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Re: Docket EPA-HQ-ORD-2021-0561

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On behalf of the Natural Resources Defense Council (NRDC), I appreciate this opportunity to submit comments on EPA's Draft Toxicological Review for Perfluorohexanoic Acid (PFHxA).[1] I have reviewed and commented on the scientific and technical aspects of many federal and state level PFAS risk assessments including the EPA's assessments of PFOA, PFOS, GenX, PFBS, and PFBA, ATSDR's toxicological profile for perfluoroalkyls, and state assessments in CA, IL, ME, NH, NY, VT, and WA. In addition, I am the founder and co-creator of the PFAS-Tox Database (available at www.PFASToxDatabase.org), a systematic evidence map of the health and toxicological research available for 29 PFAS, including PFHxA.[2] To date, the publicly available, interactive PFAS-Tox Database contains 1,068 peer reviewed studies retrieved from PubMed Database (literature search last updated January 25, 2021). Through our searches, which are similar to those used by EPA, we have identified 120 studies on PFHxA (34 human studies, 24 animal studies, and 66 in vitro studies). I have included a listing of these studies and a comparison to EPA's findings in an attached document. It should be noted that the PECO (Populations, Exposures, Comparators, and Outcomes) statement used to guide the development of the PFAS-Tox Database is broader than was used by EPA (for example, we did not limit the routes of exposure in animal studies and we included observational animal studies in additional to experimental animal studies, we also included case-control epidemiological studies).

PFHxA is part of the massive family of synthetic per- and poly- fluorinated alkyl substances (PFAS). US EPA's CompTox program now lists over 12,000 PFAS in the PFAS Master List, over 10,000 of which have a structure assigned.[3,4] PFAS are characterized by incredible durability, which manifests as extreme persistence in the environment. The PFAS chemicals

[1] US EPA, *Toxicological Review of Perfluorohexanoic Acid [CASRN 307244] and Related Salts.* 2022. Washington DC. Available from: https://www.regulations.gov/document/EPA-HQ-ORD-2021-0561-0001.
[2] Pelch, K.E., et al., *PFAS Health Database: A Systematic Evidence Map*. 2021. Available from: https://osf.io/f9upx/.
[3] US EPA. *PFAS structures in DSSTox (update August 2021, Version 4)* 2021 August 10, 2021 [cited 2022 April 4]; Available from: https://comptox.epa.gov/dashboard/chemical-lists/PFASSTRUCTV4.
[4] US EPA. *PFAS Master List of PFAS Substances*. 2021 August 10, 2021 [cited 2022 February 10]; Available from: https://comptox.epa.gov/dashboard/chemical-lists/pfasmaster.

that have been studied show potent toxicity to internal organs, lipid metabolism, as well as the immune and endocrine systems.[5]

Given the number of people exposed to these chemicals, their persistence in the environment, and the public concern about them, it is critical that this toxicological review provides the information necessary to guide regulators and communities in their efforts to protect themselves. In this letter, I outline areas where the EPA has taken steps in the right direction as well as areas that need to be strengthened. I recognize the importance of this assessment and that communities exposed to these chemicals are eager for the EPA to complete this toxicological review, but I strongly urge the EPA to:

(1) update and strengthen this review by ensuring that it relies upon a more robust, up-to-date data set and
(2) account for cumulative risks that may occur from coexposure to additional PFAS, as is often the case in real-world exposure scenarios - where people are exposed to PFAS mixtures.

I applaud the EPA for the use of transparent systematic review practices in the development of this draft toxicological review. Systematic review has long been used to inform evidence-based choices about health interventions in clinical settings. Though the application of systematic review to questions in environmental health is still relatively new by comparison, the Integrated Risk Information System (IRIS) program at US EPA has been steadily implementing systematic review practices since receiving feedback in 2011 from the National Academies of Sciences, Engineering, and Medicine suggesting the need for programmatic reform.[6]

In particular, I support the use of the study confidence rating, which is in line with best practices for assessing risk of bias and closely aligns to the methods used by the National Toxicology Program's Office of Health Assessment and Translation (OHAT).[7] Importantly, the PECO (populations, exposures, comparators and outcomes) statement clearly outlines the criteria for inclusion and exclusion of studies in the assessment. I also support the transparent GRADE-like methods used for evidence integration in the draft PFHxA assessment. Finally, I appreciate the display of extracted PFHxA data in HAWC and Tableau, which made it very easy to evaluate the statements made in the draft PFHxA toxicological review.

