**Department of Defense (DoD)**
**Comments on the Interagency Science Discussion**
**Draft IRIS Toxicological Review of Perfluorohexanoic Acid (PFHxA) and Related Salts**
**February 2023**
(Date Received March 10, 2023)

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**Comments on the IRIS Toxicological Review of Perfluorohexanoic Acid (PFHxA) and Related Salts (February 2023)**

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

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| **Overall comment** | NA | The first PFHxA draft was one of the first publicly full IRIS assessments following the release of the Draft ORD Staff Handbook for Conducting IRIS Assessments (the "Handbook"). The Handbook was finalized in December 2022, not long before the release of the revised PFHxA assessment. While the draft and final Handbook are similar, there were some substantive changes - most notably, the minimum evidence integration judgment needed to advance specific effects to toxicity value derivation has increased to only those with "evidence indicates" or "evidence demonstrates" judgments; in the draft Handbook, EPA had indicated that effects with weaker judgments (e.g., "evidence suggests") might, in some cases, be advanced for toxicity value derivation.<br><br>These changes to the Handbook may have affected EPA's decisions regarding strength of the evidence for specific health effects of PFHxA – most notably, endocrine effects (see below comments). | N/A | E/S |

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| **Overall comment** | NA | Overall, the draft provides clear descriptions of the methodology and EPA's analysis of the evidence. The PFHxA IRIS assessment reflects many of the revised guidelines for IRIS assessments, including increased transparency and increased used of graphical representation of EPA's conclusions. In particular, the use of an "Evidence Integration" narrative and tabular summary of all evidence streams (evidence profile tables) for each health endpoint allows the reader to better identify and follow EPA's decision process, which is an important improvement in IRIS toxicological reviews. | N/A | S |
| **1.1.2 Sources, Production, and Uses** | 1-3 | EPA points out there is a small concentration of PFHxA in AFFF; however, no other specific uses are discussed for PFHxA. Is there any information about use patterns over time? Most of the data on uses and exposure are from 8-10 years ago; is it still used in the same frequency in AFFF or other products in more recent years? | More information should be provided about current uses (and if possible, volume of production/ use in the United States) for PFHxA specifically. | S |
| **1.2.4 Evidence Synthesis and Integration** | 1-3 | The document states, "Not all studies that meet the PECO criteria go through data extraction: For example, studies evaluated as being uninformative are not considered further and therefore do not undergo data extraction." Further, the revised assessment states that evidence synthesis is based primarily on studies of high and medium confidence and only in instances when "few or no studies of higher confidence are available" will low confidence studies be used to evaluate the weight of the evidence for an effect. While it is critical to evaluate and consider how biases in the evidence may be affecting results and overall consistency across studies, systematic review guidelines do not typically recommend study quality ratings as a criterion for exclusion of studies. There may be concern for a substance like PFHxA that the weight of the evidence is skewed by limiting data extraction and discussion of most/all studies. | EPA should consider providing additional justification for any studies that were not extracted/reviewed further and defining how EPA determines whether there are a sufficient number of higher-quality studies to warrant excluding low-quality studies. | S |
| **2.2 Study Evaluation Results** | 2-3 | We appreciate that EPA provides a high-level summary of the number of epidemiological studies and animal studies and their quality judgments at the outset of the study evaluation section. | N/A | E |

