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Re: Docket EPA-HQ-ORD-2021-0562 Draft IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS, CASRN 335-46-4) and Related Salts

September 22, 2023

On behalf of the Natural Resources Defense Council (NRDC), we appreciate this opportunity to submit comments on EPA's Draft Toxicological Review for Perfluorohexanesulfonic Acid (PFHxS) and Related Salts.[1] We 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, PFBA, PFHxA, PFDA, ATSDR's toxicological profile for perfluoroalkyls, and state assessments in CA, IL, ME, NH, NY, VT, and WA. In addition, we are the founders and co-creators 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 PFHxS.[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).

PFHxS is part of the massive family of synthetic per- and polyfluoroalkyl substances (PFAS). US EPA's CompTox program now lists over 14,000 PFAS structures.[3] PFAS are characterized by incredible durability, which manifests as extreme persistence in the environment. The PFAS

[1] US EPA. "IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS, CASRN 335-46-4) and Related Salts." External Review Draft, July 24, 2023.
[2] Pelch, Katherine E., Anna Reade, Carol F. Kwiatkowski, Francheska M. Merced-Nieves, Haleigh Cavalier, Kim Schultz, Taylor Wolffe, and Julia Varshavsky. "The PFAS-Tox Database: A Systematic Evidence Map of Health Studies on 29 per- and Polyfluoroalkyl Substances." *Environment International* 167 (September 1, 2022): 107408. https://doi.org/10.1016/j.envint.2022.107408; Pelch, Katherine E., Anna Reade, Carol F. Kwiatkowski, Francheska M. Merced-Nieves, Haleigh Cavalier, Kim Schulz, Keshia Rose, and Julia R. Varshavsky. "PFAS-Tox Database." PFAS-Tox Database, April 20, 2021. https://doi.org/10.17605/OSF.IO/F9UPX.
[3] US EPA. "CompTox Chemicals Dashboard - Navigation Panel to PFAS Structure Lists," August 18, 2022. https://comptox.epa.gov/dashboard/chemical-lists/pfasstruct.

chemicals that have been well-studied show potent toxicity to internal organs, lipid metabolism, as well as the immune and endocrine systems.[4]

PFHxS exposure is widespread, likely in part due to its historic use in aqueous film forming foams for firefighting. PFHxS is one of the most frequently detected PFAS in biomonitoring studies, with studies reporting ≥99% detection frequencies.[5] The EPA recently sought input on potentially listing PFHxS as a hazardous substance under CERCLA, highlighting the importance of this toxicological review.[6] The EPA also recently sought input on regulating PFHxS in drinking water using a hazard index approach in which the additive effects of PFHxS, PFNA, GenX and PFBS were acknowledged.[7] The health based water concentration for the proposed drinking water regulation for PFHxS was based on the minimum risk level calculated by ATSDR.[8] We argue here that the EPA has conducted a much more thorough and transparent review of the PFHxS health and toxicological data than was previously conducted by ATSDR, supporting the need for a stricter reference dose as reflected in this draft Toxicological Review.

Given the number of people exposed to PFAS, 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 these comments we outline areas where the EPA has taken steps in the right direction as well as areas that need to be strengthened. We recognize the importance of this assessment and that communities exposed to these chemicals are eager for the EPA to complete this toxicological review. We strongly urge the EPA to finalize this toxicological assessment as quickly as possible. In its finalization of this assessment, please consider the following comments provided here. Furthermore, as EPA continues to work on PFAS, we urge EPA to take concrete steps towards accounting for cumulative risks that may occur from coexposure to additional PFAS, as

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[4] Kwiatkowski, Carol F., David Q. Andrews, Linda S. Birnbaum, Thomas A. Bruton, Jamie C. DeWitt, Detlef R. U. Knappe, Maricel V. Maffini, et al. "Scientific Basis for Managing PFAS as a Chemical Class." *Environmental Science & Technology Letters* 7, no. 8 (August 11, 2020): 532–43. https://doi.org/10.1021/acs.estlett.0c00255.

