Division of the National Toxicology Program, National Institute of Environmental Health Sciences

Comments on the Interagency Science Consultation Draft

IRIS Assessment of Perfluorohexanoic Acid and Related Compounds Ammonium and Sodium ! Perfluorohexanoic Acid !

(Date Received August 24, 2021) !

NIEHS/DNTP's technical corrections and suggestions by question and page number are provided in **BOLD**.

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.

The methods are well document, but as presented it is challenging to pull all the details together without having multiple documents open. Although the protocol is available for download, suggest that EPA provide the protocol to reviewers with the main document and supplemental information. Understanding that the authors are trying to set a balance for presenting critical information in the main document relative to appendices, suggest adding a PFHxA-only version of the PECO to the main document.

Tier 2: Page 1-10, line 4-7: "Not all studies that meet …" describes how data are not extracted from all studies. This process is not well explained in the main document and text should be added to the main document with simple descriptions of the impact of using the uninformative category and the low confidence category for the PFHxA evaluation (does that apply to this evaluation? If so, to which references?).

Tier 2: Page 2-1, line 10: "..animal toxicological studies" Why are only the animal studies in the heatmap? And the tableau readme on page 1, left side lower corner, states that human studies are part of the visualizations. Suggest that EPA either add the human studies (which would be the preferred solution), or remove the human readme and add to the text on page 2-1, why there is not a human study heatmap.

Tier 2: Page2-3, line 4: "Thirteen epi…" Figure 2-1 shows 14 human studies – correct or clarify.

Tier 2: Page 2-3, line 8-9: "The remaining nine studies were rated medium or low confidence…" Suggest adding how this impacted potential consideration for synthesis relative to the methods. The methods and the protocol in Appendix A page 8-1, line 12 state that not all low confidence studies undergo data extraction if there are enough medium confidence studies. This is where EPA should be clear on the impact of the body of evidence and what was subsequently done in terms of extraction for PFHxA to help the reader through the methods and the details of the appendix and protocol.

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.

Mostly described well, some edits are suggested (see below)

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.

Tier 2: Page 3-2, line 23-24: "The researchers also noticed that Tmax slightly…" suggest rewording to "The data indicate that…" as we didn't highlight this, and the difference is marginal (10-13 min difference between high and low dose). The NTP study only highlights the longer Tmax with dose in male rats administered PFOA.

Page 3-3, line 11-13: suggest rewording as accumulation could be taken in a variety of ways. Suggest using total vs ongoing accumulation.

Page 3-6, paragraph starting on line 8: Suggest including Dzierlenga et al. 2019 [DOI: 10.1080/00498254.2019.1683776] data that measure liver, kidney, and brain concentrations at multiple time points after a single dose in male and female rats.

Table 3-1: Female 40 mg/kg dose, note that the error for the half-life is large and truly 213, not 2.13.

Page 3-22, line 15-16: Note that necrosis was also observed at a low incidence in the NTP 2018 study (TOX-97, https://ntp.niehs.nih.gov/go/tox097abs) in males at 1000 mg/kg (1/10) (data were not brought forward into the report, but are present in the histopathology data tables (P03): https://tools.niehs.nih.gov/cebs3/views/?action=main.dataReview&bin_id=3875

Page 3-29, line 9-10: Globulins were decreased at greater and equal to 125 mg/kg (not at 250 as stated). Believe there was a decrease at 100 mg/kg in the Loveless study too.

Table 3-8: Unclear whether the table is calculated based on total protein or globulin; only see values for one or the other and believe the data is for total protein. Need to add a table for globulins.

Table 3-34: Brain weight was not measured in the NTP 2018 28-day toxicity study; need to remove confidence for this endpoint.

Page 3-51, line 3-4: The increase in kidney weight was in females, not males. Line 4-5: Believe there was an increase in relative weight in males, possibly driven by the decrease in body weight.

Table 3-17: The increase in kidney weight occurred in female rats and needs correction. This corresponds to an increase in chronic progressive nephropathy (CPN) in female rats, which increases confidence in the effect, although it only occurred at the highest exposure (1000 mg/kg).

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 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.

Agree overall, but note that the reduction in total protein and globulins in the NTP study occurred at 125 mg/kg and not 250 mg/kg as stated in the report. This is consistent with Loveless et al. showing effects at 100 mg/kg. This section would change statements that hepatic effects occurred at 200+ mg/kg (for example, line 13 on page 3-35).

