DRAFT External Peer Review Charge Questions for the

IRIS Toxicological Review of *Perfluorohexanoic Acid and Related Compounds Ammonium and Sodium Perfluorohexanoic Acid*

August 2021

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of the draft Toxicological Review of Perfluorohexanoic Acid and Related Compound Ammonium and Sodium Perfluorohexanoic Acid developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's Center for Public Health and Environmental Assessment (CPHEA) within the Office of Research and Development (ORD). IRIS assessments contain information for chemicals that can be used to support hazard identification and dose-response assessment, two of the four steps in the human health risk assessment process. When used by risk managers in combination with information on human exposure and other considerations, IRIS assessments support the Agency's regulatory activities and decisions to protect public health.

There is no existing IRIS assessment for PFHxA. The draft Toxicological Review of PFHxA is based on a comprehensive review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to PFHxA or the related compounds, ammonium and sodium PFHxA. Additionally, the protocol for PFHxA as well as other appendices for toxicokinetic information, dose-response modeling, and other supporting materials are provided as *Supplemental Information* (see Appendices A to E) to the draft Toxicological Review.

Charge questions on the draft Toxicological Review of *PFHxA*

In response to the numbered charge questions below, the advice provided as part of this peer review would be most useful when prioritized to indicate its relative importance as follows:

- Tier 1: *Recommended Revisions* – Key major recommendations that are necessary for strengthening the scientific basis for the Toxicological Review of PFHxA. The implication of such key Tier 1 recommendations is that the assessment conclusions are not adequately supported without addressing the recommendations and need to be reconsidered or better substantiated. For Tier 1 recommendations, please describe the specific revisions necessary to modify or better substantiate the most scientifically appropriate assessment conclusions.
- Tier 2: *Suggestions* – Recommendations that are encouraged in order to strengthen the scientific analyses and conclusions in the Toxicological Review of PFHxA. It is understood that other factors (e.g., timeliness) may also be considered before deciding to address and/or incorporate Tier 2 suggestions. For Tier 2 recommendations, please provide specific suggestions to strengthen the scientific basis for assessment conclusions or improve the clarity of the analyses and presentation.
- Tier 3: *Future Considerations* – Scientific exploration that may inform future work. These recommendations are outside the immediate scope and/or needs of the current document under review but may inform future Toxicological Reviews or research efforts.

1. The Toxicological Review for PFHxA describes and applies a systematic review protocol for identifying and screening the pertinent studies. The protocol is described in brief detail in Section 1.2.1 (*Literature Searching and Screening*) and in full detail in Appendix A (*Systematic Review Protocol for the PFBA PFHxA, PFHxS, PFNA, and PFDA IRIS Assessments*). Please comment on whether the literature search strategy and screening criteria for PFHxA literature are clearly described. If applicable, please identify additional peer-reviewed studies that the assessment should consider.
2. The Toxicological Review provides an overview of individual study evaluations and the results of those evaluations are made available in the Health Assessment Workplace Collaborative linked here HAWC. Note that there exists a "HAWC FAQ for assessment readers" document linked here (scroll to the bottom of the page and the document is available for download under "attachments") that is intended to assist the reviewer in navigating this on-line resource. Data from studies that were considered informative to the assessment are synthesized in the relevant health effect-specific sections and study data are available in HAWC. Note that the reviewer will need HAWC assessment "reviewer" status to access the PFHxA assessment in HAWC linked here.
    a. Please comment on whether the study confidence conclusions for the pertinent studies are scientifically justified and clearly described, considering the important methodological features of the assessed outcomes. Please indicate any study confidence conclusions that are not justified and explain any alternative study evaluation decisions.
    b. Results from individual studies are presented and synthesized in the health system-specific sections. Please comment on whether the presentation and analysis of study results is clear, appropriate and effective to allow for scientifically supported syntheses of the findings across sets of studies.