The decisions that lead to EPA's choice of critical studies and endpoints for a quantitative assessment of health risks were clearly presented and well supported. Therefore, based on the

[5] Kwiatkowski, C.F., et al., *Scientific Basis for Managing PFAS as a Chemical Class.* Environ Sci Technol Lett, 2020. **7**(8): p. 532-543.
[6] National Academies of Sciences, Engineering, and Medicine., *Progress Toward Transforming the Integrated Risk Information System (IRIS) Program: A 2018 Evaluation*. 2018, Washington, DC: The National Academies Press.
[7] Office of Health Assessment and Translation, *Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration.* 2015. Available from: https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf.

available information, I support the conclusions reached by the EPA that the evidence evaluated within the toxicological review supports the conclusions that PFHxA likely causes hepatic, hematopoietic, and developmental effects in humans. Additionally, I support the conclusion these effects, though based in large part on rodent toxicological studies, represent adverse effects that are relevant to human health.

Though I largely support the conclusions reached by EPA, I also believe it is inappropriate for EPA to attempt to estimate the risks posed by PFHxA individually. I appreciate that EPA highlighted the utility of deriving organ/system-specific values as "the osRfDs can be useful for subsequent cumulative risk assessments."[8] However, EPA ultimately falls short of making use of these values, despite that similar values have already been derived by EPA for other PFAS, such as PFOA, PFOS, GenX, PFBS and PFBA. Americans most at risk of exposure to PFHxA will generally have greater than typical exposures to legacy PFAS chemicals as well. The available data suggests that PFHxA impacts the same body systems as other, better-studied PFAS. Given this, EPA should include a section on PFAS cumulative risks.

My specific comments address three major issues. Section 1 outlines methodological concerns regarding the conduct and reporting of the literature search, screening, and study selection. Section 2 addresses EPA's use of chemical-specific toxicokinetic parameters for the derivation of human relevant doses. Section 3 provides minor comments and recommendations for improvements in EPA's draft toxicological review of PFHxA.

1. Methodology and Reporting Concerns

A. EPA's draft toxicological assessment for PFHxA is out of date.

A concern with the draft toxicological review is that it is already a year out of date, with the last literature search conducted in April 2021. Moving forward, the EPA should ensure that all literature searches are conducted within six months of final publication, which would reflect best practices in systematic review. Basing toxicological reviews on the most current literature is essential given the rapid pace in which studies on PFAS are being published.

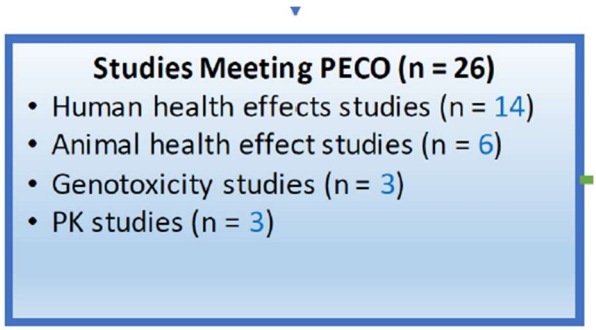
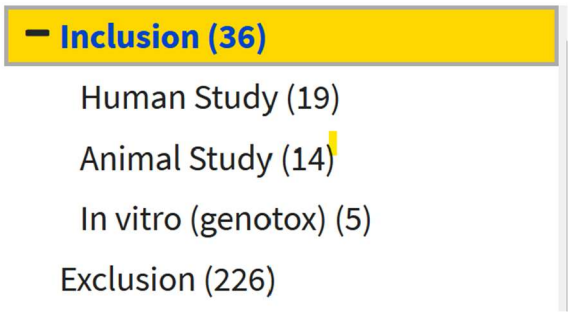
B. EPA's draft toxicological review contains unexplained inconsistencies.