[5] Boronow, Katherine E., Julia Green Brody, Laurel A. Schaider, Graham F. Peaslee, Laurie Havas, and Barbara A. Cohn. "Serum Concentrations of PFASs and Exposure-Related Behaviors in African American and Non-Hispanic White Women." *Journal of Exposure Science & Environmental Epidemiology* 29, no. 2 (March 2019): 206–17. https://doi.org/10.1038/s41370-018-0109-y; Calafat, Antonia M., Kayoko Kato, Kendra Hubbard, Tao Jia, Julianne Cook Botelho, and Lee-Yang Wong. "Legacy and Alternative Per- and Polyfluoroalkyl Substances in the U.S. General Population: Paired Serum-Urine Data from the 2013-2014 National Health and Nutrition Examination Survey." *Environment International* 131 (October 2019): 105048. https://doi.org/10.1016/j.envint.2019.105048; Ding, Ning, Carrie A. Karvonen-Gutierrez, William H. Herman, Antonia M. Calafat, Bhramar Mukherjee, and Sung Kyun Park. "Perfluoroalkyl and Polyfluoroalkyl Substances and Body Size and Composition Trajectories in Midlife Women: The Study of Women's Health across the Nation 1999–2018." *International Journal of Obesity* 45, no. 9 (September 2021): 1937–48. https://doi.org/10.1038/s41366-021-00848-9.

[6] "Addressing PFAS in the Environment." Federal Register. Proposed Rule, April 13, 2023. https://www.federalregister.gov/documents/2023/04/13/2023-07535/addressing-pfas-in-the-environment.

[7] US EPA. "PFAS National Primary Drinking Water Regulation Rulemaking." *Federal Register*, Proposed Rules, 88, no. 60 (March 29, 2023): 18638.

[8] ATSDR. "Toxicological Profile for Perfluoroalkyls," May 2021. https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf.

is often the case in real-world exposure scenarios - where people are exposed to PFAS mixtures.

Overall, this assessment is transparent and follows best practices

We 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 the 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.[9]

In particular, we 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).[10] Importantly, the PECO (populations, exposures, comparators and outcomes) statement clearly outlines the criteria for inclusion and exclusion of studies in the assessment. We also support the transparent GRADE-like methods used for evidence integration in the draft PFHxS assessment. Finally, we appreciate the display of extracted PFHxS data in HAWC, which made it very easy to evaluate the statements made in the draft PFHxS toxicological review. We also appreciate that the EPA has made available the complete study list within HAWC so that it is possible to understand and evaluate decisions made to include, exclude, or mark as supplemental individual studies.

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 available information, we support the conclusions reached by the EPA that the evidence evaluated within the toxicological review supports the conclusions that PFHxS likely causes thyroid and developmental immune effects. We further support the conclusion that early life represents a susceptible life stage for the effects of PFHxS exposure.

In particular, we support the EPA's evidence integration conclusions regarding the immunotoxicity of PFHxS. The EPA determined there is moderate evidence for immune system effects based on epidemiological studies that evaluate antibody levels in response to vaccines. This evidence of immunosuppression is corroborated by additional studies that find a higher rate of infectious diseases with increasing PFHxS exposure. We support the EPA's conclusion on this endpoint and disagree with critiques on this topic that have recently been published, which

[9] 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.
[10] 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.

question the biological significance of a decreased antibody response.[11] To require data directly linking PFHxS exposure to both altered vaccine response and increased rates of those vaccine-controlled diseases would be unreasonable given the widespread uptake of vaccines and other public health measures that keep disease like tetanus and diphtheria at a minimum.

We also support EPA's decision to calculate and present multiple candidate organ specific reference doses (osRfD) based on several identified critical endpoints from medium and high confidence studies. Our analysis of reference dose derivation for PFAS across multiple agencies highlights that simply choosing the lowest human equivalent dose ("HED") to derive a RfD does not necessarily guarantee that the RfD will protect against all health effects. A less sensitive HED could reasonably result in a lower RfD due to differences in study design and overall application of uncertainty. The IRIS PFAS assessments, including this assessment of PFHxS, are transparent and follow best practices in calculating osRfDs for multiple identified health effects.