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| **3.1.2 Distribution in animal and in vitro studies** | 3-6 (lines 3-7) | EPA states, “Serum concentrations of PFHxA were up to 17-fold higher for male than female rats after i.v. dosing, and the AUC after oral dosing was over 4-fold higher in males than females given a 50 mg/kg gavage dose. The half-life in males, however, was only 2.5 times greater than females after i.v. dosing and was similar to that in females after oral dosing. Together these lead to the conclusion of higher $V_d$ for females than for males."<br><br>It is reasonable that the lower serum concentrations in female rats indicate a greater volume of distribution. But the same passage states that the half-life is greater in male rats. The half-life equation is as follows: $t1/2 = (0.7 \times V_d)/Cl$<br><br>The larger the $V_d$, the longer the half-life. It is unclear why male rats would have a longer half-life than females, but a smaller $V_d$. In humans, half-life in females is reduced due to menstruation, but female rats do not menstruate. | EPA should re-visit this discussion of $V_d$ and consider whether there are reasons why female animals would have a higher $V_d$ but a shorter half-life. | S |
| **3.2 Noncancer Evidence Synthesis and Integration** | 3-19 | EPA notes: “Some organs/systems for which animal data were available are summarized in the animal literature inventory, but these data were not synthesized due to insufficient evidence to draw hazard judgments (i.e., evidence is inadequate). Specifically, for these health effects there were either few studies with null results (i.e., dermal, musculoskeletal, sensory, ocular) or few studies with sporadic findings of unclear toxicological significance (i.e., respiratory, gastrointestinal system, cardiovascular, and metabolic effects), including small changes in indirect outcome measures and other effects of unclear biological significance in isolation (e.g., decreases in cholesterol).”<br><br>The tables presented in the literature inventory online provide helpful summaries of studies not summarized in detail in the narrative. It would be helpful, however, if EPA provided the NOAELs/LOAELs in order of magnitude (or perhaps even plotted the results), so the range of NOAELs/LOAELs in these studies could be more easily scanned. | Consider re-arranging the tables in order of NOAEL/LOAEL magnitude. | E |

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| **3.2.1 Hepatic Effects** | 3-19 | EPA stated, "Liu et al. (2022) reported positive associations (i.e., higher liver enzyme levels with higher PFHxA exposure) for serum albumin ($p<0.05$) and alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (not statistically significant), but no association with aspartate aminotransferase (AST), total protein, or γ-glutamyl transferase (GGT)."<br><br>EPA rated this study as medium confidence. Liu et al. (2022) is a cross-sectional study meaning the exposure and outcomes are measured at the same time. Thus, because temporality cannot be established, these studies cannot be used to assess causal relationships. Furthermore, the human half life of PFHxA in the serum is estimated to be as short as 3 hours.<br><br>Further, the second study (Nian et al., 2019) reported no associations between PFHxA and any liver function biomarkers. | Given the limitations of the studies and inconsistent findings both within and across studies, EPA's conclusion of "indeterminate evidence" for hepatic effects in humans is supported; however, EPA should consider re-evaluating its "medium confidence" determination for the Liu et al. (2022) study, considering issues with serum measurement of PFHxA, among other limitations. | |
| **3.2.1 Hepatic Effects** | 3-20 | Section 3.2.1 is the first mention of histopathology, but comments that follow are equally applicable to all sections and figures/tables describing and summarizing histopathological data and images from the primary literature. Studies that comprise histopathological evidence of a toxicological insult need to be considered very carefully against the INHAND recommendations and guidance. The histopathology so reported in any cited article in the IRIS assessment might be incorrect or poorly interpreted. This can lead to incorrect assessments, recommendations and decision-making. It is recommended that IRIS authors need to evaluate the histopathology carefully and systematically themselves and not to necessarily accept the data and findings of the published histopathology data and images. It is clearly evident that IRIS authors have not made determinations based on the INHAND best practices and guidance. This is a potential weakness. IRIS authors can analyze the provided histopathology images in cited primary literature in accord with INHAND recommendations - had this been observed, it might have excluded – to some extent, any risk of bias and subjectivity. | Major recommendations are provided below for future practice by the U.S. EPA and their assessment of histopathological data according to the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) recommendations and guidance.<br><br>Please see for example:<br><br>Mann P.C. et al. (2012). International Harmonization of Toxicologic Pathology Nomenclature: An Overview and Review of Basic Principles | |

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| | | | (Updated). (Found Here: https://journals.sagepub.com/doi/full/10.1177/0192623312438738)<br><br>Thoolen R. et al (2010). Proliferative and Nonproliferative Lesions of the Rat and Mouse Hepatobiliary System. (Found Here: https://journals.sagepub.com/doi/abs/10.1177/0192623310386499)<br><br>U.S. EPA IRIS contributing authors need to review these guidance recommendations for best practice approaches, and do so for any major organ system evaluated, to include: Cardiovascular; Central and Peripheral Nervous System; Endocrine; Hepatobilliary; Reproductive (male and female); Respiratory, etc., etc.<br><br>This suggested recommendation becomes even more pressing because in the IRIS assessment, sweeping statements like "Histopathology for Chengelis et al. (2009b) was rated low confidence because of issues related to observational bias, concerns about endpoint sensitivity | |