I noted several inconsistencies and/or oversights in the reporting of the literature search and screening results, and the use of relevant supplemental information for evidence integration which I outline here.

[8] US EPA, *Toxicological Review of Perfluorohexanoic Acid [CASRN 307244] and Related Salts.* 2022. Washington DC. Available from: https://www.regulations.gov/document/EPA-HQ-ORD-2021-0561-0001.

i. The overall numbers are inconsistent across different pieces of the report.

The document states that there are 14 human studies, 6 animal studies, 3 genotoxicity studies, and 3 PK studies included (Figure 2-1). The text on page 34 in Section 2.2 also states that there are 14 human studies, but then only lists 13 studies (4 uninformative studies and 9 medium or low confidence studies). In comparison, however, the literature inventory in HAWC lists 19 human studies as included. Surprisingly, the visual on Tableau Public does not contain a tab for the human data. It is also unclear why Figure 2-1 shows four categories of included study types (human, animal, genotoxicity, and PK) and HAWC shows only three categories of included studies (human, animal, in vitro (genotox)). I strongly recommend that EPA confirm the number of included studies throughout the document, ensure there is consistent reporting across the various data display platforms, and provide an explanation for why human studies are not included in the Tableau Public display.

Screenshot of Included Studies Listed in Figure 2-1	Screenshot of References Tagged for Inclusion in HAWC
	

ii. There are inconsistencies in which human studies are reported as included.

I also note the following inconsistencies in studies listed in the document and those listed as included in HAWC: Qin et al., (2017) is discussed on page 134 in Section 3.2.8 but is not mentioned in Section 2.2 on page 34 where the study evaluation results are first discussed.[9] I also note that the Zhou et al., (2017) study listed as an included human study in HAWC[10] is not the same Zhou et al., (2017) study cited and hyperlinked in the

[9] Qin, X.D., et al., *Association of perfluoroalkyl substances exposure with impaired lung function in children.* Environ Res, 2017. **155**: p. 15-21.
[10] Zhou, Y., et al., *Perfluoroalkyl substance exposure and urine CC16 levels among asthmatics: A case-control study of children.* Ibid. **159**: p. 158-163.

PDF on page 134 in Section 3.2.8.[11] Two additional studies are listed in the included human studies in HAWC, but are not discussed in the document, Fan et al., (2014)[12] and Qin et al., (2016).[13]

Two additional human studies are listed as included in HAWC, but are never cited in the document.[14,15] Table 1 of the Maekawa et al., (2017) study lists "Perfluorohexanoic acid", but this seems to be a mistake, as the abbreviation in the table is provided as PFHxS (perfluorohexane sulfonic acid) and the authors then refer to the chemical throughout the remainder of the study as PFHxS. If possible, EPA should confirm the identity of this chemical with the study authors in order to determine if this study should be included in this toxicological review or not. Mamsen et al., (2017) investigates the relationship between maternal smoking and transfer of PFAS from the mother to the fetus. We did not include this study in the PFAS-Tox Database, as we determined it to not contain information on a health effect.

iii. There are several potentially relevant human studies unaccounted for.

In comparing the results of EPA's literature search to those we obtained when compiling the PFAS-Tox Database, I noticed several studies that were not included in the EPA review but that were part of the PFAS-Tox Database. Because it is difficult to access EPA's lists of supplemental and excluded studies, it is not obvious if these studies were reviewed and excluded, reviewed and considered supplemental, or not reviewed at all. I have attached an Excel workbook containing all of the available PFHxA data from PFAS-Tox Database, and noted which studies were part of the EPA systematic review. EPA should review the list of additional studies and clarify how these studies were processed during their systematic review. EPA should also improve the user access to included, excluded, and supplemental study lists to improve transparency in the screening and review process. I have not evaluated these studies to determine whether or not they would be deemed informative to the review, but am providing them as potential studies for consideration.