Though we largely support the conclusions reached by the EPA, we also believe the risks of exposure to PFHxS is an underestimate, as this analysis does not account for cumulative exposure to multiple PFAS. We appreciate that the EPA has previously highlighted the utility of deriving organ/system-specific values as "the osRfDs can be useful for subsequent cumulative risk assessments."[12] However, the EPA ultimately falls short of making use of these values, despite that similar values have already been derived by the EPA for other PFAS, such as PFOA, PFOS, GenX, PFBS, PFBA, PFHxA, and PFDA. Americans most at risk of exposure to PFHxS will generally have greater than typical exposures to other legacy PFAS chemicals as well. The available data suggests that PFHxS impacts the same body systems as other PFAS. Given this, the EPA should include a section on PFAS cumulative risks.

Suggested improvements

1. EPA's draft toxicological assessment for PFHxS may be missing relevant health studies.

In Section 2.1 Lines 6-8, EPA indicates that there are 446 studies meeting the PECO criteria, specifically 415 epidemiological studies and 20 animal studies.[13] This is consistent with the information presented in Figure 2-1. However, the text immediately following in Section 2.2. Lines 1-6 states that there are 117 epidemiological studies and 8 animal studies meeting the PECO criteria. There is no indication as to why these values are so different, but we suspect

[11] Antoniou, Evangelia, Thomas Colnot, Maurice Zeegers, and Wolfgang Dekant. "Immunomodulation and Exposure to Per- and Polyfluoroalkyl Substances: An Overview of the Current Evidence from Animal and Human Studies." *Archives of Toxicology* 96, no. 8 (August 2022): 2261–85. https://doi.org/10.1007/s00204-022-03303-4.
[12] 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
[13] US EPA. "IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS, CASRN 335-46-4) and Related Salts." External Review Draft, July 24, 2023.

that the information presented in Section 2.2. likely is outdated and or reflects a typographical error. HAWC, for example, indicates that 20 animal studies were included (though we note that the study by Chang et al. is probably a double entry with HAWC IDs of 100518054 and 101366633).[14] We come to this conclusion because there are animal studies listed in HAWC as included, but are not subsequently listed in Section 2.2 Lines 1-6, yet these studies are cited later in the document in Evidence Synthesis and Integration summaries in Section 3.[15] We also note there are other studies listed in Section 2.2 that are not provided in the HAWC list of included studies.[16] We hope that these are, in fact, typographical errors and that EPA has not improperly and without justification excluded more than half of the evidence base from further discussion. We look forward to further clarification on this important issue.

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[14] https://hawc.epa.gov/lit/assessment/100500074/references/?tag_id=100502208

