National Institute of Environmental Health Sciences (NIEHS)
Comments on the Interagency Science Consultation
Draft IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS)
January 2023
(Date Received January 30, 2023)

General comments:
There are a number of acronyms not found in the list of abbreviations and acronyms (e.g. ASD, ADHD).

1.1 Background Information on Perfluorohexanesulfonic acid (PFHxS)

Suggested Revisions:

- Page 1-5 (Air and Dust) and Page 1-7 (Populations with Potentially Greater Exposures), EPA is missing an important reference suggesting much higher exposures to PFHxS in firefighters via dust.
    - See Table 1 in: Hall SM, Patton S, Petreas M, Zhang S, Phillips AL, Hoffman K, Stapleton HM. Per- and Polyfluoroalkyl Substances in Dust Collected from Residential Homes and Fire Stations in North America. Environ Sci Technol. 2020 Nov 17;54(22):14558-14567. doi: 10.1021/acs.est.0c04869. Epub 2020 Nov 4. PMID: 33143410; PMCID: PMC7939574.
- Page 1-6 (Water). This section suggests, due to limited content, that PFHxS is a dated issue (old references used). There are two contemporary community studies that suggest PFHxS is a current issue due to modern waste practices and difficulty in their removal by point-of-use and point-of-entry residential drinking water filters. These are important points to make here – it is an ongoing health hazard, even if companies stopped making it or putting it into products. Also, UCMR3 (the third unregulated contaminant monitoring rule, EPA) limited the available data by the 10,000-person community cut-offs. Including these references enhances that dataset in that they include some smaller (and large) communities.
    1. Assessing the Effectiveness of Point-of-Use Residential Drinking Water Filters for Perfluoroalkyl Substances (PFASs). Nicholas J. Herkert, John Merrill, Cara Peters, David Bollinger, Sharon Zhang, Kate Hoffman, P. Lee Ferguson, Detlef R. U. Knappe, and Heather M. Stapleton. Environmental Science & Technology Letters 2020 7 (3), 178-184. DOI: 10.1021/acs.estlett.0c00004
    2. Kotlarz N, McCord J, Collier D, Lea CS, Strynar M, Lindstrom AB, Wilkie AA, Islam JY, Matney K, Tarte P, Polera ME, Burdette K, DeWitt J, May K, Smart RC, Knappe DRU, Hoppin JA. Measurement of Novel, Drinking Water-Associated PFAS in Blood from Adults and Children in Wilmington, North Carolina. Environ Health Perspect. 2020 Jul;128(7):77005. doi: 10.1289/EHP6837. Epub 2020 Jul 22. Erratum in: Environ Health Perspect. 2020 Aug;128(8):89002. PMID: 32697103.
- Page 1-7 (Other Exposures and/or Populations with Greater Susceptibility). The recent publications demonstrating measured PFHxS globally in breast milk and infant formula strengthen the approach of focusing on children as the most susceptible population and this publication should be included in one of these sections.
    - LaKind JS, Naiman J, Verner MA, Lévêque L, Fenton S. Per- and polyfluoroalkyl substances (PFAS) in breast milk and infant formula: A global issue. Environ Res. 2023 Feb 15;219:115042. doi: 10.1016/j.envres.2022.115042. Epub 2022 Dec 16. PMID: 36529330.

1.2 Summary of Assessment Methods

EPA's Toxicological Review (TR) of PFHxS is part of a coordinated systematic review of five select PFAS (PFBA, PFHxA, PFHxS, PFHA, and PFDA) with a single protocol published by EPA in 2019 and updated in 2020 in response to public comments and again in 2021. The protocol for these Tox Reviews is to be commended for following best practices and presenting EPA's systematic review methods in alignment with their standard IRIS Systematic Review Handbook. The methods overall increase transparency and consistency over previous approaches. Although the protocol is available as appendix A, it would be helpful for reviewers if the protocol is posted together with the draft PFHxS Tox Review and other appendices and included with the other documents when sent to reviewers.

EPA also directly describes the challenge of addressing potential confounding across PFAS exposures in section 1.2.2. The approach taken on addressing confounding is both reasonable and clearly presented both in the methods and in the evaluation of the immune evidence in section 3.2.2.

2. Literature Search and Study Evaluation

The literature search and screening transparently presented using best practices such as a clear PECO criteria and the development of studies categorized in what EPA refers to as literature inventories. Similarly, EPA's study evaluation (risk of bias, reporting and sensitivity assessment) is transparently reported following the detailed criteria outlined in the protocol.