- In Section 3.2.2 there may be some additional human studies that could be informative to the human evidence of developmental effects. Specifically studies

[11] Zhou, Y., et al., *Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma among children.* Sci Rep, 2017. **7**(1): p. 899.
[12] Fan, H., A. Ducatman, and J. Zhang, *Perfluorocarbons and Gilbert syndrome (phenotype) in the C8 Health Study Population.* Environ Res, 2014. **135**: p. 70-5.
[13] Qin, X.D., Z. Qian, and M.G. Vaughn, *Positive associations of serum perfluoroalkyl substances with uric acid and hyperuricemia in children from Taiwan.* Environ Pollut, 2016. **212**(519-524): p. 10–1016-10–1016.
[14] Maekawa, R., et al., *Evidence of exposure to chemicals and heavy metals during pregnancy in Japanese women.* Reprod Med Biol, 2017. **16**(4): p. 337-348.
[15] Mamsen, L.S., et al., *Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma.* Sci Total Environ, 2017. **596-597**: p. 97-105.

by Antignac et al., 2013 (birth weight),[16] Gao et al., 2019 (birth weight, length, and ponderal index),[17] Jin et al., 2020, (infant weight growth rate, infant length growth rate)[18] and Li et al., 2020 (pre-term delivery).[19]

- In Section 3.2.3, there may be some additional human studies that could be informative to the human evidence for renal effects. Specifically, studies that investigated serum uric acid levels and hyperuricemia in children from Taiwan,[20] kidney function in a pediatric population with chronic kidney disease in sub-Saharan Africa,[21] and kidney function stage, hyperuricemia, uric acid in Chinese adults.[22]
- In Section 3.2.6, Emerce and Cetin (2018) evaluated PFHxA and other PFAS ability to induce genotoxic damage to human sperm cells, but this was not discussed in the section on male reproductive effects.[23] It is plausible this study was tagged by EPA as potentially relevant material.

[16] Antignac, J.P., et al., *Occurrence of perfluorinated alkylated substances in breast milk of French women and relation with socio-demographical and clinical parameters: results of the ELFE pilot study.* Chemosphere, 2013. **91**(6): p. 802-8.
[17] Gao, K., et al., *Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes.* Environ Sci Technol, 2019. **53**(11): p. 6529-6538.
[18] Jin, H., et al., *Poly- and perfluoroalkyl substance concentrations in human breast milk and their associations with postnatal infant growth.* Sci Total Environ, 2020. **713**: p. 136417.
[19] Li, J., et al., *Transplacental Transfer of Per- and Polyfluoroalkyl Substances (PFASs): Differences between Preterm and Full-Term Deliveries and Associations with Placental Transporter mRNA Expression.* Environ Sci Technol, 2020. **54**(8): p. 5062-5070.
[20] Qin, X.D., Z. Qian, and M.G. Vaughn, *Positive associations of serum perfluoroalkyl substances with uric acid and hyperuricemia in children from Taiwan.* Environ Pollut, 2016. **212**(519-524): p. 10–1016-10–1016.
[21] Sood, S., et al., *Association Between Perfluoroalkyl Substance Exposure and Renal Function in Children With CKD Enrolled in H3Africa Kidney Disease Research Network.* Kidney Int Rep, 2019. **4**(11): p. 1641-1645.
[22] Zeng, X.W., et al., *Isomers of per- and polyfluoroalkyl substances and uric acid in adults: Isomers of C8 Health Project in China.* Environ Int, 2019. **133**(Pt A): p. 105160.
[23] Emerce, E. and O. Cetin, *Genotoxicity assessment of perfluoroalkyl substances on human sperm.* Toxicol Ind Health, 2018. **34**(12): p. 884-890.

- Other potentially relevant human studies include those that looked at body mass index,[24,25,26,27,28] gestational diabetes,[29,30] breast cancer risk,[31] eye disease,[32] osteoporosis,[33] liver phenotype in morbidly obese,[34] and the estrogen receptor activity of human serum from exposed subjects.[35]

iv. There are inconsistencies in which animal studies are included.

