National Institute of Environmental Health Sciences (NIEHS)
Comments on the Interagency Science Consultation
Draft IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA)
March 2022
(Date Received April 21, 2022)

Note: The scientific review and comments are provided by National Institute of Environmental Health Sciences (NIEHS) Division of the National Toxicology Program scientists and are not intended to represent formal agency position or opinion.

1.2. Summary of Assessment Methods

1.2.1. Literature Search and Screening

NIEHS Comments: The summary of the literature search, screening, and systematic review methodology was clear and transparent. It was helpful to include a link to the protocol in the systematic review, and it was evident that the EPA's IRIS program took great care in addressing comments on the protocol and improving their process to reach their final methods.

There were a few instances where the methods section of the systematic review needs further clarification. It was not clear how the studies with topics considered potentially relevant, identified as supplemental material, were documented in the study flow diagram. Are they considered excluded? (see additional comments below on same topic in Section 2.1). Second, the methods indicate that other studies of other PFAS were to be marked as supplemental materials (Page 1-11, lines 6-7). This statement was confusing. Was it related to the fact that EPA's IRIS program was working on a total of 5 PFAS at the same time? It appeared that each of the 5 PFAS under evaluation had a separate literature search and thus a separate literature screening. Thirdly, the exposure of interest is not mentioned in the title sentence of the first paragraph of the results (Page 1-18, lines 1-3).

- Tier 2 o Please clarify how supplemental material will be screened and documented in the study flow diagram, and whether all these studies will undergo full-text screening.
  - o Please clarify what is meant by "Studies of other PFAS (e.g., perfluorooctanoic acid [PFOA] and perfluorooctane sulfonate (PFOS)" being identified as supplemental material (Page 1-11, lines 6-7).
  - o Please include the name of the PFAS under evaluation in the title sentence of the first paragraph of the results (Page 1-18, lines 1-3). This will keep the reader focused on the PFAS under review.
  - o Minor edits:
    - Page 1-11, lines 22-23: The punctuation around the insensitivity issue is hard to follow. It appears that a parenthesis is missing after "false negative" on line 23.
    - Page 1-12, line 32: - Please doublecheck the grammar of the phrase "...may be evident of a biologic gradient". It should probably read, "...may be evidence of a..".
    - Page 1-14, line 2: Can you provide example(s) of an outcome(s) that were determined to be less relevant?

2. Literature Search and Study Evaluation

NIEHS Comments: Overall, this section was clearly written. However, the number of studies in Figure 2-1 (Page 2-2) does not add up the total of unique studies – there are 104 studies missing at title and abstract level and 19 missing at full text review. There are no comments on individual studies or categories of PECO-relevant studies presented in section 2.2, as the information is presented within Section 3 by health outcome categories.

 Tier 2 o The discrepancy in the number of studies in the literature screening figure should be addressed with better explanation of the identification of studies identified with supplemental information. Please clarify if these studies were also screened at the full-text review. Also, it would be helpful to identify the number of studies that contain data relevant to multiple evidence streams as well as potentially useful supplemental data. These details should, at minimum, be included in the text. Consider the inclusion of a figure caption. 3. Pharmacokinetics, Evidence Synthesis, and Integration

NIEHS Comments:

Appendix A identifies the potential for pharmacokinetic (PK) differences across species and sexes as a key science issue and lays out a hierarchy for using relevant PK data in extrapolating doses between laboratory animals and humans. Section 3.1 evaluates and synthesizes the PK data in relevant species and sexes, and among human life stages, up to the derivation of key PK parameters used in the subsequent modeling. Section 5.2.1 describes the various approaches considered for PK extrapolation and the rationale for the selected approach. Given what is known and not known about the potential interspecies differences in pharmacokinetics of PFDA, EPA used a single compartment PK model to adjust the POD to estimate a human equivalent dose in the derivation of the respective RfDs.

a. Is applying the single compartment PK model for PFDA scientifically justified? If not, please provide an explanation and detail on a more appropriate approach.