3.1 Pharmacokinetics

Studies utilized to make decisions were appropriate and adequate information and justifications were provided in the document. There are some redundancies in the information in different sections of the document and suggest eliminating although it may be challenging to articulate the reasons and decisions made without repeating the information.

The Bayesian PK analysis conducted that combines the data from across the studies and doses available in the literature to generate overall mean values and ranges for PK parameters for species and sexes is appropriate given the variability of parameters between studies. The document adequately highlights the flaws in the available PK and PBPK models and their potential to overestimate clearance, compared to estimates from empirical in vivo human data, and the challenges in using EPA's high throughput toxicokinetics computational model predict clearance since the EPA model fails to account for transporters. Therefore, the alternate approach (the use of data derived extrapolation factors (DDEF) calculated from the estimated population average of total clearance) is a reasonable approach to estimate human equivalent dose (HED) for an endpoint with an identified point of departure (POD) from animal toxicity studies. The justification provided to use the lowest clearance value for humans as a more conservative (health protective) approach is appropriate.

Suggested Revisions:

- Page 3-16 (Breast Milk). Numerous papers have quantified PFHxS in breast milk (e.g., LaKind et al. 2023 (below) This section deserves updating and it is not clear why so many papers would be excluded.
    - LaKind JS, Naiman J, Verner MA, Lévêque L, Fenton S. Per- and polyfluoroalkyl substances (PFAS) in breast milk and infant formula: A global issue. Environ Res. 2023 Feb 15;219:115042. doi: 10.1016/j.envres.2022.115042. Epub 2022 Dec 16. PMID: 36529330.

- Page 3-102, Figure 3-23: The top is missing. The legend indicates that 22 studies are included and only 19 are shown. Also, there is no header for the figure.
- Ensure that the chemical name is stated correctly. In some places it is named as perfluorohexane sulfuric acid. For example, see page 3-1, line 1; page 4-1, line 2; page 5-1, line 1.
- Higher total AUC in blood (AUC0-∞) estimated after oral compared with IV doses (4 mg/kg PFHxS) in rats by Kim et al (2016b) was also observed by Huang et al. (2019a) for 4 and 16 mg/kg females. Consider adding this information in section 3.1.1 as appropriate and revising the statement regarding the data by from Kim et al (2016b)—currently stated as an experimental artifact which doesn't seem be the case as it has been observed in multiple studies. Regardless, the assumption of 100% bioavailability for the low dose extrapolation from rats to humans is appropriate.
- Check sentence on page 3-6, line 34 for accuracy.
- Pages 3-10 to 3-12 (Fetal Blood and Placenta): It is important to note that the below reference (which has been included for transplacental transfer) also indicates that breast feeding elevates infant PFHxS exposures over time. The authors noted elevated blood level measures after several months of breast feeding. This could be mentioned in the last part of the section, as there is limited data, but seems important if the infant or child are the susceptible population of choice.
    - Pre- and Postnatal Exposure to Perfluorinated Compounds (PFCs). Hermann **Fromme**, Christine Mosch, Maria Morovitz, Irene Alba-Alejandre, Sigrun Boehmer, Mandy Kiranoglu, Fabienne Faber, Iris Hannibal, Orsolya Genzel-Boroviczény, Berthold Koletzko, and Wolfgang Völkel. Environmental Science & Technology 2010: 44 (18), 7123-7129. DOI: 10.1021/es101184f.
- Page 3-16 (Trend in Pregnacy). The **Fromme et al.** paper 2010 (above) also evaluated the levels of PFHxS in mothers during and after pregnancy (during lactation) and should be included here.
- On page 3-18, lines 13-15, when clearance data from studies are compared, it is not clear why IV data from Kim et al. (2018b) is compared to oral data from Huang et al (2019a) instead of the data from the same route. Please clarify.
- There is significant variability in the animal data between studies as highlighted in the IRIS document. Data from repeated exposures as well as mixture studies are not available. Additionally, non-linearity of PFHxS ADME has been reported in single administration studies. Collectively, these areas need future scientific exploration.

3.2. Noncancer Health Effects

3.2.1. Thyroid Effects

The available data have been clearly and appropriately synthesized to describe the strengths and limitations. The weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified.

Suggested Revisions:

- Page 3-52, Figure 3-10: It seems like there are missing red triangles for T4 at the top dose; instead, there's a grey mark instead of a circle or triangle.
- Page 3-55, line 12: Should this be Butenhoff et al. (2009) and NTP (2018a)? Chang et al. (2018) is not discussed further in the paragraph about adrenal gland weights, though NTP is included without citation.

