

Charge Question: #5D. Uncertainty Factors

EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) for PFOA and PFOS.

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

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

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

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

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

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

that this UF is considered when developing RfDs for use in MCLGs for “suggestive carcinogens” such as PFOS and explain why it did not need to be included in the PFOS RfD.

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

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

One potential framework to explore is the Mixture Assessment Factor (MAF). The use of MAFs is discussed in Kortenkamp and Faust (2018) and is currently being explored by the European Commission (2020) for the assessment and management of chemical mixtures. A potential starting place for incorporating an MAF would be to create an UF_M that accounts for mixtures, with a default value equal to 10. The default value assumes that only a small number of chemicals contribute to a particular effect. The value of the UF_M could be increased or decreased based upon the number of chemicals (and to the extent possible, non-chemical stressors) expected to co-occur with the chemical being evaluated. Given that PFAS often co-occur, PFOA and PFOS are ideal chemicals for which to utilize a mixture-associated uncertainty factor with the default value of 10.

However, the Panel did not reach consensus on advising this approach for the current assessments because of divergent views on the appropriate methods of accounting for the effects of mixtures on chemical toxicity. Noting that the reference dose is specific to the intrinsic behavior of the chemicals being evaluated, in the absence of other chemicals that cause the same effect or inhibit its effect, there was concern about using this approach. Further stating that an uncertainty factor accounting for the effects of other chemicals on the intrinsic behavior of the chemical being studied was deemed inappropriate. The approaches discussed in the draft EPA Mixtures Framework document – in which co-occurring PFAS that cause the same toxic effect are considered – were seen as more appropriate for accounting for toxicity of mixtures of PFAS. In these approaches, a toxicity factor or relative potency is developed for each individual PFAS, and their additive effects are considered.

Others noted that uncertainty factors should have a clear conceptual basis before being applied. There were concerns that identifying an agreed-upon framework for developing a mixture uncertainty factor, should one exist, was beyond the scope of the charge. Furthermore, it could be argued that the concerns about mixtures are already covered by the UF_H, since human variability incorporates not just genetic variability, but variability in background and co-exposures, nutrition, etc. (Zeise *et al.* 2013).

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

Recommendations

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

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

Additional comments on draft document - "Proposed Approaches to the Derivation of a Draft MCLG for PFOA in Drinking Water"

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

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

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

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

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

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

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

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

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

p. 30, last paragraph. The relevance of exposure sources needs clarification. It is unclear how exposure source can affect the maternal:cord serum ratio, unless this refers to the proportion exposure to branched vs linear isomers, which can vary with exposure sources. If that is what is meant, it should be so stated.

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

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

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

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

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

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

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

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

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

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

p. 95, 3.3.1.2.5. Were any studies that evaluated whether PFOA causes structural malformation identified other than Lau et al. (2006)? If this is the only such study, it appears to be an important data gap.

Additionally, suggest distinguishing structural malformations (e.g., limb and tail defects) from developmental delays (e.g., delayed ossification) in this writeup.

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

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

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

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

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

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

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

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

p. 142, Section 3.3.32.3. Suggest adding discussion of steatosis caused by PFOA reported by Das et al. (2017, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5994610/>). This effect may be relevant to the potential for PFOA to impact fatty liver in humans. It is also suggested that Quist et al. (2015, <https://journals.sagepub.com/doi/full/10.1177/0192623314551841>), which reported histopathological changes in mouse liver that are observable only with electron microscopy at very PFOA low doses, be discussed.

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

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

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

p. 146, first paragraph. Should “cystoid” be “cystic”?

p. 151. First full paragraph beginning with “Of the studies, ...”, and last paragraph, three sentences beginning with “The Faroe Islands studies {Grandjean et al., 2012, 1248827; Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889} observed associations...” and ending with “...at later time periods from children at age 5 years, age 7 years, and age 13 years.” In the four studies from the Faroe Islands cited here (Grandjean et al., 2012; 2017a, 2017b; Mogensen et al., 2015), two different cohorts were evaluated, and associations of maternal and/or child (at different age points) serum PFAS levels and antibody response to vaccination at different ages are reported. The text here is difficult to follow, and does not clearly and accurately indicate that, among these studies, maternal serum PFAS were measured late in pregnancy or two weeks after delivery (depending on the cohort), offspring serum PFAS levels were measured at 18 months, 5 years, 7 years, and/or 13 years (depending on the cohort), and vaccine antibodies were measured at 5 years pre-booster, 5 years post-booster, 7 years, and 13 years (depending on the cohort). I needed to make my own table of the cohort(s), age points for serum PFAS, and age points for vaccine response analyzed in each study in order to review this information. Adding such a table here would be helpful, especially since these studies and this endpoint were selected as the critical study and endpoint for the RfD.

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

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

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

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

Suggest qualifying this statement by adding that globulin consists of several components, most of which are not immunoglobulins. "The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells." See: Busher (1990 , <https://www.ncbi.nlm.nih.gov/books/NBK204/>) Therefore, a decrease in total globulin does not necessarily indicate decreased immunoglobulin.