   The one compartment pharmacokinetic model is the simplest way to describe the disposition of the test article in the body. In general, it is applied to IV bolus model where the target test article is injected all at once into the body or compartment, and the test article distributes/equilibrates instantaneously and rapidly throughout the compartment. It is also assumed that the elimination from the compartment also occurs immediately after the IV bolus injection. If the PK model is going to be used to estimate plasma levels following oral administration in humans and the intention is not to predict the actual test article levels in the tissues (e.g., assumption of the changes in plasma levels are proportional in tissue levels), this model should be acceptable. However, it should be explained with more detail why the decision was based on half-lives only, and why a two compartmental model is not considered, bearing in mind the two studies in the literature (Kim et al 2019, and Dzierlenga et al 2019). Is this approach selected based on assumption of linear pharmacokinetics (e.g., first order elimination and no dose effect on volume of distribution and clearance)? Is the preference made also considering the concerns on distribution throughout the body and the preferential accumulation within the mother or fetus? In other words, is the PK model used mainly focusing on the developmental exposures or predictions in human in general regardless of exposure, sex, species etc.? Section 5.2.1 provides a more detailed explanation to the reasoning behind the one compartment model decision. Some of the conclusions could be brought forward or additional referrals to the section 5.2.1 could be made indicating that the in-depth explanations are provided in a later section.

b. Are the selections of PK model parameter values for rats, mice, and humans adequately described and reasonable?

   While the selections of PK model parameter values for rats, mice, and humans are adequately described and reasonable, it would be best to provide examples for some of the ‘key pharmacokinetic data’ inconsistencies highlighted in the text. Are the inconsistencies referring to published data or the assumptions? (Page 3-12, line 33). Any additional insight provided would be beneficial for the reader. Some of the points made in the pharmacokinetics section become clearer after reading section 5.2 since additional insight and detailed evaluations are provided there.

c. Have the uncertainties in these parameters been adequately evaluated and described?

   The uncertainties in these parameters have been adequately evaluated and described considering the limited studies and available data on PDFA (especially on distribution from mother to fetus). Section 5.2 clearly and adequately evaluated, described, and addressed the uncertainties in the PK parameters, in particular for humans (beginning on Page 5-14).

d. Do the methods used to derive toxicity values for PFDA appropriately account for uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans?

The methods used to derive toxicity values for PFDA appropriately account for uncertainties in evaluating the pharmacokinetic differences between experimental animal data and humans and were highlighted and described in detail. The methods used to derive toxicity values for PFDA are appropriate and accounted for the PK uncertainties as well as species differences.

Additional comments:

- Section 3.1., Page 3.1, lines 20-22: "calculating the average clearance across studies…". Please provide references for the studies from which the averages were calculated. From the sentence, it is unclear what the standard errors (SE) are since only mean values are provided. Would the SE values change the conclusion and statement of 30% lower clearance in females since only mean values were highlighted? 3.2.1. Hepatic Effects

NIEHS Comments:

- Page 3-27, Table 3-5: Are references to Butenhoff et al., 2012 and van Otterdijk, 2007a intentionally included in the first column?
- Page 3-28, Figure 3-4: The hepatocyte cytoplasmic alteration should be statistically significant for the 0.625 mg/kgday groups in male and female.
- Page 3-34, Table 3-9: Are references to Butenhoff et al., 2012 and van Otterdijk, 2007a intentionally included in the first column?
- Page 3-36, line 8: Please note the typographical error - PXR = Pregnane X Receptor rather than pregnant x receptor
- Page 3-37, line 12: The study described isn't related to the NTP 28-day study, which used the rat for its animal model. Is PFD (line 12) supposed to be PFDA? If not, it should be defined.
- Page 3-41, Line 27-30: What is meant by "all hepatic effects of PFDA exposure have been adequately characterized"? Would it be better to say that longer term studies would likely characterize a higher severity of PFDA hepatotoxicity? Given the data set, is it possible to consider this robust evaluation? We recommend similar language as on Page 5-34, lines 12 -14 regarding length of exposure to be consistent.