- Page 3-58, line 1: Change “available thyroid hormones data…” to “available thyroid hormone data…”
- Page 3-61, line 33: Suggest adding justification as to why this moderate call is not considered robust as multiple animal studies show thyroid hormone disruption. Is it the lack of mechanistic data or limited evidence of histological lesions?
- Page 3-59, lines 4 – 9: Appreciated the observation that the ToxCast data for PFHxS are largely focused on the thyroid hormone receptor-based assays, while the thyroid hormone system has many other components including the sodium iodide transporter.

3.2.2. Immune Effects

The available data have been clearly and appropriately synthesized to describe the strengths and limitations.

Suggested Revisions:

- For Figures 3-16 (page 3-66) and 3-17 (page 3-76), the Stein references should be designated “a” or “b”, as appropriate.
- Page 3-66, Figure 3-16: According to the figure, the Goudarzi et al. (2017) reference in the text should be 2016 (pages 3-71 to 3-72).
- Page 3-75 cites 12 studies in Figure 3-17 (page 3-76), but 13 are listed.
- Page 3-76, Figure 3-17: It is not clear from the text for the sections on Sensitization or allergic response and Asthma (beginning 3-74 through 3-78) why the following 4 references are in Figure 3-17 since they are not mentioned in the text or Table 3-12 (Dalsager, 2016; Grandjean 2017 a, b; Kielsen, 2016)?
- Page 3-76, line 3: Missing a close parenthesis after “(see Table 3-12.”
- Page 3-76, lines 3 – 4: States that “All studies were medium confidence, with the exception, of Timmermann et al. (2017)…” but Table 3-12 (page 3-80) indicates that both Timmermann et al. (2017) and Humblet et al. (2014) are considered of low confidence.
- Page 3-77, line 4 and in Table 3-12 (page 3-79), is there supposed to be ‘a and b’ for Zeng et al., (2019)? The text on page 3-77 indicates Zeng et al. (2019a) while Figure 3-17 just says Zeng, 2019.
- Beginning page 3-74, Sensitization or allergic response section: It is confusing as to what are “studies” vs. “publications”.
- Page 3-87: It could be noted that the asthma effect is more tied to chronic inflammation than immunosuppression.

3.2.3. Developmental Effects

The available data have been clearly and appropriately synthesized to describe the strengths and limitations. The weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified.

Suggested Revisions:

- Page 3-100, lines 8-10: There are some extra letters (ca) at the beginning of a string of citations that prevented the citations from formatting properly.
- Page 3-96, Line 19: Consider reformatting Xu et al., 2019 to display as Xu et al. (2019).
- Page 3-115, Table 3-16: Please spell out the abbreviations used for AGD and related measurements are not spelled out in the footnotes.

3.2.2. Hepatic Effects

The available data have been clearly and appropriately synthesized to describe the strengths and limitations. The weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified.

Suggested Revisions:

- Page 3-131 (Hepatic Effects, Human Studies and Animal Studies, page 3-135): Since the cut off date for your search, a couple of papers providing strong evidence for links between PFHxS and liver disease and its biomarkers (ALT especially) have been published. They are noted here and should be considered. In fact, the latter study may change the conclusion on page 3-152, as it confirms disease progression in a C57 mouse model. There are also several publications in mice from Angela Slitt's research group that are relevant.
  1. Borghese MM, Liang CL, Owen J, Fisher M. Individual and mixture associations of perfluoroalkyl substances on liver function biomarkers in the Canadian Health Measures Survey. Environ Health. 2022 Sep 14;21(1):85. doi: 10.1186/s12940-022-00892-6. PMID: 36104725.
  2. 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 Jun 25;827:153900. doi: 10.1016/j.scitotenv.2022.153900. Epub 2022 Feb 23. PMID: 35218824.
  3. He X, Jiang J, Zhang XX. Environmental exposure to low-dose perfluorohexanesulfonate promotes obesity and non-alcoholic fatty liver disease in mice fed a high-fat diet. Environ Sci Pollut Res Int. 2022 Jul;29(32):49279-49290. doi: 10.1007/s11356-022-19369-7. Epub 2022 Feb 25. PMID: 35217953.
- Page 3-136, lines 15 - 17: Suggest mentioning higher exposure levels to account for PK differences in females (NTP, 2018a) is the reason for the increased liver weights (both absolute and relative); that is, this is not an inconsistency with the 3M (2000a) study, but rather a design difference.
- Page 3-143, line 2-3: Please add a verb to this sentence: "As in epidemiological studies, two serum measures of clinical markers which inform of potential liver damage in experimental studies:…"
- Page 3-146, line 3: Change "EPAs" to "EPA's".
- Page 3-146, Figure 3-41: The NTP, 2018, study showed an increase in A/G ratio in male rats at the highest dose which should be indicated by a green triangle (missing from the figure)
- Page 3-147, line 3: Correct "reponses" to "responses".
- Page 3-149, line 22: Change "EPAs" to "EPA's".
- Page 3-136, line 26: Suggest adding the age of sampling (PND 21 and PND 36) for the Chang et al., 2018, paper.
- Page 3-139, line 18: the phrase "…but hepatocellular cell necrosis was not affected" doesn't seem correct; rather, should it say "… but hepatocellular cell necrosis was not increased"?
- Page 3-139, lines 25 – 29: Similar to above comment, odd wording; suggest changing "was not affected" (line 29) to "not increased".
- Page 3-143, footnote 9: Consider adding this background information to the description of endpoints found in the beginning of the hepatic effects section. Presumably, these descriptions of ALP are also relevant to humans.
- Page 3-150, lines 7 and 10: Please clarify if the text should read "organellar growth" as in line 7 or "organelle growth" as in line 10.

