TASK ORDER 68HERH20F0407 UNDER
CONTRACT EP-C-17-017

EXTERNAL PEER REVIEW OF EPA'S "DRAFT IRIS TOXICOLOGICAL REVIEW OF PERFLUORONONANOIC ACID (PFNA, CASRN 375-95-1) AND RELATED SALTS"

FINAL PEER REVIEW SUMMARY REPORT

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Submitted to:
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1.0 INTRODUCTION

This report documents the results of an external independent peer review of the U.S. Environmental Protection Agency's (EPA's) draft "IRIS Toxicological Review of Perfluorononanoic Acid (PFNA, CASRN 375-95-1) and Related Salts." ERG, a contractor to EPA, organized this review and developed this report. The peer review included a virtual meeting that was open to the public as observers and included an opportunity for oral public comment (in addition to opportunity for the public to submit written comments to EPA via the PFNA docket).

Section 1.0 provides background about the review. Section 2.0 provides a high-level summary of reviewer comments. Section 3.0 presents reviewer final individual post-meeting comments. In Section 3.0, reviewers' final written comments are organized by charge question and presented exactly as submitted, without editing or correction of typographical errors (if any). Section 4.0 presents any additional comments provided by reviewers. Appendices A, B, and C, respectively, provide the list of reviewers, EPA's charge to reviewers, and the peer review meeting agenda.

1.1 Background

In 2024, ERG organized and managed an external peer review of EPA's draft "IRIS Toxicological Review of Perfluorononanoic Acid (PFNA, CASRN 375-95-1) and Related Salts," developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). 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 PFNA. EPA's draft Toxicological Review of PFNA 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 PFNA or related salts.

1.2 Peer Review Process

During the first stage of the peer review process, ERG assembled a set of experts interested in serving as reviewers, who collectively spanned three key areas of expertise required by EPA for this and four other related peer reviews: environmental epidemiology, experimental toxicology, and the use of quantitative methods (e.g., dose-response modeling, PBPK model development) important for the derivation of toxicity values in human health assessments of environmental chemicals. To identify candidates, ERG used standard search processes and considered experts nominated by the public in response to a Federal Register Notice (FRN) requesting nominations. After considering comments on these candidates submitted by members of the public in response to a second FRN, ERG assembled a final pool of 20 experts from which to select reviewers for this and the four other related peer reviews. For this PFNA review, ERG selected the following nine experts after confirming they had no conflict of interest for this review:

- Courtney C. Carignan, Ph.D.
- Alan M. Ducatman, M.D., M.S.
- Elaine M. Faustman, Ph.D., DABT (Panel Chair)
- Panagiotis G. Georgopoulos, Ph.D.
- Joseph T. Haney, Jr., M.S.
- Angela M. Leung, M.D., M.S.

- Zhoumeng Lin, Ph.D., DABT
- David A. Savitz, Ph.D.
- R. Thomas Zoeller, Ph.D.

See Appendix A for a detailed list of reviewers.

ERG provided reviewers with the draft PFNA toxicological review document and with EPA's charge to reviewers (Appendix B), which asked reviewers to address each of the eight questions and multiple sub questions and to categorize their advice to EPA into three tiers:

- 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 PFNA 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 PFNA Toxicological Review, they could inform future reviews or research efforts.

For their consideration, ERG also provided reviewers with written public comments submitted to EPA's docket for this review and with a table developed by EPA that listed published literature identified (1) during EPA literature search updates after release of the draft PFNA toxicological review for public comment or (2) in public comments received through the EPA docket.

Working individually, each reviewer prepared written pre-meeting comments in response to the charge questions (Appendeix B), and ERG compiled and distributed these preliminary comments to all reviewers a few days prior to the peer review meeting to help them prepare for discussions at the meeting.

ERG organized and facilitated a virtual peer review meeting, which took place via Zoom.gov on July 30, 31, and August 1, 2024. The meeting was open to members of the public to attend as observers and provided an opportunity for members of the public to make an oral comment. During this meeting, reviewers discussed and commented on EPA's draft PFNA Toxicological Review, with discussion structured by EPA's charge questions. Appendix C provides the meeting agenda. After the meeting, reviewers prepared their individual final post-meeting comments (see Sections 3.0 and 4.0) and ERG prepared a high-level summary (Section 2.0) of reviewer comments.

2.0 SUMMARY OF KEY REVIEWER COMMENTS BY CHARGE QUESTION

This section provides a high-level summary of reviewer comments organized by charge question. For reference, a brief summary of each of EPA's charge questions is shown in bold font.[1] This summary section focuses on key comments. Therefore, it summarizes selected Tier 1, 2, and 3 comments, as well as key comments that reviewers did not categorize into a tier. Editorial comments are not included even if the reviewed designated the comment as Tier 1, 2, or 3. For all tiered comments, the tier in which the reviewer categorized the summarized comment is provided in parentheses. If "no tier" is indicated, the reviewer did not assign a tier to that comment.

[1] For simplicity, the bold text does not include charge question text that provides background or summarizes EPA's rationale. For the full text of EPA's charge to reviewers, see Appendix B.

Please see Section 3 for the full text of all reviewers' post-meeting comments, including comments not included in this summary and the original and complete text of comments that are summarized here.

1a. Comment on whether the literature search strategy and screening criteria for PFNA are appropriate and clearly described.

- Carignan, Ducatman, Faustman, Georgopoulos, Leung, Lin, Savitz, and Zoeller commented that the literature search strategy and screening criteria appear appropriate and clearly described.
- Georgopoulos also suggested providing references to documents relevant to PFNA risk characterization that have been developed by U.S. and international regulatory agencies (Tier 2).
- Haney commented that the literature search strategy and screening criteria were largely appropriate and clearly described, although he recommended that the PECO for human/epidemiological studies should indicate that studies were only included for derivation of toxicity factors when study subjects were exposed to the single PFAS of interest (Tier 1).

1b. Identify additional peer-reviewed studies of PFNA that EPA should consider incorporating prior to finalizing the assessment.

- Haney, Leung, Savitz, and Zoeller did not provide any further articles for consideration.
- Carignan recommended incorporating four new studies on inhalation and dermal exposures (Tier 2).
- Ducatman recommended that EPA add discussion of PFNA in mixtures, although he noted that this would not likely alter any relevant points of departure (Tier 2).
- Ducatman identified several articles on breastfeeding and lactation, although he noted that these articles were not likely to change EPA's conclusions (Tier 2 [metanalyses], Tier 3 [primary publications]).
- Faustman did not provide additional references but noted agreement with EPA's stated intention of including musculoskeletal effects based on their review of new references (no tier).
- Georgopoulos provided a list of 27 articles for EPA's consideration (no tier).
- Lin commented that while he did not have any articles to recommend that would materially change the draft's conclusions, he provided three studies that may improve understanding of the tissue distribution of PFAS in aquatic species and the interaction effects of PFAS mixtures, or that were not available in the HERO database (Tier 3).

2. For each health effect considered in the assessment and outlined below, please comment on the (1) evidence synthesis, (2) study confidence conclusions, (3) weight-of-evidence decisions, and (4) additional unincorporated studies of material impact to weight-of-evidence decisions.

2a. Developmental effects

- Carignan, Faustman, Georgopoulos, Leung, Lin, Savitz, and Zoeller commented that the conclusions on the developmental effects were scientifically justified.
- Ducatman made several comments about confounding by other PFAS and PFNA as part of PFAS mixtures. He suggested that EPA's reservations on confounding by PFDA were overstated (Tier 2). Further, he commented that Surflon is primarily PFNA, and as such, Surflon studies should be considered (Tier 1). Finally, he noted that EPA should consider noting the presence of PFNA

contamination in AFFF contamination (Tier 2) and clarify language around the relative contributions of dam-pup and maternal-child contribution of PFAS in offspring (Tier 2).

- Faustman commented that additional discussion is needed on postnatal growth, body weight, and survival data comparisons across human epidemiological and animal outcome data.
- Haney commented that confounding from other PFAS was not adequately addressed in epidemiology studies (Tier 1).
- Zoeller suggested defining the word "precision" in relation to data sets to clarify EPA's conclusions about study quality (Tier 2).

2ai. Robustness of the epidemiological evidence

- Carignan, Georgopoulos, Lin, and Zoeller agreed that the evidence is robust and that the weight-of-evidence decisions were scientifically justified.
- Haney commented that epidemiology studies should not be used as the basis for quantitative dose-response assessments or toxicity factor derivation (Tier 1). He also commented that if EPA chooses to use epidemiology studies as the basis for toxicity factors, the Agency should include an explanation as to why they are deviating from the IRIS Handbook and ATSDR guidelines (Tier 1).

2b. Liver effects

- Carignan, Faustman, Georgopoulos, Leung, and Lin commented that data appear clearly and appropriately synthesized, agreed that the weight of evidence decisions are clearly described and scientifically justified, and concurred with EPA's conclusions.
- Ducatman concurred with EPA's conclusions but recommended adding adverse alterations in human cholesterol more explicitly as evidence of liver toxicity (Tier 1) and adding additional framing for the limits of knowledge on PFNA and biomarker associations (Tier 1). He suggested adding uric acid effects as a topic separate from the kidneys (Tier1) and adding uric acid to the reasons for considering PFNA as a cause of hepatotoxicity (Tier 2). Further, Ducatman suggested that EPA consider the clinical impact when characterizing associations with biomarkers.
- Haney commented that he disagreed with the use of Kim et al. (2023) as the basis for a quantitative dose-response assessment or toxicity factor derivation (Tier 1).

2bi. Comment on EPA's conclusions regarding confounding across PFAS and concurrent outcome and exposure measurement

- Carignan, Ducatman, Lin, Savitz, and Zoeller commented in agreement with the determination of moderate evidence in human studies and the conclusion that observed effects are unlikely to be explained by confounding across PFAS or concurrent measurement of exposure and outcome.
- Ducatman suggested adding context about whether and how human population and experimental data inform each other, including consistency or inconsistency across studies and any evidence that PFNA adds or detracts from toxicity (Tier 2).
- Haney commented that it is not possible to accurately determine a point of departure for toxicity factor derivation from an epidemiological mixture study (no tier).

- Zoeller further commented that the document would be improved by an explanation of the designation of moderate evidence in epidemiologic studies, given the consistent relationship between PFNA and ALT despite the number of factors that can affect serum levels of hepatobiliary markers (Tier 2).

2bii. Comment on the basis of the judgement of human relevance of liver effects observed in animals (PPARα-dependent and independent pathways)

- Carignan, Ducatman, Faustman, Georgopoulos, Haney, Leung, Lin, and Zoeller commented in agreement with EPA's judgement on the relevance of experimental studies to humans, including the relevance of both PPARα-dependent and -independent pathways.
- Savitz declined to comment.

2biii. Comment on the basis for determination that the hepatotoxic effects observed in rodents are considered adverse

- Carignan, Ducatman, Faustman, Georgopoulos, Haney, Leung, Lin, and Zoeller commented in agreement with EPA's determination that the hepatotoxic effects observed in rodents are considered adverse.
- Ducatman suggested adding language to clarify that the absence of long-term experimental studies is a "data gap" and use precise language to specify biomarker use and limitations as much as possible for ALT and bilirubin (Tier 2).

2c. Male reproductive effects

- Carignan, Ducatman, Faustman, Georgopoulos, Haney, Leung, Savitz, and Zoeller concurred with EPA's conclusions and presentation of the data in the male reproductive effects section.
- Lin declined to comment.

2d. Immune effects

- Carignan, Ducatman, Georgopoulos, Leung, Savitz, and Zoeller commented that data appear clearly and appropriately synthesized and concurred with EPA's conclusions in the immune effects section.
- Ducatman commented that discussing inconsistency of findings across multiple studies regarding an association between PFAS and COVID-19 would be a clearer way to summarize the current state of scientific knowledge about PFAS and COVID (Tier 2).
- Faustman commented that more details should be provided about the Faroe Island sub-analysis showing a positive Ab response association with PFNA in children at age 5 when exposure was measured in infancy (Tier 1).
- Faustman also suggested that EPA consider expanding the discussion of confounding with other PFAS related compounds (Tier 1).
- Lin declined to comment.

2di. Comment on the weight-of-evidence decisions for immunosuppression

- Carignan, Faustman, Haney, and Leung commented that the weight-of-evidence is clearly described and scientifically justified.

- Carignan recommended that EPA consider noting the conclusions for other PFAS (e.g., PFOA, PFHxS) in this section (Tier 2).
- Georgopoulos commented that the substantial concerns regarding imprecision and potential residual confounding by other PFAS are explained adequately.
- Haney commented that the level of serum antibodies corresponding to a clinically protective level is assay-specific, which could be more clearly described in the text (Tier 1).
- Lin declined to comment.

2e. Thyroid effects

- Carignan, Faustman, Georgopoulos, Haney, and Savitz commented that data appear clearly and appropriately synthesized and concurred with EPA's conclusion that "the available evidence suggests, but is not sufficient to infer, that PFNA exposure may have the potential to cause effects on the thyroid in humans."
- Carignan commented that additional clarity could be added to this section by improving the figures by grouping studies by indicator and pregnancy status (Tier 2) and adding an indication of the strength of evidence (Tier 2).
- While Ducatman agreed with EPA's conclusion, he noted that the rationale provided was confusing (Tier 1).
- Faustman suggested adding a discussion of the potency of different PFAS compounds and their known impact on thyroid pathways (Tier 1).
- Leung commented that studies not providing time of day of thyroid hormone measurement should not be downgraded to deficient (Tier 2). Leung also suggested including a discussion of the potential mechanisms for adverse thyroid effects, as not all hypothyroxinemia is pathologic (Tier 1). In addition, Leung noted that concurrent measurements of multiple thyroid markers provide stronger evidence, and these studies should be discussed earlier in the section (Tier 2).
- Leung further commented on the necessity of separating studies with pregnant and non-pregnant women because of the physiologic effects of pregnancy on thyroid function (Tier 1).
- Zoeller recommended carrying through the point of departure for thyroid to develop a subchronic RfD for comparison with the RfDs developed for other PFAS that the agency has performed (Tier 2). However, he also noted that EPA's decision not to carry the POD forward to a subchronic RfD was scientifically justified within the context of this review. Zoeller further suggested defining the uncertainties in the dataset (Tier 2) and reconsider the current argument about the use of analog assays for measuring free T4 (Tier 2).
- Lin declined to comment.

2f. Cardiometabolic effects

- Carignan, Faustman, Georgopoulos, Haney, Leung, and Savitz commented that data appear clearly and appropriately synthesized, agreed that the weight of evidence decisions are clearly described and scientifically justified, and concurred with EPA's conclusions in the cardiometabolic effects section.
- Ducatman commented that underestimation bias in cross sectional studies measuring albuminuria may be worth discussing in this section (Tier 2).

- Faustman suggested incorporating a discussion of the mechanistic and supplemental information be added to Table 3-47 for evidence integration (Tier 2).
- Zoeller suggested including an analysis of endpoints that are considered adverse in absence of clear evidence of an apical endpoint which could be used for a point of departure (Tier 3).
- Lin declined to comment.

2g. Neurodevelopmental effects

- Carignan, Faustman, Georgopoulos, Haney, Leung, Savitz, and Zoeller commented that data appear clearly and appropriately synthesized and concurred with EPA's conclusions in the neurodevelopmental effects section.
- Carignan suggested updating Table 3-35 to clarify the outcome measured (Tier 2).
- Ducatman commented that the summary was reasonable, although he also noted that the downgrading of cross-sectional studies with exposure variables related to childhood rather than maternal serum PFAS presumes that gestation is the more relevant exposure window (Tier 2). Ducatman also suggested two possibly useful studies of PFNA and neurodevelopmental endpoints (Tier2).
- Lin declined to comment.

2h. Female reproductive, urinary, adrenal, and other non-cancer effects

- Carignan, Faustman, Georgopoulos, Haney, and Zoeller commented that data appear clearly and appropriately synthesized and concurred with EPA's conclusions for these outcomes.
- Carignan suggested the following changes to improve the clarity of this section:
  - Update Table 3-35 to clarify the outcome measured (Tier 2).
  - State whether or which studies excluded individuals with kidney disease, an important source of negative bias for renal studies (Tier 2).
  - Provide more explanation of the concern for reverse causation for renal effects (Tier 2).
  - Add statistical significance to the fecundability table (Tier 2).
  - Add developmental effects on breast bud development in experimental studies (Tier 2).
- Ducatman commented in agreement with EPA's conclusions about female reproductive outcomes but suggested that this organizational grouping did not do each topic justice and commented that the wording on the section heading may imply that EPA is considering female urinary, female adrenal, and female other non-cancer effects, but not the male counterparts (Tier 3).
- Ducatman suggested the following changes to improve this section:
  - Include a discussion of uric acid, although uric acid should ideally have its own section (Tier 1). This can include the multiple aspects of excretion and how non-causal excretion considerations interact with population findings (Tier 2).
  - Include a section on bone mineral density and osteoporosis (Tier 1).
- Lin and Savitz declined to comment.

3. For PFNA, no reference concentration (RfC) was derived for inhalation exposures. A reference dose (RfD) was derived based on a meta-analysis (Wright, 2023, 10699259) examining reduced birth weight in humans from 10 studies with biomarkers collected early in pregnancy. Note that the

selected RfD based on developmental effects is further supported by the lifetime oral hepatic organ-specific osRfD, based on Kim et al. (2023).

3a. Are the selected 10 epidemiological studies for developmental effects used in deriving the RfD values for PFNA scientifically justified?

- Carignan, Faustman, Georgopoulos, and Lin concurred with EPA's scientific justification of the studies chosen for deriving the RfD values for developmental effects.
- Haney suggested moving the results of the meta-analysis to table D-19 to reflect that it includes epidemiological studies (Tier 2). Haney did not agree with the use of this meta-analysis as the basis for a quantitative dose-response assessment (Tier 1).
- Lin commented that based on Table D-9 and Table D-10, there are dose-response analysis results from five epidemiological studies rather than 10 and EPA should add clarification as to where the other five epidemiological studies are (Tier 2).
- Ducatman and Leung declined to comment.

3ai. Please comment on whether the effect selected is appropriate for use in deriving the lifetime RfD, including considerations regarding adversity and the scientific support for its selection.

- Carignan, Georgopoulos, Haney, Lin, and Zoeller concurred with the selection of birthweight as the outcome for deriving the lifetime RfD.
- However, Haney also suggested that the text note that the POD for developmental effects is well below the 50th percentile for U.S. women based on the most recent NHANES data and state whether adverse developmental effects are expected to be currently occurring in the U.S. population (Tier 2).
- Savitz suggested explaining the logic for interpreting small biological changes as an endpoint of health concern, as a biological change is not always of pathological consequence (Tier 3).
- Leung declined to comment.

3aii. Are the modeling and the meta-analysis for decreased birth weight based on 10 studies with biomarkers collected early in pregnancy appropriate for use in derivation for the RfD? Are the selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for developmental effects scientifically justified and clearly described?

- Carignan, Georgopoulos, and Savitz agreed that the modeling and meta-analysis for decreased birthweight were appropriate for use in derivation of the RfD and EPA; the selection and justification of benchmark response levels, the selection of the BMD models, and the POD were scientifically justified and clearly described.
- Haney commented that he disagrees with EPA's choice to derive an RfD based on the meta-analysis or based on any of the epidemiological studies; sufficient animal studies should be used (Tier 1). However, he did agree that the modeling itself, aside from the studies used for derivation, was scientifically justified.
- Lin suggested providing the plots showing the comparisons of observed versus model-predicted results in this section (Tier 2).
- Leung and Zoeller declined to comment.

3b. Are the modeling approaches for the liver effects, selection of cutoff for abnormal, selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for hepatic effects scientifically justified and clearly described?

- Carignan, Faustman, Georgopoulos, Lin, and Savitz agreed that the modeling approaches for liver effects, selection of cutoff for abnormal, selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for hepatic effects were scientifically justified and clearly described.
- Haney commented that he disagrees with EPA's choice to derive an RfD based on any of the epidemiological studies. However, he did agree that the modeling itself, aside from the studies used for derivation, was scientifically justified (no tier).
- Haney commented that after the uncertainty factor is applied, the resulting PFNA serum POD_{HEC} is below the 50^{th} percentile for the US population based on the most recent NHANES data and EPA could strengthen the document by acknowledging this and stating if adverse liver effects are expected to be currently occurring in the US population (Tier 2).
- Ducatman, Leung, and Zoeller declined to comment.

3c. Please comment on whether the provided scientific rationale supports the decision to consider male reproductive effects for the subchronic RfD? Are the selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for male reproductive effects scientifically justified and clearly described?

- Carignan, Faustman, Georgopoulos, and Lin agreed that the provided scientific rationale supports the decision to consider the male reproductive effects for the subchronic RfD. The selection and justification of benchmark response levels, selection of the BMD models, and selected POD were scientifically justified and clearly described.
- Haney commented that he disagrees with EPA's choice to derive an RfD based on epidemiological studies. However, he did agree that the modeling itself was scientifically justified.
- Ducatman, Leung, Savitz, and Zoeller declined to comment.

3d. For immune and thyroid effects, no reference values were derived given uncertainties in the databases that were judged to indicate suggestive evidence of effects. However, while a dose-response assessment is typically not conducted for health effect judgments of "evidence suggests," when the database includes at least one well-conducted study, quantitative analysis may still be useful for some purposes, such as providing a sense of the magnitude and uncertainty of estimates for health effects of concern, informing responses in potentially susceptible populations, or setting research priorities (U.S. EPA, 2005; U.S. EPA, 2020). For this assessment, immunosuppression in children and reduced serum T4 in adult female rats were advanced for dose-response modeling to facilitate comparisons with other PODs and to inform uncertainty factor (UF) selection given that effects have been observed for several other PFAS.

3di. Are the modeling approaches for immune endpoints appropriate and scientifically justified, and is the decision to not advance the modeling for derivation of reference values supported?

- Carignan, Faustman, Georgopoulos, Lin, and Savitz concurred that the modeling approaches for immune effects are appropriate and scientifically justified, as was the decision not to advance the modeling for derivation of reference values.
- Ducatman suggested that EPA move the discussion of Kim (2023b) from the supplemental material to the main text, given its relevance to EPA's decision making (Tier 2).
- Haney agreed with the decision not to advance the modeling and suggested an independent BMD analysis by EPA or others to ensure that the results ultimately relied upon are consistent with best practices and are scientifically justifiable (Tier 1).
- Leung and Zoeller declined to comment.

3dii. Is the approach taken for thyroid effects appropriate and scientifically justified, and is the decision to not advance the reductions in serum total T4 in female rats for derivation of a subchronic reference value supported?

- Carignan, Faustman, Georgopoulos, Lin, and Zoeller concurred that the modeling approaches for thyroid effects are appropriate and scientifically justified, as was the decision not to advance the modeling for derivation of a subchronic reference value.
- Haney agreed that quantitative analyses should not be carried forward where there is only suggestive evidence of an effect, and the available science is insufficient to infer a hazard (applicable to immune and thyroid effects in this case).
- Leung and Savitz declined to comment.

3e. Given the lack of studies on inhalation exposure to PFNA, no RfC is derived. Please comment on this decision.

- Carignan, Ducatman, Faustman, Georgopoulos, Lin, Savitz, and Zoeller concurred that an RfC cannot be derived for inhalation exposure at this time due to the lack of studies.
- Carignan and Faustman noted that inhalation is a large and highly relevant data gap and should be a priority for future studies (Tier 3).
- Haney suggested adding a statement that a validated PBPK model is not available for route-to-route extrapolation of oral toxicity study results to the inhalation route of exposure (Tier 2).
- Lin suggested a PBPK model be developed for PFNA in rodents and humans following different routes of exposure, including oral and inhalational routes. Once this model is available, it will greatly help to derive RfC for inhalational exposure based on toxicity data from oral exposure by conducting route-to-route extrapolation (Tier 3).
- Leung declined to comment.

4. In addition, for PFNA, an RfD for less-than-lifetime ("subchronic") exposures is derived. No subchronic RfC was derived. The same studies and outcome used in deriving the lifetime RfD for developmental effects were chosen for use in deriving the developmental subchronic RfD.

4a. Please comment on whether the selection of these studies and these effects for the derivation of the subchronic RfD for PFNA is scientifically justified.

- Carignan, Faustman, Georgopoulos, Lin, and Zoeller agreed that the selection of studies for the derivation of the subchronic RfD was scientifically justified.

- Haney commented that the text should include reasoning on why EPA is choosing to use epidemiology studies for the purposes of deriving subchronic RfDs while other agencies such as ATSDR advise against this practice (Tier 1).
- Ducatman, Leung, and Savitz declined to comment.

4b. 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.

- Haney commented that animal studies would be more appropriate for subchronic and lifetime RfD derivation, but did not identify a specific study (Tier 1).

4c. As part of the recommendations in "a" or "b" above, please comment on whether the effect selected is 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.

- Carignan, Faustman, Georgopoulos, Lin, and Zoeller agreed that the selected effect is appropriate for use in deriving the subchronic RfD.
- Haney commented that while the effects may be appropriate, the studies used to derive the RfD are not.
- Ducatman, Leung, and Savitz declined to comment.

4d. Please comment on the other subchronic osRfDs (i.e., for liver and male reproductive effects).

- Carignan, Faustman, Georgopoulos, and Lin agreed that the other subchronic osRfDs were appropriate.
- Haney commented that the other subchronic osRfDs were based on more reliable laboratory animal toxicity studies (no tier).
- Ducatman, Leung, and Savitz declined to comment.

4e. Given the lack of studies on inhalation exposure to PFNA, no subchronic RfC is derived. Please comment on this decision.

- Carignan, Faustman, Georgopoulos, Haney, Lin, and Zoeller agreed that a subchronic RfC cannot be derived due to the lack of studies.
- Haney also suggested adding a statement that a validated PBPK model is not available for route-to-route extrapolation of oral toxicity study results to the inhalation route of exposure (Tier 2).
- Ducatman, Leung, and Savitz declined to comment.

5. The evaluation of existing physiologically based pharmacokinetic (PBPK) models and a classic PK model described in Appendix E.4 found that these options were not sufficiently reliable for use. For PODs derived from laboratory animal studies, given the information available on potential interspecies differences in PFNA PK and the results of comparing PK model predictions to bioassay data, EPA concluded that a hybrid approach for extrapolation of POD values in animals to estimate corresponding human equivalent doses (HEDs) was the best option in the derivation of the respective RfDs.

5a. Are the methods for calculating POD_{int} values for PFNA for endpoints in rats (adult animals) vs. mice (adult females and pups) scientifically justified for conversion of PODs from animal toxicity studies to HEDs? If not, please provide an explanation and detail on a more appropriate approach.

- Carignan, Faustman, Georgopoulos, Haney, and Lin agreed that the methods for calculating POD_{int} values for PFNA for endpoints in rats compared to mice are scientifically justified for conversion of PODs from animal toxicity studies to HEDs.
- Ducatman commented that the approach seemed mostly reasonable.
- Lin suggested clarifying that by using the same approach for both animal and human POD_{int}, it is assumed that the same internal dose in animals and humans would result in the same toxicity response (Tier 2).
- Leung, Savitz, and Zoeller declined to comment.

5b. Is the use of maternal clearance (in women of reproductive age) to calculate HED values for gestational and early postnatal endpoints appropriate and scientifically justified? If not, please provide specific alternatives for extrapolation of these endpoints.

- Faustman, Georgopoulos, Haney, and Savitz commented that the approach was well documented and justified in application.
- Carignan commented that the use of maternal clearance to calculate HED values is not sufficiently protective, and application of male clearance rates would assure protection of non-menstruating women, and nulliparous women and their offspring (Tier 2).
- Lin commented that while the approach was appropriate, the description was not clear. Lin suggested clarifying the calculation and final value of human maternal clearance (Tier 2) and clarifying the difference in the importance of menstrual clearance between PFNA and other PFAS (Tier 2).
- Leung and Zoeller declined to comment.

5c. Are the selected values of CL_H, specifically the 95% lower CI of the geometric mean from Chiu et al. (2022), 0.090 mL/kg-day for males of all ages and females below 12.4 and above 40 years of age, and 0.124 mL/kg-day for women 12.4-40 years of age (Subsection: Total clearance in humans), appropriate and scientifically justified?

- Faustman, Georgopoulos, Haney, Lin, and Savitz agreed that EPA's approach was conservative, appropriate, and scientifically justified.
- Carignan commented that protection of the full population requires selection of the lowest observed human clearance value; to this extent Carignan suggests considering nulliparous non-menstruating women (Tier 2).
- Ducatman commented that although the male-female differences limited to ages 12.4-40 are not defensible (Tier 1), it is misleading to dismiss the role of menstruation and misleading to infer that PFNA is different from other PFAS in relation to sex-by-age comparisons (Tier 1). He provided several examples from the document of statements on sex differences and the role of menstruation that he found to be confusing, incorrect, or unjustified (Tier 1).
- Leung and Zoeller declined to comment.

5d. Is application of CL_H to estimate POD_{HED} values from POD_{int} values (from animal or epidemiological studies as summarized above) scientifically justified? If not, please provide an explanation and detail on a more appropriate approach.

- Carignan, Faustman, Georgopoulos, Haney, Lin, and Savitz commented in agreement that the application of CL_H to estimate POD_{HED} values is an appropriate and scientifically justified approach.
- Lin suggested explicitly stating that by using the same approach for both animal and human POD_{int}, it is assumed that the same internal dose in animals and humans would result in the same toxicity response (Tier 2).
- Leung and Zoeller declined to comment.

5e. Have the uncertainties in the POD_{int} estimates for animal studies and CLH been adequately evaluated and clearly described?

- Carignan, Faustman, Georgopoulos, and Haney agreed that the uncertainties were adequately evaluated and clearly described.
- Carignan suggested clarifying the more prominent sex difference in excretion for rats compared to mice and the notable short-term elevation among infants (Tier 2).
- Georgopoulos commented that EPA should consider clarifying the statement made on page 3-36 about the validity of the assumption of "most existing PBPK models" that PFAS distribution to tissues and clearance are limited to the fraction unbound in blood (Tier 1). He also suggested eliminating contradictory statements and statements that are too "strong" in section 3.1 and Appendix E (Tier 1). Finally, Georgopoulos suggested evaluating and discussing the consistency of the derived human pharmacokinetic parameters and the pharmacokinetic parameters reported in Yu et al., 2022 (Tier 2).
- Lin suggested improving the clarity of the discussion of the uncertainties in the POD_{int} estimates by providing ranges of clearance values for different subpopulations of humans in Table 3-3 (Tier 2). Lin also suggested explaining the statement that the PBPK assumption that distribution to tissues and clearance are strictly limited to the fraction unbound in blood is incorrect (Tier 2).
- Leung, Savitz, and Zoeller declined to comment.

6. EPA has evaluated and applied, where appropriate, UFs to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL (lowest-observed-adverse-effect level to no-observed-adverse-effect level) extrapolation (UFL) for PFNA. For a–f below, please comment on whether the uncertainty in the derivation of the candidate and selected toxicity values is scientifically justified and clearly described.