As noted above, HAWC indicates there are 14 included animal studies, but figure 2-1 indicates there are only 6 animal studies. At least 9 of the studies listed in HAWC but not mentioned in the document are either NTP reports or archival data or are industry technical reports. It is unclear to the reader if these represent multiple reports of the same data. EPA should clarify how it counts and evaluates multiple reports of the same data that appears in multiple publications and/or provide further explanation as to why these studies were not further discussed in the toxicological review.

v. There are inconsistencies in the way supplemental materials are tagged and used for evidence synthesis.

Another important issue is that studies presumably marked as supplemental materials are not consistently referred to and discussed in the document. EPA should provide further guidance for when it will make use of available supplemental materials. Two

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[24] Duan, Y., et al., *Distribution of novel and legacy per-/polyfluoroalkyl substances in serum and its associations with two glycemic biomarkers among Chinese adult men and women with normal blood glucose levels.* Environ Int, 2020. **134**: p. 105295.
[25] Li, J., et al., *Urine concentrations of perfluoroalkyl acids in children and contributions of dietary factors: a cross-sectional study from Shanghai, China.* Environ Sci Pollut Res Int, 2021.
[26] Ramli, M.R., et al., *Level and determinants of serum perfluoroalkyl acids (PFAAs) in a population in Klang Valley, Malaysia.* Int J Hyg Environ Health, 2020. **223**(1): p. 179-186.
[27] Siebenaler, R., et al., *Serum perfluoroalkyl acids (PFAAs) and associations with behavioral attributes.* Chemosphere, 2017. **184**: p. 687-693.
[28] Tian, Y.P., et al., *Isomers of perfluoroalkyl substances and overweight status among Chinese by sex status: Isomers of C8 Health Project in China.* Environ Int, 2019. **124**: p. 130-138.
[29] Liu, X., et al., *Structure-based investigation on the association between perfluoroalkyl acids exposure and both gestational diabetes mellitus and glucose homeostasis in pregnant women.* Ibid. **127**: p. 85-93.
[30] Liu, X., et al., *Identification and prioritization of the potent components for combined exposure of multiple persistent organic pollutants associated with gestational diabetes mellitus.* J Hazard Mater, 2021. **409**: p. 124905.
[31] Tsai, M.S., et al., *A case-control study of perfluoroalkyl substances and the risk of breast cancer in Taiwanese women.* Environ Int, 2020. **142**: p. 105850.
[32] Zeeshan, M., et al., *Incidence of ocular conditions associated with perfluoroalkyl substances exposure: Isomers of C8 Health Project in China.* Ibid. **137**: p. 105555.
[33] Banjabi, A.A., et al., *Serum concentrations of perfluoroalkyl substances and their association with osteoporosis in a population in Jeddah, Saudi Arabia.* Environ Res, 2020. **187**: p. 109676.
[34] Rantakokko, P., et al., *Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a cohort study.* Environ Health, 2015. **14**: p. 79.
[35] Bjerregaard-Olesen, C., et al., *Extraction of perfluorinated alkyl acids from human serum for determination of the combined xenoestrogenic transactivity: a method development.* Chemosphere, 2015. **129**: p. 232-8.

animal studies are marked in HAWC as being excluded for having a non-PECO route of exposure.[36,37] The Kudo et al., (2006) study is a mouse study in which animals were exposed by intraperitoneal injection and appears to contain hepatic outcomes that are relevant to the discussion in section 3.2.1. It is understandable that this study was marked as "potentially relevant supplemental material" during the literature search and screening. However it is not clear why it was not discussed in the evidence synthesis step (Section 3), as other mechanistic and i*n vitro* studies are described in this section. It is also unclear why Kudo et al., (2011) was not marked as supplemental material for non-PECO route of exposure as it too describes potential hepatic effects in mice exposed to PFHxA via intraperitoneal injection.[38]

In addition to these two studies that were marked as non-PECO routes of exposure, there are at least 16 additional animal studies that could be considered potentially relevant supplemental materials. This list includes several observational wildlife studies,[39,40,41,42,43,44] mechanistic studies in species such as Daphnia,[45] rotifer,[46] and fish,[47,48] and studies that may be relevant to the evidence profiles for PFHxA (described below):