- There are also several molecular initiating event (MIE) and mode of action (MoA) papers in liver cells and tissues that have been published since 2019 that suggest there may be sex-specific MOA and that sex-specific effects should be better defined. hER and sex-specific hormone regulated genes may be involved.

3.2.5. Neurodevelopmental Effects

Suggested Revisions:

- Page 3-170, line 2: Why is the Butenhoff et al. (2009) publication considered medium confidence, when Figure 3-43 (page 3-171) gives it an overall high confidence?
- Page 3-171, lines 8 – 13: All in the same sentence, line 11 cites 3 doses of PFHxS (single oral) given on PND10, but on line 13, cites only the highest dose given on PND9. This sentence needs to be reviewed for accuracy and edited for increased clarity.
- Page 3-172, line 1: Should read: "…litter effects in their…" (the "i" is capitalized in the report).
- Page 3-172, line 10: Referring to the above comment on page 3-171, please verify that dosing was on PND9. Do the authors comment on the choice of PND9 for dosing? Also, please verify that the dose cited in line 10 is correct, as the earlier study design used 6.1 rather than 6.2 mg/kg-d.
- Page 3-172, line28: This sentence is confusing and the authors should consider revision, especially if this sentence is linked to the previous sentence where prenatal exposure is discussed and some of the same literature is cited.
- Page 3-174, Table 3-23, 5th row, 4th column: Please verify that the information is correct. States: "Inverse associations between cognition and PFHsX exposure were observed in X confidence? Studies, but…" (questionable language found in table highlighted by reviewer)
- Page 3-162: Why are there specific tables reporting cognition and ADHD and not one for ASD effects.
- Table 3-22, beginning page 3-167: In the "Study name" column, please check the font size for the hyperlinks to studies. They do not appear to be a consistent font size.
- Page 3-170: In general, expanded discussion on dose selection as it relates to human exposure levels would be helpful. In the cited literature, did any of the exposures cause any general toxicity to the dams or offspring? Were internal measures of exposure quantified in dams or offspring? This information would aid in overall interpretation of the results.
- Page 3-172, lines 6 – 7: When using the phrase "using the same study design," is this the same animals or an entirely new study?

3.2.6. Cardiometabolic Effects

Suggested Revisions:

- Page 3-176. There are some recent papers that reflect consistent associations of PFHxS with adverse change in cholesterol levels in adults and children that should be considered.
  1. Batzella E, Girardi P, Russo F, Pitter G, Da Re F, Fletcher T, Canova C. Perfluoroalkyl substance mixtures and cardio-metabolic outcomes in highly exposed male workers in the Veneto Region: A mixture-based approach. Environ Res. 2022 Sep;212(Pt A):113225. doi: 10.1016/j.envres.2022.113225. Epub 2022 Apr 4. PMID: 35390304.
  2. 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 Jun 25;827:153900. doi: 10.1016/j.scitotenv.2022.153900. Epub 2022 Feb 23. PMID: 35218824.

