External Peer Review Charge Questions for the
Draft IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS)
and Related Salts

January 2024

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of the draft *IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS) and Related Salts* developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is housed within EPA's Center for Public Health and Environmental Assessment within the Office of Research and Development. IRIS assessments contain information about chemicals that encompasses 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 PFHxS. The draft Toxicological Review of PFHxS is based on a comprehensive review of the available scientific literature on the potential for noncancer and cancer health effects in humans exposed to PFHxS or salts of PFHxS. The systematic review protocol for PFHxS and other appendices for toxicokinetic information, dose-response modeling, and other supporting materials are provided as *Supplemental Information* (see Appendices A to G) to the draft Toxicological Review.

REVIEW MATERIALS PROVIDED

- Draft PFHxS Toxicological Assessment
- Supplemental Information (PFHxS Appendices)

CHARGE QUESTIONS

In response to the numbered charge questions below organized by topic area (italicized headers), the advice provided as part of this peer review would be most useful when prioritized to indicate its relative importance 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.
- **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 PFHxS 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 PFHxS Toxicological Review, they could inform future reviews or research efforts. Advice with no Tier may be considered by EPA as Tier 3.

Literature Search Methods and Documentation

1. The Toxicological Review for PFHxS 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:

    a. Comment on whether the literature search strategy and screening criteria for PFHxS are appropriate and clearly described.

    b. Identify additional peer-reviewed studies of PFHxS that EPA should consider incorporating prior to finalizing the assessment.

    c. EPA fully synthesized the literature published through April 2022 in the PFHxS external review draft. EPA also screened studies published through April 2023, but only incorporated into the external review draft those studies interpreted to have a material impact on the draft conclusions (i.e., changing which hazards are identified or notably affecting the RfDs) or directly informing the identified key science issues. These decisions are documented in a tabular format in Appendix B.3 (Table B-5). Specifically, EPA identified five pharmacokinetic studies from the April 2023 literature search update that inform an identified key science issue and thus incorporated these studies into the PFHxS PK analysis in the PFHxS external review draft. No other supplemental studies from the 2023 search were interpreted to warrant incorporation. EPA also characterized the epidemiological studies meeting the PECO criteria from the April 2023 literature search and determined that none of these epidemiological studies published since April 2022 would have a material impact on the draft conclusions. No animal studies meeting the PECO criteria were identified in the April 2023 literature search review. Please review EPA's characterization and provide tiered recommendations regarding which additional studies, if any, would have a material impact on the draft's conclusions and should be incorporated into the assessment before finalizing, as well as your interpretation of the impact of those studies to be incorporated.

Noncancer Hazard Identification

2. 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, including 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. Please comment on whether the study confidence conclusions for the PFHxS studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes[1]. Please specify any study confidence conclusions that are not justified and explain any alternative study evaluation decisions. For each, please also comment on whether the weight-of-evidence decisions for hazard

[1] The Toxicological Review provides an overview of individual study evaluations within each evidence synthesis section, and the results of those outcome-specific 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.

identification have been clearly described and scientifically justified. Note that the data from studies considered informative to the assessment are synthesized in the relevant health effect-specific sections and available in the Health Assessment Workspace Collaborative (HAWC).

a. For immune effects, the Toxicological Review concludes the available ***evidence indicates*** PFHxS exposure is likely to cause immunosuppression in humans given sufficient exposure conditions, primarily on the basis of consistent evidence of reduced antibody responses from epidemiological studies in children and adults. For nearly all epidemiology studies of PFHxS, there is potential that exposure to other highly correlated PFAS could contribute to the observed effects. Thus, the synthesis of epidemiology studies on immune effects included an evaluation of the potential for confounding across PFAS as well as other sources of confounding. After considering these factors on the basis of the available data, it was determined that there was minimal concern for substantial confounding, and it was unlikely to fully explain the associations seen in the literature. Although immunotoxicity-specific animal studies were not identified, general toxicity or developmental toxicity studies that included immune-related endpoints (i.e., basophil cell counts) were identified. However, these endpoints in animals were nonspecific and not informative to the immune effects hazard judgment.

b. For thyroid effects, the Toxicological Review concludes the available ***evidence indicates*** PFHxS exposure is likely to cause thyroid effects in humans given sufficient exposure conditions, on the basis of a series of short-term studies in rats demonstrating consistent and coherent effects with a clear biological gradient. The thyroid findings for PFHxS were similar to those observed for other structurally related long-chain PFAS and determined to be adverse and relevant to humans.