6a. Please comment specifically on whether the methods used to derive toxicity values for PFNA appropriately account for uncertainties in pharmacokinetics, including accounting for differences between the experimental animal data and humans.

- Carignan, Ducatman, Faustman, Georgopoulos, Haney, Lin, and Savitz commented that the UFs appropriately account for uncertainty in pharmacokinetics, including differences between the experimental animal data and humans.
- Ducatman suggested adding a brief description about the inverted U-shaped curve that describes the relationship between eGFR in humans and serum PFAS as well as its likely

explanation (Tier 2). He also commented that while there may be more to learn about the excretion curve, the document implies that the curve is not understood, which is not true (Tier 2).

- Leung and Zoeller declined to comment.

6b. For developmental effects, a UF_A of 1 was used since the value was based on human data. 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). Uncertainties with regard to additional susceptible lifestages (e.g., other early-life developmental stages) are addressed as part of the UFD. Does the provided scientific rationale support this decision? If not, please explain.

- Carignan, Faustman, Georgopoulos, Haney, and Lin commented that the provided scientific rationale supports UF decisions for developmental effects.
- Zoeller suggested increasing the UF_D from 3 to 10 (Tier 1).
- Leung declined to comment.

6c. For liver effects and derivation of the lifetime osRfD using human studies, a UF_A of 1 was applied as the liver effects were reported in epidemiological studies and the value was based on human adult data. Does the provided scientific rationale support this decision? If not, please explain.

- Carignan, Faustman, Georgopoulos, Haney, Lin, and Zoeller commented that the provided scientific rationale support the decision to apply a UF_A of 1.
- Leung declined to comment.

6d. For liver effects and derivation of the subchronic osRfD using animal studies, a value of 3 is applied to extrapolate between effects in laboratory animals and in humans during the derivation of the subchronic RfD. Although PPARα dependence might support a value of UF_A = 1 for hepatotoxicity if that were the sole pathway leading to these effects, evidence for the involvement of non-PPARα pathways is available in the PFNA database. Thus, uncertainty remains regarding the potential differences in sensitivity across species because of the involvement of both PPARα-dependent and PPARα-independent mechanisms. As such, the Toxicological Review concludes the available data are not adequate to determine whether humans are likely to be equally or less sensitive compared to laboratory animals with respect to the observed liver 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 and Derivation of Toxicity Values is clearly documented.

- Carignan, Faustman, Georgopoulos, Haney, Lin, and Zoeller commented that the available animal and mechanistic studies support the conclusion to apply a UF_A of 3 and the analysis is clearly documented.
- Ducatman declined to comment but concurred that PPARα is not the only relevant pathway.
- Leung declined to comment.

6e. For liver and male reproductive effects, a value of 10 is applied for the UF_S when extrapolating from 28-day animal data to a subchronic exposure. Considering the potential for some health effects (decreased reproductive organ weights and sperm measures, liver enlargement and concurrent effects) to worsen with increasing duration and the large uncertainty associated with the lack of

existing or reliable chemical-specific data to evaluate the effects of subchronic exposure on liver and male reproductive outcomes, respectively, the Toxicological Review concludes that application of a UF_S of 10 is supported for the purpose of deriving the subchronic RfD from the 28-day toxicity data. Does the provided scientific rationale support this decision? If not, please explain.

- Carignan, Faustman, Georgopoulos, Haney, Lin, and Zoeller commented that the provided scientific rationale support the decision to apply a value of 10 for the UF_S.
- Ducatman and Leung declined to comment.

6f. Are the provided rationales for the remaining UF values (UF_L, UF_D, UF_H) scientifically justified and clearly described (to inform the UF_H, the assessment evaluates and considers the available evidence on potential susceptibility to PFNA within different populations or lifestages, including any potential impacts from early-life exposure to PFNA on lifelong health, although few studies on susceptibility were available)? If not, please explain.

- Carignan, Faustman, Georgopoulos, Haney, Lin, Savitz, and Zoeller commented that the rationale for the remaining UF values is scientifically justified and clearly described.
- Ducatman and Leung declined to comment.

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

- All nine reviewers agreed that there is currently inadequate information to assess carcinogenic potential for PFNA.
- Ducatman also commented that it is important to note findings in several studies that investigated carcinogenicity, such as Feng, et al., 2022 (Tier 2), Shearer et al., 2020 (Tier 2), Benninghoff et al., 2012, and Rhee et al., 2023 (Tier 1).

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

- Carignan, Faustman, Georgopoulos, Haney, Leung, Lin, Savitz, and Zoeller agreed that quantitative estimates for cancer effects of oral and inhalation exposures could not be described.
- Ducatman commented that the discussion may benefit from including the references provided in response to charge question 7, but ultimately agreed with EPA's conclusion (Tier 2).

3.0 REVIEWER RESPONSE TO CHARGE QUESTIONS

3.1 The Toxicological Review for PFNA 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 PFNA are appropriate and clearly described.

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

i. EPA fully synthesized the literature published through April 2022 in the external review draft and has been monitoring newly identified studies (i.e., studies identified by EPA or the public that meet the PECO (population, exposure, comparator, and outcome) criteria or otherwise inform key assessment conclusions but that were not addressed in the external review draft—for example, due to publication after April 2022). EPA characterizes these studies in a tabular format in Appendix B.2 (studies identified in the most recent literature search update or public comments on PFDA or PFHxS) and in a second table provided to peer reviewers (studies identified from the PFHxS peer review or PFNA public comment). In both tables, the characterization focuses 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. Studies that were classified as having a possible material impact on the conclusions (e.g., epidemiological studies of hepatic effects and breastfeeding duration; absorption, distribution, metabolism, and excretion/pharmacokinetic [ADME/PK] studies that informed clearance values or otherwise were helpful in the interpretation of the available ADME/PK data) were incorporated into the evidence synthesis. Please review EPA's characterizations 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.

Literature Search Methods and Documentation	
Reviewer	**Comments**
Carignan	a. The literature search strategy and screening criteria appear appropriate and clearly described. b. Recommend summarizing new studies on inhalation and dermal exposures. **(Tier 2)**. This is recommended to improve the document and is not expected to impact overall conclusions. • Kissel J, Titaley I, Muensterman D, Field J. Evaluating neutral PFAS for potential dermal absorption phase. 2023. https://pubs.acs.org/doi/10.1021/acs.est.2c08835

	• Zhu Y, Pan X, Jia Y, Yang X, Song X, Ding J, et al. Exploring route-specific pharmacokinetics of PFAS in mice by coupling in vivo tests and physiologically based toxicokinetic models. Enviro Health Perspect. 2023. 131(12): 127012. doi: 10.1289/EHP11969 • Ragnarsdóttir O, Abdallah MA, Harrad S. Dermal bioaccessibility of perfluoroalkyl substances from household dust; influence of topically applied cosmetics. Environ Res. 2023 Dec 1;238(Pt 1):117093. doi: 10.1016/j.envres.2023.117093. Epub 2023 Sep 6. PMID: 37683793. • Ragnarsdóttir O, Abdallah M, Harrad S. Dermal bioavailability of perfluoroalkyl substances using in vitro 3D human skin equivalent models. 2024. Environ Int. 188. doi: 10.1016/j.envint.2024.108772 Recommend future research and assessments on dermal and inhalation exposure to PFNA and other PFAS (legacy and current use) individually and as mixtures. **(Tier 3)** b. No additional studies are noted at this time. Please see the end of this review for comments and recommended studies to improve the Section 1.1.4. exposure summary. Overall, I want express gratitude and commend IRIS on producing a clear and detailed report that will be extremely useful for the scientific and regulatory community.
Ducatman	a. No concerns. about the approach and description. Looking ahead. **Tier 3:** EPA might consider doing something more formal about categorizing data in the circumstance when a study is low confidence because it has some built-in source of bias, yet the result is in the other direction from the bias. (Clinicians regard this as a Bayesian topic. The result is not of lesser confidence when the bias is in the other direction, and downgrading of the study due to the bias can confuse readers about the outcome implication if the outcome is consistent with other literature and present despite a bias that makes the outcome – whether adverse or beneficial- less likely.) In the document, EPA sometimes does note that the study deficiency does not affect the interpretation, but is not consistent. I have commented on an example in the text. **Tier 3:** EPA provided a very interesting and potentially important discussion about a potential bias from logarithmic transformations. This is greatly appreciated. Because many PFAS-associated outcomes are not normally distributed (suggesting possible saturation effects??), logarithmic transformations are a common tool. In future, EPA might consider this topic more broadly concerning (either) quality or impact on the size of associations. If this is important, it would be great to learn more about EPAs perspective in future. **Tier 1**: The UCMR data (p 1-5) could be updated. This is simply a referencing detail.

	b. EPA has been thorough, and the section is generally strong. The reviewer appreciates and commends the approach. (for this topic and more broadly). I am especially appreciative of the detailed description of several organs as candidates. This is very helpful and commended. The review is about potential improvements There is no point in iteratively focusing on points of agreement. Discussion: Key words do not always work and there is some evidence that important documents were perhaps seen, often (and not always) put in an appendix, and possibly not always fully considered. A reference list of potentially useful references is at the end of this document, and is organized in the text according to the question posed, a topic with considerable overlap. I do not think recommendations and comments will likely alter relevant points of departure. They can improve the science. **Tier 2**: The presence of PFNA in mixtures (including mixtures that feature PFNA such as (**Surflon**) could be discussed. **Tier 3** About organization. The current document organization means that there will be repetition in thorough reviews, as topics appear in several places. This is hard to minimize, it is just a condition. • EPA is alert that it is distributing one topic into more than one section, and that responses in a section may again come up in subsequent sections. In reviews that may involve edits, EPA will need to go back to the topics and references that pertain across sections in order to obtain consistency. • In addition, some of the most important recommendations do not have a particularly good home in the questions posed. Efforts were made to put relevant topics in the best place, and EPA will need to decide where the topic belongs. **Tier 3**. Literature updates for Breast feeding as an outcome. Breastfeeding , is mentioned in this section (there could be more logical places). For PFNA, there are a number of relevant studies with null findings, and two (that I know about) with adverse associations, including one that is not within and one within the time-frame of the review (Romano et al., 2024; Rosen et al., 2018). I do not think that the two studies showing adverse associations are enough to change the approach, there are at least equal numbers of studies with null findings (for PFNA). Discussion: On p. 3.2, the following quotation about excretion in females and the topic of menstruation and other sex-specific secretion/excretion/transfer characteristics is a mash up of explanations, trying to pull too many concepts together, some of it just incorrect, and much of it unintentionally misleading. Addressing the confusion in the following quotation will not alter conclusions, only improve the science.

In general, EPA has staked out an unusual position as regards the role of menstruation. A careful reader would believe that EPA has data that menstruation plays no role in the lower serum PFNA during reproductive years, but has also failed to present the information to support that unusual perspective.

Since the topic is not central to anything else in the document, it is not clear why such strong statements are made. The topic could be omitted and the document would be the same (better). Alternatively, better evidence is needed.

Quoting from the document:

> *"The NHANES data do indicate that PFNA clearance is higher in women of childbearing age than in men over the same age range**, although the mechanism for this difference is unclear. This additional excretion in women appears to occur between 12.4 and 40 years of age** and results in a lower half-life, estimated at 2.9 years, compared with 4.0 years for men and younger and older women (analysis below). Measured concentrations of PFNA in breastmilk (see Section 3.1.2, "Human distribution during gestation and childhood") and the correlation of PFNA concentration in young children with length of breastfeeding (Koponen et al., 2018) clearly show that lactation is a route of excretion for the mother and exposure for the infant."*

Initial Discussion of the topic of this quotation, sex differences in human excretion and half-lives. (Initial because some similar and more detailed statements recur at other places in the document, where they are also addressed).

- **Tier 1**: The age range quoted here and elsewhere in the document for sex differences is not correct. See also more detailed comments for similar and more detailed EPA quotations from section 3.1. These may also help to revise the quotation above.)

- **Tier 1**: In the quotation above, the comment that the reason for the differences is "unclear" is overstated. It is a surprising position, not particularly supported by the literature including literature cited by EPA. The unusual perspective does not appear to affect any conclusions it is possible that EPA authors are trying to convey that they are interested to find out if there are other contributors in addition to the generally accepted effects of menstruation, pregnancy, and lactation. That would be reasonable.

Discussion: EPA cites (Jain & Ducatman, 2022) data, which is about NHANES data. That yearly male-female sex comparison commences at age 12. At that point, the differences are small compared to older ages yet visible. This is not surprising given the variability of age of menarche and the time it takes to make a dent in serum PFAS.

Perhaps this is the origin of the misleading quotation. The average age of menarche in the US was considered to be 12.4 in the past (possibly where EPA mixed in the slightly misleading thought that it begins at 12.4, but that is not quite right. The difference is already visible at age 12.) However, the mean age of menarche is a moving target trending towards earlier in successive generations. This trend is often linked to nutrition (and access to dense sources of calories). The onset of

	menstruation in the US is now probably just under 12, is known to be decreasing in age over time, and has a distribution from 10-16 in healthy children (Marques, Madeira, & Gama, 2022; Z. Wang et al., 2024). From the onset of menarche, an average time to menstrual regularity is around two years, with expected variability among individuals At either end of the female reproductive cycle, the amount of menstrual fluid excreted is less than at peak in an age stratified population. The average age of menopause in the US is 52, and there is most often a decrease of menstrual blood in the preceding years (and some women have hysterectomies before that age). However, sex differences in excretion are not known to vanish at age 40. In the section "Excretion in Humans," the EPA document acknowledges the role of menstruation for other PFAS, and then confusingly (confusing in my view) also questions the role of menstruation for PFNA alone, although it is possible that authors hold similar beliefs about other PFAS. So far as any rationale is presented, it is mostly based on what I think are some questionable interpretations concerning age of onset of sex differences and, also, on PFDA data. Additional discussion. The PFNA, analogy to PFDA is a lot of inference, based on very little. First, the PFDA data do not refute the common inference based on data for a role of menstruation for PFNA, (nor by the same logic PFOA, PFOS, and PFHxS). Second, data for PFNA, PFOA, PFOS, and PFHxS are compatible with a role for menstruation for these four compounds. Third, the PFDA data are also compatible with a role for menstruation concerning PFDA specifically, but better characterized as not reliably contributing to the discussion due to nondetects, to instability between survey year concentrations, and more limited range. The PFDA data comparisons in the cited literature are unstable for the purposes cited (I think even in the eyes of the authors who published the cited PFDA data who discuss limitations and interpretations.) They are in the range of but less stable than PFNA data. In our paper (also cited in the EPA document) comparing male and females by age-year, PFDA was deliberately excluded. It was excluded because the combination of nondetects, and narrow range of values, and unstable appearing differences between survey years, did not create a conditions for a reliable comparison. This choice about unreliability is illustrated in the CDC survey year contaminant tables in which the highest serum concentration survey year for PFDA, the geometric mean is <0.4 ng/mL (again, that is the highest year) and it is 0.193 by 2017-8, with medians rounded off to increment of 0.1 (which is the reasonable thing to do for data so near the detection limit). The curious dip in mean/median PFDA values in 2015-6 survey cycle compared to 2017-8 also shows the danger of making confident statements when the detection limit and much of the range are this close. (As technology advances, the wonderful chemists at CDC will make ever more accurate comparisons. If frozen samples exist and are split, frozen, and retested, and the freeze thaw cycles and the container chosen do not affect the < tenth nanogram/ml differences that appear to be needed for PFDA sex comparisons, perhaps the PFDA topic can be revisited and something can eventually be made of it.) less interpretable.

	Also contrary to what is implied in the quotation, section 3.1.2 does not clear up or meaningfully address how or why the skepticism about a role for menstruation (and possibly also skepticism about a role for pregnancy?) in affecting serum PFAS was derived. The apparently relevant reference in the section (Friss-Hansen, 1961) is not an explanation. In the relevant publication cited by EPA which permits sex comparison by year of age, the age-years of difference between females and males for PFNA in NHANES data are visible at the first year of comparison - age 12, gradually increase to an inflection point (at around age 37), and then decrease with sex equalization at around age 51 (The inflection point is age 37 or near age 40 as written by EPA, but that is still not a guarantee that either age 37 or age 40 describes the age when sex differences in excretion vanish. In fact, the data suggest otherwise. Can there be other, additional contributing explanations for sex differences.? Of course! However, so far none are demonstrated. (I am interested in/sympathetic to EPA's perspective that there may be causes that are not menstruation, and have published data concerning a possible role for estrogen as a possible contributing modifier to some of the sex differences (Jain & Ducatman, 2023), not otherwise explained exclusively by pregnancy, and lactation (excretion, secretion and transfers). Nevertheless, the male-female comparisons in NHANES data are frankly highly compatible with an explanation that is wholly or mostly about menstruation, pregnancy, and lactation, with "tails" at both ends related to both the variability in life stage events (menarche and menopause are life events with an age range distribution around the mean age of onset as well as increasing/decreasing impacts on loss of menstrual fluid, which is assumed by all researchers to contain PFAS.). The age range of the normal physiologic events of the menstrual cycle are excellent explanations for the shape of the curve at both ends of the reproductive life cycle, whilst pregnancy and lactation are more likely to make population contributions away from the tale ends of this age distribution. Concerning the lactation in humans discussion (begins on p. 3-30). • **Tier 2**: Meta analyses are very useful At least one brand new paper implicates PFNA (Romano et al., 2024), and a meta-analysis (Timmermann et al., 2023) can provide useful summary guidance (and also relevant papers to this important topic deserving consideration). Again, no suggestion is made that conclusions need change. It is simply good to use the advantage of a meta-analysis when availab.e Concerning the distribution of maternal PFNA during and after pregnancy, Quoting: "*In summary,* ***the observed pattern of PFNA decline during and after pregnancy is considered likely to result from the relatively rapid increase in maternal and fetal body weight during pregnancy****, so the PFNA body burden distributes into a larger volume, and lactational transfer from the breastfeeding mother*" (from EPA p. 3-33) **Discussion.** The quotation is defensible for what it says but potentially misleading because also incomplete. The intra-pregnancy alterations are about more than just

	weight gain, they also reflect rapid and important changes in fluid compartment distributions that are not necessarily proportional to weight gain. Further, EPA is aware but did not explicitly state that PFAS are transferred to the fetus. **Tier 2**: Please consider a more complete presentation of the reasons for serum decrements during pregnancy. Weight gain is part of the picture. There is more.
Faustman	a. USEPA provided detailed information on their literature search strategy which is summarized in Chapter 2. Figure 2-1 Shows this strategy and identifies the number and type of studies which met the PECO criteria (N=585). It also shows the number of excluded literature (N=404) and provides details on reasons why these articles were excluded. Literature "tagged" as Supplemental (N=946) was also listed and it was noted that at least 231 were identified as Mechanistic or MOA. This information was helpful and appropriate. b. This reviewer examined the additional studies that were identified after Aprill 2022 as well as additional references identified by public comment. In Table 1 the USEPA provided these citations as well as an evaluation of potential of the specific references to impact the characterization of the impact. This reviewer agrees with these comments. The majority were deemed to have minimal to no impact on the current draft syntheses. However, USEPA discussed the new references on musculoskeletal effects in humans identified in the Peer review of PFHxS and indicates that these references will be added and identifies that this endpoint may represent a previously omitted effect area. This reviewer agrees with the addition of these new references on bone mineralization and bone density. This reviewer did not have additional references to add.
Georgopoulos	a. The literature search strategy and screening criteria related to pharmacokinetics and health effects of PFNA are clearly described in Section 1.2 and in Appendix B of the EPA Draft Toxicological Review, as well as in the updated Protocol available (via link provided in Appendix A) at https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=345065 The methods used for the PFNA Draft Toxicological Review are appropriate and consistent with established scientific standards and practices. **Note I:** It has been recognized on multiple occasions that the process for identifying and screening pertinent studies for the Toxicological Reviews of PFAS (including the current Draft Toxicological Review for PFNA) poses significant challenges. The consideration of multiple health effects potentially associated with exposures to individual PFAS and PFAS mixtures has led to numerous studies worldwide, other completed and many on-going, with related publications appearing at increasing rates. It is therefore necessary to implement a process for efficiently "aligning" the information presented in documents, such as the present Toxicological Review, with the content of regularly updated online resources, such as the Health & Environmental Research Online portal for PFNA (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2633), the Health Assessment Workspace Collaborative (HAWC) portal for PFNA(https://hawc.epa.gov/assessment/100500071/), and the CompTox Chemicals

	Dashboard (https://comptox.epa.gov/dashboard/) so as to allow new information to appear on the online portals without creating inconsistencies with statements included in the documents. As an example, though the Draft PFNA Toxicological Review states (on page 2-1) that 3,316 unique records were identified (April 2023) for PFNA, the HERO portal PFNA page currently (July 2024) lists 5,088 records. **Note II:** A useful addition to the Toxicological Review would be a summary, e.g., as a table, of assessments and activities of regulatory agencies, both in the US and internationally, related to PFNA. This summary could also list organizations actively collecting risk-relevant information on PFNA and other PFAS: representative examples are The European Chemicals Agency document on PFNA [EC Number 206-801-3, CAS Numbers 375-95-1, 21049-39-8, 4149-60-4; Proposing authority: Sweden, Reason for proposing: Toxic for reproduction (Article 57 c); PBT (Article 57 d)] Proposal for Identification of a Substance of Very High Concern on the Basis of the Criteria Set Out in Reach Article 57; ECHA, 2015 • The European Human Biomonitoring (HBM4EU) Platform https://www.hbm4eu.eu/what-we-do/european-hbm-platform/ • The Interstate Technology and Regulatory Council (ITRC) Per- and Polyfluoroalkyl Substances Technical and Regulatory Guidance (https://pfas-1.itrcweb.org/) • The CDC/ATSDR PFAS Multi-site Study (MSS) (https://www.atsdr.cdc.gov/pfas/activities/studies/multi-site.html) • The HHEAR (Human Health Exposure Analysis Resource) data Center (https://hheardatacenter.mssm.edu) that currently assembles information from multiple the PFAS studies, etc. **Note III:** On page 1-12 of the PFNA Toxicological Review it is stated that "In addition to those studies meeting the PECO criteria and studies excluded as not relevant to the assessment, studies containing supplemental material potentially relevant to the specific aims of the assessment were inventoried during the literature screening process. Although these studies did not meet PECO criteria, they were not excluded. Rather, they were considered for use in addressing the identified key science issues (see Appendix A, Section 2.4 of the protocol) and other scientific uncertainties identified during assessment development." It would be very useful to identify and systematically organize supplemental PFNA-related studies with respect to both the methods used (in vitro, in silico, in vivo, etc.) and the bioactivity endpoints considered. Although in silico, in vitro and non-mammalian model organism studies may not have the same weight as human epidemiological and rodent laboratory and studies, they can provide valuable mechanistic insights, both for hypothesis development and for animal study evaluation, as well as corroborate hypotheses derived from rodent and epidemiological studies when their results consistently

	"point to the same direction". It would also be useful to identify and explicitly list consistencies as well as inconsistencies appearing not only in the "PECO literature" but also in the "supplemental literature". Examples are a. the characterization of PPAR binding affinities of PFNA (and of other PFAS) presented in the studies of Ishibashi et al. (2019), Khazaee et al. (2021), Evans et al. (2023), Sun et al. (2023), etc. b. the characterization of PFNA (and other PFAS) organic anion transporter interactions presented in the studies of Louisse et al. (2023) and Ryu et al. (2024b). c. the characterization of PFNA binding affinities to serum proteins presented in the studies of Allendorf et al. (2019), Fedorenko (2019), Allendorf (2021), Alesio et al. (2022), Starnes et al. (2024), and Ryu et al. (2024a) *[Citations for the studies listed above are provided in Response 1b].* **Note IV:** As in previous PFAS Toxicological Reviews, the Background Information section (section 1.1) is the weakest component of the document under review. Although it is clear that PFNA sources, environmental fate and transport, and exposures are not the focus of this (or any other) Toxicological Review, and therefore a more detailed discussion of these topics is not required, the cited references should be up-to-date and basic information on PFNA bioactivity (e.g. serum protein binding affinities) should be provided. As an example, the citation to the CompTox Chemicals Dashboard on page 2-1 is (U.S. EPA, 2019), where a 2019 date is assigned to the CompTox Chemicals Dashboard citation in relation to data retrieved in 2023. Furthermore, Section 1 would benefit from thorough editing to correct typographical errors (for example, Surflon S-111 appears as "Sulflon S-111" and fluorine as "flourine") and to clarify statements regarding the environmental properties of PFNA. Some examples of statements that need to be revised are: a. The statement "PFNA would be expected to have limited mobility in soil given its soil adsorption coefficient" (page 1-4, line 30) appears to be inconsistent with the statement "[d]eposition from air emissions to soil and subsequent migration to groundwater is another potential transport pathway," that appears in the previous paragraph (page 1-4, lines 18-19). b. The origin (computational estimate) and any experimental support/verification, if available, of the reported 31-day atmospheric half-life (page 1-4, line 20) of PFNA, should be provided. c. The information on drinking water exposures in the US, derived from Uncontaminated Monitoring Rule (UCMR) data, that appears on page 1-5 (lines 14-27) should be edited: UCMR5 commenced in 2023 and the preliminary data reported include all public water systems serving more than 3,300 people (not 10,000, as stated on line 16 of page 1-5) and a representative sample of smaller

systems. EPA should consider also reporting data from UCMR3, that cover the 2013-15 period and included all public water systems serving more than 10,000 people (using a higher PFNA reporting level of 20 ng/L than the 4 ng/L used in UCMR5).

d. The statement (page 1-6, lines 27-28) "PFNA was also detected above the MRL (0.096 µg/L) in groundwater near an industrial site in New Jersey," should be revised: A PFNA concentration of 0.096 µg/L was measured in a public drinking water well, while the MRL reported for that study was 0.004 µg/L (Post et al., 2013).

Suggested Revisions and Future Considerations

Tier 1 Necessary Revision: Thoroughly edit Section 1 (Note IV, above, lists specific examples of statements that require editing) and check Table 1-1 for consistency with information currently available online (on portals such as EPA's CompTox Chemicals Dashboard); evaluate the feasibility of expanding Table 1-1 to include values of PFNA properties that are critical for is pharmacokinetics (e.g., binding affinities to serum proteins).

Tier 2 Suggested Revision: Include references to documents (and online portals) relevant to PFNA risk characterization that have been developed by US and international regulatory agencies; compile a summary (e.g., in the form of a table) of established or proposed values for metrics of reference doses/concentrations if such metrics are identified.

Tier 3 Future Consideration: Develop a plan for the systematic and regular updating, as well as evaluating the consistency, of portals (specifically HERO and HAWC) tracking information relevant to the Toxicological Review(s); furthermore, specify criteria for new information that would require re-evaluation of the PFAS Toxicological Reviews.

b. The "supplemental table" with additional studies, that was provided for the review, states that "[a] total of 157 studies were submitted by the State of New Jersey Department of Environmental Protection (NJDEP) and the Natural Resources Defense Council (NRDC). Of the 157 studies, 10 were evaluated for potential incorporation and impact on the assessment's conclusions as stated above, with the other 147 being already cited in the draft assessment and/or considered to provide supplemental information under the PFAS PECO criteria." The table also states that "[t]oxicology studies conducted in laboratory animals exposed to S-111-S-WB (a commercial mixture that is mostly PFNA but also other PFAS) were considered to provide supplemental information for hazard considerations under the PFAS PECO criteria." This obviously refers to the studies of Stump et al. (2008) and Mertens et al. (2010); however, these studies are not listed in the "supplemental table" (nor in Appendix B of the Review), and, from the preceding statement, it is not clear how they were used as supplemental information by EPA.

Below, in addition to the Stump et al. (2008) and Mertens et al. (2010) studies (which are included in the HERO database for PFNA), is a list of references for

	potential consideration by EPA. The list also includes studies mentioned above, in Response 1a. • Alesio, J. L., Slitt, A., & Bothun, G. D. (2022). Critical new insights into the binding of poly-and perfluoroalkyl substances (PFAS) to albumin protein. Chemosphere, 287, 131979. • Allendorf, F., Berger, U., Goss, K. U., & Ulrich, N. (2019). Partition coefficients of four perfluoroalkyl acid alternatives between bovine serum albumin (BSA) and water in comparison to ten classical perfluoroalkyl acids. Environmental Science: Processes & Impacts, 21(11), 1852-1863 • Allendorf, F. (2021). Equilibrium sorption of perfluoroalkyl acids (PFAAs) and four of their alternatives in mammals (Doctoral dissertation, Dissertation, Halle (Saale), Martin-Luther-Universität Halle-Wittenberg, 2021). • Beglarian, E., Costello, E., Walker, D. I., Wang, H., Alderete, T. L., Chen, Z., ... & Chatzi, L. (2024). Exposure to perfluoroalkyl substances and longitudinal changes in bone mineral density in adolescents and young adults: a multi-cohort study. Environmental Research, 244, 117611. • Evans, N., Conley, J. M., Cardon, M., Hartig, P., Medlock-Kakaley, E., & Gray Jr, L. E. (2022). In vitro activity of a panel of per-and polyfluoroalkyl substances (PFAS), fatty acids, and pharmaceuticals in peroxisome proliferator-activated receptor (PPAR) alpha, PPAR gamma, and estrogen receptor assays. Toxicology and applied pharmacology, 449, 116136. • Fedorenko, M. (2019). Molecular Mechanisms of Protein Binding by Perfluoroalkyl Substances (PFASs). MS Thesis, University of Rhode Island. • Forsthuber, M., Kaiser, A. M., Granitzer, S., Hassl, I., Hengstschläger, M., Stangl, H., & Gundacker, C. (2020). Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. Environment international, 137, 105324. • Goodrich, J. A., Walker, D. I., He, J., Lin, X., Baumert, B. O., Hu, X., ... & Chatzi, L. (2023). Metabolic signatures of youth exposure to mixtures of per-and polyfluoroalkyl substances: a multi-cohort study. Environmental health perspectives, 131(2), 027005. • Ishibashi, H., Hirano, M., Kim, E. Y., & Iwata, H. (2019). In vitro and in silico evaluations of binding affinities of perfluoroalkyl substances to Baikal seal and human peroxisome proliferator-activated receptor α. Environmental science & technology, 53(4), 2181-2188. • Khazaee, M., Christie, E., Cheng, W., Michalsen, M., Field, J., & Ng, C. (2021). Perfluoroalkyl acid binding with peroxisome proliferator-activated receptors α, γ, and δ, and fatty acid binding proteins by equilibrium dialysis with a comparison of methods. Toxics, 9(3), 45.

