burdens, increased serum triglycerides were observed and at high PFOA body burdens (above those measured even in fluorochemical workers in the US), decreased serum triglycerides were observed. Furthermore, in a study with male cynomolgus monkeys, significant increases in serum triglycerides were observed following exposure to PFOA at serum levels less than 90 µg/mL [15]; this study was not included in the serum lipid analysis in the EPA Proposed Approaches document.

When only studies that used exposure scenarios resulting in human-relevant serum PFOA levels are considered, **PFOA exposure consistently results in increased serum triglycerides.**

Serum cholesterol

Only two studies of effects of PFOA on serum cholesterol were discussed in depth in the EPA Proposed Approaches document provided to the SAB. In a short term (10-16 day) gestational exposure, serum PFOA was associated with only a trend toward increasing cholesterol in maternal serum of CD-1 mice [16]. In a 28-day PFOA exposure in male BALB/c mice, HDL cholesterol (“good” cholesterol) was significantly decreased [3]. Further, it is stated, *“Conclusions from these studies are met with limitations as the difference in serum lipid composition between humans and commonly used rodent models may impact the relevance to human exposures {Getz et al., 2012, 1065480; Oppi et al., 2019, 5926372}. Additionally, food consumption may confound these results, as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection.”* It is true that studying cholesterol biology in rodents has several challenges. Diet influences serum cholesterol levels [17]. Cholesterol homeostasis differs depending on mouse strain and sex [18]. Species differ in the distribution of cholesterol among the different cholesterol particle types [19]. However, with careful model and experimental design, these challenges can be addressed.

We agree that studies using rodents fed a standard, low fat/low cholesterol rodent diet and exposed to PFOA for 6 weeks show decreased serum cholesterol levels (reviewed in [20]). However, when mice are fed a cholesterol and fat-containing diet, PFOA does increase serum cholesterol levels [20], particularly in males and in C57BL/6 mice at a serum concentration of approximately 30 µg PFOA/mL. In a dose response analysis in male APOE*3-Leiden.CETP mice treated with PFOA for 4 weeks, serum cholesterol concentrations were only decreased in mice with a PFOA serum concentration of 144 µg/mL [14]. Importantly, in mice expressing human PPARα, PFOA increased serum cholesterol. Nakamura et al [13] exposed male hPPARα (Sv/129 strain) to 0.3 mg PFOA/kg/day for 2 weeks (no serum PFOA concentration was reported) and Schlezinger et al. [21, 22] exposed male hPPARα mice to 0.7 mg PFOA/kg/day for 6 weeks (47 µg PFOA/mL serum). In both studies, PFOA exposure was associated with increased serum cholesterol, particularly low density lipoprotein cholesterol [13, 21, 22]. No study in rodents to date has investigated the relationship of PFAS at steady state exposures to effects on serum lipids; however, increased serum cholesterol with associated with serum PFAS concentrations in household cats [23].

We conclude that when studies used human-relevant diets and exposure scenarios, PFOA exposure consistently results in increased serum cholesterol in sensitive rodent strains, including one that expresses human PPARα.

Sex Differences

In the EPA Approach document, sex was noted as a variable when discussing only a single study, *“In a study conducted by NTP, sex differences were observed in Sprague-Dawley*

rats exposed to PFOA by gavage for 28 days {NTP, 2019, 5400977}. Males had significantly decreased serum TC and triglyceride levels at exposure concentrations as low as 0.625 mg/kg/day. Female rats in the same study were exposed to 10-fold higher doses than their male counterparts due to sex differences in PFOA excretion (Section D.4). Females had significant increases in both serum TC and triglyceride levels at the two highest doses (50 and 100 mg/kg/day)." Of the 30+ studies investigating the effect of PFOA on liver and/or lipid homeostasis in animal models published since 1992, only four have included females (not including gestational exposure studies). Of the two studies that investigated effects on both male and female mice, significant sex differences were observed, which were not dependent on differences in pharmacokinetics [20, 24]. Rebholz et al. [20] was the first to investigate the influence of sex on the serum lipid response to PFOA and showed that male mice were most sensitive to modulation of cholesterol homeostasis by PFOA across the C57BL/6 and BALB/c strains but that female C57BL/6 mice had the greatest increase in serum cholesterol in response to PFOA. Similarly, Schlezinger et al. [22] showed that male hPPAR α (Sv/129 strain) were more sensitive to PFOA-induced increases in serum cholesterol than females. In contrast, female PPAR α null mice were more sensitive to PFOA-induced increases in serum triglycerides [21]. Differences in serum lipid responses to PFOA between sexes were accompanied by significant sex-dependent differences in changes in hepatic gene expression, as well [20–22]. In a very recent study of exposure to a mixture of PFAS, alteration of serum cholesterol homeostasis also was shown to be sex-dependent [25].