3.2.2. Immune Effects

NIEHS Comments:

- For Frawley et al 2018 (a NIEHS DNTP study), confidence level was downgraded for not reporting metrics regarding blinding of those involved in assessing immunotoxicity endpoints. Blinding is not routine practice for these types of studies and additional detail on methods is available on request.
- Regarding the two NIEHS Division of the NTP studies that are compared, the hematology results are considered "unclear" and inconsistent. We believe that the results are consistent if the doses that were evaluated in common are considered. Study 1 showed significant changes not identified by Study 2 because Study 1 reported on doses as high as 2 mg/kg. There is no review of the potential acute toxicity. In Study 2, animals exposed to 1 and 2 mg/kg developed acute toxicity, and some were euthanized early due to moribundity. All animals at those doses were removed from the study. The impact that acute toxicity may have had on animals in Study 1 is not clear. Although this is eventually acknowledged, it would be better to identify the complications of acute toxicity at the same time as the immune results, instead of at the end of the paragraph.

3.2.3. Developmental Effects

NIEHS Comments: Page 3-116, line 37: Please edit to "… for the delayed brain case ossification,…"

3.2.5. Female Reproductive Effects

NIEHS Comments: In general, this section of the review was well written, comprehensive given the data cut-off, and information was clearly presented. There are some missing references that may add weight of evidence for this section

(these were the most important examples found). The first two provide further support for the RfD and should be evaluated.

- Li K, Zhao Q, Fan Z, Jia S, Liu Q, Liu F, Liu S. The toxicity of perfluorodecanoic acid is mainly manifested as a deflected immune function. Mol Biol Rep. 2022 Mar 2. doi: 10.1007/s11033-022-07272-w. Epub ahead of print. PMID: 35233679.
- Timmermann CAG, Pedersen HS, Weihe P, Bjerregaard P, Nielsen F, Heilmann C, Grandjean P. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7-12 years exposed to marine pollutants, a cross sectional study. Environ Res. 2022 Jan;203:111712. doi: 10.1016/j.envres.2021.111712. Epub 2021 Jul 31. PMID: 34343554.
- Petersen KU, Hærvig KK, Flachs EM, Bonde JP, Lindh C, Hougaard KS, Toft G, Ramlau-Hansen CH, Tøttenborg SS. Per- and polyfluoroalkyl substances (PFAS) and male reproductive function in young adulthood; a cross-sectional study. Environ Res. 2022 Mar 19;212(Pt A):113157. doi: 10.1016/j.envres.2022.113157. Epub ahead of print. PMID: 35318009.
