

**DRAFT External Peer Review Charge Questions for the
IRIS Toxicological Review of Perfluorohexanoic Acid
and Related Salts**

April 2022

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of the draft *IRIS Toxicological Review of Perfluorohexanoic Acid and Related Salts* 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 within the Office of Research and Development. IRIS assessments contain information about 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 perfluorohexanoic acid (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 related salts. The systematic review protocol for PFHxA and 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

When responding to the charge questions below, categorize any recommendations for EPA as part of this peer review into one of three categories (Tier 1, 2, or 3). The categories are useful for prioritizing the relative importance of comments, as follows:

- Tier 1: *Necessary Revisions* – Use this category for any revisions you believe are necessary to adequately support and substantiate the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the PFHxA Toxicological Review
- Tier 2: *Suggested Revisions*– Use this category for any revisions you encourage EPA to implement to strengthen the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the PFHxA Toxicological Review
- Tier 3: *Future Considerations* – Use this category for any advice you have for scientific exploration that might inform future work. While these recommendations are generally outside the immediate scope or needs of the PFHxA Toxicological Review, they could inform future reviews or research efforts.

Systematic Review Methods and Documentation

1. The Toxicological Review for PFHxA describes and applies a systematic review protocol for identifying and screening 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 PFAS IRIS Assessments*). Please comment on whether the search strategy and screening criteria for PFHxA literature are clearly described. If applicable, please identify additional peer-reviewed studies of PFHxA that the assessment should incorporate¹.
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 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”), is intended to help the reviewer navigate this on-line resource. Data from studies considered informative to the assessment are synthesized in the relevant health effect-specific sections, and study data are available in HAWC.
 - a. Please comment on whether the study confidence conclusions for the PFHxA 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 PFHxA studies are presented and synthesized in the health system-specific sections. Please comment on whether the presentation and analysis of study results are clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies.

Noncancer Hazard Identification

3. For each health effect 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 available **evidence indicates** PFHxA likely causes hepatic effects in humans under relevant exposure circumstances. This conclusion is based on studies of rats showing increased liver weight, hepatocellular hypertrophy, increased serum enzymes, and decreased serum globulins. The hepatic findings for PFHxA were similar for other PFAS and determined to be adverse and relevant to humans.
 - i. Additional considerations influenced the hepatic effects hazard identification decisions. Appendix A (*Systematic Review Protocol for the PFAS IRIS*)

¹ Newly identified studies (i.e., studies identified by EPA or the public that meet PECO criteria but were not addressed in the external review draft, for example due to recent publication) will be characterized by EPA and presented to the peer review panel. This characterization will focus on EPA’s judgment of whether the studies would have a material impact on the conclusions (i.e., identified hazards or toxicity values) in the external review draft. The peer review panel is asked to review EPA’s characterization and, based on the panel’s interpretation of the expected impacts, provide tiered recommendations to EPA regarding which studies, if any, to incorporate into the assessment before finalizing.

Assessments) outlines the human relevance of hepatic effects in animals that involve PPAR α receptors as a key science issue. To the extent supported by the PFHxA literature (and to a lesser extent, literature for other PFAS), the Toxicological Review evaluates the evidence relevant to the potential involvement of PPAR α and non-PPAR α pathways with respect to the reported hepatic effects. The Toxicological Review ultimately concludes 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 from PFHxA exposure, but 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.

- b. For developmental effects, the Toxicological Review concludes the available **evidence indicates** 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 exposed throughout gestation and lactation, showing increased perinatal mortality, decreased offspring body weight, and delayed eye opening. These effects are similar to those observed for other PFAS following developmental exposure and were determined to be adverse and relevant to humans.
- c. For hematopoietic effects, the Toxicological Review concludes the available **evidence indicates** PFHxA likely causes hematopoietic effects in humans under relevant exposure circumstances. This judgment is based on consistent findings, including decreased red blood cells [RBCs], hematocrit, and hemoglobin, across study designs that, when interpreted together, signifies PFHxA-related hematological effects such as anemia. These findings were determined to be adverse and relevant to humans.
- d. For endocrine effects, the Toxicological Review concludes the available evidence suggests, but is not sufficient to infer, that PFHxA may cause endocrine effects in humans under relevant exposure circumstances. This conclusion is based on some evidence of thyroid effects based on hormone and histopathological changes in two rat studies; however, the data is limited, lacking consistency across studies, and histopathological changes may be explained by non-thyroid related effects
- e. For all other potential health effects (i.e., renal, male and female reproductive, immune, and nervous system), the Toxicological Review concluded the available evidence is inadequate to assess whether PFHxA may cause effects in humans under relevant exposure circumstances. In general, these conclusions were driven by sparse evidence bases or data that were largely null.

Noncancer Toxicity Value Data Selection

4. 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) 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.
5. In addition, for PFHxA, an 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.

Noncancer Toxicity Value Derivation

6. EPA used benchmark dose modeling (USEPA, 2012) to identify 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?
7. Appendix A identifies the potential for pharmacokinetic differences across species and sexes as a key science issue and lays out a hierarchy for using relevant pharmacokinetic data in extrapolating doses between laboratory animals and humans. Section 5.2.1 describes the various approaches considered and the rationale for the selected approach. Given what is known and not known about the potential interspecies differences in PFHxA pharmacokinetics, EPA used the ratio of human-to-animal serum clearance values assuming the volume of distribution (V_d) in humans is equivalent to that in 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 pharmacokinetic differences between the experimental animal data and humans?

8. EPA has evaluated and applied 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.
- Is uncertainty in the derivation of the toxicity values scientifically justified and clearly described? Please describe and provide comments, if needed.
 - For uncertainty in interspecies differences (UF_A), a value of 3 is applied to account for remaining uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between laboratory animals and humans after calculation of the HED. For developmental and hematopoietic outcomes, the evidence base lacked chemical- and species-specific information that would have been useful for informing the UF_A ; for hepatic outcomes, however, available mechanistic and supplemental information was useful for further evaluating the interspecies uncertainty factor. Some data indicate a PPAR α -dependent pathway that might support a UF_A of 1. Evidence for non-PPAR α modes of action, however, is available in the PFHxA (and larger PFAS) database. Thus, uncertainty remains regarding the potential differences in sensitivity across species due to the involvement of both PPAR α -dependent and -independent pathways. Further, data are lacking to determine with confidence the relative contribution of each of these pathways. As such, the Toxicological Review concludes 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 pharmacodynamic 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.
 - To inform uncertainty in intraspecies variability (UF_H), the assessment evaluates and considers the available evidence on potential susceptibility to PFHxA within different populations or lifestages, including any potential human health impacts from early life exposure. Are the available information and data appropriately considered and the resultant UF_H values scientifically justified and clearly described?
 - Are the provided rationales for the remaining uncertainty factors (UF_L , UF_D , UF_S) scientifically justified and clearly described? If not, please explain.

Carcinogenicity Hazard Identification and Toxicity Value Derivation

9. The Toxicological Review concludes that there is *inadequate information 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.
10. Given the conclusion there was *inadequate information 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?