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[36] Kudo, N., et al., *Responses of the liver to perfluorinated fatty acids with different carbon chain length in male and female mice:in relation to induction of hepatomegaly, peroxisomal beta-oxidation and microsomal 1-acylglycerophosphocholine acyltransferase.* Biol Pharm Bull, 2006. **29**(9): p. 1952-7.
[37] Cassone, C.G., et al., *In ovo effects of perfluorohexane sulfonate and perfluorohexanoate on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos.* Toxicol Sci, 2012. **127**(1): p. 216-24.
[38] Kudo, N., et al., *Effects of perfluorinated fatty acids with different carbon chain length on fatty acid profiles of hepatic lipids in mice.* Biol Pharm Bull, 2011. **34**(6): p. 856-64.
[39] Robuck, A.R., et al., *Legacy and Novel Per- and Polyfluoroalkyl Substances in Juvenile Seabirds from the U.S. Atlantic Coast.* Environ Sci Technol, 2020. **54**(20): p. 12938-12948.
[40] Parolini, M., et al., *Within- and Among-Clutch Variation of Yolk Perfluoroalkyl Acids in a Seabird from the Northern Adriatic Sea.* Environ Toxicol Chem, 2020.
[41] Soloff, A.C., et al., *Environmental perfluorooctane sulfonate exposure drives T cell activation in bottlenose dolphins.* J Appl Toxicol, 2017. **37**(9): p. 1108-1116.
[42] Levengood, J.M., et al., *Interspecific and Spatial Comparisons of Perfluorinated Compounds in Bighead and Silver Carp in the Illinois River, Illinois, USA.* Bull Environ Contam Toxicol, 2015. **95**(5): p. 561-6.
[43] Routti, H., et al., *Spatial and temporal trends in perfluoroalkyl substances (PFASs) in ringed seals (Pusa hispida) from Svalbard.* Environ Pollut, 2016. **214**: p. 230-238.
[44] Dassuncao, C., et al., *Phospholipid Levels Predict the Tissue Distribution of Poly- and Perfluoroalkyl Substances in a Marine Mammal.* Environ Sci Technol Lett, 2019. **6**(3): p. 119-125.
[45] Barmentlo, S.H., et al., *Acute and chronic toxicity of short chained perfluoroalkyl substances to Daphnia magna.* Environ Pollut, 2015. **198**: p. 47-53.
[46] Wang, Y., et al., *Toxicity assessment of perfluorinated carboxylic acids (PFCAs) towards the rotifer Brachionus calyciflorus.* Sci Total Environ, 2014. **491-492**: p. 266-70.
[47] Blanc, M., et al., *Mixture-specific gene expression in zebrafish (Danio rerio) embryos exposed to perfluorooctane sulfonic acid (PFOS), perfluorohexanoic acid (PFHxA) and 3,3',4,4',5-pentachlorobiphenyl (PCB126).* Ibid.2017. **590-591**: p. 249-257.
[48] Benninghoff, A.D., et al., *Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro.* Toxicol Sci, 2011. **120**(1): p. 42-58.

- Table 3-16 provides the evidence profile for developmental effects. The last line in the table indicates that there is no supporting mechanistic evidence or supplemental information for developmental endpoints but this is not accurate. There are studies on developmental effects and early life mortality from other species, primarily fish[49,50,51,52,53] and frogs.[54] As per the text on page 27, non-PECO populations, including non mammalian models would be considered "potentially relevant supplemental material."
- In Section 3.2.5, there are a few additional *in vitro* studies that are related to thyroid hormone activity that were not mentioned in the document that should be evaluated as potentially relevant supplemental material.[55,56,57]
- Section 3.2.5 discusses endocrine effects of PFHxA, but focuses primarily on the potential for PFHxA to disrupt thyroid hormone signaling. I also note that other endocrine-related activities are explored in the *in vitro*/mechanistic data beyond thyroid hormone activity, including androgen activity, estrogen activity, glucocorticoid activity, aryl hydrocarbon receptor activity, and progesterone receptor activity. However these endpoints are not discussed, presumably because there is an absence of corresponding epidemiological or toxicological studies. EPA should at least mention and summarize these mechanistic studies, in order to more fully describe the potential endocrine effects of PFHxA. Further, it is unclear why Table 3-29 does not summarize the available mechanistic evidence and supplemental information for endocrine effects that is discussed in the evidence integration (on page 114, lines 29-32) for this health effect category.