3. For each of the health effects considered in the assessment and outlined below, please comment on whether the available data have been clearly and appropriately synthesized to describe the strengths and limitations. For each, please also comment on whether the weight of evidence decisions for hazard identification are scientifically justified and clearly described.
    a. For hepatic effects, the Toxicological Review concludes 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 occuring 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.
    b. For developmental effects, the Toxicological Review concludes the available ***evidence indicates*** that PFHxA likely causes developmental effects in humans under relevant exposure circumstances. . This judgment is based primarily on gestational exposure experiments in mice, with supportive findings in rats), showing increased perinatal mortality, decreased offspring body weight, and delayed eye opening. These effects are similar to those observed for other PFAS. These findings are interpreted as relevant to humans based on similarities in the anatomy and physiology of the developmental systems across rodents and humans.

c. For hematopoietic effects, the Toxicological Review concludes the currently available ***evidence indicates*** that PFHxA likely causes hematopoietic effects in humans under relevant exposure circumstances. This judgement is based on several consistent findings (i.e., decreased red blood cells [RBCs], hematocrit, and hemoglobin) across study designs that when interpreted together suggest PFHxA related adverse hematologic effects such as anemia. There were also indications that red blood cells were swollen and making up a larger proportion of the blood volume (increased mean corpuscular volume [MCV, a measure of the average red blood cell size]). These changes were correlated with potential secondary erythrogenic responses to PFHxA exposure including increased reticulocyte (immature RBCs) counts that were consistently increased across study designs and exposure durations, even in the females (that received a dose two-times the male dose) of chronic study. These findings are interpreted as relevant to humans based on similarities in the anatomy and physiology of the developmental systems across rodents and humans

d. For endocrine effects, the Toxicological Review concludes that the currently available ***evidence suggests***, but not sufficient to infer, that PFHxA may cause endocrine effects in humans under relevant exposure circumstances. This conclusion is based on four animal studies generally rated high confidence that reported treatment related changes in thyroid hormone levels, thyroid histopathology after exposure to PFHxA at ≥ 62.5 mg/kg-day.

e. For all other potential health effects (i.e., renal, male and female reproductive, immune, and nervous system), the Toxicological Review concluded that the currently available ***evidence is inadequate*** to assess whether PFHxA may cause effects in humans under relevant exposure circumstances. These conclusions (except for renal) were driven by sparse evidence bases and/or data that were largely null. For renal there was a report that selected renal endpoints as the critical effect with an RfD of 0.25 mg/kg-day (Luz, 2019, 5080589). In the chronic study the incidence of papillary necrosis and tubular degeneration were increased in females compared to controls at the highest dose (200 mg/kg-day, two times the highest male dose). Urinalysis findings suggested decreased urine concentration ability and were specific to females.

4. Appendix A (Systematic Review Protocol for the PFBA PFHxA, PFHxS, PFNA, and PFDA IRIS Assessments) outlines the human relevance of hepatic effects in animals that involve peroxisome proliferator-activated receptor alpha (PPARα) receptors as a key science issue. To the extent supported by the PFHxA literature (and to a lesser extent, other PFAS), the Toxicological Review evaluates the evidence relevant to the potential involvement of PPARα and non-PPARα pathways with respect to the reported hepatic effects. The Toxicological Review ultimately concludes that evidence from *in vivo* (including genetic mouse models) and *in vitro* studies support a potential role for multiple pathways operant in the induction of hepatic effects by PFHxA exposure, however those pathways cannot be specifically determined. Please comment on whether the conclusions regarding the available animal and mechanistic studies are scientifically justified and clearly described.

5. The Toxicological Review concludes that there is inadequate evidence to assess carcinogenic potential for PFHxA and that this descriptor applies to oral and inhalation routes of human exposure. Please comment on whether the available animal and mechanistic studies, and the analysis presented in the Toxicological Review are scientifically justified and clearly described.