Also relevant to this point, (2002, <https://www.fda.gov/media/72228/download>) states: "Decreases in serum globulin levels (often detected, where seen, as an increase in the serum albumin/globulin ratio) may indicate impairment of immunoglobulin production. However, decreased basal serum globulin level is a relatively insensitive indicator, because under normal circumstances the immune system should be challenged with antigen and a particular antibody response evaluated to detect immunosuppression. When decreased serum globulin level is observed, the protein components affected should be determined using appropriate assays (Duncan et al., 1994; Hall, 2001; Weingand et al., 1996)."

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

p. 167. 3.3.4.4. Evidence Integration for immune effects.

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

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

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

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

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

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

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

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

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

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

p. 189, last sentence. It is suggested that the differences in the effect of PFOA on serum lipids in rodents versus humans may arise from the fact that the animals were not fasted in some studies before serum collection. Other potentially important factors include difference in exposure levels (see comment on Convertino et al., 2018, above), and differences in the fat content of human diets versus rodent lab diet. This is discussed in studies reviewed in DWQI (2017 <https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>) including Tan et al. (2012, <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061409>) and Rebholz et al. (2016 <https://www.sciencedirect.com/science/article/pii/S2214750015300822?via%3Dihub>), as well as newer studies such as Schlezinger et al. (2020, <https://www.sciencedirect.com/science/article/abs/pii/S0041008X20303306?via%3Dihub>).

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

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

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

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

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

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

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

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

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

p. 289, third paragraph, and p. 290, second to last paragraph. If Onishchenko et al. (2011) is discussed, Koskela et al. (2011) should also be included (Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation.

<https://pubmed.ncbi.nlm.nih.gov/27068293/>)

This study looked at effects on bone in the same group of mice used in Onishchenko et al. (2011). This study is the basis for the ATSDR MRL for PFOA, although it included only one dose level.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

p. D-3. Section D.1.4. Dermal Exposure. An additional study relevant to dermal absorption of PFOA is: Fairley, K.J., Purdy, R., Kearns, S., Anderson, S.E., Meade, B.J. (2007). Exposure to the

immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol. Sci. 97: 375-383.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

D-23, third paragraph. “. Factors such as exposure sources, parity, and other maternal demographics can potentially account for these variations [in cord:serum ratio]. For example, nulliparous mothers generally have significantly higher serum PFOA than parous women (Kato et al., 2014, 2851230). Conversely, younger women tend to have lower serum PFOA than older women (Kato et al., 2014, 2851230). Therefore, studies with high percentages of young, multiparous women may report lower levels of PFOA in maternal and cord blood.” The rationale for this statement is not clear. While young, nulliparous women may have lower levels of PFOA in maternal and cord blood, the topic here is the cord:maternal blood ratio, and it is unclear why it would differ in women with lower maternal and cord blood levels than in women with higher levels.

“A summary of recent studies examining RCM is presented in Table D-10. The percentages of maternal PFOA that accumulate in cord blood ranged from 57 to 133% and did not strictly correlate to maternal serum values. This variability suggests that TTE may differ across populations likely due to maternal characteristics or differing levels of exposure.” Again, it is not clear why the ratio between maternal and cord blood would be expected to correlate with maternal serum levels. And it is not clear why differing exposure levels would impact the TTE.

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

p. D-28, first paragraph. “PFOA is highly soluble in water relative to PFOS (solubilities of 3.4 g/L and 0.68 g/L, respectively). Since amniotic fluid is 94% water, the solubility properties may account for the observation that the PFOA concentration (0.044 ng/mL) was twice as much as PFOS (0.02 ng/mL) in this matrix.” This explanation does not appear to be plausible, since the concentrations in amniotic fluid mentioned here are many orders of magnitude below the water solubilities of PFOA and PFOS. Therefore, it is highly unlikely that solubility is the limiting factor here.

p. D-28, second paragraph. “...of comparing the portioning of PFOA from mother to fetus across studies.” “Portioning” should be “partitioning.”

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

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

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

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

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

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

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

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

p. D-46, first full paragraph. The Vd used by Thompson et al. (2010) and Lorber and Egeghy (2011) does not come from NHANES data, as stated here. It comes from data from the C8 study population in West Virginia and Ohio, a population with exposure to drinking water contaminated with PFOA by releases from an industrial facility.

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

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

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

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

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

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

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

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

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

p. D-70, Section D.4.5.1, paragraph 1. Is there evidence that the half-life of PFOA is shorter in populations exposed to contaminated drinking water or occupationally? Any studies relevant to this issue should be evaluated and discussed here. Also, it is unclear why exposure to contaminated drinking

water is referred to as occurring "under acute conditions," since exposure to contaminated drinking water generally has occurred for a considerable period of time (years) before it is discovered.

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

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

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

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

Additional Comments on "Draft Framework for Estimating Noncancer Health Risks Associated with Mixtures of PFAS"

Gloria B. Post

December 30, 2021

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

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

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

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

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

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

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

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

[standards-68854/](#) and <https://www.seacoastonline.com/story/news/2020/09/04/judge-rules-for-nh-in-3mrsquos-bid-to-block-pfas-protections/42435483/>.

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

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

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

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

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

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