[15] Tetzlaff, Cecilie Nethe Ramskov, Louise Ramhøj, Aurélie Lardenois, Marta Axelstad, Bertrand Evrard, Frédéric Chalmel, Camilla Taxvig, and Terje Svingen. "Adult Female Rats Perinatally Exposed to Perfluorohexane Sulfonate (PFHxS) and a Mixture of Endocrine Disruptors Display Increased Body/Fat Weights without a Transcriptional Footprint in Fat Cells." *Toxicology Letters* 339 (March 15, 2021): 78–87. https://doi.org/10.1016/j.toxlet.2020.12.018; Viberg, Henrik, Iwa Lee, and Per Eriksson. "Adult Dose-Dependent Behavioral and Cognitive Disturbances after a Single Neonatal PFHxS Dose." *Toxicology* 304 (February 8, 2013): 185–91. https://doi.org/10.1016/j.tox.2012.12.013; Yin, Xiaorui, Tingting Di, Xinyuan Cao, Zhengnan Liu, Jingyan Xie, and Suyun Zhang. "Chronic Exposure to Perfluorohexane Sulfonate Leads to a Reproduction Deficit by Suppressing Hypothalamic Kisspeptin Expression in Mice." *Journal of Ovarian Research* 14, no. 1 (October 27, 2021): 141. https://doi.org/10.1186/s13048-021-00903-z; He, Xiwei, Jinhong Jiang, and Xu-Xiang Zhang. "Environmental Exposure to Low-Dose Perfluorohexanesulfonate Promotes Obesity and Non-Alcoholic Fatty Liver Disease in Mice Fed a High-Fat Diet." *Environmental Science and Pollution Research* 29, no. 32 (July 1, 2022): 49279–90. https://doi.org/10.1007/s11356-022-19369-7; Das, Kaberi P., Carmen R. Wood, Mimi T. Lin, Anatoly A. Starkov, Christopher Lau, Kendall B. Wallace, J. Christopher Corton, and Barbara D. Abbott. "Perfluoroalkyl Acids-Induced Liver Steatosis: Effects on Genes Controlling Lipid Homeostasis." *Toxicology* 378 (March 1, 2017): 37–52. https://doi.org/10.1016/j.tox.2016.12.007; Sim, Kyeong Hwa, and Youn Ju Lee. "Perfluorohexane Sulfonate Induces Memory Impairment and Downregulation of Neuroproteins via NMDA Receptor-Mediated PKC-ERK/AMPK Signaling Pathway." *Chemosphere* 288 (February 1, 2022): 132503. https://doi.org/10.1016/j.chemosphere.2021.132503; Pfohl, Marisa, Lishann Ingram, Emily Marques, Adam Auclair, Benjamin Barlock, Rohitash Jamwal, Dwight Anderson, Brian S. Cummings, and Angela L. Slitt. "Perfluorooctanesulfonic Acid and Perfluorohexanesulfonic Acid Alter the Blood Lipidome and the Hepatic Proteome in a Murine Model of Diet-Induced Obesity." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 178, no. 2 (December 1, 2020): 311–24. https://doi.org/10.1093/toxsci/kfaa148; Rosen, Mitchell B., Kaberi P. Das, John Rooney, Barbara Abbott, Christopher Lau, and J. Christopher Corton. "PPARα-Independent Transcriptional Targets of Perfluoroalkyl Acids Revealed by Transcript Profiling." *Toxicology* 387 (July 15, 2017): 95–107. https://doi.org/10.1016/j.tox.2017.05.013; "Support: Metabolism, Toxicity and Epidemiological Studies of Fluorochemicals, with Attachments and Cover Letter Dated 04/25/2000." Hazleton Labs. America, Inc., Madison, WI.; Environmental Protection Agency, Washington, DC. Office of Toxic, 2000. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS020492910.xhtml; Zeng, Guowei, Qi Zhang, Xiaowei Wang, and Kai-Hong Wu. "The Relationship between Multiple Perfluoroalkyl Substances and Cardiorespiratory Fitness in Male Adolescents." *Environmental Science and Pollution Research* 29, no. 35 (July 1, 2022): 53433–43. https://doi.org/10.1007/s11356-022-19685-y; Marques, Emily S., Juliana Agudelo, Emily M. Kaye, Seyed Mohamad Sadegh Modaresi, Marisa Pfohl, Jitka Bečanová, Wei Wei, Marianne Polunas, Michael Goedken, and Angela L. Slitt. "The Role of Maternal High Fat Diet on Mouse Pup Metabolic Endpoints Following Perinatal PFAS and PFAS Mixture Exposure." *Toxicology* 462 (October 2021): 152921. https://doi.org/10.1016/j.tox.2021.152921.

[16] 3M. "Oral (Gavage) Combined Repeated Dose Toxicity Study of T-7706 with the Reproduction/Developmental Toxicity Screening Test," 2003. https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/4241233.

In addition, we have additional concerns about studies that may have been erroneously excluded from the analysis either by excluding studies at the title and abstract level or by marking studies as "supplemental". These concerns stem from our comparison of studies that were listed as "included" in HAWC[17] with studies that are currently available in the PFAS-Tox Database. [18] The PFAS-Tox Database was built using literature searches and a PECO statement similar to that used by EPA[19]. The PFAS-Tox Database currently indicates there are 578 studies that evaluate a health or toxicological endpoints for PFHxS (literature search through January 2021); specifically, there are 449 human studies, 59 animal studies, and 71 *in vitro* studies.

We have included an attachment with a listing of the human studies that were included in the PFAS-Tox Database but were not tagged as included in HAWC (Worksheet labeled "PFASTox Studies Not Included" in the attachment). The attachment contains a brief summary of the endpoints that are relevant to human health (column S). We encourage peer reviewers to also review this attachment, specifically noting if there are any studies that would be of importance to the health effects that they are charged with reviewing in detail. We agree that some of the studies that were included in the PFAS-Tox Database may be out of the scope of the EPA's analysis (for example if the only noted health effect is body mass index (BMI)). However, reviewers may be particularly interested in studies that EPA marked as supplemental, and we suggest they be checked to see whether they contain additional information on health outcomes relevant to this assessment.