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| | | | and specificity, and results presentation……." OK – but On what guidance standards or set of principles were those confidence levels, and critiques of the study made? This and similar statements in the IRIS assessment appear subjective and arbitrary. | |
| **3.2.1 Hepatic Effects** | 3-35 | EPA concluded, "Overall, the currently available evidence indicates that PFHxA likely causes hepatic effects in humans under relevant exposure circumstances. This conclusion is based on studies of animals showing increased liver weight, hepatocellular hypertrophy, increased serum enzymes (>2-fold ALT) and decreased serum globulins generally occurring at ≥ 200 mg/kg-day (with some effects noted at lower doses) within the evidence base of four primarily high confidence studies of short-term, subchronic, and chronic PFHxA exposure in (primarily male) Sprague-Dawley rats. The findings in rats were determined to be adverse and relevant to humans, with the likely involvement of both PPARα-dependent and -independent pathways."<br><br>It is unclear how EPA justifies "relevant exposure circumstances." Most liver effects are reported at or above 200 mg/kg/day in animal studies, well above expected human exposure levels. | Recommend that the EPA clarify what they define as "under relevant exposure conditions," as it is unclear how it's currently written. | |
| **3.2.2 Developmental Effects** | 3-42 | EPA states that decreased offspring body weights were observed in several animal studies. In some cases, these body weight changes resolved after weaning. The one-generation reproductive/developmental study by Loveless et al. (2009) reported statistically significant reductions in body weight at 500 mg/kg/day postnatally (but not after weaning); however, maternal toxicity was also apparent at this dose (body weight loss); indicating this is not a selective developmental effect. Iwai and Hoberman (2014) reported pup body weight losses in mice at all doses, but these effects only persisted at doses also causing substantial maternal toxicity (≥350 mg/kg/day). The authors indeed | It is recommended to add context to the doses and effects observed on postnatal body weight as related to doses that elicit maternal toxicity. With regard to maternal toxicity, please consider the limitations of using reduced pup weight as a basis for an RfD and/or identification of PFHxA as a developmental toxicant. | S, Major |

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| | | stated, "Results of this study support what has been generally observed for other PFAAs in that developmental toxicity has generally only been seen in the presence of maternal toxicity."<br><br>Similar conclusions - that PFHxA is not a selective reproductive or developmental toxicant - were reached in a comprehensive toxicity review of PFHxA conducted by Luz et al. (2019). While EPA cited this article in reference to pharmacokinetics, it did not cite this review anywhere else in the assessment when discussing any of the critical endpoints, including developmental toxicity. | Luz, AL; Anderson, JK; Goodrum, P; Durda, J. (2019). Perfluorohexanoic acid toxicity, part I: Development of a chronic human health toxicity value for use in risk assessment. Regul Toxicol Pharmacol 103: 41-55. http://dx.doi.org/10.1016/j.yrtph.2019.01.019. | |
| **3.2.2 Developmental Effects** | 3-46 | The conclusion that "PFHxA likely causes developmental effects in humans" overstates the weight of the evidence. In animal studies, developmental effects were reported at high doses that are not likely to be applicable to exposure in humans. Notably no discussion of doses at which maternal toxicity was observed was integrated into the weight of evidence discussion. Epidemiological studies of developmental toxicity were uninformative. | Evidence integration for developmental toxicity as a specific adverse effect associated with PFHxA must incorporate consideration of maternal toxicity, currently absent from this assessment. | S, Major |
| **3.2.3 Renal Effects** | 3-55 | EPA states that two of three epidemiological studies of renal effects were considered uninformative "due to critical deficiencies in multiple study evaluation domains" (Seo et al., 2018; Zhang et al., 2019). EPA provided no summary whatsoever of the findings of these studies; the HAWC link was broken so study details could not be reviewed.<br><br>While it is very important to thoroughly review the quality of studies, the exclusion of low-quality studies for hazard identification may not be appropriate, particularly for a chemical such as PFHxA, which has relatively little information. Very low-quality studies clearly should not be used for any quantitative analysis, but it seems EPA could retain and summarize them, providing the caveat that there is low confidence in this study. Further, because EPA's quality evaluation system does not weigh any particular study quality domain more than others, epidemiological studies with deficiencies in a single domain (e.g., | Consider summarizing, at least briefly, all the epidemiological evidence for renal effects, including lower quality studies. | S |