3. Canova C, Di Nisio A, Barbieri G, Russo F, Fletcher T, Batzella E, Dalla Zuanna T, Pitter G. PFAS Concentrations and Cardiometabolic Traits in Highly Exposed Children and Adolescents. Int J Environ Res Public Health. 2021 Dec 7;18(24):12881. doi: 10.3390/ijerph182412881. PMID: 34948492.
4. Sinisalu L, Yeung LWY, Wang J, Pan Y, Dai J, Hyötyläinen T. Prenatal exposure to poly-/per-fluoroalkyl substances is associated with alteration of lipid profiles in cord-blood. Metabolomics. 2021 Nov 24;17(12):103. doi: 10.1007/s11306-021-01853-9. PMID: 34816353.
5. Zare Jeddi M, Dalla Zuanna T, Barbieri G, Fabricio ASC, Daprà F, Fletcher T, Russo F, Pitter G, Canova C. Associations of Perfluoroalkyl Substances with Prevalence of Metabolic Syndrome in Highly Exposed Young Adult Community Residents-A Cross-Sectional Study in Veneto Region, Italy. Int J Environ Res Public Health. 2021 Jan 29;18(3):1194. doi: 10.3390/ijerph18031194. PMID: 33572770.
6. Dalla Zuanna T, Savitz DA, Barbieri G, Pitter G, Zare Jeddi M, Daprà F, Fabricio ASC, Russo F, Fletcher T, Canova C. The association between perfluoroalkyl substances and lipid profile in exposed pregnant women in the Veneto region, Italy. Ecotoxicol Environ Saf. 2021 Feb;209:111805. doi: 10.1016/j.ecoenv.2020.111805. Epub 2020 Dec 24. PMID: 33360787.

- Also consider the following for potential diabetic tendency:
  1. Zeeshan M, Zhang YT, Yu S, Huang WZ, Zhou Y, Vinothkumar R, Chu C, Li QQ, Wu QZ, Ye WL, Zhou P, Dong P, Zeng XW, Hu LW, Yang BY, Shen X, Zhou Y, Dong GH. Exposure to isomers of per- and polyfluoroalkyl substances increases the risk of diabetes and impairs glucose-homeostasis in Chinese adults: Isomers of C8 health project. Chemosphere. 2021 Sep;278:130486. doi: 10.1016/j.chemosphere.2021.130486. Epub 2021 Apr 5. PMID: 34126693.
- Page 3-202 (Considerations for interpreting the human relevance of the animal cardiometabolic evidence): The effect of a higher fat diet in humans is not mentioned and is potentially a reason for human-rodent disconnect, based on some recent publications suggesting a more human-like response in blood lipids following PFHxS exposures when a high fat diet is administered.
  1. Marques ES, Agudelo J, Kaye EM, Modaresi SMS, Pfohl M, Bečanová J, Wei W, Polunas M, Goedken M, Slitt AL. The role of maternal high fat diet on mouse pup metabolic endpoints following perinatal PFAS and PFAS mixture exposure. Toxicology. 2021 Oct;462:152921. doi: 10.1016/j.tox.2021.152921. Epub 2021 Aug 28. PMID: 34464680.
  2. Pfohl M, Ingram L, Marques E, Auclair A, Barlock B, Jamwal R, Anderson D, Cummings BS, Slitt AL. Perfluorooctanesulfonic Acid and Perfluorohexanesulfonic Acid Alter the Blood Lipidome and the Hepatic Proteome in a Murine Model of Diet-Induced Obesity. Toxicol Sci. 2020 Dec 1;178(2):311-324. doi: 10.1093/toxsci/kfaa148. PMID: 32991729.

3.2.7. Hematopoietic Effects

Suggested Revisions:

- Page 3-210, figure 3-55: For the NTP, 2018, study, reticulocytes were significantly decrease at the highest dose (10 mg/kg) in males; there should be a red triangle to indicate significant decrease in males at the 10 mg/kg dose.
- Page 3-206, line 4: Is something missing after “confounding”? “confounding factors”?
- Page 3-209, line 1: Rather than “did not report” (which sounds like it was not measured), suggest using “did not observe”.

3.2.8. Female Reproductive Effects

Suggested Revisions:

- Section 3.2.8. lacked any new references to enhance the weight of evidence for female reproduction. However, two important points should be considered. First, in some cases the authors are comparing effects in exposed adult females to female offspring exposed developmental/lactationally. These exposures should not be assumed to be same, as differences due to timing of exposure have been substantiated in female reproductive outcomes for other PFAS (PFOA) in rodents and humans. This important point is noted in the conclusions on page 4-3, yet disregard this in the data presented in Section 3.2.8. Second, did the studies in rodents compare hormones and reproductive tissue weights within the same stage of the estrous cycle? In rodents, stage of cycle is reported to alter uterine weights, steroid outcomes, and mammary gland developmental status. This is a critical point and if not controlled for, then the studies should be noted with deficits.
- Page 3-221, line 2: There is a typo; '..health women…" should be "…healthy women.."