	• Li, Y., Baumert, B. O., Stratakis, N., Goodrich, J. A., Wu, H., Liu, S. H., ... & Chatzi, L. (2024). Exposure to per-and polyfluoroalkyl substances and alterations in plasma microRNA profiles in children. Environmental Research, 119496. • Lin, C. Y., Lee, H. L., Wang, C., Sung, F. C., & Su, T. C. (2024). Examining the Impact of Polyfluoroalkyl Substance Exposure on Erythrocyte Profiles and Its Related Nutrients: Insights from a Prospective Study on Young Taiwanese. Environmental Pollution, 124576. • Louisse, J., Dellafiora, L., van den Heuvel, J. J., Rijkers, D., Leenders, L., Dorne, J. L. C., ... & Koenderink, J. B. (2023). Perfluoroalkyl substances (PFASs) are substrates of the renal human organic anion transporter 4 (OAT4). Archives of Toxicology, 97(3), 685-696. • Mertens, J. J., Sved, D. W., Marit, G. B., Myers, N. R., Stetson, P. L., Murphy, S. R., ... & Farr, C. H. (2010). Subchronic toxicity of S-111-S-WB in Sprague Dawley rats. International journal of toxicology, 29(4), 358-371. • Moon, J., & Mun, Y. (2024). The association between per-and polyfluoroalkyl substances (PFASs) and brain, esophageal, melanomatous skin, prostate, and lung cancer using the 2003–2018 US National Health and Nutrition Examination Survey (NHANES) datasets. Heliyon, 10(2). • Narizzano, A. M., May Lent, E., East, A. G., Bohannon, M. E., & Quinn Jr, M. J. (2024). Threshold for increased liver weight is protective of other effects in peromyscus exposed to PFNA. Toxicological Sciences, kfae077. • Ryu, S., Burchett, W., Zhang, S., Modaresi, S. M. S., Agudelo Areiza, J., Kaye, E., ... & Slitt, A. L. (2024a). Species-Specific Unbound Fraction Differences in Highly Bound PFAS: A Comparative Study across Human, Rat, and Mouse Plasma and Albumin. Toxics, 12(4), 253. • Ryu, S., Yamaguchi, E., Modaresi, S. M. S., Agudelo, J., Costales, C., West, M. A., ... & Slitt, A. L. (2024b). Evaluation of 14 PFAS for Permeability and Organic Anion Transporter Interactions: Implications for Renal Clearance in Humans. Chemosphere, 142390. • Smorada, C. M., Sima, M. W., & Jaffé, P. R. (2024). Bacterial degradation of perfluoroalkyl acids. Current Opinion in Biotechnology, 88, 103170. • Starnes, H. M., Jackson, T. W., Rock, K. D., & Belcher, S. M. (2024). Quantitative cross-species comparison of serum albumin binding of per-and polyfluoroalkyl substances from five structural classes. Toxicological Sciences, 199(1), 132-149. • Stump, D. G., Holson, J. F., Murphy, S. R., Farr, C. H., Schmit, B., & Shinohara, M. (2008). An oral two-generation reproductive toxicity study of S-111-S-WB in rats. Reproductive Toxicology, 25(1), 7-20. • Tian, Q., Yang, Y., An, Q., Li, Y., Wang, Q., Zhang, P., ... & Lei, L. (2024). Association of exposure to multiple perfluoroalkyl and polyfluoroalkyl

	substances and glucose metabolism in National Health and Nutrition Examination Survey 2017–2018. Frontiers in Public Health, 12, 1370971. • Wang, T., Yang, J., Han, Y., & Wāng, Y. (2024). Unveiling the intricate connection between per-and polyfluoroalkyl substances and prostate hyperplasia. Science of The Total Environment, 932, 173085. • Wang, Z. H., Liang, F. Z., Chen, X. R., Wu, P., & Wu, W. (2024). Determination of seven perfluoroalkyl and polyfluoroalkyl substances in serum of pregnant women and evaluation of neonatal neurobehavior based on high performance liquid chromatography-tandem mass spectrometry. Se pu= Chinese Journal of Chromatography, 42(2), 194-202. • Wittkopp, S., Wu, F., Windheim, J., Robinson, M., Kannan, K., Katz, S. D., ... & Newman, J. D. (2022). Vascular endothelium as a target for perfluroalkyl substances (PFAs). Environmental research, 212, 113339. • Yu, C. H., Weisel, C. P., Alimokhtari, S., Georgopoulos, P. G., & Fan, Z. T. (2021). Biomonitoring: a tool to assess PFNA body burdens and evaluate the effectiveness of drinking water intervention for communities in New Jersey. International Journal of Hygiene and Environmental Health, 235, 113757. • Zhao, Y., Jin, H., Hu, S., Zhang, S., Zhao, M., & Xue, J. (2024). The impact of perfluoroalkyl substances on the clinical manifestations of primary Sjögren syndrome. (BMC Immunology under review)
Haney	a. The literature search strategy and screening criteria for PFNA appear largely appropriate and clearly described (see comment below). In regard to the appropriateness of the PECO criteria, Table 1-4 indicates that mixture studies in laboratory animals are only included if they employ an experimental arm that involves exposure to a single PFNA. This is entirely appropriate when toxicity factors are being derived for a single PFAS, as in this case for PFNA, and not a specific PFAS mixture. Quantitatively attributing all or part of mixture effects to a single chemical is problematic even in animal studies. With thousands of PFAS and other environmental chemical exposures and associated issues (e.g., confounding correlated co-exposures, both quantified and unquantified), this is even more true for epidemiological studies. For example, when there are numerous significant exposures to both PFAS and other chemicals known or suspected to affect the same endpoint(s), with most such exposures not even quantified in these studies, it is not possible to derive a scientifically defensible toxicity factor for a single chemical as a reasonably accurate numerical estimate since it is not possible to accurately quantify the specific degree to which a given chemical contributed to producing the observed mixture effect (assuming causality). In such mixture studies, all effect-relevant chemical exposures are not being accounted for, and their relative potencies as to the effect in question are unknown. For these types of reasons, epidemiological studies where populations are exposed to PFAS mixtures cannot be relied upon for derivation of toxicity factors for a single PFAS (e.g., PFNA), consistent with conclusions of other agencies (e.g., ATSDR 2021). Based on these considerations, as with animal studies, the PECO for human/epidemiological studies

	should indicate that such studies are only included (e.g., for potential use in quantitative dose-response assessment) if people were only exposed to the single PFAS of interest (e.g., PFNA) (**Tier 1 necessary revision**), although they may still have use for hazard identification. b. This reviewer has no further comments on EPA's characterizations of whether the studies referenced by this charge question would have a material impact on the conclusions (i.e., identified hazards or toxicity values) in the external review draft other than to say that this is a reasonable and practical criterion. Furthermore, this reviewer personally knows of no additional peer-reviewed studies of PFNA that the assessment should have incorporated.
Leung	a. The systematic review protocol used in this assessment initially searched and screened pertinent studies in 2017. Annual updates to the search have been formally performed since, in addition to the ongoing monitoring for pertinent new studies since published. I agree that the PECO criteria used for the search are appropriate and the literature search method were overall appropriate and clearly described. Figures 2-1 (flow diagram) and 2-2 (literature tagtree) for the overall search, and the interactive Health Assessment Workspace Collaborative schematic heatmap of study quality for each endpoint, were especially helpful to clearly and effectively summarize the findings. In addition, as each health effect is individually examined, the reference to other health effect sections with potentially overlapping data outlines the broader understanding that PFNA exposure may have multiorgan, integrated, and parallel effects. b. Appendix B.1 is a clear summary of the additional studies found since the last formal update to the PECO search was performed in April 2022. I agree with the EPA's disposition of these studies as summarized in Table B-1, and do not have suggestions for any other peer-reviewed studies that should be included in the final draft assessment.
Lin	a. The literature search strategy and screening criteria for PFNA are appropriate and clearly described. Specifically, the literature search strategy, including key words (i.e., query strings), inclusion/exclusion criteria, timelines and annual updates is described in detail in the risk assessment document and the supplemental information (i.e., Appendices A and B). The literature search key words are listed in Table A-1 (Appendix A). The PECO (populations, exposures, comparators, and outcomes) criteria are listed in Table 1-4, and additional inclusion and exclusion criteria are discussed in Section 1.2.1. Literature Search and Screening. The literature search is also very comprehensive. Specifically, the literature search was done in four databases, including PubMed, Web of Science, Toxline, and TSCATS (Toxic Substances Control Act Test Submissions). In addition, relevant literature not found through these four databases were also checked, including review of studies cited in studies that were identified from the four databases, searches of published PFAS SEMs (systematic evidence maps), review of studies

	submitted to regulatory agencies, and other studies found during screening for other PFAS assessments, as well as gray literature. The literature search is also up-to-date. The original literature search was done in 2017. However, literature update search was done annually from 2017 to 2022. In addition, many newer studies were considered, including studies from the most recent 2023 literature update for PFNA, studies identified from public comments on PFNA draft assessment, and studies identified in public comments on the EPA IRIS assessments on other PFAS, such as PFDA and PFHxS. Relevant literature are available in EPA's HERO (Health and Environmental Research Online) database, and the link is provided in the risk assessment document (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2633). b. This reviewer is not aware of additional peer-reviewed studies of PFNA that may have a material impact on the risk assessment draft's conclusions. My area of expertise is in the development and application of PBPK models to support human health risk assessment of environmental chemicals. The available PBPK model for PFNA in rats and humans by Kim et al. (2019) has been considered in this risk assessment. There are other generic PBPK models for multiple chemicals, including PFNA (Fabrega et al. 2015; Breen et al., 2021; Pearce et al., 2017). These models have been evaluated by EPA, and were considered as not useful due to low accuracies in model predictions. There is another PBPK model for PFAS, including PFNA, in zebrafish by Golosovskaia et al. (2024). This model was published in November 2023. This study provides a useful tool to improve the understanding of the tissue distribution of PFAS in aquatic species and also the interaction effects of PFAS mixtures. However, this model is in zebrafish and will not have a material impact on the conclusions of the current risk assessment. Therefore, I suggest this reference be considered as a **Tier 3: Future Considerations**. **Tier 3: Future Considerations:** • Golosovskaia E, Örn S, Ahrens L, Chelcea I, Andersson PL. Studying mixture effects on uptake and tissue distribution of PFAS in zebrafish (Danio rerio) using physiologically based kinetic (PBK) modelling. Sci Total Environ. 2024 Feb 20;912:168738. doi: 10.1016/j.scitotenv.2023.168738. Epub 2023 Nov 27. PMID: 38030006. There are also a few studies that were published after March 2023 and thus not available in the HERO database, but are very relevant to this assessment. I suggest these studies as **Tier 3: Future Considerations**. For example: **Tier 3: Future Considerations:** • Lynch MT, Lay CR, Sokolinski S, Antezana A, Ghio C, Chiu WA, Rogers R. Community-facing toxicokineticmodels to estimate PFAS serum levels based on life history and drinking water exposures. Environment International. 2023 Jun;176:107974. doi: 10.1016/j.envint.2023.107974. Epub 2023 May 13. PMID: 37245445.

	• Qin XD, Zhou Y, Bloom MS, Qian ZM, Geiger SD, Vaughn MG, Chu C, Li QQ, Yang BY, Hu LW, Yu Y, Zeng XW, Dong GH. Prenatal Exposure to PFAS, Associations with Preterm Birth and Modification by Maternal Estrogen Levels: The Maoming Birth Study. Environmental Health Perspectives. 2023 Nov;131(11):117006. doi: 10.1289/EHP11377. Epub 2023 Nov 14. PMID: 37962440; PMCID: PMC10644897. • Kuo KY, Chen Y, Chuang Y, Lin P, Lin YJ. Worldwide serum concentration-based probabilistic mixture risk assessment of perfluoroalkyl substances among pregnant women, infants, and children. Ecotoxicology and Environmental Safety. 2023 Dec;268:115712. doi: 10.1016/j.ecoenv.2023.115712. Epub 2023 Nov 24. PMID: 38000299. • Li L, Guo Y, Ma S, Wen H, Li Y, Qiao J. Association between exposure to per- and perfluoroalkyl substances (PFAS) and reproductive hormones in human: A systematic review and meta-analysis. Environmental Research. 2024 Jan 15;241:117553. doi: 10.1016/j.envres.2023.117553. Epub 2023 Nov 4. PMID: 37931739. References cited in this response: • Breen M, Ring CL, Kreutz A, Goldsmith MR, Wambaugh JF. High-throughput PBTK models for in vitro to in vivo extrapolation. Expert Opin Drug Metab Toxicol. 2021 Aug;17(8):903-921. doi: 10.1080/17425255.2021.1935867. Epub 2021 Jun 15. PMID: 34056988; PMCID: PMC9703392. • Fàbrega, F., Kumar, V., Benfenati, E., Schuhmacher, M., Domingo, J. L., & Nadal, M. (2015). Physiologically based pharmacokinetic modeling of perfluoroalkyl substances in the human body. Toxicological & Environmental Chemistry, 97(6), 814–827. https://doi.org/10.1080/02772248.2015.1060976 • Kim SJ, Choi EJ, Choi GW, Lee YB, Cho HY. Exploring sex differences in human health risk assessment for PFNA and PFDA using a PBPK model. Arch Toxicol. 2019 Feb;93(2):311-330. doi: 10.1007/s00204-018-2365-y. Epub 2018 Nov 27. PMID: 30483840. • Pearce RG, Setzer RW, Strope CL, Wambaugh JF, Sipes NS. httk: R Package for High-Throughput Toxicokinetics. J Stat Softw. 2017 Jul 17;79(4):1-26. doi: 10.18637/jss.v079.i04. PMID: 30220889; PMCID: PMC6134854.
Savitz	a. The literature search strategy and screening criteria appear to be complete and are very unlikely to have missed any publications that would have a material impact on the overall assessment and conclusions. **No revisions recommended.** b. I am not aware of any additional peer-reviewed studies of PFNA that address the topic. **No revisions.** b.i. This is a reasonable, practical approach to the evolving literature and there is good reason to be confident that it has effectively identified all relevant studies and that any that may have been missed would not have an impact on the overall

	assessment. It makes sense to focus on studies that would change the conclusions and to be as certain as possible that no such studies exist. Furthermore, this is documented in a way that is fully transparent. **No revisions.**
Zoeller	a. The literature search strategy and screening criteria for PFNA appear to be appropriate and mostly well-described, although it is not clear how "regular" updates were/are made and how new information may be integrated into the analysis. In the areas of my critical expertise, I do not identify additional studies that would affect the conclusions in the review. **No Recommendation.** b. I have no further comment from 1a.

3.2. For each health effect considered in the assessment and outlined below, please comment on the following:

- **Are the available data 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.**
- **Are the study confidence conclusions for the PFNA studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes. Please identify any study confidence conclusions that are not justified and explain any alternative study evaluation decisions.**
- **Are the weight-of-evidence decisions for hazard identification clearly described and scientifically justified.**
- **Are there any studies not considered in the assessment that would be expected to materially impact the weight-of-evidence decisions. Please describe the scientific rationale for any recommended inclusions.**

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

a. For developmental effects, the Toxicological Review concludes that the available evidence demonstrates that PFNA exposure causes developmental effects in humans given sufficient exposure conditions, based primarily on growth impairments observed in epidemiological studies. It was determined that there was robust evidence of decreased birth weight in studies of exposed humans, with support from generally coherent epidemiological findings for other fetal and postnatal growth restriction endpoints (e.g., birth length, postnatal weight and height). In further support, cross-stream coherence is provided by moderate animal evidence for PFNA-induced developmental effects in gestationally exposed rodent offspring that included reduced postnatal survival and body weights, and delays in attaining developmental milestones.

i. The evidence synthesis and integration for potential PFNA-induced developmental effects included a meta-analysis (see Appendix C.1) conducted by EPA (Wright et al., 2023) that considered the findings of birth weight deficit to be

statistically robust across all sampling periods and study confidence levels, indicating there are demonstrated birth weight deficits as PFNA exposure levels increase. Although the epidemiological data were ultimately judged as robust, there is residual uncertainty regarding some potential for confounding by other per- and polyfluoroalkyl substances (PFAS) and sample timing; however, these factors were not interpreted by EPA to substantially reduce confidence in the evidence base. Please comment on whether the determination that the epidemiological evidence is robust is scientifically justified.

b. For liver effects, the Toxicological Review concludes that the available evidence indicates PFNA exposure is likely to cause liver effects in humans given sufficient exposure conditions, based on consistent and coherent evidence from human, animal, and mechanistic studies. There is moderate evidence in human studies that PFNA is associated with liver injury based on increased ALT, AST GGT, and bilirubin. In animals, there was robust evidence from a series of short-term studies in rats and mice demonstrating consistent and coherent effects on liver weight, clinical pathology, and histopathology that included hepatocellular necrosis, cholestasis, and triglyceride accumulation. The liver findings for PFNA were similar to those for other structurally related long-chain PFAS and were determined to be adverse.

i. The judgment that there is moderate evidence in human studies was based primarily on cross-sectional studies in general population adults. For nearly all epidemiological studies of PFNA exposure, there is potential that exposure to other highly correlated PFAS could contribute to the observed effects. The evidence synthesis for potential PFNA-induced hepatic effects included evaluation of the adequacy of studies with exposure and outcome measured concurrently as well as the likelihood of confounding across PFAS. It was concluded that these sources of uncertainty were unlikely to explain the observed effects. Please comment on whether these conclusions are scientifically justified.

ii. Additional considerations influenced the liver effects hazard identification decisions. Appendix A (Systematic Review Protocol for the PFAS 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. For PFNA, there is evidence of both PPARα-dependent and -independent (e.g., CAR/PXR) pathways contributing to hepatotoxic effects, consistent with the judgment drawn for several other PFAS. The Toxicological Review evaluates the evidence relevant to the potential involvement of PPARα and non-PPARα pathways with respect to the reported liver effects. The Toxicological Review ultimately concludes that evidence from in vivo and in vitro studies supports a potential role for multiple pathways operant in the induction of hepatic effects from PFNA exposure and that the effects are potentially relevant to humans. Detailed information is provided in the Mechanistic and Supplemental Information of Section 3.2.4, Hepatic Effects. Please comment on the basis for the judgment of human relevance of the liver effects and whether it is scientifically justified.

iii. In judging that the animal evidence for hepatic effects is robust, the Toxicological Review concludes that the hepatic effects in animals were adverse (vs. adaptive), based in part on consideration of criteria from Hall et al. (2012). The liver enlargement from short-term testing in rats and mice was accompanied by histopathological lesions, including adverse lesions such as necrosis. However,

the lack of longer-duration exposures was a substantial source of uncertainty. Therefore, although the linkage between liver hypertrophy and histological evidence of necrotic changes was found to support adversity, the short-term data were further evaluated based on additional criteria set forth in Hall et al. (2012) that considers dose-dependent and biologically significant changes in at least two clinical pathology parameters (see Hall et al., 2012) as confirmatory indicators of hepatocellular damage. The PFNA database was found to meet at least two of the additional criteria set forth by Hall et al. (2012), including large increases in ALT and AST in mice (effects in rats were mild); large increases in bile acids and bilirubin in male rats considered by the National Toxicology Program (NTP) to be indicators of intrahepatic cholestasis; in addition to reductions in blood proteins, increasing triglyceride accumulations and disrupted lipid homeostasis. Please comment on the basis for determination under the criteria set forth in Hall et al. (2012) and others (e.g., U.S. EPA, 2002; EMEA, 2008; Thoolen et al., 2010; Boone et al., 2005) that the hepatoxic effects observed in rodents are considered adverse.

c. **For male reproductive effects, the Toxicological Review concludes that the available evidence indicates PFNA exposure is likely to cause male reproductive effects in humans given sufficient exposure conditions. This conclusion is based primarily on a high confidence 28-day oral toxicity study in adult rats that reported a consistent and coherent pattern of adverse male reproductive effects, with additional support from medium confidence, short-term studies in adult rats and prepubertal mice observing effects at similar doses.**

d. **For immune effects, the Toxicological Review concludes that the available evidence suggests, but is not sufficient to infer, that PFNA exposure has the potential to cause immunosuppression in humans. This conclusion is primarily based on epidemiological studies (see Table 3-22) providing evidence of reduced antibody response with PFNA exposure, and possible evidence for effects on asthma and asthma-related outcomes, but with concerns regarding imprecision and potential residual confounding by other PFAS. The human evidence was considered slight and the animal evidence indeterminate.**

    i. **The evidence for immune effects for PFNA differs from that of other long-chain PFAS (e.g., perfluorodecanoic acid [PFDA] and perfluorohexanesulfonic acid [PFHxS]), which found stronger evidence of immunosuppression. Please comment specifically on whether the weight-of-evidence decisions for immunosuppression have been clearly described and are scientifically justified.**

e. **For thyroid effects, the Toxicological Review concludes that the available evidence suggests, but is not sufficient to infer, that PFNA exposure may have the potential to cause effects on the thyroid in humans. This was a complex evidence base to interpret, and the judgment was based primarily on moderate animal evidence from a high confidence 28-day study in adult rats that showed large, dose-dependent reductions in serum free and total T4 in females and in serum free T4 in males. Although this study provided evidence of effects on T4 homeostasis, there were uncertainties surrounding the reliability of methods used for measuring free T4 in both sexes. There were also body weight losses in males at higher doses that challenged interpretation of the T4 reductions, as well as additional responses in males that were difficult to decipher (i.e., decrease in thyroid-stimulating hormone [TSH], including at doses absent substantial body weight loss). The epidemiological database was slight and did not demonstrate coherence with the animal evidence, with the strongest evidence showing positive**

associations with T4 in children/adolescents, although effect sizes were small. However, there was considerable uncertainty in the human evidence because of inconsistent directions of association and concerns related to study sensitivity.

f. For cardiometabolic effects, the Toxicological Review concludes that the available evidence suggests, but is not sufficient to infer, that PFNA exposure may have the potential to cause cardiometabolic effects in humans. This conclusion was based on studies in humans that showed generally increased serum lipids and some potentially supportive but mixed results for other increased risk factors for cardiovascular disease. However, the evidence has unexplained inconsistencies within and across studies and concerns for imprecision, which add considerable uncertainty. Evidence in experimental animals was indeterminate.

g. For neurodevelopmental effects, the Toxicological Review concludes that the available evidence suggests, but is not sufficient to infer, that PFNA exposure may have the potential to cause neurobehavioral effects in humans, based on associations between PFNA and outcomes related to attention and behavior in epidemiological studies. However, there is considerable uncertainty in this association, including imprecision in all the estimates from the three studies evaluating attention-deficit/hyperactivity disorder (ADHD) diagnosis, the most specific outcome, and some unexplained inconsistency. There was no relevant evidence in experimental animals to inform this outcome.

h. For female reproductive, urinary, adrenal, and other noncancer effects (i.e., hematological, respiratory, digestive, dermal, and musculoskeletal), the Toxicological Review concludes there is inadequate evidence to determine whether PFNA exposure has the potential to cause these effects in humans based on the sparsity and/or uncertainties of available evidence.

Noncancer Hazard Identification	
Reviewer	**Comments**
Carignan	a. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results appear clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. The conclusion, "that the available *evidence demonstrates* that PFNA exposure causes developmental effects in humans," is scientifically justified. The weight-of-evidence decisions are clearly described and scientifically justified. I agree IRIS that the evidence is robust, a conclusion that is further supported by the detailed meta analysis of the epidemiological studies of PFNA and birth weight as well as by experimental animal studies. b. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results appear clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies.

	The conclusion, "that the available *evidence indicates* PFNA exposure is likely to cause liver effects in humans," is scientifically justified, as the conclusion of minimal concern that substantial confounding that would fully explain the observed associations. The weight-of-evidence decisions are clearly described and scientifically justified. b.i. I agree that the determination of moderate evidence in human studies is scientifically justified, as is the conclusion that the observed effects are unlikely to be explained by confounding across PFAS or concurrent measurement of exposure and outcome. b.ii. I agree that the judgement of human relevance of the liver effects is scientifically justified as experimental studies support a potential role for multiple pathways operant in the induction of hepatic effects from PFNA exposure relevant to humans. b.iii. I agree that the hepatic effects are adverse (vs. adaptive) and that the observed effects in offspring are indicative of early life susceptibility. c. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results appear clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. The conclusion, "that the available *evidence indicates* PFNA exposure is likely to cause male reproductive effects in humans," is scientifically justified. The weight-of-evidence of are clearly described and scientifically justified. Recommend future research and assessments on male reproductive effects of PFNA and other PFAS (legacy and current use) individually and as mixtures. **(Tier 3)** d. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results appear clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. The conclusion, "that the available *evidence suggests*, but is not sufficient to infer, that PFNA exposure has the potential to cause immunosuppression in humans," is scientifically justified. d.i. The weight-of-evidence is clearly described and scientifically justified. Suggest noting conclusion of immunosuppression for PFOA, PFHxS, ect. **(Tier 2**) Future epidemiologic studies should investigate relevant immune endpoints for PFNA alone and as commonly observed mixtures. **(Tier 3)** e. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results appear clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies.

	The conclusion, "the available *evidence suggests*, but is not sufficient to infer, that PFNA exposure may have the potential to cause effects on the thyroid in humans," is scientifically justified. The weight-of-evidence is clearly described and scientifically justified. The human evidence is variable (not slight) **(Tier 2)**. It would be helpful to group the pregnancy studies together and to indicate which is the preconception study in the figure **(Tier 2)**. Clarity could be further improved by grouping T3, T4 and TSH as directionality may differ between these markers **(Tier 2).** Some figures do not note the strength of evidence, which would be helpful **(Tier 2)**. The thyroid disease studies appear underpowered based on the consistent directionality and wide confidence intervals. Future epidemiology studies investigating thyroid disease of PFNA with sufficient statistical power are needed **(Tier 3)**. Future epidemiology and animal studies should investigate thyroid effects of PFNA, especially in children. **(Tier 3)** Future epidemiology and animal studies should investigate adrenal effects of PFNA. **(Tier 3)** f. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results appear clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. The conclusion, "that the available *evidence suggests*, but is not sufficient to infer, that PFNA exposure may have the potential to cause cardiometabolic effects in humans," is scientifically justified. The weight-of-evidence is clearly described and scientifically justified. Future epidemiology and animal studies should investigate cardiometabolic effects of PFNA individually and with common PFAS mixtures; these should include perinatal exposures in sufficiently powered studies **(Tier 3)**. g. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results appear clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. Suggest updating Table 3-35 to clarify the outcome measured **(Tier 2)**. The conclusion, "that the available *evidence suggests*, but is not sufficient to infer, that PFNA exposure may have the potential to cause neurobehavioral effects in humans," is scientifically justified.

	Future epidemiologic and animal studies should investigate neurodevelopmental effects of PFNA individually and with common PFAS mixtures; especially perinatal exposures and outcomes related to ADHD, autism and cerebral palsy. **(Tier 3)** h. The conclusion, "that the available *evidence suggests*, but is not sufficient to infer, that PFNA exposure may have the potential to cause neurobehavioral effects in humans," is scientifically justified. The available data appear clearly and appropriately synthesized to describe the strengths and limitations. Presentation and analysis of study results are generally clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. I have some suggestions for improvement: Suggest updating Table 3-35 to clarify the outcome measured **(Tier 2)**. Please state whether/which studies excluded individuals with kidney disease, an important source of negative bias for renal studies of PFAS **(Tier 2)**. Please provide more explanation of the reverse causation concern for renal effects **(Tier 2)**. The fecundability table should note the results that are statistically significant, which appear to be the Greenland and LIFE studies **(Tier 2)**. I'm pleased IRIS has added a section on breastfeeding duration. It would be helpful to provide context by citing developmental effects on breast bud development in experimental studies. **(Tier 2)** Future epidemiological and animal studies should investigate female reproductive, urinary and adrenal effects of PFNA alone and as commonly observed mixtures. **(Tier 3)**
Ducatman	a. This is a nice review, and the EPA meta-analysis adds confidence. Thank you for doing that! The summary section beginning on p. 5-3 is helpful. EPA is especially to be congratulated for prioritizing serum results from early pregnancy over other considerations concerning sources of bias. The explanation of log conversion bias and the consideration of it (see p. 5-24) is very helpful. **Tier 3:** The very recent study (Save-Soderbergh et al., 2024) interestingly does <u>not</u> show <u>longitudinal</u> PFAS (including PFNA) associations to birthweight based on assigned doses from exposure modeling in Sweden. The model is semi-ecological and perhaps that is the explanation for a different finding but the study appears to me to be reasonably done, and modeled exposures have the theoretical advantage of being independent of the uncertainties that go with fluid shift effects upon PFAS measures intra-pregnancy. (They gain this advantage by being less specific to the individual, however.) I do <u>not</u> think this finding in an important population changes the current document conclusions, I do think that the Ronneby exposure population is important and that EPA should be alert that the topic of birthweight has many variables affecting studies and is a topic to keep reconsidering based on evidence going forward.