- Dunder L, Lind PM, Salihovic S, Stubleski J, Kärrman A, Lind L. Changes in plasma levels of per- and polyfluoroalkyl substances (PFAS) are associated with changes in plasma lipids - A longitudinal study over 10 years. Environ Res.
  2022 Feb 26;211:112903. doi: 10.1016/j.envres.2022.112903. Epub ahead of print. PMID: 35231461.
- Cakmak S, Lukina A, Karthikeyan S, Atlas E, Dales R. The association between blood PFAS concentrations and clinical biochemical measures of organ function and metabolism in participants of the Canadian Health Measures Survey (CHMS). Sci Total Environ. 2022 Feb 23;827:153900. doi: 10.1016/j.scitotenv.2022.153900. Epub ahead of print. PMID: 35218824.
- Yang J, Wang H, Du H, Fang H, Han M, Wang Y, Xu L, Liu S, Yi J, Chen Y, Jiang Q, He G. Exposure to perfluoroalkyl substances was associated with estrogen homeostasis in pregnant women. Sci Total Environ. 2022 Jan 20;805:150360. doi: 10.1016/j.scitotenv.2021.150360. Epub 2021 Sep 16. PMID: 34818773.
- Bommarito PA, Ferguson KK, Meeker JD, McElrath TF, Cantonwine DE. Maternal Levels of Perfluoroalkyl Substances (PFAS) during Early Pregnancy in Relation to Preeclampsia Subtypes and Biomarkers of Preeclampsia Risk. Environ Health Perspect. 2021 Oct;129(10):107004. doi: 10.1289/EHP9091. Epub 2021 Oct 12. PMID: 34637358; PMCID: PMC8509361.
- Bloom MS, Commodore S, Ferguson PL, Neelon B, Pearce JL, Baumer A, Newman RB, Grobman W, Tita A, Roberts J, Skupski D, Palomares K, Nageotte M, Kannan K, Zhang C, Wapner R, Vena JE, Hunt KJ. Association between gestational PFAS exposure and Children's adiposity in a diverse population. Environ Res. 2022 Jan;203:111820. doi:
  10.1016/j.envres.2021.111820. Epub 2021 Jul 31. PMID: 34343551; PMCID: PMC8616804.
- Christensen JVR, Bangash KK, Weihe P, Grandjean P, Nielsen F, Jensen TK, Petersen MS. Maternal exposure to perfluoroalkyl chemicals and anogenital distance in the offspring: A Faroese cohort study. Reprod Toxicol. 2021 Sep;104:52-57. doi: 10.1016/j.reprotox.2021.06.016. Epub 2021 Jun 25. PMID: 34182087; PMCID: PMC8403157.
- Blomberg AJ, Shih YH, Messerlian C, Jørgensen LH, Weihe P, Grandjean P. Early-life associations between per- and polyfluoroalkyl substances and serum lipids in a longitudinal birth cohort. Environ Res. 2021 Sep;200:111400. doi:
  10.1016/j.envres.2021.111400. Epub 2021 May 31. PMID: 34081971; PMCID: PMC8403652.
- Ou Y, Zeng X, Lin S, Bloom MS, Han F, Xiao X, Wang H, Matala R, Li X, Qu Y, Nie Z, Dong G, Liu X. Gestational exposure to perfluoroalkyl substances and congenital heart defects: A nested case-control pilot study. Environ Int. 2021 Sep;154:106567. doi: 10.1016/j.envint.2021.106567. Epub 2021 Apr 23. PMID: 33882431.