We did identify one study that appeared to be marked as included by EPA, but did not have an evidence stream tag (i.e. human) applied to it, and it was therefore missing from the list of included studies we downloaded from HAWC.[20] We discuss below other studies that may warrant additional review, either because they were marked as excluded at TIAB level or because EPA marked them as supplemental:

- An epidemiological study by Mogensen et al. (2015) that appears to have information on PFHxS and vaccine-related antibody response[21]

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[17] https://hawc.epa.gov/lit/assessment/100500074/references/?tag_id=100512631; https://hawc.epa.gov/lit/assessment/100500074/references/?tag_id=100502208; or https://hawc.epa.gov/lit/assessment/100500074/references/?tag_id=100513255

[18] Pelch, Katherine E., Anna Reade, Carol F. Kwiatkowski, Francheska M. Merced-Nieves, Haleigh Cavalier, Kim Schulz, Keshia Rose, and Julia R. Varshavsky. "PFAS-Tox Database." PFAS-Tox Database, April 20, 2021. https://doi.org/10.17605/OSF.IO/F9UPX.

[19] Pelch, Katherine E., and Carol F. Kwiatkowski. "Invited Perspective: The Promise of Fit-for-Purpose Systematic Evidence Maps for Supporting Regulatory Health Assessment." *Environmental Health Perspectives* 130, no. 5 (May 2022): 051303. https://doi.org/10.1289/EHP10743.

[20] Workman, Clare E., Allan B. Becker, Meghan B. Azad, Theo J. Moraes, Piush J. Mandhane, Stuart E. Turvey, Padmaja Subbarao, Jeffrey R. Brook, Malcolm R. Sears, and Charles S. Wong. "Associations between Concentrations of Perfluoroalkyl Substances in Human Plasma and Maternal, Infant, and Home Characteristics in Winnipeg, Canada." *Environmental Pollution* 249 (June 1, 2019): 758–66. https://doi.org/10.1016/j.envpol.2019.03.054.

[21] Mogensen, Ulla B., Philippe Grandjean, Carsten Heilmann, Flemming Nielsen, Pál Weihe, and Esben Budtz-Jørgensen. "Structural Equation Modeling of Immunotoxicity Associated with Exposure to

- An epidemiological study by Wen et al. (2019) that evaluates the association between cord plasma PFHxS and atopic dermatitis, an immune relevant endpoint[22]
- An experimental rodent study that investigated endpoints relevant to behavioral outcomes[23]
- Two experimental rodent studies relevant to mechanistic hepatic effects[24]
- Four in vitro studies have endpoints that should be evaluated for their relevance to the carcinogenicity study (DNA damage, generation of reactive oxygen species)[25]

Additionally, a study by Buttenhoff et al. (2009), which was included by EPA, was left out of Section 3.2.2. on page 133 where animal evidence for immunotoxicity is discussed. This study describes a >28 day exposure in rats the measurement of immune relevant organs (spleen, thymus, bone marrow, lymph nodes)[26] and discussion of this paper should be added to the immune effects section.

Perfluorinated Alkylates." *Environmental Health* 14, no. 1 (June 5, 2015): 47. https://doi.org/10.1186/s12940-015-0032-9.

[22] Wen, Hui-Ju, Shu-Li Wang, Pau-Chung Chen, and Yue Leon Guo. "Prenatal Perfluorooctanoic Acid Exposure and Glutathione S-Transferase T1/M1 Genotypes and Their Association with Atopic Dermatitis at 2 Years of Age." *PloS One* 14, no. 1 (2019): e0210708. https://doi.org/10.1371/journal.pone.0210708.

[23] Zhang, Qian, Wei Liu, Qiao Niu, Yu Wang, Huimin Zhao, Huifang Zhang, Jing Song, Shuji Tsuda, and Norimitsu Saito. "Effects of Perfluorooctane Sulfonate and Its Alternatives on Long-Term Potentiation in the Hippocampus CA1 Region of Adult Rats in Vivo." *Toxicology Research* 5, no. 2 (January 7, 2016): 539–46. https://doi.org/10.1039/c5tx00184f.