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| | | "selective reporting") but that are otherwise strong, may be useful; in contrast, studies with many critically deficient judgments, or critical deficiencies in very important domains such as exposure measurement may indeed be relatively uninformative. | | |
| **3.2.5 Endocrine Effects** | 3-79 | Only two epidemiological studies evaluated PFHxA and thyroid hormone changes. One was considered uninformative and was not evaluated further.<br><br>The second study, Li et al. (2017), reported inverse associations between PFHxA and with free T3 (not statistically significant) and thyroid stimulating hormone (TSH) (statistically significant). There were no associations between PFHxA and FT4. Typically, when T3 or T4 are decreased, there is a compensatory *increase* in TSH, rather than the decrease observed in this study, calling into question the clinical significance of these findings. Further, this study reported very low exposures to PFHxA (0.01 [LOD-1.1]) with 47% of samples below the limit of detection. The issue of few participants with measurable serum PFAS concentrations is common for some of the short-chain PFAS with short half-lives (and in some cases, less widespread use). Given that low or no detection often precludes analysis, this is a substantial uncertainty regarding the relevance of PFHxA effects measured in animals at relatively high doses to the human population at these low exposure levels. | Suggest providing additional discussion regarding the uncertainty of the human relevance of high-dose animal studies considering the epidemiological studies reporting low or non-detect for PFHxA in serum. | S |
| **3.2.5 Endocrine Effects** | 3-80 | Endocrine effects were evaluated in four short-term (28-day) studies in adult rats. However, thyroid hormone levels were only evaluated in the 28-day NTP study (NTP, 2018). Thyroid hormones were altered only in males, and with only free T4 (FT4) exhibiting a dose-response relationship. Thyroid epithelial cell hypertrophy was observed in a different study of rats exposed to 500 mg/kg/day PFHxA for 90 days (Loveless, et. Al, 2009), but there was no clear dose-response relationship. Similarly, there were no clear treatment-related findings for organ weights. | EPA should revise the discussion of the NTP (2018) study of thyroid hormones to more thoroughly outline the uncertainties in the biological relevance of decreased T4 in adult male rats, given that TSH levels were not changed, histopathological changes were not observed, and mechanistic evidence is weak. These | S, Major |

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| | | T4 is produced in higher quantities relative to triiodothyronine (T3); however, T3 is the more active hormone, and it provides negative feedback to maintain homeostasis in the hypothalamic-pituitary axis (HPT). Thyroid stimulating hormone (TSH), another clinical indicator, stimulates the thyroid to produce more thyroid hormones. In clinical primary thyroid disease, T4 is typically decreased with TSH increased (hypothyroidism), or T4 is increased and TSH is decreased (hyperthyroidism). Changes in T3 or T4 absent changes in TSH may not be a biologically relevant effect. Early or mild hypo- or hyperthyroidism are typically indicated by alterations in TSH.[1] Changes in T4 alone are typically only informative in pregnant and developing offspring, as T4 is important to brain development. EPA indicates that it believes changes in T4 do not "translate perfectly to human clinical definitions" but asserts they are biological relevant. The studies it cites to support this assertion (Crofton et al., 2004; Lau et al., 2003) pertain to *maternal* hypothyroxinemia, a condition associated with pregnancy and neurodevelopment that are therefore completely irrelevant for interpreting findings exclusively observed in adult male rats.<br><br>NTP (2018) did not observe histopathological findings in the thyroid of affected rats, even at doses of 1000 mg/kg-day. The histopathological findings were observed only in a single study, and only at 500 mg/kg-day. Two other studies (Klaunig et al., 2015; Chengelis et al., 2009b) also reported no changes to thyroid histopathology after subchronic exposure or chronic exposure of up to 200 mg/kg-day.<br><br>Finally, the mechanistic studies that EPA cites to support possible mechanisms for thyroid hormone disruption are mixed and limited by relevance of the species and endpoint. For example, Zhang et al. (2022) | considerations should also inform the overall evidence integration judgment, discussed below.<br><br>Findlay KAB, Kaptein E, Visser TJ, Burchell B. 2000. Characterization of the Uridine Diphosphate-Glucuronosyltransferase-Catalyzing Thyroid Hormone Glucuronidation in Man. The Journal of Clinical Endocrinology & Metabolism, Volume 85, Issue 8, pp. 2879–2883. Accessed online at: https://doi.org/10.1210/jcem.85.8.6715<br><br>Regulatory Sciences Associates (RSA). 2018. A literature review of the current state of the science regarding species differences in the control of, and response to, thyroid hormone perturbations. Part 1: A human health perspective. Prepared on behalf of the European Crop Protection Association, Report Number RSA/ECPA001_Thyroid. Accessed online at http://cefic-lri.org/wpcontent/uploads/2018/06/ | |