6. For PFHxA the study chosen for use in deriving the RfD is the Loveless et al. (2009) one-generation reproductive toxicity study based on decreased offspring body weight in rats

exposed continuously throughout gestation and lactation to PFHxA sodium salt via the dam. Is the selection of this study and these effects for use in deriving the RfD for PFHxA scientifically justified and clearly described?

   a. If yes, please provide an explanation.

   b. If no, please provide an alternative study(ies) and/or effect(s) that should be used to support the derivation of the RfD and detail the rationale for use of such an alternative.

   c. As part of the recommendations in "a" or "b" above, please comment on whether the effects selected are appropriate for use in deriving the RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection.

7. In addition, for PFHxA, a RfD for less-than-lifetime ("subchronic") exposures is derived. The same study and outcome were chosen for use in deriving the RfD. Is the selection of this study and these effects for the derivation of the subchronic RfD for PFHxA scientifically justified and clearly described?

   a. If yes, please provide an explanation.

   b. If no, please provide an alternative study(ies) and/or effect(s) that should be used to support the derivation of the subchronic RfD and detail the rationale for use of such an alternative.

   c. As part of the recommendations in "a" or "b" above, please comment on whether the effects selected are appropriate for use in deriving the RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection.

8. EPA employed benchmark dose modeling (USEPA, 2012) in the identification of points-of-departure (PODs) for PFHxA. Are the modeling approaches used, selection and justification of benchmark response levels, and the selected models used to identify each POD for toxicity value derivation scientifically justified and clearly described?

9. Appendix A identifies the potential for toxicokinetic differences across species and sexes as a key science issue and lays out a hierarchy for using relevant toxicokinetic data in extrapolating doses between laboratory animals and humans. Section 5.2.1 describes the different approaches that were considered and the rationale for the selected approach. Given what is known and not known about the potential interspecies differences in toxicokinetics of PFHxA, EPA used the ratio of human-to-animal serum clearance values based on the assumption that the volume of distribution (Vd) in humans is equivalent to monkeys to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs.

   a. Is applying the ratio of human-to-animal serum clearance values for PFHxA scientifically justified and clearly described? If not, please provide an explanation and detail the preferred alternative approach.

   b. Does the Toxicological Review clearly describe the uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?

10. EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), exposure duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) for PFHxA.

   a. Is uncertainty in the derivation of the toxicity values scientifically justified and clearly described? Please describe and provide comments, if needed.

b. For uncertainty in interspecies differences (UF_A), a value of 3 is applied to account for remaining uncertainty in characterizing the toxicokinetic and toxicodynamic differences between laboratory animals and humans after calculation of the HED. For developmental and hematopoietic outcomes the evidence based lacked chemical- and species-specific information that would have been useful for informing the UF_A, however for hepatic, mechanistic and supplemental information were available that were useful for further evaluating the interspecies uncertainty factor. There is some data indicating a PPARα-dependent pathway that might support a UF_A of 1, however evidence for non-PPARα modes of action is available in the PFHxA (and larger PFAS) database. Hence, uncertainty remains regarding the potential differences in sensitivity across species due to the involvement of both PPARα-dependent and -independent pathways. Further, data is lacking to determine with confidence the relative contribution of each of these pathways. As such, the Toxicological Review concludes that the available data are not adequate to determine if humans are likely to be equally or less sensitive than laboratory animals with respect to the observed hepatic effects and that a value of $UF_A = 3$ is warranted to account for the residual uncertainty in toxicodynamic differences across species. Please comment on whether the available animal and mechanistic studies support this conclusion and whether the analysis presented in the Toxicological Review is scientifically justified and clearly described.

c. Are the provided rationales for the remaining uncertainty factors (UF_L, UF_H, UF_D, UF_S) scientifically justified and clearly described? If not, please explain.

11. Given the conclusion that there was inadequate evidence to assess carcinogenic potential for PFHxA (Charge Question #5), the Toxicological Review does not derive quantitative estimates for cancer effects for either oral or inhalation exposures. Is this decision scientifically justified and clearly described?