	Tier 3: eGFR consideration as a primary means of rating studies in the maternal/child study environment. I am less confident than EPA that eGFR is sufficiently reliable as a population measure during pregnancy that it should be a primary means to rate excellence of studies. (I proposed this advice for the PFHxS document as well, along with rationale and alternatives). Specific advice for EPA is to reconsider whether reliance on eGFR for sorting study excellence is really a good idea in the setting of pregnancy.(see for example P. 3-52) While large intra-pregnancy decreases in eGFR are clinically/sequentially useful in an individual, the use of eGFR as an adjustment in maternal/child population studies relies on and is likely less important than very accurate recording of pregnancy dates and comparisons calibrated/stratified by-dates comparisons. Elsewhere in the document, EPA has done a great job pointing out how important it is that maternal and maternal/child studies are more valuable when they have synchronized blood draws, with priority for early pregnancy (and even immediately pre conception if possible). **Tier 2:** These timing topics are more important than eGFR for ranking. **Tier 2:** The table3-13 on p. 3-139 appears to suggest that PFDA and PFNA exposures are similar. EPA also appears to be concerned that correlation of PFNA with PFDA could be a source of confounding. Confounding by mixtures is an inevitable, reasonable concern in general. While it is the case that PFNA and PFDA are "the same order of magnitude" (sort of, if one is looking for a full log increase rather than the next decimal). so is PFHxS in the survey tables over successive years. Is EPA also questioning a role for menstruation for PFHxS based on this extended analogy? By the way, in some survey years, PFOA and PFOS are also of the same order of magnitude. The concern is unfounded in my view. The PFNA measures appear to be stable and have relatively few nondetects while the PFDA appear to be unstable by trend in some survey years, have relatively more non-detected biomarkers, and have been rounded off appropriately. It is up to EPA if, after looking at the underlying table of contaminant levels, this specific reservation (About PFDA data having some kind of impact on PFNA knowledge) is really important. I suggest it is misleading. • **Tier 1**. I think Surflon as a mixture is mostly PFNA and the surflon toxicology studies have relevant PFNA information. Adding consideration of these studies (Mertens et al., 2010; Stump et al., 2008) in mixture context is a reasonable addition to the text. • **Tier 2.** The role of fluorotelomer alcohols in creating PFNA exposure could be mentioned. The presence of PFNA contamination in AFFF use/manufacturing sites could be mentioned. (See for example a report on the Battelle PFAS website https://www.battelle.org/docs/default-source/hidden/2022-chlorinated-conference-abstracts/f4_938.pdf?sfvrsn=548b20d9_3) • **Tier 2:** On page 5-28, EPA makes some assumptions about relative contributions of dam-pup and maternal child contribution of PFAS in offspring. The language and thought train are dense and outside of my expertise. At the end, I think EPA

	is making an assumption about relative contributions. Perhaps the text can be rewritten for greater clarity so that readers can be more sure of the point. • **Tier 2**. Are the appendixes all correctly listed in the text? I was confused at one point (See 3-52). b. The reviewer appreciates the review and concurs with the conclusion. There are a number of thoughtful explanations for readers such as the meaning of different types of bilirubin measures, and the specific implications of different types of biomarkers, and possible different ways that ROS may be part of the picture. This is excellent. The conclusion and summary in section 5 are helpful. There are also a few concerns, noted below, about language and organization. The concerns do not affect conclusions, they are simply addressed to avoiding misunderstanding and to organizing what belongs to liver under liver and not in other categories, These details can scientifically important and assist readers to understand the document, as follows: • **Discussion: Cholesterol** is metabolized primarily in the liver. Cholesterol should be explicitly mentioned as evidence of liver toxicity, and excess abnormal values eligible for lipid lowering agents as an outcome. Papers by Steenland for example that address this topic. Deep in the text but not so much in the headlines, the EPA document expresses awareness that cholesterol is metabolized in the liver, and that disrupted cholesterol metabolism associated with PFNA is about hepatotoxicity (and does not occur in the heart, where it is discussed). The text does a better job, however, however, in acknowledging that disruptions in cholesterol (and other lipid) metabolism for experimental studies than for human studies. • **Tier 1:** Please add adverse alterations in <u>human</u> cholesterol including abnormal values more explicitly as another piece of the evidence of liver toxicity, or at least of cardiac toxicity if the goal is to unfriend the liver's role in lipid metabolism. (Remember, humans get treated with consequential medications when lipid levels cross thresholds, based on evidence of preventive benefit.) **Discussion:** Similarly, Uric acid metabolism is distributed in several organs. Some is up to kidney as noted by EPA. However, much of the metabolism of uric acid manufacture occurs in the liver, and the data from a 360 perspective is consistent with hepatotoxicity and not with renal toxicity as a cause of uric acid associations to PFAS, including PFNA Discussion of deficiencies: First uric acid is discussed only in the context of evidence for/against a role of PFNA causing kidney disease. This is a deficiency, an incomplete understanding For several reasons. When the bias introduced by possible reverse causation is adjusted or better still stratified, the association to uric acid remains. The more sophisticated the treatment, the more the association persists and deepens. Details: studying the topic requires awareness that, in moderate-severe kidney disease, serum PFAS tend to decrease while uric acid increases, creating a double

	nidus of underestimation bias (underestimation bias if the renal excretion physiology is not understood and accounted for in the study design) in the presence of kidney disease. This problem is particularly pertinent for cross-sectional studies, and less problematic in longitudinal designs. Since PFNA is associated with worse uric acid including hyperuricemia and that association is also seen in longitudinal studies (Feng, Fu, et al., 2022), it is reasonable to think that alterations seen in uric acid with PFNA (and with other long-chain PFAS) are potentially causal. In addition, the association may be attributable, at least in part, to liver toxicity. In its table on p. 3-394, EPA has stated that all kidney studies are low confidence. This illustrates the problem of considering uric acid in the wrong place. **Tier 1**: please reconsider for uric acid studies (which may or may not be included in this table, but which are not considered on their own independent of the concept that they are an outcome of kidney disease). Discussion: The bias for these uric acid studies is likely in the direction of underestimation, the cause need not be kidney disease, there is a corroborative longitudinal study, and the findings appear consistent with other PFAS studies such as PFOA. The current organization notes the association of uric acid to PFNA exposure, and then dismisses it because it is not deemed as sufficient evidence of urinary system effects (P. 383). Again, this illustrates the problem of not thinking through the best way to consider a topic. There is no a priori reason to think that higher uric acid reasonably consistently associated to PFAS exposure (including to PFNA) should be dismissed based on the presence/absence of causal associations of PFAS to kidney disease. Liver toxicity affecting the urea cycle is the more likely explanation. **Tier 1** Please consider uric acid as its own topic. Whatever the conclusion, please note the presence of longitudinal evidence of higher uric acid and clinical hyperuricemia (Feng, Fu, et al., 2022). **Tier 2**. Please consider adding adverse alterations in human uric acid to the reasons for considering PFNA as a cause of hepatoxicity. A number of articles have shown that the uric acid associations for other PFAS are robust to the presence of kidney disease, and not explained by the presence of kidney disease. (This is especially the case in the presence of albuminuria, which decreases serum PFAS including PFNA, or moderate-severe kidney disease, which decreases serum PFAS including PFNA). Thus, reverse causation is not the explanation. EPA's logic on this detail is not fully consistent with the literature. It is the case that reduced renal function causally raises serum uric acid, yet about half of the individuals who do have hyperuricemia do have and about half do not have seriously reduced kidney function (eGFR < 60mL/1.73m$^{2.}$ (Y. Wang et al., 2021) the range at which PFAS also decrease in serum . In its review of liver, EPA characterizes the size of associations such as transaminase associations as "small." This occurs in several places Examples, p. 3-185, 3-188, not a complete list. **Tier 2**: EPA is asked to find these places in the text, and to consider a better, less confusing description that is more compatible with the clinical impact (which EPA discusses nicely in section 5. The impact on abnormal values is what matters clinically, and it is not "small." To understand why this happens, it helps to understand the actual distribution of the biomarker).

	Discussion: This characterization is misleading, potentially confusing, and EPA is asked to consider if it should close the loop so readers understand that the "small" adverse change is predictably not small when abnormal values are considered. The "small" changes are predicted to have outsized impact on abnormal values, just as similar "small" changes were predicted to have and then demonstrated to have outsized impact on abnormal values for PFOA exposure. (This is mathematically inexorable for a change in the biomarkers based on the distribution of the biomarker such as ALT or AST and not dependent on specific PFAS species.) Our normal values cluster at the top of the normal range in any adult population, few have values at the bottom end of the range, the normal range that begins at zero is clinically correct but no one has zero and few are under ten.) "Small changes" in PFOA to ALT lead to appreciable changes in abnormal values as a result. (Darrow et al., 2016; Ducatman, Tan, Nadeau, & Steenland, 2023). (. Consider the SAB's (provided in the document as USEPA 2022c)) similar perspective. The changes characterized as "small" are meaningful and have population/public health significance in other PFAS settings The suggestion is therefore to alter the adjective or explain the impact to avoid the impression of internal contradiction. This following quotation is similarly problematic or at least incomplete given a 360 view of the evidence. *"There is some uncertainty as to the biological significance of the observed changes due to the small magnitude of effect in most studies, particularly given that the two available studies of more functional hepatic endpoints (e.g., histopathology) are low confidence and the functional results are inconsistent across studies." From p. 3-153* **Discussion:** it is fair to say that mechanisms are still being worked out. It is reasonable to discuss the difficulty of creating large longitudinal studies with invasive endpoints such as biopsy/histopathology, and reasonable to mention that this is an important literature gap that leaves unanswered questions. However, it is misleading to leave an impression that the adverse associations might not be significant biologically (could be trivial and unimportant). it is misleading/unjustified to have a conclusion statement that can be read to say that the PFNA-liver associations could be biologically unimportant based on inconsistency, when the literature is in fact more consistent than acknowledged. **Tier 1**: Please frame the very real limits of what we know and the gaps of what we need to know about PFNA more carefully, without implying that the biomarkers associations could have unique exemptions from adversity in the case of PFNA. We do need to know more, yet the associations are definitively adverse and biologically significant, even if we can't yet state the mechanism. Please consider (**Tier 2**) also mentioning the uric acid findings (which reflect on liver and on kidney but kidney reverse causation is not a likely explanation) , as additional evidence. **Tier 1** please do mention especially the cholesterol associations (which are likely causal and primarily about hepatotoxicity), and the biologic and public health implications.

	Tier 3: A recent review pools estimates from already extant PFNA (and other PFAS) including a focus on abnormal outcome values (Song et al., 2024). While the review and integration work is recent, outside of the timeframe of the review, the articles cited and their clinical/population implications are not new. The cholesterol data (and the uric acid data, which are more likely due to liver than to kidney toxicity in this setting) predict additional medical interventions. There are parallel experimental data. The reviewer believes the reference list in the review could be useful to EPA. b.i. EPA is reasonably concerned with confounding, and the document uses a reasonable approach and made the right judgement expressed with reasonable scientific language concerning inevitable uncertainty about mixture effects. In a world where PFAS will always be a mixture and PFNA will usually not be the dominant compound, EPA reasonably notes the degree of uncertainty (and could be clearer that the experimental evidence supports the human evidence, and that the mixture evidence is reasonably consistent). **Tier 2**: The EPA document could benefit from a summary paragraph about whether and how the human population and experimental data inform each other, including consistency/inconsistency across studies of legacy PFAS including PFNA. In mixtures, is there any evidence that PFNA adds/detracts from toxicity? **Discussion of the difficult problem of mixtures vs single compounds.** The mixture conundrum will never go away but it could be finessed if stakeholders agreed to one uniform approach and not the circular firing squad we sometimes see today. An irony for future consideration is the decision inconsistency created by the mixture vs single chemical approach. Opposite approaches are taken, and both exonerate toxicants.. When we have a reliable mixture effect that includes the chemical of interest, we note uncertainty because we do not know if the mixture might not be equally as adverse in the absence of that contribution. When we have a single chemical study, we express uncertainty because the reality is that exposures are mixtures (and some of the compounds will predict the presence of others) even if strong efforts are made to isolate relative contributions.. While more dismissive decisions are individually epidemiologically conservative and reasonable, one wonders if the decision to minimize for both considerations is reasonable from a science perspective. **Tier 3:** If we believe that mixtures are indeed important, perhaps future efforts can ask stakeholders to provide a consistent approach that is not inadvertently dismissive both ways. In addition, EPA does an excellent job explaining that the (inevitable appearance of PFAS in) mixtures do not materially decrease confidence in reasonably consistent associations. (see for example the summary discussion on p 5-40.) **Tier 3:** Would stakeholders consider it confidence building to actually cite the many papers in which the compound of interest (example PFNA) is included as a mixture component, and the summary mixture findings? This happens sort of organically (and helpfully for understanding!), but is hidden away in a table not systematically, hidden away in a table on p. 5-46. b.ii. Concur that it is justified. Both PPAR-alpha-dependent and -independent pathways is correct. Humans do have PPAR-alpha activity. The presence of receptors in

	human organs (and including especially in developing human organs) is illustrative of the folly of dismissing PPAR-alpha (Abbott, 2009; Abbott, Wood, Watkins, Das, & Lau, 2010), although it is likely that other pathways (including other PPAR pathways) are reasons for some of the most obvious hepatic toxicity. Further, and noted above, adverse lipid (**Tier 1**) (and uric acid **Tier 2**)) evidence for PFNA is consistent with other long-chain PFAS liver outcomes and such additional evidence and should be mentioned as part of the evidence basis for liver effects. These outcomes have definite (cholesterol, LDL-cholesterol) and probable (uric acid) liver origins and further speak to the mechanisms. b.iii. Concur (and it is a bit unclear why the Hall effect is given so much review time in any case, as the criteria for application are obviously not met. The Hall considerations are for short term exposures in the absence of evidence of chronic toxicity and are irrelevant in the face of parallel human long term exposure and human/experimental evidence of harm). There are some problematic details that do not change conclusions. The document will be stronger scientifically if they are addressed. Quoting from the document "The evidence integration judgment for hepatic health effects is based on *moderate* epidemiological evidence of increased serum markers (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and bilirubin) in humans." Reviewer Comment: Elsewhere in the document EPA expresses awareness of several reasons that total bilirubin is a complex and problematic biomarker of liver damage. The comment on p. xx which states "bilirubin" could be read to implicate total bilirubin only. Consider (Kasarala & Tillmann, 2016), in the Journal Clinical Liver Disease (presented as "an official learning resource of the American Association for the Study of Liver Disease, "AASLD"). Quoting from this external learning resource: "In healthy individuals, conjugated bilirubin comprises a small proportion of total bilirubin in adults, unconjugated bilirubin elevation is most often of extrahepatic origin, mainly caused by hemolysis" (Kasarala & Tillmann, 2016) **Tier 2:** My advice is that EPA can make the language identifying biomarkers and their use/limitations as precise as possible in the introduction, or at least note in the introduction the clarification presented in section 5. (which appears hundreds of pages later). Noted in section 5 of the document, ALT is our most specific liver biomarker. EPA is aware (noted elsewhere in the document). Total bilirubin can be abnormal in liver disease, and has other common causes. P. 3-184 contains the following quotation, which seems to this reviewer to give credence to the concept of harmless adaptive response which is reasonably rejected elsewhere in the document. Quoting:

	p. 3-184 The lack of longer duration exposures was a substantial source of uncertainty. This data gap prevented full consideration of whether the liver enlargements and concurrent effects (e.g., hepatobiliary markers) at lower PFNA doses could elicit adaptive mechanisms or, as indicated by the histopathology data at higher PFNA doses, could progress to more severe liver disease. **Reviewer comment**: This quotation raises the concept that the 360 review of liver effects could be fully adaptive and therefore unimportant, because there are no long-term exposures. This are data gaps, but the totality of gaps do not admit for adaptive responses that explain parallel human/experimental data. Later, EPA provides convincing evidence that the 360 effects are not adaptive. **Tier 2** Since the language quoted does not have that larger context and appears to contradict other conclusions, a clarification is suggested to avoid confusion. It is reasonable to point out that absence or paucity of long term experimental studies are a "data gap" without ambiguous language endorsing a low probability adaptive/harmless terminology. It is also reasonable to point out that other evidence supports liver toxicity. c. No concerns with the conclusions concerning humans or in experiments. (Further, the reviewer explicitly agrees with EPA that sperm concentration, motility, and morphology are reasonable endpoints for evaluating the concern about reproductive toxicity.) **Tier 3** consideration of a detail: It is important to use systematic review process as EPA has done and this practice is encouraged. The choices made for these deliberations are not easy. EPA reasonably mentions that it detected sources of (mostly underestimation) bias (such as short exposure duration) in several experimental studies of sperm counts. The short term exposure reduced confidence in the study(ies). It also mentioned that it still detected an effect of the exposure despite the potential for underestimation bias (see draft EPA document, p. 3-213),. While the a priori rankings of study quality are logical and consistent, clinicians are also accustomed to Bayesian logic patterns that address results. (Humans are intuitive Bayesian thinkers, We change our perspective when we have new evidence to do so,.) Such consideration is suggested for the benefit of all readers. The forward-looking, **Tier 3** consideration is about a systematic way to routinely state the evidence is <u>not</u> less despite an a priori study design weakness, when the outcome is despite rather than because of the weakness. EPA does a great job of illustrating this topic in fecundity (which it classifies as pertaining to females, please do fix that) (p3-323), but does not have a consistent approach and could use a notation for this circumstance in tables. Perhaps such a notation (asterisk or other finding is not lessened by the a priori study weakness) would be useful. **Tier 2** inquiry about language: Quoting from the document concerning male reproductive toxicity: *Results of animal testing with PFNA provide moderate evidence of reproductive toxicity based on generally consistent, dose-dependent, and coherent pattern of perturbations to the male reproductive system of adult and prepubertal rodents*

	following short-term and developmental exposures. PFNA exposure caused decreased reproductive organ weights and serum and testicular testosterone as well as impaired spermatogenesis, and with more uncertain evidence for corresponding structural changes to reproductive tissues. Coherent with reduced testosterone in prepubertal mice, a dose-dependent delay in preputial separation was also observed in male mouse offspring exposed gestationally to PFNA. There is some mechanistic evidence supporting biological plausibility, primarily related to findings related to disrupted spermatogenesis. **Tier 2 question**. Is "moderate" about human evidence, animal evidence, or a synthesis? **Tier 3 suggestion**: A question about male reproductive effects is appropriate, and leads the reviewer to inquire about the organization that separates male reproduction (section c) from female reproduction (section h) in the reviewer response. (Female reproduction is also expertly reviewed in the document, Further, some important measurable outcomes such as fecundity reflect on male and female reproduction effects, and not on female reproductive health only. d. The notation in the introduction to the section concerning immune effects (p. 3-244) announcing overlap across sections and how it was handled is helpful. In addition, the decision to consider immune as immune per se and not as some alternative downstream outcome is reasonable.The scientific description in this section is clear. The judgment of reasonably consistent adverse outcomes for vaccine response and the discussion of uncertainty concerning the interpretation of adversity of vaccine response in humans is reasonable. The discussion of experimental evidence is organized and helpful. **Tier 2 discussion**: On p. 3-253, the critique of the Grandjean Covid study is fair as regards disease severity but deserves additional explanation as there is no reason to think that serum PFAS is somehow affected by enrollment bias in the registry (unlikely). The subsequent sentence, that COVID severity is multifactorial is correct yet an objectionable rationale for the purpose of downgrading evidence. (Malignant mesothelioma is my short list of single causes.) The multifactorial statement is true, trivial (because true for everything). Further, the dismissal is inappropriate and jarring since immune suppression is understood to be quite important to COVID severity (although, as noted, immune status is not the only relevant factor, it is an important factor and we do not dismiss important factors based on adjectives). Subsequent PFAS/COVID studies exist and do <u>not</u> show the same association; that inconsistency of findings (more studies find no adverse association) represent a better way to summarize the current state of scientific knowledge concerning PFAS and COVID than the approach quoted above. **Tier 2 suggestion**: Omit or better explain multifactorial. e. The approach and conclusions appear reasonable. The mechanistic discussion that beings on p. 3-312 and discusses binding on p. 313 (especially) is strong and very helpful. It is important to use systematic review process as EPA has done and this practice is encouraged. The choices made for these deliberations are not easy. The reviewer appreciates that studies were <u>not</u> arbitrarily downgraded for biases that

	were unlikely to have changed results {other than to make presence or absence of associations stronger if the bias were avoided), and commends that perspective as a routine **Tier 3** policy. The separate consideration of thyroid alterations in pregnancy is also a strength of the approach. Some details to consider: The question posed to reviewers states that the epidemiological database "was slight." (quoting from the question posed to reviewers. This is misleading. There are a number of available studies It is justified to say it is inconsistent and suffers from study design questions, and not justified to call it "slight.".) This thought appears on p. 3-315 of the EPA document. Quoting: "*This was a complex evidence base to interpret as **the human evidence was slight** and the only animal study available provided moderate evidence but also had some uncertainties related to the free T4 analytical method used and body weight loss in males at higher doses. Despite these uncertainties, the large, dose-dependent reductions in free and total T4 in female rats and free T4 in male rats suggest some level of concern."* **Discussion:** I think EPA's conclusion is correct but the rationale and description of the human evidence is muddled.. **Tier 1.** There is plenty of thyroid epidemiology for PFNA (not "slight"). The document correctly reviews a fair amount of literature and reasonably concludes that the available literature does not provide a consistent picture. (Correct conclusion, confusing and refutable rationale as regards the extent of relevant literature.) Circulating autoantibodies to thyroid peroxidase (TPOAb) are the common cause of clinically important thyroid disease (detectable in about 90% of adults who truly need thyroid replacement hormone). A **Tier 3 suggestion** is separately evaluate and to place a priority on human studies that can provide TPOAb longitudinal data, as it is closer to the proximate cause and less subject to other temporal variables compared to thyroid and TSH measurements. It was difficult to interpret this sentence from p. 3-307. *Although it is difficult to interpret the TSH reductions in males and patterns of TH changes in animals may not translate directly to human clinical definitions, the observed decreases in total or free T4 in females in the absence of increases in TSH indicate thyroid perturbations and are considered biologically relevant to humans …* I think I parsed the sentence and believe it leads up to/contains a deliberative conclusion based on two lines of evidence. **Tier 2:** Please consider rewriting the point about relevance as several sentences for clarity. **Tier 3:** In future, please consider placing document consideration of sterol hormones and questions to reviewers about sterol hormones in a fully separate place from thyroid hormones. These topics are poorly related.

	f. While the reviewer regrets the organization, which shifts important liver outcomes into downstream cardiac consequences (and assumes that uric acid is relevant only if it contributes to kidney disease), the factual presentation is mostly reasonable. The discussion of future study needs beginning on p 3-379 is excellent. Suggestions follow: • **Discussion about organization:** Lipids are not metabolized in the heart, and "metabolic" is a broad category that can include a very wide range of outcomes. **Tier 3** for future: In future documents, EPA should parse these outcomes to most relevant organs. Explanation: A regrettable weakness of this approach is that the human lipid data, which unlike the animal data are based on long-term exposures at relevant dose, are not considered alongside the transaminase data, even though they are complementary and inform each other (as do the uric acid data. This 'atomization of evidence' problem is also noted under liver noncancer health effects.) The closest the detailed review in the document comes to integrating the evidence is in a mechanistic and supplemental information paragraph (in the cardiometabolic section on p. 3-379 which is focused on animal data only.) Section 5 of the document pulls together evidence to some degree, yet it is a lot to ask readers to get through hundreds of pages before there is synthesis. This suggested consideration is not expected to alter points of departure. The goal is to improve the review and reader understanding. • The mishmash of evidence in Table 3-47 illustrates an aspect of this problem. It deploys an absence of other CV risk factors to minimize liver/lipid conclusions, leaving a misleading impression that is then partly contradictory to the synthesis in section five of the document. In the age of effective lipid lowering agents, first introduced in the 1980s and improving ever since, the decision is questionable for heart and confusing for liver. (Are the lipid lowering medications taken as a result of more abnormal liver metabolism somehow unimportant?) It also emphasizes inconsistency of findings, yet for lipids and transaminases, the most relevant liver outcomes, PFNA data are reasonably consistent with PFOA and PFOS data. An improvement from previous evaluations is that the document acknowledges that the "cardiometabolic" topic "overlaps" with liver. This acknowledgement is a step in the right direction and does not substitute for an integrated view of all the liver data. A big improvement is the synthesis in section five of the document, but that still leaves out much of the converging evidence. **Tier 3:** Downgrading of papers which gathered non fasting lipid data. Discussion: It is important to use systematic review process as EPA has done. The choices made for these deliberations are not easy. The reviewer apologized for asking questions about some of them. Most of the world has moved past requiring fasting cholesterol. (This requirement can also be an IRB issue.) Downgrading papers on this basis has a theoretical basis which is adequately described in the text (3-337). A suggestion is that this consideration is a far less important source of weakness than studies with a small "n" and narrow range of measured exposure. (A decent dose distribution from low to high and not all clustered at one end is more

	important than considering fasting status). A question for EPA is whether it thinks confounding from the occasional postprandial hypotriglyceridemic participant is expected to be nondifferential for PFAS serum concentrations in general and PFNA specifically? From their careful wording, I do think EPA authors are likely aware that the standard of care has moved past this fasting consideration. In the real world, fasting is required only of those who have the genetic condition, a consideration that can be challenging to shoehorn into ethical study designs. The **tier 3 question**: is EPA sure upon reflection that it wants to prioritize this topic in future? Wouldn't population size and good representation across a wide range of exposures be better? Discussion of a hypertension consideration. In the hypertension discussion (begins p 3-348), it might be useful to note that cross-sectional PFAS/hypertension studies can have a built-in underestimation bias. Albuminuria is (casually) associated with lower serum PFAS. (It is also possible that PFAS cause kidney disease, a different discussion. This is a discussion of reverse causation. Similarly, deterioration of glomerular filtration (stage 3b CKD or worse) does the same thing to a lesser extent, urine PFAS excretion increases and serum PFAS declines. Further, hypertension is well understood to be a cause of albuminuria and of worse eGFR including as a cause of CKD, thus leading to lower serum PFAS and underestimation bias for the disease state. The **tier 2** question to EPA authors is whether this source of underestimation bias for the outcome of concern is worth mentioning? The discussion of diabetes begins on p. 3-353. This discussion is similar to the discussion of hypertension and albuminuria/CKD, and diabetes is an even stronger risk factor for kidney disease manifested as anormal low eGFR and/or albuminuria. Diabetes very commonly causes albuminuria (and sometimes does so before diagnosis, so that albuminuria is seen in retrospect as the presenting finding. **Tier 2** question for EPA authors is whether this source of underestimation bias in cross sectional studies (and theoretically capable of influencing longitudinal studies because of presenting albuminuria before diagnosis) is worth mentioning? g. The summary is reasonable. The review of relevant literature inclusion and grading of articles raises some detail questions:. **Tier 2:** Possibly useful studies of PFNA and human neurodevelopmental endpoints include (Carrizosa et al., 2021; Enright et al., 2023). **Tier 2** The downgrading of cross-sectional studies with exposure variables related to childhood rather than to maternal serum PFAS (see p. 3-319) presumes that we know which is the more relevant exposure window. It is up to EPA if they want to give this decision more thought. **Tier 3:** A very recent maternal-child article described 177 families with high risk of autism. It quantified serum PFAS in first and third trimesters of pregnancy and found PFNA associations to externalizing behaviors and to and aggressive behaviors (Choi et al., 2024)

h. From the reviewer perspective, the work on reproductive outcomes is reasonable, and the inclusion of a potpourri of miscellaneous outcomes hides the good work and diminishes the value of trying to respond. **Tier 3 consideration**. In future, please consider avoiding this kind of organization. Female reproduction, adrenal, hematological, digestive (a word that could include liver!), dermal, and musculoskeletal is a lot of ground to cover. The discussion of urinary outcomes beginning on p. 3-383 is not sex specific as the document implies.

Revisions: As noted elsewhere in this review, there are suggested improvement in the discussion of renal effects (I think the even broader category of urinary is meant to encompass renal effects) which are:

- Not limited to females (as the question to reviewers implies. Is there a parallel question about males? A search of the charge questions is not revealing concerning kidney disease in general, EPA addressed this topic, but it is un clear if there is interest in reviewer impressions of it.)
- **Tier 1:** Could include a discussion of uric acid, but uric acid should really be its own topic as the adverse data are consistent with other PFAS and probably not due to kidney.
- **Tier 2:** Associations are more consistent (Less inconsistent than implied by EPA document) when the several difficult aspects of excretion (Inverted U-shaped relationship to stages of eGFR decrements, direct relationship of urine PFAS excretion to albuminuria leading to inverse relationship to serum PFAS including PFNA).
- **Tier 2:** Discuss how these noncausal excretion considerations (inverted U-shaped curve, albuminuria) interact with population findings, including underestimation bias for kidney disease, diabetes, and hypertension associations.
- **Tier 2:** The summary discussion of animal studies concerning kidney disease on P. 3-388 could more completely describe what was found.

Further discussion of several of these points is also distributed to the other parts of the document, where they also appeared. (It is uncertain if suggestions will change conclusions. The intention is that suggestions improve the improve consideration of the available data.

Important topics with relevant literature such as osteoporosis deserve consideration.

Tier 1. Please consider adding a section on bone mineral density and osteoporosis.