[24] Zhang, Qian, Wei Liu, Qiao Niu, Yu Wang, Huimin Zhao, Huifang Zhang, Jing Song, Shuji Tsuda, and Norimitsu Saito. "Effects of Perfluorooctane Sulfonate and Its Alternatives on Long-Term Potentiation in the Hippocampus CA1 Region of Adult Rats in Vivo." *Toxicology Research* 5, no. 2 (January 7, 2016): 539–46. https://doi.org/10.1039/c5tx00184f.

[25] Bjork, James A., and Kendall B. Wallace. "Structure-Activity Relationships and Human Relevance for Perfluoroalkyl Acid-Induced Transcriptional Activation of Peroxisome Proliferation in Liver Cell Cultures." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 111, no. 1 (September 2009): 89–99. https://doi.org/10.1093/toxsci/kfp093; Martínez-Quezada, R., G. González-Castañeda, I. Bahena, A. Domínguez, P. Domínguez-López, E. Casas, M. Betancourt, et al. "Effect of Perfluorohexane Sulfonate on Pig Oocyte Maturation, Gap-Junctional Intercellular Communication, Mitochondrial Membrane Potential and DNA Damage in Cumulus Cells in Vitro." *Toxicology in Vitro: An International Journal Published in Association with BIBRA* 70 (February 2021): 105011. https://doi.org/10.1016/j.tiv.2020.105011; Oseguera-López, Iván, Serafín Pérez-Cerezales, Paola Berenice Ortiz-Sánchez, Oscar Mondragon-Payne, Raúl Sánchez-Sánchez, Irma Jiménez-Morales, Reyna Fierro, and Humberto González-Márquez. "Perfluorooctane Sulfonate (PFOS) and Perfluorohexane Sulfonate (PFHxS) Alters Protein Phosphorylation, Increase ROS Levels and DNA Fragmentation during In Vitro Capacitation of Boar Spermatozoa." *Animals: An Open Access Journal from MDPI* 10, no. 10 (October 21, 2020): 1934. https://doi.org/10.3390/ani10101934; Wielsøe, Maria, Manhai Long, Mandana Ghisari, and Eva C. Bonefeld-Jørgensen. "Perfluoroalkylated Substances (PFAS) Affect Oxidative Stress Biomarkers in Vitro." *Chemosphere* 129 (June 2015): 239–45. https://doi.org/10.1016/j.chemosphere.2014.10.014.

[26] Butenhoff, John L., Shu-Ching Chang, David J. Ehresman, and Raymond G. York. "Evaluation of Potential Reproductive and Developmental Toxicity of Potassium Perfluorohexanesulfonate in Sprague Dawley Rats." *Reproductive Toxicology (Elmsford, N.Y.)* 27, no. 3–4 (June 2009): 331–41. https://doi.org/10.1016/j.reprotox.2009.01.004.

2. EPA's draft toxicological assessment for PFHxS could be strengthened by considering additional supplemental studies.

We encourage EPA to make use of (i.e. summarizing) relevant supplemental evidence, in particular animal studies that are observational or use non-mammalian species.

- In Section 3.2.1 Thyroid Effects, specifically on page 104, we suggest considering additional supplemental studies that pertain to PFHxS binding to thyroid hormone transport proteins[27] or thyroid hormone T3 receptor.[28]
- In Section 3.2.1 Thyroid Effects, specifically on page 108 where evidence integration is discussed, EPA notes a significant data gap in that there is a lack of studies evaluating "brain development and bone growth during early childhood and adolescence." We note that one study[29] excluded at TIAB and three studies marked as supplemental[30] may provide additional mechanistic information relative to this data gap.
- In Section 3.2.2 Immune Effects, specifically on page 137 where mechanistic and supplemental studies are discussed, the EPA ignores a body of evidence that has investigated immune relevant endpoints in species beyond rats and mice, for example in observational studies of cats, striped bass, birds, and dolphins[31] and has missed a

[27] Ren, Xiao-Min, Wei-Ping Qin, Lin-Ying Cao, Jing Zhang, Yu Yang, Bin Wan, and Liang-Hong Guo. "Binding Interactions of Perfluoroalkyl Substances with Thyroid Hormone Transport Proteins and Potential Toxicological Implications." *Toxicology* 366–367 (July 29, 2016): 32–42. https://doi.org/10.1016/j.tox.2016.08.011; Weiss, Jana M., Patrik L. Andersson, Marja H. Lamoree, Pim E. G. Leonards, Stefan P. J. van Leeuwen, and Timo Hamers. "Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 109, no. 2 (June 2009): 206–16. https://doi.org/10.1093/toxsci/kfp055.