c. For developmental effects, the Toxicological Review concludes the available ***evidence suggests*** but is not sufficient to infer whether PFHxS exposure has the potential to cause developmental effects in humans given sufficient exposure conditions. This judgement is based primarily on mostly consistent but notably uncertain evidence of decreased birth weight and length from studies of exposed humans in which PFHxS was measured during or shortly after pregnancy (see Sections 3.2.3 and Appendix C.1). This judgment was also supported by results from a meta-analysis of birth weight conducted by EPA. However, the strength of the epidemiology evidence is reduced due to concern for potential bias due to sample timing (pregnancy hemodynamics) especially among studies with later sampling (see Appendix Section C.14). Evidence in experimental animals from two *high* confidence and three *medium* confidence studies in rats and mice was *indeterminate*.

d. For hepatic effects, the Toxicological Review concludes the available ***evidence suggests*** but is not sufficient to infer whether PFHxS exposure has the potential to cause liver effects in humans given sufficient exposure conditions. This conclusion is based on *slight* evidence of an association between PFHxS exposure and small changes in serum markers of liver disease in humans that was inconsistent across studies, and evidence from experimental animal studies in rats and mice that was also considered *slight*.

e. For neurodevelopmental effects, the Toxicological Review concludes the available ***evidence suggests*** but is not sufficient to infer whether PFHxS exposure has the potential to cause neurodevelopmental effects in humans given sufficient exposure conditions. This conclusion is based on *slight* epidemiological evidence of some positive associations between PFHxS exposure and ADHD or behaviors potentially related to ADHD. Evidence from experimental animal studies was *indeterminate*.

f. For cardiometabolic effects, the Toxicological Review concludes the available ***evidence suggests*** but is not sufficient to infer whether PFHxS exposure has the potential to cause cardiometabolic effects in humans given sufficient exposure conditions. This conclusion is based primarily on consistent increases in cholesterol in humans. However, limitations in the available epidemiological studies introduced significant uncertainty. Evidence from experimental animal studies was *indeterminate*.

g. For hematopoietic effects, male and female reproductive effects, and renal effects the Toxicological Review concludes there is ***inadequate evidence*** to determine whether PFHxS exposure has the potential to cause these effects in humans on the basis of the sparsity of the available evidence.

Noncancer Toxicity Value Data Selection and Modeling

3. For PFHxS, no RfC was derived for inhalation exposure. Organ/system-specific RfDs were derived for immune and thyroid effects and considered for use in deriving the oral RfD. The RfD was based on immune effects observed in humans. The study chosen for use in deriving the immune osRfD was Grandjean et al. (2012) with additional analyses by Budtz-Jorgensen and Grandjean (2018), which reported decreased serum anti-tetanus antibody concentrations in children (male and female) at age seven years and PFHxS measured at age five years (see Appendix D and Section 5.2.1). Are the selection of the studies for the immune effects (Butz-Jørgensen and Grandjean, 2018; and Grandjean, 2012) for use in deriving the RfD for PFHxS scientifically justified? Are the modeling approaches appropriate?

    a. If so, please provide an explanation.

    b. If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the lifetime 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 lifetime RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection[2]. Please also see charge questions 2b and 2c.

[2] For the decreased antibody responses, Selgrade (Tox Sci 2007;100:328–332) suggests that these specific immunotoxic effects may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards.

d. EPA used benchmark dose modeling (BMD) (U.S. EPA, 2012) to identify points-of-departure (PODs) for PFHxS. Are the BMD modeling approaches, selection, and justification of benchmark response levels, and selection of the BMD models used to identify each POD for toxicity value derivation scientifically justified and clearly described?

e. Given the lack of studies on inhalation exposure to PFHxS, no reference concentration (RfC) is derived. Please comment on this decision.

4. In addition, for PFHxS, an RfD for less-than-lifetime ("subchronic") exposures is derived in Section 5.2.2. No "subchronic" RfC is derived. The same studies, outcomes, and comparisons were chosen for use in deriving the lifetime and subchronic RfD. Are the selection of these studies and these effects for the derivation of the subchronic RfD for PFHxS scientifically justified?

    a. If so, please provide an explanation.

    b. If not, please provide an alternative study(ies) 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 subchronic RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection.

    d. Given the lack of studies on inhalation exposure to PFHxS, no "subchronic" RfC is derived. Please comment on this decision.

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors

5. Appendix E describes a Bayesian pharmacokinetic (PK) analysis of the available data for mice, rats, and monkeys, in order to obtain key PK parameters with rigorous confidence ranges. Appendix E also describes the application of the resulting parameters in a one-compartment (1-C) PK model for rats to evaluate its potential for use in extrapolating PFHxS toxicity data to humans. Section 3.1 evaluates and synthesizes the PK data in relevant species and sexes, and among human life stages, up to the derivation of key PK parameters used in the subsequent analysis. The evaluation of existing PBPK models and the 1-C PK model found that the PBPK model was not sufficiently reliable for use. While the 1-C PK model significantly overpredicts concentrations observed in adult male and female rats in the NTP bioassay at high doses (see Figure E-8 of Appendix E), the predictions were considered by EPA to be acceptably close to the observed NTP bioassay concentrations in the range of the rat PODs being evaluated (0.684 mg/kg-d in male rats, and 0.051 and 5.5 mg/kg-d in female rats).