While the comments on hormone measurements in women (should be collected under fasting conditions and controlled for in some way or deemed lower confidence) are appropriate, the same is true for animal studies, and it is unclear if the authors held the animal studies to the same rigor (which is easier to control for than in women). If the animal study controlled for fasting and time of day (controls and treated animals all necropsied within the same general amount of time since fast started, i.e., 4-6 hr), then that should be qualified “as adjusted for” or “as a limitation”, thus lowering the confidence in hormone measures.

- Page 3-150, lines 8-18: There is no evidence that Tanner stage 2 and 3 are under the same endocrine control as Tanner stage 4 and 5 (T2/T3 happens before menarche and the T4/5 generally occurs at the same time or after menarche). We recommend not linking those outcomes. A suggested re-write of this section: "In girls, age at Tanner stages two and three for breast development were lower with higher exposure. These differences were not statistically significant…" Also, line 18 needs to be adjusted to avoid suggesting that these outcomes are linked.
- Page 3-150, line 23-29: The potential for reverse causality should not make a study low confidence. It may make it unreasonable to choose as the point of departure, but it doesn't change the confidence in the study, just the interpretation. This should be modified throughout this section. Furthermore, there are some studies that have controlled for reverse causality, but it does not appear if the authors took that into consideration. Some reevaluation of these studies is needed – was contraception, parity, time since last child, or other metrics that may explain the outcome considered in the evaluation?
- Page 3-151, line 6: Suggest use of "less severe or asymptomatic cases amongst control". Cases don't have to be asymptomatic.
- Page 3-151, lines 31-36: These lines are repetitive and should be combined. A suggested re-write: "…28 d study, and the mean estrous cycle length is 4.4 d amongst multiple sub-strains of Sprague Dawley rats (Marty et al 2009)." Delete the remaining text.
- Page 3-153, Table 3-27, and those like it: These tables are hard to understand as the percentage doesn't add up within the dose groups for all the cycle stages. These artificial values are hard to understand when the reader is looking for a change in percent time spent in cycle stages across groups. This may need a bit more explanation.
- Page 3-153, line 8: Is it possible to relax the statement on fertility by saying "the potential influence that lack of cyclicity may have on fertility"? It is possible that once this very high dose (that caused weight loss) was stopped, cyclicity may resume. This dose is far outside what a human might be exposed to chronically. Additionally, women who lose dramatic amounts of weight may also become acyclic for short periods of time, and this information is missing from this section.
- Page 3-154: There is a problem with the organ weights as described.
  Female reproductive organ weight should only be compared within an estrous cycle stage. There is a biologically significant reason for this: the uterus in diestrus is a fraction the size of that in any other cycle stage. Comparison of the uteri across stages doesn't make sense and the data should be re-analyzed or stated as a deficiency, lowering the confidence. Of course, the uterine weights will be lower if the animals are all in diestrus, and the other dose groups are not. This is not an independent line of evidence. It is the effect of the PFDA on the ovaries and the unmeasured hormones that cause the uterus to remain in the static phase. This is not a strong argument.
- Page 3-156, lines 9-12: Consider leaving out the Wyeth drug data as this doesn't add to the evidence and may suggest a direction that is irrelevant.
- Page 3-156, lines 25-27: Consider rephrasing the comment on the anogenital distance measures in the NTP study for two reasons: 1) There was no developmental exposure in the study, so why measure it, and 2) Those endpoints are not indicated in an adult dosing 28-day study.
- P3-157, line 10: "indicative of infertility" is too strong a term. Consider the language used on Page 3-158, line 1 ("reductions or delays in fertility"). Also, please note that it is unclear that the uteri weight data were analyzed correctly, taking stage effects into consideration. This is a deficit that could be corrected for in re-analysis of the data.
- Page 3-159, lines 9-12: Note that these are likely at high doses. From the discussion presented here, it is not evident that longer exposures were needed, but possibly developmental exposures were needed to address the several points brought up.

3.2.6. Endocrine Effects

NIEHS Comments:

- Page 3-163, line 5: There is a mistake on the Blake study confidence level within this entire section. In Figure 3-45 the study was given medium confidence, yet it is repeatedly rated as low confidence in the narrative (lines 5, 23).

- Page 3-164, line 7, the Kang study is reported incorrectly as low, but it is medium. This was also a problem in the urinary section – some studies, such as the Blake et al. 2018 study, are noted as low confidence and are medium confidence and yet the Blake study was noted correctly in the obesity section as medium confidence.
- The Blake 2018 study is the only longitudinal study included, and the strength of the study design is not mentioned in the discussion, especially in the hormone section. Unlike TSH, which was collected repeatedly over a period of 18 years for individuals and was indicative of progressive disease in thyroid or kidney over time, T4 was only available at the first visit. This paper reports several significant outcomes for PFDA and should be looked at more closely, specifically, the interquartile dose dependent increase in eGFR.

3.2.7. Urinary Effects

NIEHS Comments:

- Page 3-189, Table 3-33: Are references to Butenhoff et al., 2012 and van Otterdijk, 2007a intentionally included in the first column by the study names? Could this be a systemic issue?
- Page 3-190, Figure 3-56: Minor comment, is it possible to extend the shading across rows into the dot plot for improved readability?
- Page 3-191: It is probably worth noting in the summary that male and female rats responded similarly in BUN response and Creatinine response, although those responses (BUN and Creatinine) were in different directions.