[28] Ren, Xiao-Min, Yin-Feng Zhang, Liang-Hong Guo, Zhan-Fen Qin, Qi-Yan Lv, and Lian-Ying Zhang. "Structure–Activity Relations in Binding of Perfluoroalkyl Compounds to Human Thyroid Hormone T3 Receptor." *Archives of Toxicology* 89, no. 2 (February 1, 2015): 233–42. https://doi.org/10.1007/s00204-014-1258-y

[29] Kalloo, Geetika, Gregory A. Wellenius, Lawrence McCandless, Antonia M. Calafat, Andreas Sjodin, Megan E. Romano, Margaret R. Karagas, et al. "Exposures to Chemical Mixtures during Pregnancy and Neonatal Outcomes: The HOME Study." *Environment International* 134 (January 1, 2020): 105219. https://doi.org/10.1016/j.envint.2019.105219.

[30] Buck, Catherine O., Melissa N. Eliot, Karl T. Kelsey, Antonia M. Calafat, Aimin Chen, Shelley Ehrlich, Bruce P. Lanphear, and Joseph M. Braun. "Prenatal Exposure to Perfluoroalkyl Substances and Adipocytokines: The HOME Study." *Pediatric Research* 84, no. 6 (December 2018): 854–60. https://doi.org/10.1038/s41390-018-0170-1; Gao, Ke, Taifeng Zhuang, Xian Liu, Jianjie Fu, Jingxing Zhang, Jie Fu, Liguo Wang, 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." *Environmental Science & Technology* 53, no. 11 (June 4, 2019): 6529–38. https://doi.org/10.1021/acs.est.9b00715; Hu, Janice M. Y., Tye E. Arbuckle, Patricia Janssen, Bruce P. Lanphear, Liheng H. Zhuang, Joseph M. Braun, Aimin Chen, and Lawrence C. McCandless. "Prenatal Exposure to Endocrine Disrupting Chemical Mixtures and Infant Birth Weight: A Bayesian Analysis Using Kernel Machine Regression." *Environmental Research* 195 (April 2021): 110749. https://doi.org/10.1016/j.envres.2021.110749.

[31] Bost, Phillip C., Mark J. Strynar, Jessica L. Reiner, Jerry A. Zweigenbaum, Patricia L. Secoura, Andrew B. Lindstrom, and Janice A. Dye. "U.S. Domestic Cats as Sentinels for Perfluoroalkyl Substances:

relevant mechanistic study.[32] Additionally, there are studies that EPA excluded at TIAB[33] (despite the title of this paper, it does contain analyses on PFHxS) or marked as supplemental[34] that investigate Chron's disease, ulcerative colitis, and other inflammatory bowel diseases that were not discussed in the section on immune effects but likely should have been.

3. EPA should acknowledge and protect for mammary gland effects.

We previously noted in the IRIS review of PFDA that the EPA has been overlooking an important health endpoint related to mammary gland function.[35] Here again, there is an opportunity to include discussion of decreased breastfeeding duration as a result of PFHxS exposure. These effects were recently systematically reviewed by Timmerman et al. (2023), which included four primary studies that evaluated associations between PFHxS exposure and breastfeeding duration.[36] As we previously described, a woman's ability to breastfeed is an important health outcome, not just for the baby, but also for her own health. The EPA should

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include a discussion of mammary gland function in the review of female reproductive health (Section 3.2.8).

Conclusions

In conclusion, we urge the agency to incorporate our feedback to strengthen its final toxicological review of PFHxS and to finalize this profile in a timely manner.

Respectfully submitted,

Katherine Pelch, PhD
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Natural Resources Defense Council

Attachment: Please see “NRDC_Attachment_PFHxS.xlsx”