    A third option, not presented in the draft, was suggested during the external peer review of the Toxicological Review of PFDA: direct application of the measured end-of-study serum concentrations, which are available for the NTP bioassay, but not the Ramhøj, 2018 study. This approach would interpolate among the concentrations at the NTP bioassay doses to estimate internal doses for PODs between that fall between those doses and would be limited to the specific dosing regimen and lifestage for which the serum concentration data are available.

Extrapolation of developmental lifestage (thyroid) endpoints to HEDs involves uncertainty due to the lack of PK data for pregnancy and lactation in rats and limited developmental PK data for humans for either use of the 1-C PK model or the data-derived extrapolation factor (DDEF) approach selected in the draft.

Given the information available on potential interspecies differences in PFHxS PK, EPA applied sex- and life-stage-specific DDEFs to POD values from toxicity studies in laboratory animals to estimate corresponding human equivalent doses (HEDs) in the derivation of the respective RfDs. Use of DDEFs effectively assumes that concentrations in rats reach steady state, which may be an appropriate approximation for female rats, but not male rats based on the predicted serum concentration time-courses shown in Figure E-8. Estimates using the 1-C PK model predict that the serum concentration in male rats has reached 54% of the steady state value after a 29-day exposure. However, at the lowest NTP dose of 0.625 mg/kg-d, the steady state value in male rats was only 31% greater than the observed mean concentration while the 1-C PK model prediction was 30% below.

Similarly, estimated human clearance (CL) values were used to convert internal dose POD (PODint) values from epidemiological analyses to corresponding HEDs (i.e., human dose levels that are equivalent to the identified PODint values). In selecting the CL values, EPA evaluated published data for PFHxS relevant to PFHxS dosimetry in women of childbearing age (see question 5c below), including during pregnancy and lactation.

a. Is the decision and method for applying DDEFs for rat-human extrapolation scientifically justified, given the available science or should the EPA give greater weight to the 1-C PK model, despite the noted uncertainties? Should the choice of extrapolation method depend on the rat sex in which an endpoint is observed (or when dosing occurred to dams for developmental effects, in which case the adult female PK is considered relevant)? Should EPA instead use direct interpolation of the NTP end-of-study concentration data (which are sex-specific) to estimate internal doses for that bioassay in particular? Please provide some details on your rationale for a preferred approach.

b. Is use of the human sex- and lifestage-specific clearance values to estimate HEDs from internal dose PODs in humans scientifically justified? Specifically, should EPA include estimates of total CL from studies that only measured urinary clearance, requiring an estimation of fecal (and other pathway) clearance, or only use estimates based on empirical half-lives or estimates total CL based on estimated exposure levels that do not require estimation of fecal clearance? Please provide some details supporting your recommendation.

c. Have the uncertainties in the DDEFs and human CL been adequately evaluated and described? In answering this question, please explicitly consider EPA's approach to adjusting for menstrual fluid loss in the draft assessment. If the estimated menstrual fluid clearance as a specific mechanism is not considered reliable, should EPA adjust for empirical differences observed between 1) women of childbearing age and 2) men and younger and older women, such as reported by (Jain, 2022), discussed in section 4.1.3/**Humans**/Sex differences in human PFHxS.

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

    a. Is uncertainty in the derivation of the toxicity values scientifically justified and clearly described given that the assessment evaluates and considers the available evidence on potential susceptibility to PFHxS within different populations or lifestages, including any potential impacts from early life exposure to PFHxS on children's health or health effects later in life? If not, please explain. Please describe and provide comments, if needed.

    b. Please specifically comment on whether use of the intra-human uncertainty factor, UF_H = 10, in combination with the selected sex- and lifestage-specific human CL values, appropriately accounts for both the pharmacokinetic and pharmacodynamic uncertainties and differences (variability) among humans?

    c. For immune effects, a UF_S of 1 was selected. A UF_S of 10 was not considered as the developmental period is recognized as a susceptible lifestage for these types of effects and therefore exposure during this time window can be considered more relevant than exposure in adulthood (U.S. EPA, 1991). Also important is the fact that, given PFHXS long half-life and the expectation that the children and their mothers have been exposed to elevated levels of PFHxS for many years, the observed effects on immune response are considered to be the result of a cumulative, prolonged exposure. Uncertainties with regards to additional susceptible life stages (e.g., old age) are addressed as part of the UF_D. Does the provided scientific rationale support this decision? If not, please explain.

Carcinogenicity Hazard Identification and Toxicity Value Derivation

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

8. Given the conclusion there is *inadequate information to assess carcinogenic potential* for PFHxS, the Toxicological Review does not derive quantitative estimates for cancer effects for oral or inhalation exposures. Is this decision scientifically justified and clearly described?