3.2.8. Cardiometabolic Effects

NIEHS Comments: Overall, the information is clearly presented in the cardiometabolic and overall evidence integration sections. If studies are introduced to provide context for the findings but are not included in the evaluation, consider providing more context and indicate why the studies are not included in the main analyses (e.g., Pilcher and Langley, 1986; Van Rafelghem et al 1988a).

“Other risk factors for cardiovascular disease” comments:

- Page 3-198: Regarding relative wall thickness (RWT) outcome, consider slight edit to highlight that the included Mobacke et al. 2018 study outcome is consistent with the Hashem and Biton studies that found associations between decreased RWT and ventricular geometry and tachyarrhythmia outcomes. The data suggest that changes in RWT are associated with adverse outcomes (conversely, if the endpoint is not clearly an indicator of the health effect of interest, consider not including that endpoint and document why that endpoint was not included).
- Page 3-212, starting line 10: Please expand on the rationale for the indeterminate conclusion for the high confidence animal study to include a brief description of the endpoints as it relates (or doesn’t relate in this case) to the human condition.
- Please also note if any studies/health outcomes were stratified by race/ethnicity and if there were any disparities along racial/ethnic lines.

3.2.10. Other Health Effects

NIEHS Comments: Page 3-224, lines 19-22: This is a good brief summary of hematological effects. Edits to reflect that the effects occurred at 1.25 and 2.5 mg/kg doses (as written, the text implies only a high dose effect) are suggested.

3.3. Carcinogenicity

Human Studies

NIEHS Comments:

- The conclusion, “available epidemiologic evidence on PFDA and the risks of any type of cancer is scant and does not support a conclusion currently” is appropriate. There is only one study each for two cancer sites.
- In an effort to improve the reporting quality, consider:

- Replacing Figure 3-66. Study evaluation results for epidemiological studies of PFDA and cancer (which by itself, is not very informative) with a table on the study characteristics – design, geographical region, population, outcome, results, and study evaluation conclusion (highlighting the specific biases).
- Providing a concise overview of the studies. The text does not report the study designs, relevant exposure window, the type of selection bias, etc.

Animal Studies

The text is clear.

Mechanistic

- The text is clear, and the tables are helpful.
- The conclusion, "PFDA does not appear to elicit a strong genotoxic response" and "some evidence of potential clastogenic effects" is appropriate.

Overall conclusion

EPA's conclusions that "PFDA does not appear to elicit a strong genotoxic response" and "some evidence of potential clastogenic effects" are supported.