Based on the literature cited above, in animal models, PFAS-induced health adverse health effects are sex-dependent. Therefore, it is critical that derivation of a data-driven, health protective RfD must be based on data from the most sensitive sex.

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Relative Source Contribution:

1. “EPA applies a Relative Source Contribution (RSC) when calculating the MCLG to provide a margin of safety that an individual’s total exposure from a contaminant does not exceed the RfD. The RSC is the portion of an exposure for an individual in the general U.S. population estimated to equal the RfD that is attributed to drinking water; the remainder of the exposure equal to the RfD is allocated to other potential sources. Based on the physical properties, detected levels, and available exposure information, there are significant potential sources other than drinking water ingestion for PFOA and PFOS; however, information is not available to quantitatively characterize exposure from these different sources. EPA followed Agency guidance on how to derive an RSC (U.S. EPA, 2000; available online at:

<https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf>) and recommends an RSC of 20 percent (0.20) for PFOA and PFOS. This RSC is the same as what was used in the 2016 HAs for PFOA and PFOS”.

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

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

Comment: The Relative Source Contribution of 20 percent (0.2) for PFOA and PFOS is adequately supported, but not clearly described.

While it is vitally important to recognize the large exposure burden to people in communities whose drinking water is contaminated with PFAS, it is also important to account for non-drinking water sources of PFOA and PFOS in the non-carcinogen development of the Maximum Contaminant Level. However, the language used to describe the RSC is inconsistent with the EPA definition of the RSC and perpetuates misunderstanding of the RSC. As per USEPA, 2000: *“The percentage of total exposure typically accounted for by drinking water, referred to as the relative source contribution (RSC), is applied to the RfD to determine the maximum amount of the RfD “apportioned” to drinking water reflected by the MCLG value. In using this percentage procedure, the drinking water program also applies a ceiling level of 80 percent of the RfD and a floor level of 20 percent of the RfD. That is, the MCLG cannot account for more than 80 percent of the RfD, nor less than 20 percent of the RfD.” (USEPA, 2000).*

Therefore, a lower RSC results in a more stringent (lower) drinking water guideline/standard.

At issue is how the Reference Dose (RfD) is to be apportioned and whether the RSC should be higher than 20% of the RfD or lower than 80% of the RfD, not whether the portion of an exposure for an individual in the general U.S. population is estimated to equal the RfD that is attributed to drinking water. Since the RSC represents daily exposure from drinking water as a fraction of the RfD, it is ***independent*** of the concentration that is present in contaminated drinking water. The EPA's risk-assessment guidance specifies a default RSC of 20% (the most stringent possible value) when data on exposures from nondrinking water sources needed to derive a chemical-specific value are not available.

Recent drinking water standards developed by States, including NJ, VT and MA (amongst others) have relied on a RSC of 0.2 (reviewed in Post, 2020) because relevant and accessible alternative data were not available and existing data could not justify a larger RSC. The rigorous work by MN (Goeden et al., 2019) provides a case where an RSC of 0.5 is used, based on

subtraction of upper percentile serum PFAS levels in children and adults not exposed to contaminated drinking water from a target human serum level. However, we don't yet know the full picture of PFAS serum levels in people consuming contaminated water across the US. While NHANES provides an invaluable source of US population biomonitoring data, and data show the decrease in PFOA/PFOS, these data are insufficient to characterize the serum levels of PFAS in the most susceptible populations.