[49] Wasel, O., et al., *Comparison of zebrafish in vitro and in vivo developmental toxicity assessments of perfluoroalkyl acids (PFAAs).* J Toxicol Environ Health A, 2021. **84**(3): p. 125-136.
[50] Annunziato, K.M., et al., *Subtle morphometric, behavioral and gene expression effects in larval zebrafish exposed to PFHxA, PFHxS and 6:2 FTOH.* Aquat Toxicol, 2019. **208**: p. 126-137.
[51] Gaballah, S., et al., *Evaluation of Developmental Toxicity, Developmental Neurotoxicity, and Tissue Dose in Zebrafish Exposed to GenX and Other PFAS.* Environ Health Perspect, 2020. **128**(4): p. 47005.
[52] Menger, F., et al., *Behavioural effects and bioconcentration of per- and polyfluoroalkyl substances (PFASs) in zebrafish (Danio rerio) embryos.* Chemosphere, 2020. **245**: p. 125573.
[53] Dasgupta, S., et al., *High-content screening in zebrafish identifies perfluorooctanesulfonamide as a potent developmental toxicant.* Environ Pollut, 2020. **256**: p. 113550.
[54] Kim, M., et al., *Perfluoroheptanoic acid affects amphibian embryogenesis by inducing the phosphorylation of ERK and JNK.* Int J Mol Med, 2015. **36**(6): p. 1693-700.
[55] Vongphachan, V., et al., *Effects of perfluoroalkyl compounds on mRNA expression levels of thyroid hormone-responsive genes in primary cultures of avian neuronal cells.* Toxicol Sci, 2011. **120**(2): p. 392-402.
[56] Weiss, J.M., et al., *Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin.* Ibid.2009. **109**: p. 206-16.
[57] Naile, J.E., et al., *Transcriptional effects of perfluorinated compounds in rat hepatoma cells.* Chemosphere, 2012. **86**(3): p. 270-7.

2. Support of EPA's use of chemical-specific toxicokinetic parameters

It is unfortunate that a more complete toxicokinetic profile is not currently available in the literature for PFHxA. Given that, I support EPA's overall approach to use the ratio of clearance rates. I agree with EPA that using data-informed clearance value-based dosimetric adjustment factors is preferred to using dosimetric adjustment factors relying on $BW^{3/4}$ scaling, an approach that is not specific to this class of chemicals.

3. Recommendations for improvements in EPA's derivation of toxicity values

A. Uncertainties are not appropriately accounted for.

I note that in the recent Human Health Toxicity Values derivation for PFBS EPA included a database uncertainty factor of 10, citing a lack of chronic studies and neurodevelopmental and immunotoxicity studies as well as a lack of mammary gland studies.[58] Though developmental, reproductive, and chronic exposure studies were available for PFHxA, I note that the specific endpoints noted as deficiencies for PFBS were also generally lacking in the literature for PFHxA.

It is therefore unclear why EPA drew a different conclusion in the draft toxicological review of PFHxA, deciding to only apply a partial database uncertainty factor of 3. I suggest that to be adequately protective of public health, and consistent across assessments given the same underlying concerns about the lack of data, EPA should use the same database uncertainty factor for PFHxA as was used in the finalized Human Health Toxicity Value derivation of PFBS, which is 10.

In addition, I note that biomonitoring studies demonstrate that Americans have chronic exposure to multiple PFAS chemicals throughout their lifetimes. Therefore, it is impossible to be exposed to PFHxA and no other PFAS chemicals. CDC's NHANES studies reveal that nearly every American has detectable concentrations of four PFAS chemicals in their bloodstream (PFOS,

[58] US EPA, *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)* 2021. Washington DC. Available from: https://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=542393.