Comment: This will probably not affects points of departure, yet there is highly relevant published science concerning bone/mineral health effects of PFNA. While there is no assumption concerning EPA conclusions. EPA should consider the substantial amount of human evidence, with usual focus on consistency of human evidence, a parallel and similar literature for other PFAS, biologic plausibility (for example based on known metabolic pathways such as PPAR-gamma and bone

	incorporation of PFAS. Ther is a fair amount of pathway data as well, but most of the pathways data are not explicitly linked to bone keywords in the PFAS literature, One that is easy to find interrogates yet relevant pathways are PPAR-gamma agonist impact on bone health (Grey et al., 2007). **For bone mineral density and osteoporosis, relevant population literature** includes and m*ay* not be limited to: • (Khalil et al., 2016) found some adverse association in NHANES (see references for the complete reference) • (Khalil et al., 2018) PFNA inversely (adversely) associated with bone measurements in NHANES • (Cluett et al., 2019) included PFNA in its analysis of Project Viva participants. Other PFAS adversely associated, not PFNA. • (Fan et al., 2023) No association of PFNA to bone measurements. (other PFAS associated) • (Xu, Hansson, Andersson, Jakobsson, & Li, 2023) examined PFAS as a PFAS mixture, the mixture included PFNA, association to osteoporosis measures noted. • (Xiong et al., 2022) PFNA among PFAS adversely correlated with BMD • (Jeddy et al., 2018) Equivocal adverse findings • (Hu et al., 2019) Adverse longitudinal adolescent findings in the Pounds lost study. • (Buckley et al., 2021) Adverse PFNA findings in children within a dietary intervention study • (Buckley et al., 2024) PFAS mixture and adverse BMD in adolescence within a dietary intervention study • (Kirk, DeStefano, Martin, Kirk, & Martin, 2023) Mixture study includes PFNA adverse association • (Carwile et al., 2022) PFAS mixture associated with adverse measures. • (Hojsager et al., 2022) PFNA considered, other PFAS not PFNA associated with adverse measures • (Banjabi, Li, Kumosani, Yousef, & Kannan, 2020) PFNA and other PFAS associated with measures of osteoporosis. • (Blomberg, Mortensen, Weihe, & Grandjean, 2022) Maternal child study showing adverse effects, including for PFNA exposure

	• (Zhao, Lin, Dong, Tang, & Yan, 2023) Another NHANES study, adverse association in women. (Older women are the *a priori* susceptible population. • (Beglarian et al., 2023) No association of PFAS including PFNA to measures of BMD in adolescents and young adults
Faustman	a. This reviewer agrees with the choice by the USEPA to use the available epidemiological data evaluating fetal and childhood growth restriction (including mean and standardized measures of birth weight, birth length and head circumference as well as low birth weight and small for gestational age measures), gestational duration, birth defects, fetal loss and anogenital distance to examine the potential of PFNA for developmental toxicity. The biological significance of changes in these developmental endpoints was made clear by the USEPA in the IRIS document reviewed. The epidemiological studies identified and reviewed as a part of this assessment were correctly identified as resenting a robust level of evidence and supports PFNA exposure associations with altered developmental endpoints. These endpoints are supported by a large number of epidemiological studies. For example, Figure 3-4 and Table 3-4 show an evaluation heat map of 38 epidemiologic studies of birth weight and PFNA exposure. These include 27 prospective birth cohorts and 8 cross-sectional studies. Exposure was determined in multiple ways with some using umbilical cord samples as well as some using infant heel stick blood measures after birth. Twenty-seven of the studies had preconceptual maternal blood measures with prenatal blood concentrations or had blood samples during first trimester or third trimester and some with multiple trimester sampling. To ensure compatibility between USEPA's assessments, they exampled data from "one measure, such as umbilical cord or maternal serum concentrations and when necessary, relied on other related publications or additional information provided by study authors." Thus, the birth weight conclusions were based on 35 informative studies with standardized measurement and exposure data with 15 studies identified as high confidence, 11 with medium confidence and 9 as low confidence. Evidence synthesis focused on primarily the high and medium confidence studies. EPA used 27 of these studies to conduct meta-analyses and estimated a statistically significant decrease in mean birth weight of 33 grams per Ln-unit increase in PFNA. Sensitivity analysis was done to determine the impact of using only high or medium and high confidence studies as well as determine the importance of timing of exposure measurements. Timing of exposure assessment affected the inverse relationship of larger birth weight impacts with later trimester exposure measures and this was consistent with both other PFAS studies of birth weights as well as being consistent with developmental longitudinal trajectories. The PFNA epidemiological studies were supported by animal studies which included 3 high/medium confidence studies in mice and rats which were treated during gestation. Outcomes as summarized in Evidence Table 3-13 concluded that there was moderate evidence streams for PFNA developmental impacts which included "consistent, dose-dependent and coherent reductions in preweaning neonatal survival, pre- and post-weaning body weight, and developmental milestones in two strains of mice and reduced birth weights in rats". As observed in the human epidemiology studies there

	were some reported differences across sex and lifestage. Sex differences in body weight changes after PFNA were reported and similar observations on sex differences in weight and weight trajectories were summarized for humans in Table 3-4. Two additional observations in animal studies suggested that at least a part of the developmental toxicity observed in mice were associated with PPAR alpha signaling as revealed in use of PPAR alpha null mice where developmental endpoints were largely "unaffected" by PFNA exposure. The importance of PPAR independent pathways for MOA of PFNA was not determined with the animal studies cited by USEPA in this report however Table 3-13 does summarize that some evidence for CAR/PXR activation is also available that would support PPAR alpha independent pathway involvement. Further dose and context is needed for a more full interpretation (see discussion for other endpoints). The animal studies on PFNA allowed for further support of PFNA alone being responsible for reduced body weight seen in the human epidemiological studies and not due largely to co-exposures to other PFAS exposures. USEPA investigated the importance of co-exposures to other PFAS related compounds in the observations from PFNA and this is well documents and discussed in detail in Appendix C.1 and this reviewer was convinced of the importance of PFNA exposures in mediating the developmental toxicity outcomes. USEPA has conducted excellent investigations examining the potential confounding of co-exposure by other PFAS compounds as they have individually investigated specific PFAS related compounds. With PFNA these investigations have developed compelling arguments for a critical role of PFNA alone in contributing to developmental toxicity impacts observed. Appendix C provided an excellent discussion and summary using meta-analysis of PFNA. This reviewer also noted the excellent discussion of the High-Throughput screening assays from EPA's chemicals dashboard that was presented in Appendix C in section C.2. Very well done with details at a relevant and synthesized manner. Overall, this reviewer does caution that in real life it appears that rarely do we see only PFNA exposures so what the larger impacts on humans from co-exposures on a population basis is an issue that hopefully will be addressed when the overall PFAS testing programs are synthesized. **Tier 1 Necessary Revisions**-- Additional discussion is needed on postnatal growth, body weight and survival data comparisons across the human epidemiological and animal outcomes data. For the human studies, concerns about the potential for co-exposures is one factor that appears to impact the evidence stream summary of slight for epi data versus moderate for the animal observations (where co-exposures can be ruled out). Does evidence synthesis make a stronger case for the relevance of the findings in the human epi studies? Does this change the "bottom line" PODs and potentially the Non Cancer Toxicity Values? See also comments submitted by NJDEP in the public comments document (8-9). This reviewer congratulates USEPA on their use of the ToxCast data in Appendix

	Section C.2. which used this data for informing overall results as well as specific for Hepatic Systems, reproductive and developmental signaling pathways, cell viability, etc. The activity profiles given provide the reader with potential domains of toxicological response and hence inform the biological relevancy and in many cases link with specific plausible mechanistic data across PECO statements and support overall MOA discussions as well as Characteristics of Toxicological Pathways, ie put molecular and supplemental data into context or cross organ system responses to a more wholistic pattern for PFAS associated responses. A **Tier 3 Future consideration** would be to expand the information in the current Appendix section C back into each of the final sections of the hazard identification sections—Section 4 summary reviews. This could expand from the bioactivity tables seen Appendix 2 C by providing summary Figures spanning the dose ranges tested. b. USEPA provides an integrated discussion and evaluation frame for their conclusion of a Moderate level of evidence as well as a summary evidence integration of Likely for evidence which supports their conclusion that hepatic effects result from PFNA exposure. This evidence is based on several lines of information. First, there are 9 medium confidence studies in adults which report elevated ALT levels with increasing PFNA exposure (N=6 statistically sig). These assessments indicate consistency across the studies, coherence in the direction of impact (increases in serum ALT, AST, GGT and total Bilirubin) and medium confidence in small but consistent elevations in these liver enzymes. Animal evidence for changes in liver weight, histopathology, and clinical chemistry are all reported and details on dose-response is given for PFNA. Synthesis of the human and animal evidence strengthens the USEPA evidence for PFNA association with hepatic effects. This is supported by consistency across other PFAS compounds causing such hepatic effects. Because the animal evidence is conducted under PFNA only exposures this evidence along side the human epi studies strengthens the case for PFNA being the important exposure for both. Mechanistic data available from three lines of investigation including: Molecular events, Cellular and organ effects for both hepatic lipid accumulation and steatosis and hepatobiliary cholestasis further support the integration of PFNA impact. The availability of findings from cross strain and wild type as well as null mutant mice for PPAR alpha further support the key pathways involved in these hepatic effects. USEPA provides discussion on the biological ramifications of changes in hepatic enzymes that are observed in these studies and these include functional changes as well as liver pathologies associated with liver disease. The lack of long-term exposure and response data is acknowledged however for this reviewer the available data was sufficient to justify examining these effects for characterizing PFNA's potential health impact for humans. In summary, this reviewer commends the USEPA for this detailed, section on PFNA

	and hepatic effects and is fully supportive of their conclusions. c. The USEPA provided a detailed and thorough evaluation of human studies, in vivo animal evidence and mechanistic data from both in vivo and in vitro methods for evaluating the potential of PFNA associations with male reproductive outcomes . Twelve epidemiological studies were identified that evaluated semen quality parameters, reproductive hormones and timing of pubertal development. As an example of these evaluations, Table 3-53 summarized five epidemiological investigations of PFNA and semen quality parameters. Four of these were characterized as medium confidence. Although different study designs (including pregnancy study, infertility assessments and a male military recruitment study) all used PFNA measures in serum and they used recognized analytical methods for these analyses. Measurement of semen outcomes did differ and included measurement decreases in sperm motility (2 studies), reduced percent normal morphology and reduced concentration. However, all of these assessments were not statistically significant and thus no association between PFNA and semen analyses were observed. Ten human studies (Figure 3-54) examined associations between PFNA and levels of reproductive hormones (including testosterone and estradiol however) and some associations were observed for lowered testosterone and estradiol however one one study was statistically significant for each hormone. Overall, the evidence stream for hormone effects was designated as indeterminate due to lack of consistent dose response relationship and issues in limited characterization of the dynamics of these hormone impacts and of the biological implications of such small changes. Evaluation of results from animal studies was done (Table 3-19) and two high confidence studies (28d in adult rats and 14d in adolescent rats), two medium confidence studies (90 d prepubertal to adult mice study and 14d prepubertal mice) and one low confidence study from gestationally exposure mice (exposure GD 12-20) were identified. Figures 3-55, 3-57,3-58, and 3-61 show the summary of these study evaluations for male reproductive organ weights, organ histopathology, male reproductive hormones and sperm quality respectfully. Figures 3-56, 3-59,3-60 and 3-62 show dose response data across these studies for the same endpoints as listed above for the confidence evaluations. This reviewer agrees that this rodent dataset provides a moderate level of evidence for the reproductive toxicity of PFNA. This confidence is based on consistency of findings, dose- response information, functional magnitude of effects, coherence of overall impacts across the outcomes observed for peripubertal to adult developmental trajectories and an integration of reproductive toxicity based on a systems based understanding of the functional activities of this organ system. Evidence Table 3-21 describes this in concise, informative framing that accompanies the evidence integration summary judgement with emphasis on the 28d oral toxicity study in adult rats. This reviewer agrees with the decision of USEPA to use this compiled dataset to move forward for further use in this IRIS review for PFNA. Mechanistic information from molecular and cellular effects analysis support the male reproductive outcomes observed in the in vivo rodent studies and enhance the biological plausibility.

	d. Please note that Table-26 should be listed in this review question rather than Table 3-22. USEPA provides a detailed and thorough discussion of available data that can be used to examine the association of PFNA and immune response. This section discusses the biological importance of looking at the immune system by citing the immunotoxicity guidelines from WHO. These endpoints include immunosuppression, immunostimulation, sensitization and allergic response and autoimmunity. No publications were identified for autoimmunity nor immunostimulation and PFNA exposure. USEPA correctly focused on immune functionality as critical for this hazard discussion. Figure 3-67 provides an evidence summary of the epidemiological studies of PFNA and immunosuppression and study summaries are provided in Table 3-26 for children and Table 3-27 in adults. As is seen with several other long chain PFAS type compounds the immune hazard profile is primarily associated with effects on antibody response. This response is reported in "multiple, well-conducted "studies which include six medium and 1 low confidence study in children and 1 medium and 2 low confidence studies in adults. Three distinct cohorts from the Faroe Islands provided the longitudinal and exposure measurement across time and provided details on vaccination scheduling and booster timing. Most of the responses were consistent with imunosupression and were seen at relatively low levels of PNAS (0.6 ng/ml serum) and had levels of decrease in Ab response for the majority of the findings at "…greater than 5% and several were greater than 10%". Statistical significant suppression was observed for rubella and diphtheria. The suppression was seen with higher prenatal and children's concentrations of PFNA as well as in one adult cohort. The report relates in particular some discussion on the biological significance of the diphtheria suppression with OR for decreased protection against diphtheria. As well the one medium confidence study reporting increased asthma was of interest in this discussion. Across two of the Faroe Island cohorts there was a "sub-analysis" and it was a statistically significant positive Ab response association with PFNA in children ages 5 with exposure measured in infancy. **Tier 1 Necessary Revision** is needed to provide a few more details regarding this sub-analysis and why this sub-analysis was undertaken. Although some details are provided in the report since these positive versus negative Ab response was observed it would be important to bolster this discussion, especially on page 3-247 where the various cohorts are discussed. This reviewer overall agrees with the statements of Suggestive Evidence in Table 3-32 for human studies providing immunosuppression of Ab responses in children at low PFNA exposures in serum. A strong component for this endorsement is the consistency in these types of effects across the PFAS related compounds and the potential for magnitude of impact at low PFNA exposure levels. The discussion and reality of the animals studies does little to affect the suggestive evidence category and the characterization of serious toxicity across the higher dose of PFNA used in these studies was very well described and included in the overall evidence summary.

	This reviewer requests a **Tier 1 Necessary Revision** for expansion of the discussion of confounding with other PFAS related compounds. This is also suggested for addition around Page 3-247 as well as in the section on Mechanistic and Supplemental information. For the latter it was unclear why additional discussion from CompTox was not included on Structure Activity profiles other than general statements about long chain PFAS type compounds. It would seem that this particular endpoint would be important to more fully characterize based on this structural feature. It was unclear to this reviewer but it appears from the earlier section on use and exposure profiles that it would be difficult to identify children's cohorts and experiences where PFNA was not always present with other PFAS compounds. If this is true it appears to be more of an "academic exercise" in dismissing the effects attributed to PFNA versus the other PFAS compounds. The question is not whether confounding occurred but rather that it is anticipated and would be additive at best. USEPA should address this more directly. e. This reviewer thanks USEPA for their write-up on thyroid impacts from PFNA and agrees that it is challenging with so few and so inconsistent studies to review for the hazard identification. The write-up addressed details of these studies that would enhance as well as diminish confidence in the evidence that PFNA could cause thyroid impacts. Evaluations presented in the numerous association assessments such as with graphic comparisons presented in tables such as Tables 3-78-80 were helpful in tracking these signals. The reviewer appreciated the details on these studies. The strength in known impacts on thyroid pathways is from the cross-compound comparison between the various PFAS compounds and this reviewer recommends a **Tier 1 Revision** that more explicated looks at the different potency of these larger series of compounds including both short and long chain structures in the review of PFNA. The review does specifically discuss the common pattern seen with the larger PFAS family of compounds – for example the affects on T4 without effects on TSH and does include discussion of these observations and notes that would be what would be expected for PFNA but stops short of discussion of Structural Activity relationships with longer chain PFAS compounds such as PFNA. Can any quantitative or semi-quantitative ranking of potency for thyroid effect occur that could help put the findings with PFNA into a stronger context? Because the Evidence Profile table (Table 3-34) can only determine slight evidence for the human studies from the 26 medium confidence and 11 low confidence studies and the one animal study can only provide moderate evidence (considerably impacted by toxicity at higher tested doses), increasing the contrast across the other PFAS compounds would seem to be an option to discuss further and to determine if such a discussion could improve the evidence integration summary judgment. f. USEPA provided a detailed evaluation of available human, animal and supplemental information to determine if there were associations between PFNA

	exposure and risk of cardiometabolic effects. The review methodically evaluated possible associations of PFNA exposure and insulin resistance, dyslipidemia, hypertension, adiposity and changes in heart weight and histopathology. These assessments included not only overall categorization of study evaluation for these endpoints (for example see Table 3-92 for blood lipid evaluations in epidemiological studies or Table 3-94 for cardiovascular disease risk factors). These evaluations were followed by Tables (such as Table 3-39 for blood lipids and Table 3-40 for hypertension) which provided details on the studies such as populations studied, size of populations as well as effect estimates for the various effects. These later tables provided significance and effect parameters as well as median exposure levels. Table 3-47 provided the overall evidence profiles for these effects which for human epidemiological findings was "slight" for all human endpoints. The addition of animal studies provided indeterminate evidence especially as there were only very limited animal studies available and those that were available were confounded by toxicity in the animal models. This reviewer in general is supportive of these findings but is suggesting a **Tier 2 Suggested Revision** that requests that discussion from the mechanistic and supplemental information be added to Table 3-47 for evidence integration. This reviewer did find the inclusion of the section on Page 3-379 entitled: "Considerations for Interpreting the Human Relevance of the Animal Cardiometabolic Evidence" to be a great addition as this section included identification of critical data gaps. This should be considered as a **Tier 3 Future Consideration** for inclusion in an overall section for the compounds under evaluation. It is of concern to this reviewer that we frequently have multiple epidemiology studies and animal studies that should have been informed prior to design, key components that are needed for IRIS assessment. This is a part of a large issue that arises with the overall PFAS family of compounds. g. This reviewer supports the conclusions of USEPA that the available data does not support that there is a clear association of PFNA exposure and neurodevelopmental effects. There were 25 available epidemiological studies with 18 medium confidence and 7 low confidence. Most of the studies examined were birth cohorts. Two out of four of these studies yielded some evidence for ADHA and four out of seven studies had some related behaviors reported. These associations were reported as having "considerable uncertainty" and again this reviewer would agree. Table 3-36 illustrates some of these inconsistencies. Other potential associations that were investigated included visual-motor scores, cerebral palsy and alterations in MRI activity in brain regions. No rodent animal evidence was presented but some evidence on key developmental neuro-biological endpoints was reported including elevated mortality, delayed hatching, altered neurotranmitters and malformations in zebrafish and Xenopus however this was only slight as best and was labeled as "indeterminant". Tox Cast in vitro screening assays showed some bioactivity for neurological targets. This reviewer agreed that evidence suggests but is "not sufficient to infer". h. This reviewer agrees with USEPA and feels that there is inadequate evidence to examine further the association of PFNA and endpoints such as female

	reproductive, urinary, adrenal or other noncancer effects.
Georgopoulos	a. The Draft Toxicological Review appropriately concludes that the available evidence demonstrates that PFNA exposures cause developmental effects in humans given sufficient exposure conditions. This conclusion is based primarily on growth impairments observed in epidemiological studies. The evidence of decreased birth weight in studies of exposed humans is robust and scientifically justified and is supported by generally coherent epidemiological findings for other fetal and postnatal growth restriction endpoints (e.g., birth length, postnatal weight and height). Cross-stream coherence provided by moderate animal evidence for PFNA-induced developmental effects in gestationally exposed rodent offspring that included reduced postnatal survival and body weights, and delays in attaining developmental milestones, provides additional support to this conclusion. The evidence synthesis and integration for developmental effects is presented in detail in Section 3.2.2 and Appendix C.1 and appropriately includes the meta-analysis of Wright et al. (2023) that considered the findings of birth weight deficit to be statistically robust across all sampling periods and study confidence levels, indicating there are demonstrated birth weight deficits as PFNA exposure levels increase. The Draft Review correctly recognizes the fact that there is residual uncertainty regarding some potential for confounding by other per- and polyfluoroalkyl substances (PFAS) and sample timing; EPA's interpretation that this uncertainty does not substantially reduce confidence in the evidence base appears justifiable. b. The Draft Toxicological Review appropriately concludes that the available evidence indicates PFNA exposures are likely to cause liver effects in humans given sufficient exposure conditions. This conclusion is based on consistent and coherent evidence from human, animal, and mechanistic studies. EPA recognizes that there is moderate evidence in human studies that PFNA is associated with liver injury based on increased ALT, AST GGT, and bilirubin. However, the evidence from a series of short-term studies in rats and mice that demonstrate consistent and coherent effects on liver weight, clinical pathology, and histopathology including hepatocellular necrosis, cholestasis, and triglyceride accumulation, is robust. The Review concludes that liver findings for PFNA align with those for other structurally related long-chain PFAS and were determined to be adverse. Furthermore, the Draft Review presents available evidence of both PPARα-dependent and -independent pathways contributing to PFNA hepatotoxic effects, consistent with what has been observed for several other PFAS. Based on such evidence from in vivo and in vitro studies, the Draft Review justifiably recognizes a potential role for multiple pathways involved in the induction of hepatic effects from PFNA exposure and concludes that these effects are potentially relevant to humans. The Draft Review's conclusion that hepatic effects of PFNA observed in rodent studies should be considered adverse and relevant to humans (page 3-188, lines 5-11) is justifiable. Regarding the criteria of Hall et al. (2012), it should be recognized that they emphasize the effect of expected duration of exposure in determining the adversity of hepatic effects such as increased liver weight and

	hepatocellular hypertrophy. As NJDEP (2024) points out in Comments submitted for the present Toxicological Review, in the development of chronic Reference Doses, potential reversibility is not a valid reason to discount the adversity of increased liver weight and hepatocellular hypertrophy in shorter-than-chronic rodent studies since these lesions can potentially progress with longer exposure. A clear and appropriate synthesis of available data, supporting the stated conclusions regarding liver effects, is presented in Section 3.2.3 of the Draft Review; this section adequately summarizes the strengths and limitations of the studies underlying the available data and describes weight-of-evidence decisions. c. The Draft Toxicological Review appropriately concludes that available evidence indicates PFNA exposure is likely to cause male reproductive effects in humans given sufficient exposure conditions. The Draft Review considered eight studies in rats and mice that evaluated the effects of PFNA exposures on the male reproductive system examining effects of these exposures on sperm number and quality, reproductive organ weight and histopathology, and serum hormone concentrations. The conclusion was based primarily on a high confidence 28-day oral toxicity study in adult rats that reported a consistent and coherent pattern of adverse reproductive effects, generally at ≥1.25 mg/kg-day PFNA but with some coherent changes at 0.625 mg/kg-day, with additional support from medium confidence, short-term studies in adult rats and prepubertal mice observing effects at similar doses (≥2 mg/kg-day). The Review also reasonably concludes that lack of association in most of the epidemiological studies does not decrease confidence in the animal results given the uncertainties in the epidemiological evidence base. In Section 3.2.4, the Draft Toxicological Review presents a clear and appropriate synthesis of available data supporting its conclusions regarding male reproductive effects, summarizing strengths and limitations of corresponding studies and adequately describing relevant weight-of-evidence decisions. d. The Draft Toxicological Review appropriately concludes that the available evidence suggests, but is not sufficient to infer, that PFNA exposures have the potential to cause immunosuppression in humans: the human evidence was considered slight and the animal evidence indeterminate. Section 3.2.6 of the Review presents a clear synthesis of available data supporting the conclusions regarding immune effects, summarizing strengths and limitations of underlying studies and appropriately describing relevant weight-of-evidence decisions. Epidemiological studies provide evidence of reduced antibody response with PFNA exposure, and possible evidence for effects on asthma and asthma-related outcomes, but there are substantial concerns regarding imprecision and potential residual confounding by other PFAS; these concerns are explained adequately in the Draft Toxicological Review. e. The Draft Toxicological Review appropriately concludes that the available evidence suggests, but is not sufficient to infer, that PFNA exposures may have the potential to cause thyroid effects in humans. The conclusion was based on moderate animal evidence from a high confidence 28-day study in adult rats that showed large, dose-dependent reductions in serum free and total T4 in females and in serum free T4 in males, providing evidence of PFNA effects on T4 homeostasis. However, the Review recognized that uncertainties associated with the reliability of measurement

	methods quantifying free T4 in both sexes were very large and there were body weight losses in males at higher doses that complicated assessing the T4 reductions, as well as additional responses in males that were difficult to interpret. The Review also recognized that available epidemiological data did not demonstrate coherence with the animal evidence, with the strongest evidence showing positive associations with T4 in children/adolescents, although effect sizes were small. The conclusion of the Review was also driven by the presence of large uncertainties in the human evidence, i.e. inconsistent directions of association and concerns related to study sensitivity. The synthesis of available data, presented in Section 3.2.7 of the Draft Review presents a clear summary of the studies evaluated for thyroid effects and explains weight-of-evidence decisions. f. The Draft Toxicological Review appropriately concludes that the available evidence suggests but is not sufficient to infer whether PFNA exposures have the potential to cause cardiometabolic effects in humans. This conclusion is based on a body of evidence showing increases in serum lipids (and some potentially supportive but mixed results for other increased risk factors for cardiovascular disease). However, human evidence is limited by considerable uncertainties resulting from inconsistencies within and across studies and concerns for imprecision, while evidence in experimental animals was indeterminate. Section 3.2.9 of the Draft Review presents a clear summary of the studies evaluated for cardiometabolic effects. g. The Draft Toxicological Review appropriately concludes that the available evidence suggests, but is not sufficient to infer, that PFNA exposures may have the potential to cause neurobehavioral effects in humans. The conclusion is based on epidemiological studies that found associations between PFNA and outcomes related to attention and behavior; however, these associations had considerable uncertainties. The Review in particular recognized the imprecision in the estimates from the three studies evaluating attention-deficit/hyperactivity disorder (ADHD) diagnosis, the most specific outcome. There was no relevant evidence in experimental animals to inform neurodevelopmental effects. The synthesis of data and summary of the studies evaluated for neurodevelopmental effects in section 3.2.8 of the Draft Review is clear and adequate. h. The conclusion of the Draft Toxicological Review that there is inadequate evidence to determine whether PFNA exposures have the potential to cause female reproductive, urinary, adrenal, hematological, respiratory, digestive, dermal, and musculoskeletal effects in humans is appropriate and scientifically justified given the sparsity and/or uncertainties in available data.
Haney	a. Consideration has been given to several important study attributes, and as evaluated in the draft, the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level ratings in the draft (e.g., Figures 3-4, 3-12). However, while various strengths and limitations are described, it seems that some serious limitations of epidemiology studies are not fully appreciated (see comments below). Table 3-13 is the evidence profile table for developmental effects, which among other information contains factors that increase and decrease certainty, as evaluated in the draft, along with

	evidence stream (i.e., human, animal, mechanistic) judgments/ rationales and a summary judgment. Obviously, the text of the document (Section 3.2.2) also contains information relevant to the weight of evidence (WOE) for developmental effects. While EPA concludes that the potential impact of PFAS co-exposures did not substantially reduce confidence in the evidence base or fully explain the birth weight endpoint results/ associations (p. 3-136, lines 2-6; Table 3-13), this does not mean that epidemiological studies where populations are exposed to PFAS mixtures can be relied upon for derivation of toxicity factors for a single PFAS since it is not possible to accurately quantify the specific degree to which a given chemical contributed to producing the observed mixture effect; i.e., to accurately determine a reliable point of departure (POD) from a mixture study for a given benchmark response (e.g., BMR of 5% extra risk of exceeding adversity cutoff) that can be confidently attributed to a specific PFAS (assuming causality) when: (1) "scientific consensus on how best to address PFAS co-exposures remains elusive" (p. 3-52, lines 26-27); (2) "it is unclear which statistical approach best represents independent effects of specific pollutants within complex PFAS mixtures" (p. C-2, lines 11-13); and (3) all the relevant co-exposures (e.g.,. correlated PFAS other than the relatively few analyzed for in a given study) have not even been quantified. This is a critical issue regardless of any term used to characterize the epidemiological evidence (e.g., "robust" or otherwise) that might obfuscate the issue. While PFAS co-exposures not fully explaining the birth weight endpoint results may mean that epidemiological studies have some use in hazard identification, this is not the case for dose-response assessment and toxicity factor derivation. Unlikely to explain 100% of an association does not constitute any type of acceptable scientific criterion for use of associated data for dose-response assessment and toxicity factor derivation. When conducting a dose-response assessment for a single chemical, it is difficult to envision that a regulatory agency would derive a toxicity factor for a single chemical based on a mixture animal study in which the animals had significant exposure to numerous related chemicals (both quantified and unquantified confounding exposures) stating that its acceptable because co-exposures were unlikely to be responsible for 100% of the effect. Such a mixture animal study would be excluded, and rightly so (e.g., such studies would be deficient/crucially deficient per p. 4-33 of the *IRIS Handbook*; USEPA 2022), as the resulting toxicity factor for a single component of the mixture would be unreliable. PFAS co-exposures should be considered positive confounders as they would be expected to associated with both the primary PFAS being evaluated and endpoint of interest in the same direction as the primary PFAS of interest, biasing results away from the null (considerations on p. C-1, lines 10-16 of the draft). In fact, EPA has cited data from other PFAS across draft assessments as supporting evidence for the potential of the PFAS being evaluated to cause those same effects because PFAS are expected to affect these endpoint(s) in the same direction as the primary PFAS being evaluated (e.g., due to structural and physico-chemical property similarities). The problem is that epidemiology studies of PFAS are inherently mixture exposure studies, with significant co-exposures to other PFAS being a certainty along with bias away from the null. Just as one example based on the first key study listed with a candidate POD in Table 5-9 (p. 5-22), Savig et al. (2018) examined only four PFAS, which had correlation coefficients with PFNA of 0.52

	(PFOA), 0.42 (PFHxS), and 0.61 (PFOS) (see Table 2 of the study). Savig et al. (2018) acknowledges that none of the seven analyses for PFNA and birth-weight-for-gestational age z score or birth weight were monotonic (p. 795, Table 3 and Web Table 1 of the study), and that studies have found that PFAS other than PFNA negatively affect fetal growth and birth weight (e.g., PFOS, PFOA). In contrast to the findings of Savig et al. (2018) for PFHxS: (1) EPA found "consistent and coherent epidemiological findings on fetal growth restriction including several medium and high confidence developmental epidemiological studies", with their PFHxS meta-analysis finding similar birth weight deficits (per ln-unit PFHxS increase) across all 27 studies that examined associations in the overall population (β= −7.7 g; 95%CI: −14.8, −0.5 per each ln-unit increase), although again, important uncertainties remain in such epidemiological studies (see the *Draft IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS) and Related Salts*); and (2) Maisonet et al. (2012) reported lower birth weight with exposure to PFHxS (as cited by Savig et al. 2018). Importantly, in addition to reporting nonmonotonicity for all seven PFNA birth-weight-for-gestational age z score or birth weight analyses, with 11 of the 21 (52%) nonreferent quartile β value 95% CIs containing 0 (not statistically significant), and acknowledging co-exposure to some other PFAS (i.e., the few others analyzed for) that have been reported to also negatively affect birth weight, the authors of Savig et al. (2018) state [*emphasis added*]: > *We detected associations of PFNA with birth outcomes in the current study; however*, given the low plasma concentrations of PFNA in Project Viva compared with other, more commonly studied PFAS, such as PFOS and PFOA*, these results should be interpreted with caution. Only a few other studies have examined associations of PFNA with birth outcomes,* presumably because of the relatively low PFNA concentrations, *with mixed findings*. Significant issues such as those discussed above for Savig et al. (2018) that are relevant to the reliability of study results for toxicity factor derivation (e.g., for determining chemical-specific dose-response and a reasonably accurate POD) are readily identifiable. Relevant to confounding correlated co-exposures to similarly acting chemicals (both quantified and unquantified), EPA's criterion of a confounder(s) *not completely explaining associations* is not a scientific standard making confounded study results reliable for providing an accurate POD for quantitative dose-response assessment and derivation of a toxicity factor, and certainly not a gold scientific standard to be used in chemical assessments held out as such. Correlated co-exposures, both quantified and unquantified (e.g., PFAS both measured and unmeasured in serum), to multiple other chemicals with the same or similar MOAs and endpoints should be considered among study "attributes that would be likely to have a large effect, compared to a small effect, on confidence in the study results" (Section 4.2.1 of the *IRIS Handbook*; USEPA 2022), positively biasing study results and producing mixture effects where the contributions from even the few PFAS analyzed for in serum

	cannot be accurately adjusted for.[2] The *IRIS handbook* (USEPA 2022) cites concerns about *confounding co-exposures to PFAS specifically* [*emphasis added*]: > *Coexposures* should also be considered as *potential confounders*. *Some exposures tend to be found together in the environment* or in occupational settings *and are highly correlated.* For example*, it might be difficult to distinguish the independent effects from* exposure to specific phthalate or *per- and polyfluoroalkyl substances* in drinking water, isomers of polychlorinated biphenyls in fish, or volatile organic compounds generated by a common source (e.g., benzene, toluene, ethylbenzene, xylene in traffic emissions) *due to confounding by these coexposures.* Confounding of the effect of PFNA exposure by other PFAS seems a certainty in epidemiology mixture studies, and these studies are deficient under EPA guidelines because the potential for bias to explain some of the results is high based on an inability to rule out residual confounding by key confounders of the exposure-outcome relationship (e.g., see p. 4-21 of the *IRIS Handbook*; USEPA 2022). Consequently, such epidemiology studies should not be used as the basis for quantitative dose-response assessment and/or toxicity factor derivation (**Tier 1 necessary revision**). This would not only be consistent with the *IRIS Handbook* (USEPA 2022), but also more consistent with fairly recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021). The PFNA assessment should specifically outline why ATSDR, a federal public health agency of the U.S. HSS/CDC charged with protecting communities from the harmful health effects related to exposure to hazardous substances, is wrong about the epidemiology literature being inadequate for use as the basis for toxicity factors (**Tier 1 necessary revision**). Furthermore, it is simply noted that unreliable/inadequate data (human or otherwise) cannot be justified scientifically for use in dose-response assessment by how the associated unreliable POD(s) compares to that based on reliable data (e.g., from a well conducted animal study) definitively showing single PFAS-specific cause-and-effect for an adverse effect(s), unconfounded by significant co-exposures to similar chemicals. Lastly, EPA was only able to take a limited look at relatively few PFNA and PFAS co-exposure studies (Section C.1.3, pp. C-3 to C-5), so it is not surprising that there is "not a lot of direct evidence" that confounding by other PFAS is responsible for the birth weight deficits associated with PFNA (p. C-4, lines 25-27), but again, confounding co-exposures being 100% responsible should not be the criterion for confounding significant enough to represent a significant scientific concern. This very short section of the draft (Section C.1.3) highlights, for example, statistically significantly birth weight deficit results for both PFOS and PFOA in multi-pollutant models. Despite EPA's limited look dismissing the issue (Section C.1.3, pp. C-3 to C-5), the draft nevertheless seems to acknowledge the critical need to appropriately