One of the major challenges is that data are only now emerging on the “other” sources and pathways of PFAS (and specifically PFOA and PFOS) to which people are exposed. These include exposures from stack emissions, non-point source ambient air, indoor dust and air and food (reviewed in DeSilva et al., 2021). With the uncertainty in exposure estimates of PFAS to both the general population and to those populations who may have higher exposures from non-drinking water sources, for example ambient air, indoor air and dust, and food, in the absence of reliable data, **retaining the default RSC of 20% is most defensible in the derivation of the MCLs.**

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The following comments are provided on the External Peer Review Draft: Draft Framework for Estimating Non-Cancer Health Risks Associated with Mixtures of Per- and poly-fluoroalkyl Substances (PFAS). EPA Document No. 822D-21-003. Our comments specifically address the Charge Questions to the SAB.

SAB Charge Question #1:

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

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

Comment: There are at least two important cases:

1) PFAS mixtures that act at the same molecular initiating event (MIE). PFAS can activate a number of different nuclear receptors. We have shown that PFAS activate PPAR α with different potencies and efficacies (maximal effects) and that the effects of mixtures are well predicted by generalized concentration addition (GCA) (Nielsen et al 2021), an additivity model suitable for such cases that has its roots in pharmacodynamics (Howard and Webster 2009). Although experimental confirmation is needed for other receptors, there is reason to believe that GCA will predict the effects of PFAS for all receptors of interest. This has proved true for mixtures of other compounds—including pharmaceuticals and environmental compounds—for other receptors that we have tested: AhR, PPAR γ , androgen receptor (AR) glucocorticoid receptor (GR) (Howard et al 2010, Watt et al. 2016, de la Rosa et al 2020). Regular concentration addition (also called dose addition) and relative potency factors (RPFs) are special cases of GCA (Howard and Webster 2009). There is thus good scientific support for dose additivity (or GCA) at common MIEs, as well as the downstream effects of these MIEs (Howard and Webster 2009).

2) PFAS mixtures that act at different MIEs that affect the same outcome via converging adverse outcome pathways (AOPs). The EPA document reviews evidence that converging AOPs can follow dose additivity. This has been particularly well studied in the androgen system, e.g.,

mixtures of compounds that reduce the concentration of androgens and compounds that are competitive antagonists for the AR. However, modeling by our group suggests that converging AOPs need not always follow dose addition (Webster 2013). We therefore believe that additional research into the mixtures effects of converging AOPs is needed. However, it is scientifically plausible that such cases could follow dose addition (or GCA). This assumption is thus a reasonable default for risk assessment. It is likely to be protective as it allows for “Something from Nothing” behavior compared to the alternative of independent action (Silva et al 2002)

SAB Charge Question #3

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

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

Comment: Strictly speaking, the RPF model requires that dose-response curves differ only in potency, i.e., they have the same functional form (shape) and efficacy. As discussed above for question 1a, we have shown that PFAS differ in efficacy for activation of PPARα, a nuclear receptor likely to be important for this group of compounds. Instead of RPFs, PFAS mixtures follow GCA, a model that relaxes the assumptions of RPF and dose addition (the latter can only make predictions for effect levels less than that of the least efficacious mixture component). Differences in both potency and efficacy are common for receptor ligands. It is therefore unlikely that PFAS will follow the RPF model at all doses. However, as the RPF model is a special case of GCA, it is likely to be approximately correct at low, environmentally relevant doses.

SAB Charge Question #4

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

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

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

Comment: The “Mixture BMD” model is a simple application of dose addition. The fixed proportions of the mixture define a ray from the origin. The BMDs (or other measures of effect) for the mixture components define an isobole: a straight line for two compounds, a plane for three compounds, etc. The intersection of the two provides the BMD for the mixture on that isobole. Importantly, dose addition does not require that isoboles be parallel, i.e., isoboles can differ in slopes (Howard and Webster 2009). In other words, the slope of the isobole—the amount of one compound that substitutes for another—can depend on the isobole. In a true RPF situation, the isoboles are parallel and the slopes (related to the RPFs) are the same for all effect levels. Use of a different mixture would mean a different ray and thus intersection with the isobole at a different point, but the concept is exactly the same.

The implication is that the “Mixture BMD” model is acceptable if dose addition is acceptable, which as we discuss above is a reasonable assumption (although the use of GCA might fit data better for all effect levels). An important caveat is that if isoboles are not parallel, then the Mixture BMD model can only determine whether the dose is less than the BMD or not, i.e., the difference between them is not meaningful.

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