3.2.9. Male Reproductive Effects

The available data have been clearly and appropriately synthesized to describe the strengths and limitations. The weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified.

3.2.10. Renal Effects

The available data have been clearly and appropriately synthesized to describe the strengths and limitations. The weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified.

The length of time of potential PFHxS exposures is a potential reason for the u-shaped curves and negative effects on glomerular filtration rate (GFR) in long-term studies. The Blake et al. (2018) study, for instance, was unique in that it had repeated measures from the same individuals over 10-18 years, strengthening the evidence for PFAS induced decrease in eGFR. Additionally, they conducted a sensitivity analysis examining outcome measures occurring after the first serum PFAS measurement only, and the association was consistent with the adjusted repeated measures model. Blake et al. also had PFHxS measures over that long timeframe and found consistent exposure levels over more than a decade in the community. This is a different (prospective) and more convincing set of data than some of the other cross-sectional cohorts – a fact that is not mentioned. In fact, in Fenton et al 2021 (Enviro Toxic and Chemistry), the authors state: "A propensity score approach to NHANES data (Jain and Ducatman 2019c; Zhao et al. 2020) and a study with repeated PFAS and health measures over an 18-yr period (Blake et al. 2018) recently concluded that PFAS exposure likely causes diminished renal glomerular filtration."

Suggested Revisions:

- Page 3-252, line 15: There was no increase in BUN in the NTP, 2018b study. Does this refer to the 3M, 2000a study only?
- Page 3-253, lines 12-14 has a double negative typo. "..none of the remaining studies exposing rats or mice to similar doses and durations (ranging from 28 to 44 days) did not observe significant PFHxS-induced changes in relative or absolute kidney weights." Some editing is needed.
- Page 3-248, Line 4: Is something missing after "confounding"? Confounding what?

- Page 3-248, lines 5-6: Please clarify what the numbers in parentheses refer to. It would be clearer to state "(three studies)" or "(six studies)", instead of only the number.

4. Summary of Hazard Identification Conclusions

The summary of hazard conclusions presents a clear summary of the two key health endpoints, immune effects and thyroid toxicity, as the endpoints for RfD derivation. However, it is not clear from the text in this section that an RfD for developmental effects would be derived in section 5.

Suggested Revisions:

- Page 4-1, lines 29-31 and page 4-2, lines 1-7: the text here seems to suggest that a RfD would not be derived for developmental toxicity. Suggest revising the sentence on page 4-2 lines 3-5 as follows. "However, a RfD for developmental effects was derived to convey some sense of the magnitude of an estimate for developmental effects based on the available data."

5. Derivation of Toxicity Values

5.2.1 Oral Reference Dose (RfD) Derivation

Immune effects

The derivation of an RfD for immune effects based on the data in humans as reported in Budtz-Jorgensen and Grandjean (2018) and Grandjean et al. (2012) is scientifically justified and appropriately represents a statistically significant adverse change relevant to immunosuppression (i.e., decreased serum anti-tetanus antibodies from vaccination) in children of both sexes and within 2 years of PFHxS exposure (a reasonable window after exposure). This study also increased the confidence (although not completely) that this immune effect was not due to confounding from other PFAS (PFOS and PFOA).

Suggested Revisions:

- The curve fit to the actual human antibody data should be shown in the appendix. There are fits available for the rat data, although it would be useful to show the actual data in combination with the confidence interval representation of the dose groups. The BMDs derived from the human antibody data are very low and there needs to be the utmost transparency about the quality of the data that is being modelled to derive these values. A systematic robustness/reliability analysis should also be performed on the antibody data by iteratively dropping data to determine the stability and reliability of the BMD values.
- Page 5-3, line 33-35: Add the age at outcome assessment for serum T3 levels in the NTP (2018a) study. The age was provided for the Ramhoj et al. (2020) study in the next sentence and it would be good to make the content of the sentences equivalent.
- Page 5-7, line 11: Add "changes" after "pregnancy hemodynamic": pregnancy hemodynamic changes.
- Page 5-16, line 33: The jump from thyroid to immune needs an intermediate sentence. Suggest "The immune response was also considered to have occurred under a lifetime exposure."