[2] For example: (1) Grandjean et al. (2017a) states [*emphasis added*], "*Owing to the intercorrelations* between the serum PFAS concentrations, further analysis of *the possible role of individual PFASs was not pursued*, and *the observed associations may reflect the effects of the PFAS mixtures.*"; (2) Grandjean et al. (2017b) state, "The close correlations prevented meaningful adjustment for concomitant PFAS exposures."; and (3) Budtz-Jorgensen and Grandjean (2018) acknowledge that "an important weakness of epidemiological studies is the mixed exposures" and more than one PFAS may contribute to the lowering of antibody responses.

	adjust for these and other PFAS co-exposures when stating: (1) "scientific consensus on how best to address PFAS co-exposures remains elusive" (p. 3-52, lines 26-27); and (2) "it is unclear which statistical approach best represents independent effects of specific pollutants within complex PFAS mixtures" (p. C-2, lines 11-13). These acknowledged scientific issues should be resolved prior to considering epidemiology mixture studies for use in POD determination and toxicity factor derivation (**Tier 1 necessary revision**) b. Consideration has been given to several important study attributes, and as evaluated in the draft, the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level ratings in the draft assessment (e.g., Figures 3-40, 3-41). Table 3-17 is the evidence profile table for hepatic effects, which among other information contains factors that increase and decrease certainty, as evaluated in the draft, along with evidence stream (i.e., human, animal, mechanistic) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.3) also contains information relevant to the WOE for hepatic effects. This section concludes (pp. 3-187 to 3-188, lines 35-1) that, "Together, the evidence indicates that PFNA exposure likely causes hepatotoxicity in humans given sufficient exposure conditions based on a combination of generally consistent and coherent evidence from human, animal, and mechanistic studies (see Table 3-17)." However, while various strengths and limitations are described, it seems that some significant limitations of epidemiology studies are not fully appreciated. As a brief example, the key study of Kim et al. (2023) supporting the lifetime RfD (Table 5-18, p. 5-44) enumerates "several important limitations", including: (1) cross-sectional design, precluding the determination of causal association; (2) only a single serum sample for each participant, which might not reflect the real level of exposure; (3) adjustment for only a limited number of covariates, when study authors acknowledge the existence of other confounders (e.g., diet, medications) that alter liver enzymes and could affect their findings; (4) other markers of liver function related to PFAS levels (e.g., ALP, bilirubin, cholesterols, lipids) were not evaluated; (5) next-generation PFAS were not analyzed or evaluated for contributions to the associations observed; and (6) their analyses failed to control for exposure to other environmental pollutants which might also increase liver-enzyme levels in the general population. For these types of reasons, the authors of Kim et al. (2023) conclude that, "Future studies are therefore needed to confirm the associations between PFAS levels and liver biomarkers." This study should not be used as a supporting key study for quantitative dose-response assessment and/or toxicity factor derivation (**Tier 1 necessary revision**), and ATSDR (2021) and FSANZ (2021) agree that such studies should not be used for toxicity factor derivation. By corollary, for reasons expressed in comments on Kim et al. (2023) specifically and on the use of the epidemiology studies more generally, I also disagree with the medium confidence ratings for the study underlying the hepatic effect osRfD, for quantification of the POD_{HED}, and for the overall confidence in the osRfD itself (Table 5-19). b.i. In regard to subsection "i" above, it is reasonable to conclude that while exposure to other highly correlated PFAS could contribute to the observed effects, confounding co-exposures to such similarly-acting compounds should not be expected to *fully* explain the observed effects. This, however, is only part of the

	larger issue of *the extent to which such confounding co-exposures, both quantified and unquantified, do contribute to the observed effects*. That is, when "scientific consensus on how best to address PFAS co-exposures remains elusive" (p. 3-52, lines 26-27), "it is unclear which statistical approach best represents independent effects of specific pollutants within complex PFAS mixtures" (p. C-2, lines 11-13), and all the relevant co-exposures (e.g., correlated PFAS other than the relatively few analyzed for in a given study) have not even been quantified, *it is not possible to accurately determine a reliable POD for toxicity factor derivation from an epidemiological mixture study for a given critical effect size/benchmark response that can be confidently attributed to a specific PFAS* (assuming causality). For these types of reasons (i.e., significant epidemiological study limitations, such as those enumerated above for Kim et al. 2023), robust animal data are more compelling for a given PFAS (e.g., PFNA) and better justify that the available evidence indicates PFNA exposure is likely to cause liver effects in humans given sufficient exposure conditions. b.ii. In regard to subsection "ii" above, as there is evidence of both PPARα-dependent and -independent (e.g., CAR/PXR) pathways contributing to hepatotoxic effects, until such time as there is adequately robust scientific information available to detangle the relative contributions of the key pathways leading to hepatotoxic endpoints and their relative importance across species to enable scientifically justified interspecies adjustments and/or selection of the most appropriate/representative laboratory animal model for human dose-response, it is reasonable for EPA to simply conclude that hepatic effects from PFNA exposure are potentially relevant to humans (e.g., the regulatory burden of scientific proof is to show that they are not). b.iii. In regard to "iii", in short, EPA considered adversity criteria set forth in Hall et al. (2012), which was appropriate (see *Consideration for potentially adaptive versus adverse responses* on pp. 3-184 to 3-186). c. Consideration has been given to several important study attributes for these effects, and as evaluated in the draft, the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level ratings in the draft assessment (e.g., Figures 3-53, 3-54). The overall presentation of study results appears clear and various strengths and limitations are described. Tables 3-21 is the evidence profile table for male reproductive effects, which among other information contains factors that increase and decrease certainty, as evaluated in the draft, along with evidence stream (i.e., human, animal, mechanistic) judgments/rationales and summary judgments. Obviously, the text of the document (Section 3.2.4) also contains information relevant to the WOE for these effects. The evidence integration section for male reproductive effects concludes with, "Taken together, the currently available evidence indicates that PFNA likely causes male reproductive toxicity in humans given sufficient exposure conditions (see Table 3-21). This conclusion is based primarily on a high confidence 28-day oral toxicity study in adult rats that reported a consistent and coherent pattern of adverse reproductive effects, generally at ≥1.25 mg/kg-day PFNA but with some coherent changes at 0.625 mg/kg-day, with additional support from medium confidence, short-term studies in adult rats and

	prepubertal mice observing effects at similar doses (≥2 mg/kg-day). The lack of association in most of the epidemiological studies does not decrease confidence in the animal results given the uncertainties in the epidemiological evidence base" (pp. 3-217 through 3-218). That PFNA likely causes male reproductive toxicity in humans given sufficient exposure conditions may be the most that can be said given EPA's characterizations of the human and animal evidence as indeterminate and moderate, respectively, with only limited mechanistic evidence supporting biological plausibility (see *Evidence Integration* on pp. 3-215 through 3-218). d. Table 3-22, referenced in the charge question, is not for immune effects but rather for *Associations between PFNA and time to pregnancy in epidemiology studies*. Tables 3-26 and 3-27 regard reduced antibody response, with Table 3-29 regarding hypersensitivity in humans, and Table 3-30 regarding overall evaluation results of immunotoxicity studies examining the effects of PFNA exposures in rodents. In regard to subsection "i", it appears that overall, the WOE decisions for immunosuppression have been clearly described and are scientifically justified. However, I do offer additional perspective below regarding some epidemiological study weaknesses/limitations and therefore whether the associated results should be used for quantitative dose-response assessment and toxicity factor (e.g., RfD) derivation[3], although these studies have some appropriate use in WOE for hazard identification as is the subject of this charge question. In regard to EPA's concern about residual confounding by other PFAS, the problem is that epidemiology studies of PFAS are inherently mixture exposure studies, with significant co-exposures to other PFAS being a certainty along with bias away from the null. Correlated co-exposures, both quantified and unquantified (e.g., PFAS both measured and unmeasured in serum), to multiple other chemicals with the same or similar MOAs and endpoints should be considered among study "attributes that would be likely to have a large effect, compared to a small effect, on confidence in the study results" (Section 4.2.1 of the *IRIS Handbook*; USEPA 2022), positively biasing study results and producing mixture effects where the contributions from even the few PFAS analyzed for in serum cannot be accurately adjusted for.[4] The *IRIS handbook* (USEPA 2022) cites concerns about *confounding co-exposures to PFAS specifically* [*emphasis added*]: > *Coexposures* should also be considered as *potential confounders*. *Some exposures tend to be found together in the environment* or in occupational settings *and are highly correlated.* For example*, it might be difficult to distinguish the independent effects from* exposure to specific phthalate or *per- and polyfluoroalkyl substances* in drinking water, isomers of polychlorinated biphenyls in fish, or volatile organic compounds generated by a common source

[3] More extensive comments and considerations on this subject may be found in my comments on other draft IRIS assessments, such as that for PFHxS.

[4] For example: (1) Grandjean et al. (2017a) states [*emphasis added*], "*Owing to the intercorrelations* between the serum PFAS concentrations, further analysis of *the possible role of individual PFASs was not pursued*, and *the observed associations may reflect the effects of the PFAS mixtures.*"; (2) Grandjean et al. (2017b) state, "The close correlations prevented meaningful adjustment for concomitant PFAS exposures."; and (3) Budtz-Jorgensen and Grandjean (2018) acknowledge that "an important weakness of epidemiological studies is the mixed exposures" and more than one PFAS may contribute to the lowering of antibody responses.

(e.g., benzene, toluene, ethylbenzene, xylene in traffic emissions) *due to confounding by these coexposures.*

Confounding of the effect of PFNA exposure by other PFAS seems a certainty in the Grandjean et al. studies, and these studies are deficient under EPA guidelines because the potential for bias to explain some of the results is high based on an inability to rule out residual confounding by key confounders of the exposure-outcome relationship (e.g., see p. 4-21 of the *IRIS Handbook*; USEPA 2022) since as stated by Grandjean et al. themselves: (1) "Owing to the intercorrelations between the serum PFAS concentrations, further analysis of the possible role of individual PFASs was not pursued, and the observed associations may reflect the effects of the PFAS mixtures" (Grandjean et al. 2017a); (2) "The close correlations prevented meaningful adjustment for concomitant PFAS exposures" (Grandjean et al. 2017b); and (3) "An important weakness of epidemiological studies is the mixed exposures" and more than one PFAS may contribute to the lowering of antibody responses (Budtz-Jorgensen and Grandjean 2018). As stated in the draft (p. 3-248):

> The single-PFAS model results were not statistically significant for PFNA for antitetanus antibodies, or for antidiphtheria antibodies in children at age 5 and at age 7 (see Appendix D.1). The effects of PFNA from the single-PFAS models did not show a consistent change when adding control of PFOS and PFOA across the two periods and two antibody endpoints (tetanus and diphtheria), with effect estimates sometimes increasing and at other times decreasing, and even accounting for switching signs.

Due to these and similar issues (e.g., PFAS not being statistically significant predictors of antibodies with or without adjustment for just a couple confounding PFAS co-exposures), it is reasonable to conclude that: (1) the available evidence suggests, but is not sufficient to infer, that PFNA exposure has the potential to cause immunosuppression in humans; and (2) such epidemiology studies should not be used as the basis for quantitative dose-response assessment and/or toxicity factor derivation (e.g., a reliable POD associated with a given BMR cannot be reliably determined based on epidemiology studies of mixtures of similarly acting and correlated chemical exposures, both quantified and unquantified). This would not only be consistent with the *IRIS Handbook* (USEPA 2022), but also more consistent with fairly recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021).

Moreover, the level of serum antibodies corresponding to a clinically protective level appears to be assay specific, and this should be more clearly presented and described in the draft (**Tier 1 necessary revision**). For the toxin binding inhibition (ToBI) assay apparently used in these Faroe Islands studies by Grandjean et al., ≥0.01 IU/mL is considered to be the clinically protective level, not the value of ≥0.1 IU/mL indicated by study authors. This brings into serious question the validity of any assumptions regarding the clinical relevance/adversity of these serum antibody endpoints. The clinically protective level cited by Grandjean et al. was ≥0.1 IU/mL. However:

- Grandjean et al. (2012) reported that "serum concentrations of antibodies against then tetanus toxoid were measured in coded samples by the Statens

	Serum Institut using enzyme-linked immunosorbent assay…", citing Hendriksen et al. (1988); • Hendriksen et al. (1988) describes the ToBI assay, which is a modified ELISA; and • WHO (2017) indicates that for a modified ELISA, clinical protection is achieved at ≥0.01 IU/mL, not ≥0.1 IU/mL as indicated by Grandjean et al.[5] That is, the WHO (2018, 2017) cites Hendriksen et al. (1988) as "a toxin binding inhibition (ToBI) assay has been reported and demonstrated to show good correlation with the neutralization assay (correlation coefficient = 0.95)", and for modified ELISA assays such as this further indicates [*emphasis added*] that, "*The minimum amount of circulating antibody that, in most cases, ensures immunity to tetanus is assay-specific*. *Using* in vivo neutralization tests or *modified enzyme-linked immunosorbent assays (ELISA), concentrations exceeding 0.01 IU/ml are usually considered protective*, whereas antibody concentrations of at least 0.1-0.2 IU/ml are defined as protective when using standard ELISA techniques." WHO (2018) also discusses and illustrates the timing of primary and booster vaccinations and durations of protection in the context of the minimum putatively protective level of 0.01 IU/mL.[6] Thus, the protective level cited by Grandjean et al. for the assay used is 10-fold higher than the protective level cited by WHO (2017), calling into question the biological/clinical significance and adversity of any reported results/associations for <0.1 IU/mL (e.g., eTable 4 of Grandjean et al. 2012). In regard to confounding and the ability to causally attribute associated effects to any specific PFAS (e.g., PFNA): • Grandjean et al. (2012) state [*emphasis added*], "Although all of the 5 PFCs measured showed negative associations with antibody levels, the overlapping confidence intervals and the lack of comparative toxicology studies *prevent inference in regard to causal attribution*… PFOS (most likely the linear isomer) and PFOA appear to be the main culprits." • The more recent Grandjean et al. (2017a) study states [*emphasis added*], "Owing to the *intercorrelations between the serum PFAS* concentrations, further analysis of *the possible role of individual PFASs was not pursued*, and *the observed associations may reflect the effects of the PFAS mixtures*."

[5] For example, Grandjean et al. (2012) refers to a "clinically protective level of 0.1 IU/mL" several times and further states, "An antibody concentration greater than 0.1 IU/mL is considered an important indicator of protection in accordance with the public health rationale for routine vaccinations."

[6] WHO (2018; pp. 14-15) states [*emphasis added*] that "To illustrate the kinetics of immunity among children ≥1 year, adolescents and adults following primary and booster vaccination with TTCV, Figure 2 provides a schematic diagram of the typical response. A single dose of TT in the absence of priming induces little, if any, protection. Two to four weeks after the second dose, the mean level of tetanus antitoxin typically exceeds *the minimum putatively protective level of 0.01 IU/mL.* One year after the second dose, the mean antibody levels are expected to decline and may fall to the protective threshold level. After each subsequent dose of vaccine, immunity is boosted, then persists above the protective threshold for a time, and then wanes over time. Putatively protective levels of immunity are induced by a primary series of three TTCV doses and immunity typically persists for at least 5 years. After the third dose, each additional booster dose given after at least a one-year interval increases tetanus antitoxin levels and further prolongs the duration of immunity. Immunity may persist for approximately 10 years after the fourth dose of TTCV and for at least 20 years after the fifth dose."

- Similarly, Grandjean et al. (2017b) state [*emphasis added*], "The close correlations *prevented meaningful adjustment* for concomitant PFAS exposures", and Budtz-Jorgensen and Grandjean (2018) acknowledge [*emphasis added*] that "*an important weakness of epidemiological studies is the mixed exposures*" and more than one PFAS may contribute to the lowering of antibody responses.

Thus, it appears that effects may neither rise to the level of adversity nor be attributable specifically to any PFAS. Co-exposures to multiple other PFAS (at a minimum) that are not/cannot be adequately accounted for in the analyses are likely to be significant confounders in these epidemiological studies, especially because PFAS exposures are correlated, they are chemically-similar compounds, and there appears to be little variation in exposure (i.e., low exposure contrasts) for the single PFAS being assessed (e.g., Table 2 of Grandjean et al. 2012, Table 1 of both Grandjean et al. 2017a and 2017b). For example, Grandjean et al. (2012) shows that PFOA and PFOS had a correlation coefficient of 0.5 in the blood sera of 5-year olds and interquartile range (IQR) differences in blood sera concentrations of less than 1.6-fold each (e.g., 75th percentile blood concentration of PFOA/25th percentile blood concentration of PFOA), and PFNA had correlation coefficients of ≈0.5 with both PFOS (0.48) and PFOA (0.54) in the blood sera of 5-year olds and a less than 2-fold difference in the IQR for PFNA concentration in child blood sera (see Table 2 of the study).

The Australian government (FSANZ 2021) has concluded that associations of PFAS with immunological endpoints do not provide a suitable basis for quantitative risk assessment:

> "In summary, new epidemiological studies provide some evidence of statistical associations between PFAS blood levels and impaired vaccine response, increased susceptibility to infectious disease and hypersensitivity responses. However the data are insufficient to establish causal relationships and it cannot be ruled out with reasonable confidence that the observed statistical associations may have been due to confounding, bias or chance. On the basis of the uncertainties and limitations in the evidence base, immunomodulation is not currently considered suitable as a critical endpoint for quantitative risk assessment for PFAS."

Similarly, ATSDR (2021) found the epidemiology literature inadequate for use as the basis of deriving minimal risk levels (MRLs) for PFAS, noting:

> "There are sufficient epidemiological data to identify possible sensitive targets for many of the perfluoroalkyls; however, there are two major limitations to establishing dose-response relationships for these effects and using the epidemiological studies to derive MRLs: accurate identification of environmental exposure levels producing increased risk for adverse effects (exposure estimates and routes of exposure) and likely co-exposure to mixtures of perfluoroalkyls. Other limitations include the cross-sectional design of the majority of epidemiological studies and the potential that reverse causality contributes to the observed associations… In summary, the epidemiological databases for several perfluoroalkyls provide valuable information on hazard

	identification; however, uncertainties regarding doses associated with adverse effects and possible interactions between compounds preclude use of these data to derive MRLs." All this is not to say that PFAS are incapable of causing immune effects or that these epidemiological studies have no value for hazard identification, but rather most importantly that the epidemiological data are insufficient for dose-response assessment due to significant issues such as (but not limited to) confounding by co-exposures to other PFAS that have not been (and perhaps cannot be) adequately adjusted for, and as a consequence any effects observed are mixture effects. These and other arguments in my comments on various draft IRIS PFAS assessments (e.g., PFHxS) help support EPA's decision in this case (and would in other similar cases) not to use serum antibody endpoints from these epidemiology studies as a basis for quantitative dose-response assessment and derivation of toxicity factors. e. EPA has given consideration to several important study attributes, and as evaluated in the draft, the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level ratings in the draft PFNA assessment (e.g., Figures 3-77 and 3-86). The overall presentation of study results appears clear and various strengths and limitations are described. Table 3-34 is the evidence profile table for thyroid effects, which among other information contains factors that increase and decrease certainty, as evaluated in the draft, along with evidence stream (i.e., human, animal, mechanistic) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.7) also contains information relevant to the WOE for thyroid effects. Similar to what is stated in the charge question above, p. 3-315 (lines 19-25) states, "Taken together, the available evidence suggests but is not sufficient to infer that PFNA exposure may cause thyroid toxicity in humans given sufficient exposure conditions (see Table 3-34). This was a complex evidence base to interpret as the human evidence was slight and the only animal study available provided moderate evidence but also had some uncertainties related to the free T4 analytical method used and body weight loss in males at higher doses. Despite these uncertainties, the large, dose-dependent reductions in free and total T4 in female rats and free T4 in male rats suggest some level of concern." In regard to the moderate animal evidence, in addition to the uncertainties cited by EPA, I note that some scientific public comments submitted on the prior IRIS draft assessment (i.e., PFHxS) also seem to raise doubts in the present case about the use of rodents as laboratory animal models representative of human biology for thyroid hormone-related effects[7]. I agree that the available evidence suggests but is not sufficient to infer

[7] For example, some comments report that the significance of changes in T4 levels in rodents to human risk assessment has been questioned by the National Academy of Sciences (NAS) because of the significant differences in binding proteins and affinities between species (e.g., clearance of T4 from human serum is sharply reduced compared to rats since humans have thyroxine-binding globulin with a greater than 100-fold higher binding affinity for T4 than the albumin and transthyretin binding T4 in rat serum), with NAS noting that rats are much more sensitive to agents that disturb thyroid function than are humans so the relevance of rat studies in quantitative terms to humans is limited, and that they do not agree that transient changes in serum thyroid hormone or TSH concentrations are adverse health effects but rather are simply biochemical changes that might precede adverse effects (pp. 10-11 of *Public Comments Received on Draft IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS) and Related Salts*).

	that PFNA exposure may cause thyroid toxicity in humans given sufficient exposure conditions. f. EPA has given consideration to several important study attributes, and as evaluated in the draft, the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level ratings in the draft PFNA assessment (e.g., Figures 3-92 and 3-93). The overall presentation of study results appears clear and various strengths and limitations are described. Table 3-47 is the evidence profile table for cardiometabolic effects, which among other information contains factors that increase and decrease certainty, as evaluated in the draft, along with evidence stream (i.e., human, animal) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.9) also contains information relevant to the WOE for cardiometabolic effects. Similar to what is stated in the charge question above, p. 3-380 (lines 29-34) states, "Overall, the currently available evidence suggests but is not sufficient to infer that PFNA may cause cardiometabolic impairments in humans given sufficient exposure conditions (see Table 3-47). This judgment is based primarily on studies in humans that assessed median exposure levels of 0.6-1.5 ng/mL, and showed generally increased serum lipids, and some potentially supportive but mixed results for other increased risk factors for cardiovascular disease; however, important uncertainties remain." I agree with the overall WOE that the slight epidemiological evidence and indeterminate animal evidence suggests but is not sufficient to infer whether exposure to PFNA might cause cardiometabolic effects in humans given sufficient exposure conditions (see Evidence Integration on p. 3-380). g. The draft assessment has given consideration to several important study attributes, and as evaluated in the draft, the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level ratings (i.e., Figure 3-91). The overall presentation of study results appears clear and various strengths and limitations are described. Table 3-38 is the evidence profile table for neurodevelopmental effects, which among other information contains factors that increase and decrease certainty, as evaluated in the draft, along with evidence stream (i.e., human) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.8) also contains information relevant to the WOE for neurodevelopmental effects. pp. 3-335 to 3-336 state that… "the currently available evidence suggests but is not sufficient to infer, that PFNA may cause developmental neurotoxicity in humans given sufficient exposure conditions. This conclusion is based on studies of humans exposed at PFNA median blood levels of 0.6-1.2 ng/mL. Neurotoxicological studies in adults and in experimental animals at any lifestage are a data gap." I agree with the overall WOE that the slight epidemiological evidence and indeterminate animal evidence (lack of data) suggests but is not sufficient to infer whether exposure to PFNA might cause neurodevelopmental effects in humans given sufficient exposure conditions (see Evidence Integration on pp. 3-335 to 3-336). h. Consideration has been given to several important study attributes for these effects, and as evaluated in the draft, the consideration ratings appear overall

	consistent with (i.e., provide the scientific justification needed for) the overall study confidence level ratings in the draft assessment (e.g., Figures 3-63, 3-106, 3-86 and 3-89). The overall presentation of study results appears clear and various strengths and limitations are described. Tables 3-25 and 3-49 are the evidence profile tables for female reproductive and urinary effects, respectively, which among other information contains factors that increase and decrease certainty, as evaluated in the draft, along with evidence stream (i.e., human, animal) judgments/rationales and summary judgments. Obviously, the text of the document (Sections 3.2.5, 3.2.7, 3.2.10, and 3.2.11) also contains information relevant to the WOEs for these effects. The relevant evidence integrations for these effects conclude: • P. 3-240 states, "Taken together, the available human epidemiological and animal evidence is inadequate to assess whether PFNA has the potential to cause female reproductive toxicity in humans (see 4 Table 3-25)." • P. 3-393 states, "Taken together, the currently available evidence is inadequate that PFNA may cause renal injury in humans given sufficient exposure conditions (see Table 3-49). This judgment is based on a short-term animal bioassay showing increased relative kidney weights in adult male and female rats with elevations in a serum marker of potential renal injury. However, the adversity of the animal findings is unclear." • P. 3-315 states, "The human and animal evidence are indeterminate for PFNA effects on the adrenal gland as there are no human studies and only limited testing in animals with inconclusive results. Thus, the available evidence is inadequate to inform the potential for PFNA exposure to cause adrenal toxicity in humans. An evidence profile table for adrenal effects is not presented." • P. 3-396 states, "Overall, the currently available evidence is inadequate to determine whether PFNA exposure has the potential to cause other health effects, including those related to alimentary, musculoskeletal, hematological, respiratory, and adult nervous systems. In general, the data available for these health outcomes were sparse (limited to one short-term study) and were either largely null or had little support for biological significance or coherence." I concur with the overall WOE determinations for female reproductive, urinary, adrenal, and other noncancer effects.
Leung	a. Notably, the synthesis of available data regarding developmental effects of PFNA exposure has been strengthened by several factors: 1) a separate meta-analysis of 27 included studies was completed by the EPA on birth weight and PFNA exposure in 2023, given the large amount of evidence for this outcome, and showed consistent results of an inverse relationship in sex-stratified analyses, especially females, 2) potential confounders, including physiologic alterations in pregnancy and even coexposures, were considered and adjusted for when available, and 3) outcome measures were rescaled as needed to facilitate comparison between studies. The rationale for considering birth weight and birth weight-derived measures as the most reliable of the potential endpoints is reasonable. Overall, the

	literature on various developmental effects has been clearly and appropriately synthesized. b. The literature on liver effects has been comprehensively summarized and supports the conclusion of hepatocellular injury, cholestasis, and decreased synthetic function observed with PFNA exposure, that is mostly supported by human evidence but which may confounded by co-exposures. The report acknowledges the potential for mixture effects, and clearly summarizes the animal studies (though more limited than human data) supporting a likely direct PNAS effect across multiple species, rodent strains, sexes, and life stages (including prenatal, pregnancy, and postnatal), similar to that of other PFAS. Although there are relatively sparse data, the examination of evidence for multiple potential mechanistic pathways, involving both PPAR-alpha and non PPAR-alpha mediated effects such as activation of CAR and PXR nuclear receptors, is well summarized in the document. There is also thoughtful consideration of whether these pathways may be potentially adaptive or independently adverse. The systematic review protocol used for PFBA, PFHxA, PFHxS, PFNA, and PFDA IRIS assessments contains a section summarizing the human relevance of PPAR-alpha effects that I agree is scientifically supported by available animal literature. c. The toxicological review on the male reproductive adverse effects associated with PFNA exposure is well-summarized. The mechanistic evidence and evidence integration sections are particularly clear and effective in presenting these data. The confidence level rating of studies appropriately considers parameters relevant for interpreting this system, including the importance of the appropriate timing of serum testosterone collection in the human studies, and I agree with the assessment that these epidemiologic data are inconsistent based in part on this condition. Overall, the more consistent, dose-dependent, and coherent effects across the animal studies is reasonable to support the conclusion of a likely adverse male reproductive effect. d. The evidence for the possible association between PFNA exposure and immunosuppression, particularly dampened vaccination responses seen in some human studies, is appropriately summarized. Even with these available data though, the report also presents an appropriate level of uncertainty regarding immune outcomes in general, mostly from signals suggesting that there may be possible confounding across multiple PFAS exposures, including PFNA. Overall, the weight-of-evidence conclusions for immune outcomes appears to be scientifically justified. e. Similar to my comments that I have provided in toxicological reviews for other chemicals, specifying the time of day for measuring serum thyroid hormone concentrations is not needed. I would suggest that studies which did not provide this information should not necessarily be regarded as deficient (**Tier 2 recommendation**). However, it is noted that this was not a major source of bias and not used to downgrade studies. I agree with the report that there are uncertainties regarding possible adverse thyroid function effects of PFNA exposure. The distinct entity of hypothyroxinemia that has been observed in some studies of PFNA and other PFAS exposures is nicely

	defined on pages 3-305 and 3-306, which is appropriately described as the biochemical entity of low serum free T4 and/or total T4 levels in the setting of a concurrently normal TSH. It is important to note, though, that there are specific situations in which hypothyroxinemia is not pathologic and thus not considered an adverse outcome. To determine if hypothyroxinemia is pathologic, the report would be strengthened by including a discussion of the potential mechanisms for the adverse thyroid effects (**Tier 1 recommendation**), since the mechanism informs the validity and ability for various serum thyroid hormone levels to be interpreted. There are at least two mechanisms that can be considered to explain the abnormal thyroid function trends observed (other mechanisms are also possible): Proposed mechanism 1 (central hypothyroid effect): It is possible that PFNA exposure may have a central hypothyroid effect (also termed secondary hypothyroidism). This condition refers to the impaired production and secretion of thyroid stimulating hormone (TSH) from the pituitary gland. In this scenario, serum TSH concentrations may be inaccurate/uninterpretable, as any TSH that is made by the pituitary gland may be biologically inactive. As such, serum free T4 levels becomes the valid measure, both to confirm the effect and to assess the degree of the effect. (Serum total T4 levels can also be used, but this requires the assumption that circulatory thyroid carrier proteins [75% of which is TBG] are in the normal range, which is not the situation in pregnancy). The resulting biochemical pattern of a central hypothyroid effect would thus be low serum free T4 and/or total T4 and low serum free T3 and/or total T3 levels with concurrently low, normal, or high (i.e. inaccurate/uninterpretable) TSH concentrations. The pattern of thyroid function tests from both the human epidemiologic and animal PFNA data available thus may fit this mechanism, and if informed by a consistent trend when analyzing only the non-pregnant studies (**Tier 1 recommendation**), *would suggest a pathologic effect*. If this is felt to be the underlying mechanism for adverse PFNA effects on the thyroid, then serum TSH levels would not be expected to rise in response to low T4 and/or low T3 levels. Thus, TSH levels should not be used for any modeling purposes in this scenario; only the degree of free T4 decrease (or alternatively total T4 decrease, but definitely not in pregnant women) should be used. Proposed mechanism 2 (thyroid hormone binding abnormality): It is also possible that PFNA exposure may have adverse effects on one or more aspects of various effects of thyroid hormone binding, which can also result in isolated low serum T4 (free and/or total) concentrations. In this mechanism, altered total T3 and/or total T3 levels are an effect of thyroid hormone binding and *are not pathologic*, since the free T3 and/or free T4 levels remain in the normal physiologic range. If this is the effect of PFNA on the thyroid, it may be accomplished by either: **a**) Increasing or decreasing TBG levels, thereby increasing or decreasing total T4 levels, respectively, while generally free T3 and free T4 levels should remain unchanged in the normal range. For this reason, it would be ideal to only analyze serum thyroid function test patterns in only non-pregnant human samples to consider this mechanism (**Tier 1 recommendation**), since the estrogen of pregnancy can raise TBG levels up to 2-fold, so it remains unclear if this is the mechanism in which PFNA may have an adverse thyroid function effect. It is notable that nearly a third of the human studies in this report included pregnant women. **b**) Decreasing T3 and T4 binding to carrier proteins (i.e. TBG, albumin, transthyretin), to result in decreased total T3 and total