Disagree that "Lack of Coherence across sexes" (Page 3-36) would be a factor for decreasing certainty as many studies have shown male rodents to be more sensitive than females in regards to PFAS chemicals, which may be attributed to PK differences and/or receptor-mediated response.

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

Page 3-67: Unclear regarding selection of "moderate" animal evidence of hematopoietic effects, as effects appear to be consistent across NTP and Loveless studies; there is evidence of biological response (increased reticulocytes to counter anemia). Suggest level would be "high". Lack of coherence across sexes to decrease certainty should be given lower influence because PFAS generally have a strong sex-dependent PK and toxicity profile.

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.

Agree, data are not sufficient to infer.

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.

Note the error in describing the NTP studies: Higher kidney weights in female rats corresponded with higher incidence of chronic progressive nephropathy.

Agree with the conclusion of inadequate. Page 3-97 neutrophil and basophil should describe the tissue/organ. Page 3-98 thymus weight decrease at high dose is not an immune effect, it is evidence of overall toxicity.

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.

Agree.

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.

Agree. The chronic study in rats is limited, and it appears that exposure selection could have been higher for identifying carcinogenicity. Due to the rapid elimination in rodents and minimal effects observed at 100 mg/kg (males) and 200 mg/kg (females), a higher exposure would have been justified, in addition to evaluating a second species (mice). Note that the description on page 3-103, beginning on line 27, appears to be incorrect as males AND female rats were evaluated, and as written in the manuscript, it appears that more tissues were evaluated than the four listed.

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.

Agree in principle, but the data provided are limited for full evaluation (see below).

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.

The effects on F1 growth during lactation are considered adverse, although data indicate that this effect was transitory, and according to the authors, the effects were not apparent postweaning. However, there was no direct exposure to the F1 so postweaning could be treated as a stop-exposure study.

In general, there is limited data in the report (Loveless et al.) for a full evaluation. For example, the data for number of litters evaluated, produced from mating, litter size, and developmental landmarks are not presented. Furthermore, the weight data presentation in Table 7 is selective, switching from weight gain, to absolute weight depending on time-period and generation. This also applies to the developmental toxicity study presented in Table 8. Note that there was an 11% decrease in pup weights on PND 0 in the 100 mg/kg group, which typically would be statistically and biologically significant, but it is difficult to ascertain why it is not, as litter and pup numbers are not provided. Furthermore, the decrease in weight at this time point (following parturition) suggests that fetal growth was retarded, which did not show-up in the fetal evaluation. Again it is hard to decipher due to the lack of information.

In both the Loveless 2009 and Iwai and Hoberman 2014 developmental studies, there was no direct exposure to the F1 during lactation or postweaning thus limiting the understanding of the impact of PFHxA on development including development during puberty.

Page 3-87 ln 23: The description of the uterine horn dilation in the NTP 2018 report: Findings were based on a read down of control and high dose. In-between exposures were based on gross observation during necropsy. The data are imbalanced and have a low degree of confidence that these findings are related to chemical exposure.

Page 5-02, line 8-9 and Table 5-1: An explanation may be needed in the preceding text for why the NTP 28-toxicity studies are not included for dose-response modeling and derivation of points of departure. Is 28 days considered too short compared to the 90 day study for evaluation?

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.

No comment provided.

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?

No comment provided.

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.

Agree, an approach that has been used previously with other PFAS.

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

There are marginal sex differences identified in primates, but it is not a robust data set for primates or humans. Given the quality rodent data and limited human and primate data, could be an area to further investigate sex and age differences and to what degree they exist.

10. EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), exposure duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for PFHxA.

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

Generally, agree with the uncertainty.

b. For uncertainty in interspecies differences (UFA), 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 UFA, 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 UFA 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 UFA = 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.

Agree.

c. Are the provided rationales for the remaining uncertainty factors (UFL, UFH, UFD, UFS) scientifically justified and clearly described? If not, please explain.

A subchronic to chronic uncertainty factor (UF_S) of 3 could be applied to the developmental studies, as exposure was limited and indirect to postnatal F1 (i.e., gestation only or gestation/lactation) thus limiting the developmental evaluation. Exposure directly to PFHxA post-PND 12 could lead to body weight effects.

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?

Agree.