[1] https://www.uclahealth.org/medical-services/surgery/endocrine-surgery/conditions-treated/thyroid/normal-thyroid-hormone-levels

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| | | reported decreases in deiodinases 1 and 2 in zebrafish, with a decrease in Ugt1ab. Glucuronidation may not be a significant route by which thyroid hormones are metabolized in humans under normal physiological conditions (Findlay et al., 2000). Therefore, it has been postulated that disruption of TH levels via induction of hepatic UGT enzymes may not be human relevant (RSA, 2018). As noted by EPA, the four studies evaluating PFHxA binding to thyroid hormone transport proteins and thyroid hormone receptors reported no or low binding affinities, indicating these mechanisms are likely not operating. | RSA-Draft-Thyroid-State-of-Science-Review-v4.pdf. | |
| **Several** | N/A | An external reviewer requested that EPA remove "under relevant exposure circumstances" from the evidence integration judgments and EPA heeded. However, the IRIS Handbook states that "the evidence integration narrative and summary judgment levels include the generic phrase, "**given sufficient exposure conditions**." This highlights that, for those assessment-specific health effects identified as potential hazards, the exposure conditions associated with those health effects will be defined (as will the uncertainties in the ability to define those conditions) during dose-response analysis" (EPA, 2022). EPA's decision to remove important caveats regarding exposure levels in the PFHxA draft is inconsistent with the Handbook. | EPA should update the evidence integration statements to state "given sufficient exposure conditions"<br><br>U.S. EPA. ORD Staff Handbook for Developing IRIS Assessments (2022). U.S. EPA Office of Research and Development, Washington, DC, EPA/600/R-22/268, 2022. | S |
| **3.2.6 Male Reproductive Effects; 3.2.7 Female Reproductive Effects** | 3-90; 3-98 | EPA concluded that, "Overall, the currently available ***evidence is inadequate*** to assess whether PFHxA might cause male reproductive effects in humans." Similarly, EPA concluded that "currently available ***evidence is inadequate*** to assess whether PFHxA might cause female reproductive effects in humans." These conclusions are supported by the evidence; EPA's explanations of study and endpoint limitations were concise and clear. | N/A | S |
| **5.2.1 Oral Reference Dose Derivation** | 5-13, 5-14 | The approach to deriving a dosimetric-adjustment factor appears sound. For the preferred DAF, however, what is the range of estimated clearance levels (above and below)? The derivation of the DAF and the toxicity value are highly sensitive to the clearance value used. | Given the limited information on human PFHxA pharmacokinetics (PK), the assumptions used and range of uncertainty, we | S |

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| | | | recommend a thorough discussion of the uncertainties associated with the human clearance value and DAF. | |
| **5.2.1 Oral Reference Dose Derivation** | 5-18 | EPA used data from three other PFAS (PFHxS, PFNA, and PFOA) to "check" their assumption regarding differences in clearance between humans and animals. The chain length and functional group of PFAS can affect their physicochemical properties and toxicity. The use of PFHxS clearance data to inform PFHxA may not be appropriate, given that PFHxS is a long-chain sulfonate with a very long half-life. PFNA and PFOA are also long-chain PFAS with long half-lives (several years). There appears to be substantial uncertainty in inferring these data are informative for PFHxA. | Provide additional justification for the use of long-chain PFAS PK data to inform the PK of PFHxA in humans. | S |