	T4 levels while usually free T3, free T4, and TSH levels all remain in unchanged in the normal range. This pattern (in b) of thyroid function tests appears to be less consistent with the general trend of PFNA exposure seen in the human studies, and also is not suggested by the animal data. In general, the following considerations may help strengthen the support for the report's conclusion regarding thyroid effects: 1. Separating out the epidemiologic thyroid studies between pregnant (n=10 studies) and non-pregnant women (n=16 studies) is critical (**Tier 1 recommendation**) because of multiple physiologic effects of pregnancy on thyroid function. These adaptive responses are well-established and notably include the following, which would make determining which of the above possible mechanisms that PFNA might act on the thyroid even more challenging: **a**) TSH levels may be decreased especially in early pregnancy, due to the TSH suppressive effect of beta-human chorionic gonadotropin (bHCG), a weak thyroid gland stimulator. Thus, TSH levels in isolation and without concurrent T4 levels, may not be useful in early pregnancy. **b**) TBG levels can increase up to 2-fold, thereby leading to an up to 2-fold increase in serum total T4 and total T3 levels. As such, free thyroid hormone levels are more useful than total thyroid hormone levels, but interpreting serum free T4 levels alone in pregnancy are also unfortunately problematic due to the known variability of this assay in this population, and serum free T3 levels are also more variable in general in any population. Therefore, in pregnancy, thyroid status is thus best interpreted with concurrent sampling of TSH and free and/or total T4 levels, keeping all of the above expected physiologic considerations in mind so that thyroid function tests are not necessarily regarded as abnormal. 2. Given the normal feedback between the pituitary and the thyroid gland, it would be important to analyze any trends of abnormal serum T4, T3, and TSH levels in parallel (**Tier 2 recommendation**). Thus, I would suggest to also separate out the studies that collected serum concurrently and assigning them much greater weight, from those studies that did not or for example measured only one type of thyroid function test. Since the discussion of alterations in these three serum measurements in isolation is not easily interpretable, I would suggest focusing the weight of evidence discussion (pages 3-289 and 3-290) on the 9 studies that examined more than a single thyroid function test, for example reorganizing the thyroid text to begin with and emphasize the summary of these rather than at the end. In summary, toward the goal of understanding the mechanism of a toxicant's effects on the thyroid axis (which is crucial to determine the components of the serum thyroid function test panel that are appropriate to study, which are uninterpretable values versus expected physiologic changes, and *thus which abnormal thyroid blood tests may be pathologic*), the ideal human serum thyroid function samples should be from non-pregnant individuals who have as minimal the possibility of altered TBG levels, in whom sera for the measurement of T4, T3, and TSH were collected all at the same time. Only after the mechanism of toxicant action is established may then possible adverse thyroid effects be evaluated in

	other populations, such as pregnant women who are already anticipated to have abnormal thyroid function. Thus, the EPA may consider including a research agenda in future work, outlining the limitations of available current data and need for future interpretable data (**Tier 3 recommendation**). It should be noted that although I have made several Tier 1 and 2 recommendations above that hopefully may help inform if there is (and the magnitude if so) of an effect from PFNA on the thyroid axis, the serum thyroid function trends for this chemical are overall similar to several other PFAS, such that the overall conclusion of the report related to thyroid effects ("suggests but is not sufficient to infer an effect") is unlikely to change. Additionally, the following suggestions may improve the report's presentation of the thyroid data: • Figures 3-78, 3-79, and 3-80 would be better if reorganized into stratified groups according to their pregnant/nonpregnant samples and showing all available thyroid function tests (T3, T4, and/or TSH) for each study that were hopefully all collected at the same time (**Tier 2 recommendation**). In the subset of studies containing only pregnant women, it would also be relevant to include the gestational age, or at least trimester, in which these thyroid function tests were measured, if the gestational timepoint is available (**Tier 2 recommendation**). **Minor suggestions:** • The heading of Section 3.2.7 may be better renamed from endocrine effects, to thyroid, adrenal, parathyroid, and pituitary effects (since endocrine also encompasses male and female reproduction, and diabetes, that are all separately discussed). • Page 3-303, lines 8-9: It would be clearer to specify that subclinical hyperthyroidism and subclinical hypothyroidism in the Wen 2013 study were defined as the presence of abnormal TSH levels as shown in the setting of normal T3 and T4 levels. f. The evidence synthesis on cardiometabolic adverse effects associated with PFNA exposure is well-summarized, and the conclusions are scientifically justified. There is considerable uncertainty and potential many confounders in the assessed relevant outcomes, which the report appropriately acknowledges. g. I agree with the report's conclusion that there is considerable uncertainty regarding neurodevelopmental effects associated with PFNA exposure, and the conclusion is appropriately justified. There is also the potential for low thyroid hormone levels to be a mediator in the neurocognitive outcomes, including ADHD, reported in some studies. h. The conclusion that there is inadequate evidence to determine a potential adverse effect from PFNA exposure on these other noncancer outcomes is appropriate and scientifically justified.

Lin	a. The determination that the epidemiological evidence of developmental effects of PFNA exposure during gestation and/or lactation is robust is scientifically justified. The available studies are clearly and appropriately synthesized to highlight the strengths and limitations of each of the existing studies. The presentation and analysis of study results are clear, appropriate, and effective to allow for scientifically supported syntheses of the research findings across existing studies. The study confidence conclusions for the existing PFNA studies are also clearly presented and scientifically justified. The weight-of-evidence decisions for hazard identification are clearly described and scientifically justified. To be more specific, there are many studies (i.e., 61 epidemiological publications across 59 different studies) investigating the impact of PFNA exposure on various developmental endpoints. The extensive human epidemiological study findings are also supported by several developmental toxicity studies in rodents. To systemically synthesize existing data, US EPA performed a meta-analysis and systemic review (Wright et al. 2023). The results showed that there was a statistically significant association between PFNA exposure and birth weight deficit across all sampling periods and study confidence levels. One potential uncertainty of this association is the potential confounding effects by co-exposure to other PFAS. To address this issue, EPA did a comprehensive analysis on PFAS co-exposures (and other co-occurring contaminants). The results on the PFAS co-exposure and other confounding considerations and meta-analysis of PFNA effects on birth weight are presented in Appendix C. Based on Appendix C, EPA concluded that the potential confounding factors would not substantially reduce confidence in the evidence base. This conclusion is scientifically justified. On a last point, this reviewer is not aware of any studies not considered in the assessment that would be expected to materially impact the weight-of-evidence decisions. **Reference** • Wright JM, Lee AL, Rappazzo KM, Ru H, Radke EG, Bateson TF. Systematic review and meta-analysis of birth weight and PFNA exposures. Environ Res. 2023 Apr 1;222:115357. doi: 10.1016/j.envres.2023.115357. Epub 2023 Jan 24. PMID: 36706898. b. The conclusion that there is moderate evidence that PFNA exposure will cause liver effects in humans is scientifically justified. This is because the liver effects observed in human studies were mainly based on cross-sectional studies in general population adults. The strength of evidence from cross-sectional studies is generally less than that from case-control and cohort studies. Also, there is potential that exposure to other highly correlated PFAS may contribute to the observed liver effects. These potential confounding effects are discussed in Appendix C "Supplemental Approaches and Data Analysis". The potential confounding effects are not sufficient to explain the observed liver effects. Based on all of these considerations, it is scientifically justified to conclude that there is moderate evidence of PFNA exposure on liver effects. The basis for the judgement of human relevance of hepatic effects in animals that involve peroxisome proliferator-activated receptor alpha (PPARα) receptors as a key aspect of the mechanism of action is scientifically justified. This is because the

	important role of PPARα in PFNA-induced liver effects has been demonstrated in in vivo rodent studies and in vitro studies using both human and rodent cell lines (Das et al., 2017; Rosen et al., 2017; Wolf et al., 2010; Oshida et al., 2015a; Oshida et al., 2015b). Also, studies have shown that PPARα pathway is generally well conserved across rodents and humans (Corton et al., 2014; Mcmullen et al., 2020; Rakhshandehroo et al., 2009). The basis of this judgement is further discussed in the subsection of "Mechanistic Evidence and Supplemental Information" under Section 3.2.3. The basis for determination under the criteria set forth in Hall et al. (2012) and others (e.g., EPA, 2002; EMA, 2008) that the hepatoxic effects observed in rodents are considered adverse is scientifically justified. This is because the hepatoxic effects were observed in two species of rodents, and some of the effects were observed in a dose-dependent manner and the extent of changes of some liver effects were large enough to be considered as indicators of intrahepatic cholestasis as according to the criteria from National Toxicology Program (NTP). **References:** • EMA, 2008. Non-clinical guideline on drug induced hepatotoxicity. European Medicines Agency (EMA). EMEA/CHMP/SWP/150115/2006. London, UK. • EPA, 2002. Hepatocellular hypertrophy. HED guidance document #G2002.01. U.S. Environmental Protection Agency, Washington, DC. • Oshida K, Vasani N, Thomas RS, Applegate D, Rosen M, Abbott B, Lau C, Guo G, Aleksunes LM, Klaassen C, Corton JC. Identification of modulators of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα) in a mouse liver gene expression compendium. PLoS One. 2015b Feb 17;10(2):e0112655. doi: 10.1371/journal.pone.0112655. Erratum in: PLoS One. 2015 Apr 07;10(4):e0124224. doi: 10.1371/journal.pone.0124224. PMID: 25689681; PMCID: PMC4331523. • Oshida K, Vasani N, Jones C, Moore T, Hester S, Nesnow S, Auerbach S, Geter DR, Aleksunes LM, Thomas RS, Applegate D, Klaassen CD, Corton JC. Identification of chemical modulators of the constitutive activated receptor (CAR) in a gene expression compendium. Nucl Recept Signal. 2015a Apr 27;13:e002. doi: 10.1621/nrs.13002. PMID: 25949234; PMCID: PMC4422105. • Wolf CJ, Zehr RD, Schmid JE, Lau C, Abbott BD. Developmental effects of perfluorononanoic Acid in the mouse are dependent on peroxisome proliferator-activated receptor-alpha. PPAR Res. 2010;2010:282896. doi: 10.1155/2010/282896. Epub 2010 Sep 27. PMID: 20936102; PMCID: PMC2948904. • Hall AP, Elcombe CR, Foster JR, Harada T, Kaufmann W, Knippel A, Küttler K, Malarkey DE, Maronpot RR, Nishikawa A, Nolte T, Schulte A, Strauss V, York MJ. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol.

	2012 Oct;40(7):971-94. doi: 10.1177/0192623312448935. Epub 2012 Jun 21. PMID: 22723046. • Rosen MB, Das KP, Rooney J, Abbott B, Lau C, Corton JC. PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology. 2017 Jul 15;387:95-107. doi: 10.1016/j.tox.2017.05.013. Epub 2017 May 27. PMID: 28558994; PMCID: PMC6129013. • Das KP, Wood CR, Lin MT, Starkov AA, Lau C, Wallace KB, Corton JC, Abbott BD. Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology. 2017 Mar 1;378:37-52. doi: 10.1016/j.tox.2016.12.007. Epub 2016 Dec 31. PMID: 28049043; PMCID: PMC5994610. • Rakhshandehroo M, Hooiveld G, Müller M, Kersten S. Comparative analysis of gene regulation by the transcription factor PPARalpha between mouse and human. PLoS One. 2009 Aug 27;4(8):e6796. doi: 10.1371/journal.pone.0006796. PMID: 19710929; PMCID: PMC2729378. • McMullen PD, Bhattacharya S, Woods CG, Pendse SN, McBride MT, Soldatow VY, Deisenroth C, LeCluyse EL, Clewell RA, Andersen ME. Identifying qualitative differences in PPARα signaling networks in human and rat hepatocytes and their significance for next generation chemical risk assessment methods. Toxicol In Vitro. 2020 Apr;64:104463. doi: 10.1016/j.tiv.2019.02.017. Epub 2019 Oct 15. PMID: 31628012. • Corton JC, Cunningham ML, Hummer BT, Lau C, Meek B, Peters JM, Popp JA, Rhomberg L, Seed J, Klaunig JE. Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARα) as a case study. Crit Rev Toxicol. 2014 Jan;44(1):1-49. doi: 10.3109/10408444.2013.835784. Epub 2013 Nov 4. PMID: 24180432. c. My area of expertise is toxicokinetics (TK) and PBPK modeling. I am familiar with the TK and PBPK studies of PFAS, including PFNA, but I am not familiar with the studies on male reproductive effects of PFNA. As such, I have insufficient expertise to comment on this charge question. d. My area of expertise is toxicokinetics (TK) and PBPK modeling. I am familiar with the TK and PBPK studies of PFAS, including PFNA, but I am not familiar with the studies on immune effects of PFNA. As such, I have insufficient expertise to comment on this charge question. e. My area of expertise is toxicokinetics (TK) and PBPK modeling. I am familiar with the TK and PBPK studies of PFAS, including PFNA, but I am not familiar with the studies on thyroid effects of PFNA. As such, I have insufficient expertise to comment on this charge question. f. My area of expertise is toxicokinetics (TK) and PBPK modeling. I am familiar with the TK and PBPK studies of PFAS, including PFNA, but I am not familiar with the studies

	on cardiometabolic effects of PFNA. As such, I have insufficient expertise to comment on this charge question. g. My area of expertise is toxicokinetics (TK) and PBPK modeling. I am familiar with the TK and PBPK studies of PFAS, including PFNA, but I am not familiar with the studies on neurodevelopmental effects of PFNA. As such, I have insufficient expertise to comment on this charge question. h. My area of expertise is toxicokinetics (TK) and PBPK modeling. I am familiar with the TK and PBPK studies of PFAS, including PFNA, but I am not familiar with the studies on female reproductive, urinary, adrenal, and other noncancer effects of PFNA. As such, I have insufficient expertise to comment on this charge question.
Savitz	a. Based on the replicated evidence of small decreases in birthweight associated with elevated PFNA exposure, the conclusion that the epidemiologic evidence is robust is scientifically justified. While not a challenge to the evidence or its interpretation, small shifts in birthweight are not of clinical concern even if they do suggest a biological effect. The use of data from a meta-analysis is appropriate here since the studies are all quite similar in design and the assumption of a common effect size is reasonable. The use of multiple high-quality studies is preferable to arbitrarily choosing one of them as the basis for generating estimates. **Tier 3: Future Considerations** -- The use of toxicology to help interpret the evidence for very small and potentially non-pathological effects in humans is an important general theme that would be applicable generally in assessments of this sort. This would apply to small changes in biological markers (e.g., enzymes, hormones) and in this case, small changes in a biological measure, birthweight. b.i. This is a challenging issue to interpret, namely the correlation between elevated levels of PFNA and elevated levels of liver enzymes indicative of liver damage. The key issues of concern are correlations among the specific forms of PFAS but also the potential for reverse causality (or perhaps confounding) in which subtle liver dysfunction produces both elevated levels of PFNA and elevated levels of liver enzymes. Concluding that the evidence is “moderate” is reasonable, given that it is not negligible or conclusive. **The Tier 3 recommendation noted for developmental effects applies here as well. No revisions.** b.ii. I do not have the needed biological expertise to comment on whether the specific effects observed in experimental studies tie directly to the human data on liver toxicity. b.iii. I do not have the needed expertise in toxicology to be able to comment on this assessment. c. This is based solely on toxicology, which may be justified, and the human data does not either support or argue against the conclusion. While not bearing directly on their assessment, this would seem to be an area in which further human studies are needed. **No revisions.**

	d. The topic of immunosuppression has been addressed appropriately and based on epidemiology the support is weak, particularly for clinically significant outcomes. Although there are pieces of evidence suggesting effects, overall it is quite limited. No revisions. e. For thyroid effects, the human evidence is limited, as the document indicates, and even where effects on hormone levels or disease have been reported, there are contradictory studies. Overall, there is a lack of consistent evidence of any one specific form of thyroid effect across the studies. No revisions. f. The human evidence for increased lipids is fairly strong, subject only to the potential for residual confounding due to other forms of PFAS, but that does not correspond to increased risk of cardiometabolic disease based on available studies. This may be interpreted more as an indication of liver toxicity. Combined with the lack of support in animal studies, the overall conclusion is appropriate. No revisions. g. This is a reasonable interpretation of the literature on PFNA and ADHD, with some suggestive associations but inconsistency across studies. The specific indicators are variable and the details of the findings across studies do not provide a coherent basis for inferring causal effects. No revisions. h. No comments since this is based solely on toxicology.
Zoeller	a. The Agency identified 61 epidemiological publications across 59 different studies that examined PFNA exposure in relation to various developmental endpoints. This is quite a large number of studies the Agency had to analyze and interpret, providing a rare data set from which to draw conclusions. In addition, the Agency conducted their own systematic review and meta-analysis of these studies to derive the conclusion that the evidence is robust. The Agency appears to have appropriately synthesized the relevant data and highlighted the strengths and weaknesses. The background and discussion of key science issues is clear and robust. Analysis of study confidence conclusions are clear and reasonable. The Agency evaluated the relationship between PFNA exposure and birth weight (mean and standardized measures), small for gestational age and low birth weight, birth length, head circumference, fetal growth restriction, postnatal weight and height, adiposity, gestational duration, and anogenital distance. Each of their analyses appears thorough and transparent. Their conclusions were clear and supported by the evidence they present. Overall, their conclusion that the epidemiological evidence linking PFNA exposure to developmental effects is robust is scientifically justified. **Tier 2 Recommendation.** In the Agency's granular analysis of these many epidemiological studies, they often use the term "precision" to describe data sets. Nowhere is this defined – either in the body of the review, or in the Appendix. This makes the conclusion about "precision" – and a study's quality – seem arbitrary. It would be useful to define this term in the body of the document when it is used.

	b.i. The Agency identified 16 epidemiological studies that reported on the relationship between PFNA exposure and serum markers of liver injury such as ALT, AST. GGT and/or total bilirubin. These are considered reliable markers of liver function injury. Twelve studies were determined to be of medium confidence and 4 were either low confidence or uninformative. All 9 of the medium confidence studies in adults reported positive associations between PFNA exposure and ALT with statistical significance in all but 3 studies. While effect estimates were small in most studies, 20% difference was observed for the 95th vs 25th %tile in one study and a gradient was observed across exposure estimates. The Agency addressed the issue of co-exposures with other PFAS and concluded that the evidence supported co-exposures could not account for the full measure of PFNA correlations. Given the number of factors (chemical and otherwise) that can affect serum levels of these hepatobiliary markers, it is somewhat surprising that there was a relatively consistent relationship between measures of PFNA and ALT. Perhaps because these were "medium" confidence studies, the Agency categorized the evidence as "moderate". If so, it would be useful for the Agency to explicitly describe the logic for this conclusion. Having said this, the Agency's analysis was science based both in the description of the findings as well as the interpretation of the findings. **Tier 2 Recommendation:** The Agency should define exactly why their conclusion was that there is moderate evidence in the epidemiological studies indicating that PFNA causes adverse liver effects given sufficient exposure. b.ii. The Agency appears to have thoroughly considered the mechanistic evidence that PFNA effect on the liver in animals is relevant to humans considering the PPARalpha – dependent versus independent mechanistic pathways. This evidence not only includes information from PPARalpha null mice but also from gene expression studies showing signature patterns known to be driven by PPARalpha versus CAR and other pathways. The description and analysis of these data were clear and cogent. As a result, the Agency's judgement appears strongly justified scientifically. **No recommendations.** b.iii. First, the Hall "criteria" appear too simplistic and at 12 years old, perhaps they should be revisited. The logic of having 2 criteria of "adversity" being diagnostic and anything else being considered "adaptive" seems naive to this reviewer. Having said this, the Agency has followed these criteria and found that the evidence suggests that the effect of PFNA on liver is adverse not adaptive. This conclusion was based on a very overt and transparent analysis that was scientifically justified. **No recommendations.** c. The Agency did a thorough analysis of the relevant literature including 12 human studies, the available animal studies and the in vitro and mechanistic studies. The human studies were plagued by a number of challenges highlighted in the Review, including the timing of sample collection and the issue of abstinence. As a result, there was more confidence in the animal studies, especially a single high confidence

	study from NTP. Overall, the presentation of the study results and the integration of evidence was clear and appropriate and scientifically justified. **No recommendation.** d. The Agency concluded that the evidence suggests but is insufficient to infer that PFNA exposure can cause immunosuppression in humans. The Review clearly articulates the human evidence including an analysis of the confidence of the studies and describes the strengths and weaknesses of the reported data. Some medium confidence studies provide evidence of reduced antibody response to vaccination with PFNA exposure. These results for PFNA exhibit a weaker pattern of immunosuppression than other correlated PFAS, with less consistency and smaller effect size. The data cannot rule out that the results for PFNA are driven by associations with other PFAS. There is also weak evidence for effects of PFNA on asthma and asthma-related outcomes. The animal data is weak on functional assays and longer during exposures, which represent a significant limitation in the animal literature. Mechanistic evidence was considered to be mostly limited to high dose testing and too limited to meaningly inform an MOA. Overall, I thought the scientific analysis support the conclusion. **No recommendation.** e. The Agency evaluated the evidence that PFNA exposure could cause effects on the thyroid hormone system in humans and concluded that the available evidence is not sufficient to infer that there is a causal relationship between these two. There were 45 publications reporting on the relationship between PFNA exposure and thyroid hormones and/or thyroid disease in humans. In 16 medium confidence studies, some reported inverse relationships of interest, some positive relationships, and most were close to null. There appeared to be no clear pattern. However, the studies were focused on adults (men, women, pregnant women), children/adolescents, and infants. The findings among studies of pregnant women were similarly mixed. Six studies evaluated associations between PFNA exposure and thyroid hormones in children and/or adolescents. Five of these studies were medium confident and 4 reported significant positive associations between PFNA exposure and T4 levels. Eleven studies examined associations between PFNA and thyroid hormones in infants. Four of nine medium confidence studies reported inverse associations between PFNA exposure and T4 levels. The remaining 5 studies reported no association. One high confidence NTP study reported consistent reductions in serum T4 in adult male and female rats with PFNA exposure. Serum TSH was not affected by PFNA in females but were decreased in males. The observation that both T4 and TSH were reduced in animals treated with PFNA is atypical for PFAS exposures and thus creates uncertainty. However, it is not clear why, mechanistically, PFAS and other

	chemicals (PCBs, PBDEs) cause a significant reduction in serum free and total T4 without a commensurate increase in serum TSH. Thus, it seems reasonable to conclude that PFNA reduces both serum T4 and TSH in male rats. Note: the absolute levels of T4 in this study suggest that the animals were fed a soy-based diet. This will add an element in the interpretation given that control levels are quite low – at the low end of the assay LOQ. Clearly, the scientific observations reviewed by the Agency represent a complex data set that is difficult to interpret, but the uncertainties may depend on the specific question: • Has the study found a genuine relationship of interest? It is possible that the uncertainties are related to methods that could spuriously produce the reported pattern. In contrast, if there are no specific reasons to question the validity of the data, then it should not be regarded as "uncertain". • Can conflicting results in different studies be valid? It is certainly possible that different relationships of interest can be reported in different studies – such as negative versus positive relationships of interest – without identifying methodological reasons to consider some invalid. In this case, the uncertainty lies in how this might happen. o In this case, it would seem that it can be said that there is a relationship of interest (negative or positive association) in all (or most) of the studies. o A specific point: The Agency makes the statement that the analog RIA for free T4 could have over-estimated the reductions in serum free T4 observed in male rats (P 3-306 Lines 16-30). I'm not sure this is logical. The analog method works by using a "T4 analog" that binds to the RIA antibody, but not to serum binding proteins. If PFNA displaces T4 from serum binding proteins, that increase in free T4 would be recorded as an elevation, not a decrease. Thus, if this is in fact occurring, then the reported reductions would have been greater. Although the Agency did not build this uncertainty into their final analysis, it might be useful to correct this? • By categorizing the level of analysis at which there is uncertainty, the analysis might make more sense. For example, 4 of 9 medium confidence studies of infants report a negative association between PFAS exposure and serum T4, but the remaining studies report no association. Are the 4 studies compromised methodologically? Are the remaining 5? It seems important to define where the uncertainties are, so that stronger conclusions can be made. **Tier 2 Recommendation:** The Agency developed a POD for thyroid but did not develop it further. The reasoning is sound but does not consider how this analysis compares to the IRIS analysis for the other PFAS. The Agency should consider carrying the POD for thyroid through to develop a subchronic RfD for comparison with the RfDs developed for other PFAS the Agency has performed. **Tier 2 Recommendation:** Define where the uncertainties lie in this dataset to determine whether the conclusion will remain the same or be changed.

	Tier 2 Recommendation: Reconsider and revise the argument about the use of analog assays for measuring fT4. f. The Agency reviewed the evidence that PFNA exposure is related to each of several cardiometabolic endpoints. For example, in general population studies of adults, the five medium confidence studies examining total cholesterol all reported positive associations between PFNA exposure and total cholesterol. This was not observed consistently in studies of other populations such as adolescents, children, pregnant women or in cord blood. It is not clear whether the Review interprets as inconsistent, the results on cholesterol levels across all of these studies, or whether each subpopulation is considered independently. Clearly the complete dataset reviewed presents a complex picture and it is not clear how the data are to be interpreted as "adverse". **Tier 3 Recommendation.** It would appear to be important to the Agency to have an analysis of endpoints in this complex domain that are to be considered "adverse". For example, in the absence of clear evidence of "apical" endpoint (e.g., stroke, myocardial infarction) correlation with a chemical exposure, which "intermediate" endpoints could and should be used as a point of departure. g. This is a very complex dataset reflecting a complex issue. It seems particularly challenging to relate what may be a continuous exposure to PFNA and outcomes that are age dependent. Likely, the age dependency is not known for measures of cognition, attention, ADHD, Autism, etc. Moreover, the age-exposure relationship may differ for different toxicants. Having said this, the data from a considerable number of human studies is appropriately synthesized to describe the strengths and weaknesses. The analysis is generally clearly presented with an overt description of confidence conclusions. Finally, the evidence integration is clear. However, it is not clear how uncertainties are interpreted. For example, under the category of "cognition", it appears that the dataset is uncertain in part because specific endpoints within that category are variable across studies – sometimes within the same study across ages. So, what is uncertain is whether the observed correlation is spurious or "real". It would be clearer to define the level of analysis that is uncertain. Overall, however, the conclusion that the evidence suggests, but is not sufficient to infer, that PFNA exposure has the potential to cause neurobehavioral effects in humans is clear and scientifically justified. **No recommendation.** h. The Agency has clearly reviewed the evidence that supports this conclusion. **No Recommendation**

3.3 For PFNA, no reference concentration (RfC) was derived for inhalation exposures. A reference dose (RfD) was derived based on a meta-analysis (Wright, 2023, 10699259) examining reduced birth weight in humans from 10 studies with biomarkers collected early in pregnancy. Note that the selected RfD based on developmental effects is further supported by the lifetime oral hepatic organ-specific osRfD, based on Kim et al. (2023).

a. Are the selected 10 epidemiological studies for developmental effects used in deriving the RfD values (both lifetime and subchronic) for PFNA scientifically justified? If so, please provide an explanation. If not, 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.

i. As part of the recommendations in "a" above, please comment on whether the effect selected is appropriate for use in deriving the lifetime RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for its selection. Please also see charge questions 2a and 2a(i).

ii. EPA used benchmark dose (BMD) modeling (U.S. EPA, 2012) to identify points of departure (PODs) for PFNA-induced developmental effects. In addition, different meta-analyses were performed to evaluate the relationship between PFNA and mean birth weight differences in humans (Wright, 2023, 10699259). Are the modeling and the meta-analysis for decreased birth weight based on 10 studies with biomarkers collected early in pregnancy appropriate for use in derivation of the RfD? Are the selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for developmental effects scientifically justified and clearly described?

b. For liver effects, an osRfD was derived based on the epidemiological study by Kim et al. (2023) examining biomarkers of liver functions in humans. Are the modeling approaches for the liver effects, selection of cutoff for abnormal, selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for hepatic effects scientifically justified and clearly described?

c. For male reproductive effects, quantitative information was limited to studies in animals exposed to PFNA for 28 days, and little to no information was available to evaluate the effects of chronic exposure on these health hazards. Therefore, the derivation of lifetime osRfD values was not attempted for male reproductive effects. However, this endpoint was considered for the derivation of a subchronic osRfD (see Question 4). Please comment on whether the provided scientific rationale supports the decision to consider only these effects for the subchronic RfD? Are the selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for male reproductive effects scientifically justified and clearly described?

d. For immune and thyroid effects, no reference values were derived given uncertainties in the databases that were judged to indicate suggestive evidence of effects. However, while a dose-response assessment is typically not conducted for health effect judgments of "evidence suggests," when the database includes at least one well-conducted study, quantitative analysis may still be useful for some purposes, such as

providing a sense of the magnitude and uncertainty of estimates for health effects of concern, informing responses in potentially susceptible populations, or setting research priorities (U.S. EPA, 2005; U.S. EPA, 2020). For this assessment, immunosuppression in children and reduced serum T4 in adult female rats were advanced for dose-response modeling to facilitate comparisons with other PODs and to inform uncertainty factor (UF) selection given that effects have been observed for several other PFAS.

i. **For immune effects, the BMD modeling of the selected medium confidence epidemiological studies by Grandjean et al. (2012) using untransformed PFNA concentrations by Budtz-Jørgensen et al. (2018) was null and did not show effects of PFNA on antibody concentrations in children aged five and seven years in both the single-PFAS model and in the multi-PFAS model of PFNA controlling for PFOS and PFOA. Thus, BMDs and BMDLs (benchmark dose [lower confidence limits]) for the effects of PFNA on childhood antibody concentrations to diphtheria and tetanus are provided to compare to other PODs but are not advanced further for RfD derivations. Are the modeling approaches for immune endpoints appropriate and scientifically justified, and is the decision to not advance the modeling for derivation of reference values supported?**

ii. **For thyroid effects, with emphasis on results observed in females (results in males were uncertain), the 28-day study in adult rats indicates reductions in serum T4 that are suggestive of an effect but were found insufficient to infer a hazard (see Question 2e). Despite the uncertainties, there is concern for effects given that the T4 reductions in rats from a high confidence study were large in magnitude, and there are concerns for downstream effects on neurodevelopment, which is generally a data gap for this chemical. These concerns were further informed by delays in eye opening observed in developmental toxicity studies in two strains of mice, which is a well-characterized effect of T4 insufficiency although thyroid effects were not evaluated in these studies. Given these results and observations of thyroid effects for other PFAS, PODs were derived for total T4 in adult females for comparative purposes and to inform uncertainty. Is the approach taken for thyroid effects appropriate and scientifically justified, and is the decision to not advance the reductions in serum total T4 in female rats for derivation of a subchronic reference value supported?**

e. **Given the lack of studies on inhalation exposure to PFNA, no RfC is derived. Please comment on this decision.**