PFOA, PFHxS and PFNA)[59]. Multiple other PFAS have been detected in NHANES and state biomonitoring programs.[60]

Toxicity assessment should account for simultaneous exposure to other PFAS chemicals that impact the same target organs. EPA does this for its reference dose (RfD) used to establish the present drinking water guideline for the sum of PFOS and PFOA:

> "Adverse effects observed following exposures to perfluorooctanoic acid (PFOA) and PFOS are the same or similar and include effects in humans on serum lipids, birth weight, and serum antibodies. Some of the animal studies show common effects on the liver, neonate development, and responses to immunological challenges. Both compounds were also associated with tumors in long-term animal studies. The RfDs for both PFOA and PFOS are based on similar developmental effects and are numerically identical; when these two chemicals co-occur at the same time and location in a drinking water source, a conservative and health-protective approach that EPA recommends would be to compare the sum of the concentrations ([PFOA] + [PFOS]) to the [Heath Advisory] (0.07 µg/L)."[61]

The European Food Safety Authority also allows for the consideration of additive effects for chemicals that target the same health endpoint, even when mode of action is unknown,[62] as does the National Academy of Sciences.[63,64] The Netherlands pioneered this approach for PFAS with a relative potency estimate for liver hypertrophy using experimental data for 11 perfluoroalkyl sulfonates and perfluoroalkyl carboxylates and read across assumptions for 7 additional PFAS.[65] EPA must promote similar assessments for other PFAS related health outcomes with potential for additive toxicity, including kidney and liver toxicity, lipid metabolism, birth outcomes, immunotoxicity and developmental effects. To better account for the cumulative exposure to multiple PFAS, EPA should add an additional uncertainty factor due to the high likelihood of additive effects with other PFAS.

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[59] Ye, X., et al., *Per- and polyfluoroalkyl substances in sera from children 3 to 11 years of age participating in the National Health and Nutrition Examination Survey 2013-2014.* Int J Hyg Environ Health, 2018. **221**(1): p. 9-16.

[60] California Biomonitoring. *Results for Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs)* 2020 [cited 2021 November 5]; Available from: https://biomonitoring.ca.gov/results/chemical/2183.

[61] US EPA, *Drinking Water Health Advisory for perfluorooctanoic acid (PFOA).* 2016. Washington DC. Available from: https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_health_advisory_final-plain.pdf.

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B. Minor comments.

Overall I found the document well written, easy to follow, and in agreement with the workflow that was proposed in the *a priori* published protocol. I noted the following inconsistencies and/or opportunities to provide additional clarity in the document, which should be addressed before the review is finalized.

- To help readers better understand why some studies have been included and others have not, I also recommend that EPA make available the lists of excluded studies at each level, and when possible, to provide one or more reasons for exclusion. To this end, it would be most useful if Figure 2-1 could be linked to lists of such studies, perhaps in HAWC. Figure 2-1 currently contains numbers in blue, a color that is commonly used (including within this document) to indicate hyperlinked text. Yet the numbers in Figure 2-1 are not hyperlinked and lists that directly correlate to the data presented in Figure 2-1 are either not available, or are not easily available. At the very least, these numbers should not be presented in blue text.
- Page 165, line 9, seems like it should read, “Thus, three other PFAS, including one with the same carbon-chain length as PFHxA…” rather than “...length as PFHxS.”
- It would be helpful if Table 5-5 contained a column with the DAF that was used so that the reader doesn’t have to jump back and forth to Table 5-1, given that different DAF were used based on whether the study was conducted in rats or mice and whether the endpoint was observed in males or females. Furthermore, it would be helpful if EPA could elaborate on the superscript “a” in which it is stated “DAF values for female rats and female mice were used for the respective developmental effects on combined male and female pups of each species.” Can you please elaborate on how the DAF was applied for combined effects on both sexes?

Conclusions

In conclusion, I urge the agency to strengthen its final toxicological review and have outlined several inconsistencies and deficiencies that must be corrected in the final document. I also urge the agency to move quickly to incorporate our recommendations based on the latest science, and finalize the profile in a timely manner.

Respectfully submitted,

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Attachments: Lists of studies downloaded from PFAS-Tox Database compared to those included in this draft toxicology report.