5. Derivation of Toxicity Values

5.2.1 Oral Reference Dose (RfD)

Derivation Study/Endpoint Selection

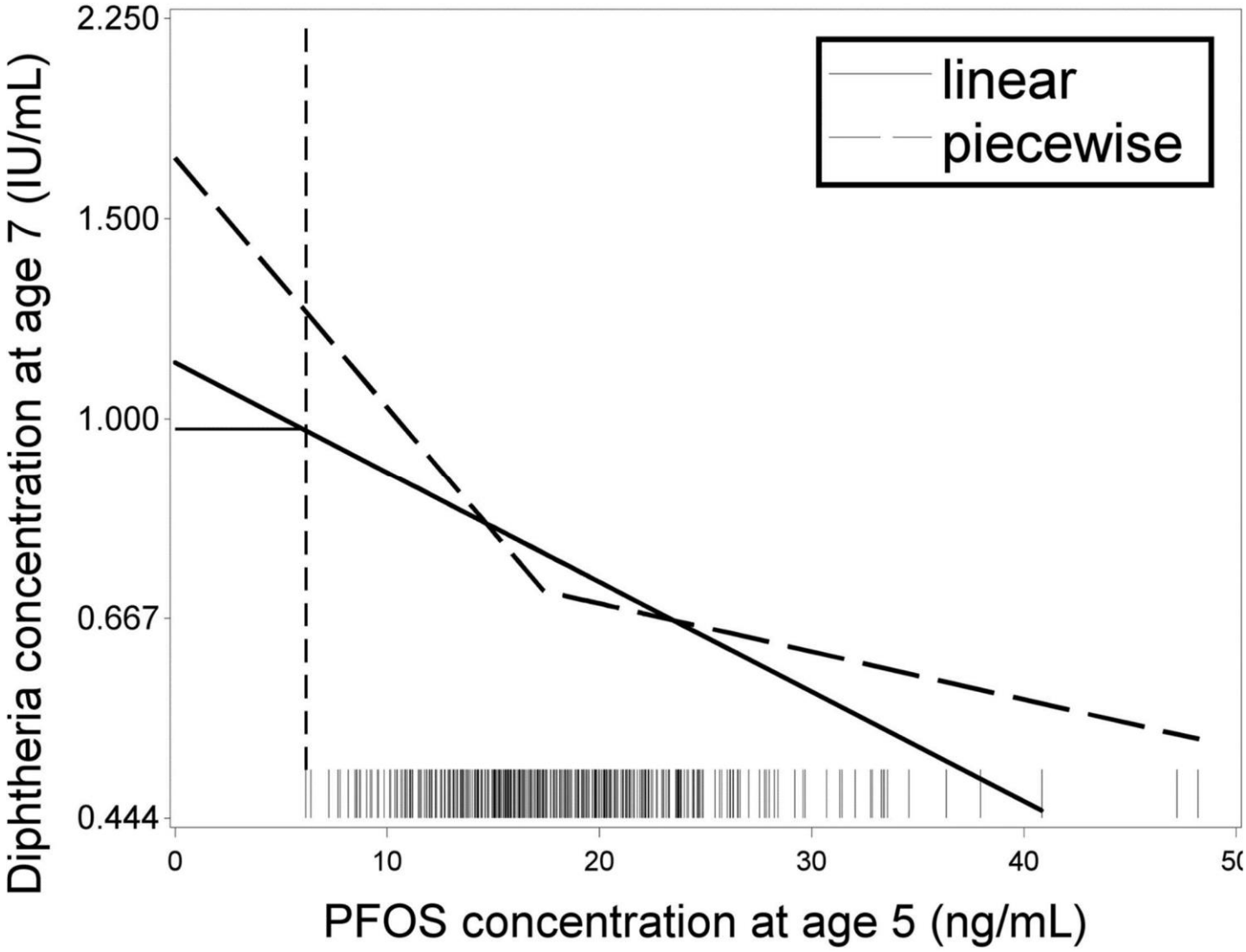
NIEHS Comments:

- Page 5-4, Table 5-1: It is unclear why POD was not derived for ALP, given the same reasoning as AST. Should the POD for ALP be "Yes" given that it was derived in table 5-9 (Page 5-31)?
- Page 5-20, line 33: The term "gender" is inappropriate here. Please use "sex" as endpoints are related to sex.

Estimation or Selection of Points of Departure (PODs) for RfD Derivation

NIEHS Comments:

- The section is well-written well and the choices and the reason for those choices are appropriate.
- Consider including the BMD plots for the human data, including the individual data values (from what it appears to be is a couple hundred to 1000 in the case of immunoglobulin levels) plotted again blood concentration and then depict the model overlayed on the data along with the BMD and BMDL estimates. The plots can be generated from BMDS. The original publication, in the case of the immune endpoint, and the actual data are not shown on the plot overlayed by the fit to the data – instead all the individual values are shown in one dimension without the corresponding response (y-axis value). Showing the actual data will increase the transparency of the study/data selection, modeling, and determination of the POD.



Derivation of Candidate Lifetime Toxicity Values for the RfD

NIEHS Comments:

- Page 5-40, line 12-13: Consider rewording: "In deciding overall confidence, confidence in the evidence base (i.e., study confidence(?)) is prioritized over the other confidences (e.g.?).
- Table 5-12, page 5-41, Developmental osRfD: In the description of Medium confidence at the bottom of page, it is unclear what "advanced to dose-response" means.