Noncancer Toxicity Value Data Selection and Modeling	
Reviewer	**Comments**
Carignan	a. Selection of the 10 epidemiologic studies used in deriving the RfD values (both lifetime and subchronic) for PFNA is scientifically justified. Those selected include the moderate and high quality studies that appropriately adjust for confounding. Those selected were also limited to those with serum PFAS measurements from earlier in pregnancy; therefore minimize potential positive bias of PFAS measurements in serum collected later in pregnancy.

	a.i. The outcome selection of birthweight is appropriate for use in deriving the lifetime RfD. At the population level, lower birth weight is negatively associated with cardiovascular disease and developmental delays. Therefore, is a measure of adversity for population level health effects. a.ii. The modeling and meta-analysis for decreased birth weight based on 10 studies with biomarkers collected early in pregnancy are appropriate for use in derivation of the RfD. The selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for developmental effects are scientifically justified and clearly described. Table 5-9 values (n, beta estimates and POD) in the last row do not appear to match those in Appendix C-17. Please clarify the values used in derivation of the RfD **(Tier 1).** b. The modeling approaches for the liver effects, selection of cutoff for abnormal, selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for hepatic effects are scientifically justified and clearly described. c. The provided scientific rationale supports the decision to consider only these effects for the subchronic RfD. The selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for male reproductive effects are scientifically justified and clearly described. Future studies should investigate chronic effects of PFNA on male reproduction. **(Tier 3)** d.i. For <u>immune effects</u>, the modeling approaches for immune endpoints are appropriate and scientifically justified, and the decision to not advance the modeling for derivation of reference values is supported. d.ii. For <u>thyroid effects,</u> the approach taken for thyroid effects is appropriate and scientifically justified, and the decision to not advance the reductions in serum total T4 in female rats for derivation of a subchronic reference value is supported. e. I agree that an RfC cannot be derived due to the lack of studies. However, note that inhalation exposures can be important, particularly for workers in certain industries. Derivation of an RfC should be a priority, particularly for PFNA and other PFAS with historic and current occupational, product-use, and/or indoor exposures. **(Tier 3)**
Ducatman	d.i This reviewer defers to risk assessors for details. Concerning the major decision points, it appears EPA has made reasonable decisions. There is one **Tier 2** addition suggested. From the questions to reviewers, it is clear that Kim (2023b) is highly relevant to decision making. However, most readers are not going to read the questions to reviewers and the discussion of this key

	reference is buried in the supplemental material. Please consider moving the explanations into the text. e. Based on available data, this is reasonable.
Faustman	a. The USEPA provided thoughtful, documented, transparent and strong justification for their choice of the developmental endpoints to further evaluate. This section 5 discusses human epidemiological studies to support their derivation of the RfD for both the lifetime and sub-chronic values for developmental impacts which included fetal growth restriction and decreased birth weight. From the 26 medium and high confidence studies that they identified, they chose 20 high (10) and medium studies (10) for modeling investigation. They correctly eliminated 3 studies from the original 26 due to limitations in modeling their impacts. They also chose not to use four additional studies that they did not model individually as they did not have statistically significant decreases in birth weight and this reviewer would support this decision. It should be noted however that three of these later studies (Buck Louis et al 2018, Xiao et al, 2019 and Chen et al 2012) were of high or medium confidence and were included in the meta-analysis (Wright et al 2023). Again, this reviewer would support this inclusion. USEPA then prioritized further these studies to focus on those that had measurements of exposure early in pregnancy, those that had consistency across sexes and those that had consistency in dose response across studies. This resulted in a final choice of 3 of the high confidence studies being chosen for individual dose response modeling. Again this reviewer would support this approach given the amazing number of high and medium confidence studies for this endpoint—robust study database. The USEPA also used a meta-analysis for examining continuous PFNA exposure in 27 of the studies and found that "all strata exampled by sample timing and confidence level showed birth weight deficits of 22 grams or larger per ln-unit increase" and this reviewer agreed that these approaches were appropriate and supported this approach for the determination of non-cancer health effects. These were supported further by animal evidence in the form of rodent bioassays with 2 high/medium confidence developmental toxicity studies in two strains of mice. Other animal evidence was available and supportive but was not used for dose-response modeling. The mouse studies revealed primarily dose related decreases in body weight as well as various growth assessments such as developmental delays (eye opening, vaginal opening and preputial separation). All of these endpoints were modeled and supported the evidence for developmental impact after PFNA exposure. The use of both the human and animal developmental endpoints are highly consistent with the well established USEPA guidelines for developmental and reproductive impacts. They are also in alignment with WHO guidance on important development impacts and trajectories that need to be examined for evidence of adverse development impacts. In particular USEPA uses the evidence surrounding early deprivation of visual clues (as observed with delayed eye opening) as an example where it is not just the timing delay but the developmental lifestage trajectories that are significantly altered and need to be considered and tracked for full appreciation of future impacts. These observations and actions are clearly laid out for reviewers to examine in Table 5-1 and 2.

	b. USEPA presents in Tables 5-3 and 4 their choice for human epidemiological studies and animal data for hepatic effects to support POD determinations. Following review of the epidemiological studies, USEPA identified consistent increased in ALT, AST and GGT associated with PFNA exposure. USEPA chose ALT for further investigation as it represented a more hepatic specific response whereas AST and GGT could have had non-hepatic signals. ALT was determined to be at high levels (easily detected) in liver tissues, more easily associated with hepatic versus non hepatic responses and was associated in a dose dependent response with PFNA exposure and hence was identified as a reliable indicator of hepatic injury. Nine medium confidence studies were identified in adult populations with significant results associating PFNA exposure with ALT changes. USEPA further evaluated only the subset of these studies where confounding of the response could be more easily be distinguished from other PFAS related compounds. USEPA explained how they did this and showed studies where the relationship with PFNA exposure was distinguished versus confounding by other PFAS related chemicals in the cohort evaluations—See Appendix section C-1. Three studies met these criteria and they were presented in Table 5-3 (Kim et al 2023, Nian er al 2019 and Cakmak et al 2022). Cakmak included both adults and children however was deemed to be less precise and had larger confidence limits so only Kim and Nian moved forward for POD determination. This reviewer agrees with USEPA decisions for the hepatic endpoint and feels that these choices were made in a transparent and scientifically justified manner. This reviewer also agreed with the approach and decisions made for the animal studies presented in Table 5-4 for modeling and POD determinations. Robust evidence was presented for hepatic injury seen with PFNA exposure in rodent studies` which included six high to medium confidence studies. USEPA chose those studies which were sensitive with the most direct and "consistent and dose-dependent increases" in response after PFNA exposure. Eight studies results were considered for POD determination and the final endpoints modeled for POD included hepatocyte lesions and increased relative liver weight. Sex and species differences were evaluated and studies used for POD determination were used when sex specific responses were consistent. This reviewer felt USEPA did a great job in defining their considerations and review criteria and in presenting a case for their approach and application for hepatic effects PODS determined in rodent studies. c. USEPA was only able to identify rodent studies for this exercise and Table 5-5 in the IRIS report lists the studies and endpoints considered for POD development. USEPA states that "only high/medium confidence" animal studies were consider for their dose response modeling activities and 5 such studies were listed and included both rat and mouse studies as well as studies conducted in adult rats, juvenile rats and one in mice with gestational exposure to PFNA (Table 5-5). USEPA used the NTP, 28-day study in adult rats (NTP, 2018; high confidence for male reproductive organ weights and histopathology, and for testosterone levels but low confidence for sperm counts) for all POD endpoint modeling except the POD determined for preputial separation where they used the Das et al, 2015 developmental study (GD 1-17) in CD-1 mice (medium confidence). Note this later study was one used for determination of POD for developmental toxicity endpoints in rodents. Remaining uncertainty in the length of observation of male

	reproductive impacts in the short 28day study and some mechanistic data examining cell data suggested that effects of PFNA maybe indirect rather than direct effects did confound interpretation of some of these studies but this reviewer felt that Table 5-5 and the specific designation of study endpoints considered for POD development were clear and scientifically justified albeit detailed regarding remaining uncertainties. d.i. For Immune effects the USEPA developed Table 5-6 that illustrated two types of human vaccine responses of antibody concentrations for diphtheria and tetanus across 5 different cohorts from the Faroe Islands. Although emphasis was placed on the un log transformed data presented in Budtz-Jorgensen and Grandjean 2018a (medium confidence studies) these studies were without significant effects of PFNA on antibody concentrations in children aged 5 and 7. The lack of significance prevented this endpoint from contributing to the larger modeling efforts, however the BMDs and BMDLs from this study provided context for other BMD comparisons. This is discussed in Appendix D.1. This reviewer appreciated these comparisons as the importance and significance of immune effects such as alterations in the vaccine response during follow-up has been a relatively consistent finding with children's cohort studies with other PFAS compounds. This reviewer also feels that these case examples should be used for such semi-quantitative comparisons to support Structure Activity Profiling and mechanistic understandings. d.ii. For the thyroid effects of PFNA, the USEPA identified a high confidence 28-day bioassay data in adult rats (NTP, 2018) and described in Table 5-7 observed dose-dependent reductions in serum free and total T4 in females and in serum free T4 in male rodents. They also noted that these observations on thyroid changes may have been complicated due to differences in timing of thyroid measurement and method of assessment of free T4 levels. This NTP study also had significant levels of toxicity at the higher test levels evaluated that contributed to uncertainty in dose-response modeling. This reviewer agrees with USEPA in including this endpoint for POD determinations and comparison due to the mechanistic consistency with other PFAS compounds and also linkage of T4 level changes and effects on growth and brain development. e. This reviewer agrees with USEPA's decision to not develop a RFC for PFNA due to lack of studies. Nevertheless, this reviewer remains concerned that lack of inhalation studies across the PFAS database remains an "missing component" across this large and growing class of compounds. Because this is critical information that is consistently missing I would suggest a **Tier 3 Future Consideration** that inhalation remains a large and highly relevant data gap in the interpretation of risk for PFAS like compounds and as such should put on a priority testing or study evaluation request list.
Georgopoulos	a. The selection of the epidemiological studies for developmental effects that were used to derive the lifetime and subchronic RfD values for PFNA is appropriate and scientifically justified. The Draft Toxicological Review provides a thorough and clear explanation of the approach employed in Section 5.2 and in Appendices C.1 and D.

	Reduced birth weight as an adverse endpoint of PFNA exposures is supported by a large number of epidemiological studies and the focus on biomarkers collected early in pregnancy reduces uncertainties in interpretation. The modeling and meta-analysis for decreased birth weight based on 10 studies with biomarkers collected early in pregnancy is appropriate for use in deriving the RfD. The selection and justification of benchmark response levels, the selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for developmental effects are all clearly described and scientifically justified. b. The modeling employed for deriving an osRfD for hepatic effects, including selection of cutoff for abnormal, selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for liver effects are all clearly described and scientifically justified. The selection of the epidemiological study by Kim et al. (2023) was appropriate. c. The decision to not derive lifetime osRfD values for male reproductive effects was justified as there are no relevant chronic exposure studies available. The information provided in Section 5.2.2 on the scientific rationale supporting the decision to derive only subchronic RfD for these effects, is appropriate and adequate. The selection and justification of benchmark response levels, the selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for male reproductive effects are all scientifically justified and clearly described in the Draft Toxicological Review. d. The modeling approaches for immune endpoints are appropriate and scientifically justified, and the decision to not advance the modeling for derivation of reference values properly reflects the available epidemiological evidence. The approaches for thyroid effects are also appropriate and scientifically justified, as is the decision to not derive a subchronic reference value based on the reductions in serum total T4 in female rats. The derivation of PODs for comparative purposes and for informing uncertainty characterizations is appropriate. e. The decision to not derive an RfC is appropriate and justifiable given the lack of PFNA inhalation exposure studies.
Haney	a. Table 5-18 (p. 5-44) shows that the candidate RfD based on developmental effects was based on EPA's meta-analysis results (i.e., the meta-analysis of 10 early sampling timepoint studies). Consistent with this, for selection of overall RfD the draft states that developmental osRfD is based on a meta-analysis of 10 studies, and that the developmental osRfD is expected to be protective across all lifestages and is based on effects observed in males and females indicating that the overall RfD would be protective for both sexes (p. 5-47-5-48). Thus, the RfD was derived based on EPA's meta-analysis. Since the meta-analysis is based on epidemiology data, consider adding those results to Table D-19 *PODs from epidemiological evidence considered for the derivation of PFNA candidate toxicity values* (**Tier 2**

	suggested revision). Comments on the use of the meta-analysis results for RfD derivation are provided below. For reasons including those cited below for the meta-analysis (in response to 3a.ii) and under Responses 2a (e.g., among other comments contains comments on Savig et al. 2018) and 2b (e.g., among others contains comments on Kim et al. 2023), and consistent with fairly recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021) published well after the key Sagiv et al. (2018) study, I do not agree with deriving an RfD based on the meta-analysis results or either of the epidemiological studies cited above (Sagiv et al. 2018, Kim et al. 2023) examining reduced birth weight and hepatic effects, respectively. Also relevant to "i", generally, while clearly adverse reduced birth weight and hepatic effects can obviously be appropriately utilized as endpoints for toxicity factor derivation and Table 5-8 contains reasoned justifications for decreased birth weight and hepatic effect benchmark response levels (critical effect size), the epidemiology studies of PFAS are confounded by significant limitations (e.g., correlated co-exposures to other PFAS, both quantified and unquantified). Moreover, a confounder(s) *not completely explaining associations* is not a scientific standard making the confounded study reliable for providing an accurate POD for quantitative dose-response assessment and derivation of a toxicity factor, and certainly not a gold scientific standard to be used in chemical assessments held out as such. Correlated co-exposures, both quantified and unquantified (e.g., PFAS both measured and unmeasured in serum), to multiple other chemicals with the same or similar MOAs and endpoints should be considered among study "attributes that would be likely to have a large effect, compared to a small effect, on confidence in the study results" (Section 4.2.1 of the *IRIS Handbook*; USEPA 2022), positively biasing study results and producing mixture effects where the contributions from even the few PFAS analyzed for in serum cannot be accurately adjusted for.[8] The *IRIS handbook* (USEPA 2022) cites concerns about *confounding co-exposures to PFAS specifically* [*emphasis added*]: > *Coexposures* should also be considered as *potential confounders*. *Some exposures tend to be found together in the environment* or in occupational settings *and are highly correlated.* For example*, it might be difficult to distinguish the independent effects from* exposure to specific phthalate or *per- and polyfluoroalkyl substances* in drinking water, isomers of polychlorinated biphenyls in fish, or volatile organic compounds generated by a common source (e.g., benzene, toluene, ethylbenzene, xylene in traffic emissions) *due to confounding by these coexposures.* Confounding of the effect of PFNA exposure by other PFAS seems a certainty in epidemiology mixture studies, and these studies are deficient under EPA guidelines because the potential for bias to explain some of the results is high based on an

[8] For example: (1) Grandjean et al. (2017a) states [*emphasis added*], "*Owing to the intercorrelations* between the serum PFAS concentrations, further analysis of *the possible role of individual PFASs was not pursued*, and *the observed associations may reflect the effects of the PFAS mixtures.*"; (2) Grandjean et al. (2017b) state, "The close correlations prevented meaningful adjustment for concomitant PFAS exposures."; and (3) Budtz-Jorgensen and Grandjean (2018) acknowledge that "an important weakness of epidemiological studies is the mixed exposures" and more than one PFAS may contribute to the lowering of antibody responses.

	inability to rule out residual confounding by key confounders of the exposure-outcome relationship (e.g., see p. 4-21 of the *IRIS Handbook*; USEPA 2022). Consequently, epidemiology studies should not be used as the basis for quantitative dose-response assessment and/or toxicity factor derivation, but rather sufficient animal studies[9] not plagued by the same types of significant limitations and uncertainties as the epidemiology studies should be relied on for RfD derivation (**Tier 1 necessary revision**). This would not only be consistent with the *IRIS Handbook* (USEPA 2022), but also more consistent with fairly recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021). As both ATSDR (2021) and FSANZ (2021) have found the epidemiology literature inadequate for use as the basis of deriving toxicity factors, the PFNA assessment should specifically outline why ATSDR, a federal public health agency of the U.S. HSS/CDC charged with protecting communities from the harmful health effects related to exposure to hazardous substances, is wrong about the epidemiology literature being inadequate for use as the basis for toxicity factors (**Tier 1 necessary revision**). In regard to "ii", use of BMD modeling to the extent possible, guided by standard statistical model fit criteria (+ visual inspection) for model selection, is essentially standard scientific procedure inside (and outside) EPA (e.g., USEPA 2012a). Putting aside all the comments herein on EPA's use of epidemiology studies for RfD derivation, the BMD modeling approaches, BMD model selection process, and BMRs used to derive PODs for developmental effects (other than immune effects in children) appear scientifically justified (e.g., see Table 5-8). However, in regard to BMD modeling for immune effects, I do have comments under Response 3d. While I do not agree with the use of the epidemiological PFAS mixture studies (or associated meta-analyses) for dose-response assessment of birth weight or hepatic effects, consistent with FSANZ (2021) and ATSDR (2021), Table 5-8 of the draft contains reasoned justifications for decreased birth weight (and hepatic) effect benchmark response levels (e.g., a BMR based on 5% extra risk of exceeding adversity cutoff (hybrid approach) for birth weight in humans is a reasoned one). Of note, EPA did not have access to the individual human study data to model decreased birth weight, therefore, the regression coefficients (β) reported in these five individual studies were used to calculate BMD and BMDL values (p. D-13). Section D.1.3 of the draft contains information needed to understand the individual study modeling methods, and Section D.1.4 contains such information for the meta-analysis of human birth weight studies. Also in regard to "ii", consistent with other comments herein, a primary and significant issue in these epidemiological PFAS mixture studies is confounding co-exposures, both quantified and unquantified, and the general lack of adjustment for them. I believe this to be one of the critical issues that preclude use of such studies for identification of a reliable PFAS-specific (e.g., PFNA) POD for toxicity factor derivation. The EPA utilized exposure-response functions quantifying the effects reported in 27 studies as a basis for their meta-analysis (p. C-8), and those

[9] Wherein laboratory animals were exposed to PFNA alone, not PFAS mixtures, demonstrating a PFNA-specific dose-response for an adverse effect (as opposed to a mixture effect) from which a reasonably accurate POD can be derived (e.g., from BMD modeling) for PFNA and that is amongst the most sensitive demonstrated in laboratory animals.

	functions have the same inherent flaw. As such, this seems to be an unfortunate unavoidable critical issue in EPA's meta-analysis of birth weight (e.g., in Appendix C.1.5). It is difficult to envision that a regulatory agency would derive a toxicity factor for a single chemical based on a mixture animal study in which the animals had significant exposure to numerous related chemicals, having both quantified and unquantified confounding exposures. Such a mixture animal study would be excluded (e.g., such studies would be deficient/crucially deficient per p. 4-33 of the *IRIS Handbook*; USEPA 2022) as the resulting POD for a single component of the mixture would be unreliable. PFAS co-exposures should be considered positive confounders as they would be expected to associated with both the primary PFAS being evaluated and endpoint of interest in the same direction as the primary PFAS of interest, biasing results away from the null (considerations on p. C-1, lines 10-16 of the draft). In fact, EPA has cited data from other PFAS across draft assessments as supporting evidence for the potential of the PFAS being evaluated to cause those same effects because PFAS are expected to affect these endpoint(s) in the same direction as the primary PFAS being evaluated (e.g., due to structural and physico-chemical property similarities). Accordingly, confounding of the effect of PFNA exposure by other PFAS seems a certainty in epidemiology mixture studies, and these studies are deficient under EPA guidelines because the potential for bias to explain some of the results is high based on an inability to rule out residual confounding by key confounders of the exposure-outcome relationship (e.g., see p. 4-21 of the *IRIS Handbook*; USEPA 2022). This issue inherently carries over into EPA's meta-analysis. While meta-analysis results provide supportive evidence of an adverse effect on birth weight from maternal exposure to PFNA that should be considered in the hazard WOE, associated results should not be used as the basis for quantitative dose-response assessment and derivation of the RfD[10] (**Tier 1 necessary revision**). By corollary, for reasons expressed in comments on the use of the epidemiology studies and the associated meta-analysis, I also disagree with the high confidence rating for the studies underlying both the lifetime and subchronic RfDs based on a "low overall risk of bias" as well as the medium-high confidence ratings for quantification of the POD_{HED} and overall confidence in these RfDs themselves (see Tables 5-19 and 5-23). Lastly, it is noted that when intrahuman variability in response and toxicokinetics (TD/TK) is accounted for through application of the UF_H of 10, the resulting PFNA serum POD_{HEC} for developmental effects (0.202 ng/mL or µg/L) is well below the 50th percentile for women in the U.S. based on the most recent NHANES data (for every survey year from 2011-2018)[11]. With such obvious public health implications, in the interest of public health and transparency, the draft assessment should acknowledge this (or something similar) and state whether adverse developmental effects are expected to be currently occurring in the U.S. population (**Tier 2 suggested revision**).

[10] For decreased birth weight associated with PFNA exposure, the POD selected from the available epidemiologic literature is 1.81 ng/mL based on the 10-study meta-analysis of high and medium confidence studies with maternal sampling collected predominately during early pregnancy that helped mitigate concerns for potential bias due to pregnancy-related hemodynamic effects (see Appendix C.1.4). (p. 5-23)

[11] Data tables may be found at https://www.cdc.gov/exposurereport/data_tables.html

	b. Again, use of BMD modeling to the extent possible, guided by standard statistical model fit criteria (+ visual inspection) for model selection, is essentially standard scientific procedure inside (and outside) EPA (e.g., USEPA 2012a). Notwithstanding my objections to use of the epidemiological PFAS mixture studies for RfD derivation, the BMD modeling approaches, BMD model selection process, and BMRs used to derive PODs for liver effects appear scientifically justified (e.g., see Table 5-8). In regard to BMD modeling for immune effects, however, I do have comments under Response 3d. While I do not agree with the use of the epidemiology studies for dose-response assessment of hepatic (or birth weight) effects, consistent with FSANZ (2021) and ATSDR (2021), Table 5-8 of the draft contains reasoned justifications for hepatic effect BMRs (e.g., a BMR based on 10% extra risk of exceeding adversity cutoff (hybrid approach) for "minimally adverse" hepatic effects in humans is a reasoned one). As with the individual human birth weight studies, EPA did not have access to the individual-level data that would be necessary to model the data from these studies with standard BMDS-based approaches, so EPA relied on the regression coefficients (β) from the linear regression models to calculate BMDs/BMDLs. Section D.1.5 of the draft contains information needed to understand the various modeling methods for increased serum ALT studies in humans. Lastly, it is noted that when intrahuman variability in response and toxicokinetics (TD/TK) is accounted for through application of the UF_H of 10, the resulting PFNA serum POD_{HEC} for liver effects (0.181 ng/mL or µg/L) is well below the 50th percentile for the U.S. population based on the most recent NHANES data (for every survey year from 2011-2018)[12]. With such obvious public health implications, in the interest of public health and transparency, the draft assessment should acknowledge this (or something similar) and state whether adverse liver effects are expected to be currently occurring in the U.S. population (**Tier 2 suggested revision**). c. The final lifetime RfD should also be protective against adverse effects that may occur due to shorter-term exposure (e.g., developmental, reproductive effects due to subacute exposure). While I disagree with the use of epidemiology studies for RfD derivation in this case, consistent with FSANZ (2021) and ATSDR (2021), examination of the POD_{HED} values for the various endpoints being considered by EPA (e.g., in Table 5-22) shows that there is no need in the context of EPA's judgments to consider these male rat reproductive effects as a possible candidate for lifetime RfD derivation. Use of BMD modeling to the extent possible, guided by standard statistical model fit criteria (+ visual inspection) for model selection, is essentially standard scientific procedure inside (and outside) EPA (e.g., USEPA 2012a). The BMD modeling approaches, BMD model selection process, and BMRs used to derive PODs for male reproductive effects appear justified scientifically or based on standard procedure/ guidance. For example, 1SD is used as the BMR for decreased absolute epididymal weight, given that biological information is not sufficient to identify the BMR (see Table 5-8). Section D.2.1 of the draft contains information needed to understand the modeling methods for animal data,

[12] Data tables may be found at https://www.cdc.gov/exposurereport/data_tables.html

	including male reproductive effects. However, in regard to BMD modeling for immune effects in children, I do have comments under Response 3d. d. In regard to "i", the decision to not advance the modeling for derivation of reference values is supported both by EPA considerations and those contained in my previous comments on the use of epidemiology PFAS mixture studies for POD and toxicity factor derivation, consistent with FSANZ (2021) and ATSDR (2021). Moreover, the modeling approaches for immune endpoints are not appropriate or scientifically justified and therefore do not support advancing the modeling. For example, EPA acknowledges in this charge question that PFNA is not a significant predictor of antibody concentrations in children aged five and seven years in both the single-PFAS model and in the multi-PFAS model of PFNA controlling for PFOS and PFOA (see Tables D-1, D-3, D-5, and D-7 of the draft). Despite Grandjean et al. (2017b) stating that the close correlations prevented meaningful adjustment for concomitant PFAS exposures, Budtz-Jørgensen and Grandjean (2018) attempts to do just that for BMD, and the results appear to help demonstrate the effects of confounding co-exposures and/or the inability to properly adjust for them. Of the many PFAS (and other) co-exposures,[13] only a few were measured in serum for any hope of adjustment. In fact, adjustment for BMD was only attempted for PFOS and PFOA (e.g., see footnote "*" in Tables 1 and 2 of Butz-Jørgensen and Grandjean 2018). Adjustments for only these two confounding co-exposures caused numerous best estimates (i.e., BMD values) for PFNA and other PFAS to go to infinity (∞). Most specific to the draft assessment, Table 1 of Budtz-Jørgensen and Grandjean (2018) reports BMD results for the five PFAS concentrations at age 5 in regard to antibody concentrations at age 7 years, both unadjusted and adjusted for PFOS/PFOA co-exposures. *For the tetanus antibody draft assessment critical effect, when adjusted for PFOS/PFOA two of the three models' (linear, piecewise, conservative) best estimates of the PFNA serum concentrations associated with a 5% change go to infinity* (although BMDLs can still be estimated). The same phenomenon occurs for diphtheria antibody concentrations; that is, *best estimates of the PFNA serum concentrations associated with a 5% change in diphtheria antibody concentrations also go to infinity for two of the three models when adjusted for PFOS/PFOA* (see Table 1 of the study). This points to methods that produce seemingly unreliable results/best estimates. Furthermore, Tables D-1 and D-3 of the draft assessment show that *serum PFNA is not even a significant predictor of antibody concentrations with or without confounding co-exposure adjustment, although an even worse predictor with adjustment and even changing signs from a negative to a positive slope*. The alternative BMD analyses in Tables D-5 and D-7 show similarly bad results. Acknowledging these latter issues, the draft concludes that, "overall, due to the poor fits for all the modeling approaches that were attempted for immune effects, the BMDs and BMDLs presented below were not interpreted as reliable" (p. D-1). Entirely aside from my own detailed comments on BMD modeling issues, Dr. Bruce Allen, apparently a well published pioneer of BMD analysis at EPA who has literally

[13] There are thousands of different PFAS (https://www.epa.gov/pfas/our-current-understanding-human-health-and-environmental-risks-pfas).

	helped EPA write the books on BMD analysis and who headed EPA's workshop on BMD modeling,[14] wrote in public comments on the draft PFHxS IRIS assessment that "the piecewise approach by Budtz-Jørgesen and Grandjean is fundamentally flawed" and that the linear analyses are lacking such that a complete re-analysis is necessary for immunotoxicity BMDs (p. 36 of *Public Comments Received on Draft IRIS Toxicological Review of Perfluorohexanesulfonic Acid (PFHxS) and Related Salts*). These comments apply equally to the present draft assessment. There are few with Dr. Allen's long-standing and extensive BMD expertise and experience, especially including the practice of acceptable BMD modeling methods and procedures at EPA, so there appears to be little doubt that his comments should be heeded (e.g., by those with less experience and expertise in this area, which includes the vast majority of chemical dose-response assessors) to help ensure the scientific defensibility of BMD modeling results being used for any purpose within the draft (e.g., simple comparison). For very important and influential assessments such as these IRIS PFAS assessments (e.g., PFNA) that *will* ultimately drive remediation efforts across the nation, including the treatment/filtration of drinking water and perhaps even the identification of new drinking water sources, surely the effort to thoroughly evaluate the BMD issues through new independent analyses by EPA or others to know that the results ultimately relied upon for some purpose within the PFNA draft assessment are consistent with best practices and fully scientifically defensible (to the extent possible) would be worth it **(Tier 1 necessary revision)**. No scientifically defensible conclusions could be drawn through comparison of results for one endpoint with results for a different endpoint ultimately found to be fundamentally flawed/lacking. Lastly, in regard to BMD modeling, Section D.2.1 of the draft contains information needed to understand the modeling methods for animal data, including thyroid effects (e.g., total and free T4 decreases in female rats; see Table D-20). In regard to both "i" and "ii", it would seem that strictly from a scientific perspective, quantitative analyses should not be carried forward where there is only suggestive evidence of an effect and the available science is insufficient to infer a hazard (applicable to immune and thyroid effects in the present case). Once it has been determined that the scientific WOE for an effect(s) is insufficient, it would seem that dose-response assessment is not then scientifically justified; e.g., the hazard identification step precedes dose-response assessment in the risk assessment process. Concerns about what science might eventually be able to more conclusively demonstrate and how results from future dose-response assessments for those effects might ultimately compare to those for which effects have already been scientifically demonstrated are understandable, but prematurely performing dose-response analyses for such effects may or may not be a fruitful exercise for regulatory scientists. In the event that future data are deemed sufficient to demonstrate a hazard, those different and currently unknown data are likely to form the basis of the future dose-response assessment anyway and better inform the basis of responsible risk communication to the public. Evaluating the "what ifs?" and their potential future health implications in the event sufficient evidence becomes available and is consistent with the current

[14] USEPA's 1996 Report on the Benchmark Dose Peer Consultation Workshop, EPA/630/R-96/011.

	limited evidence could be very time and resource consuming and quite speculatively alarmist in nature. Although EPA provides their reasons, it seems by definition to be pursuing dose-response assessments outside of what the currently available science sufficiently supports. Lowering the scientific bar for carrying adverse health effects through the risk assessment process based on concerns about what endpoints future data may ultimately sufficiently support seems a slippery slope precedent that could lead to a plethora of dose-response assessments for insufficiently supported endpoints and scientifically unjustified calls for regulation based on such assessments. e. Section 5.2.3 (p. 5-57) states that one acute, single-dose inhalation exposure study was identified (Kinney et al., 1989), but it was considered low confidence and provided inadequate evidence to draw conclusions regarding any potential health effects. Although there is a lengthy section in the draft on potential PK approaches (Section 3.1.6), further justification for not deriving an RfC would be provided by a statement (e.g., in Section 5.2.3) to the effect that a validated PBPK model (or acceptable alternative approach) is not available for route-to-route extrapolation of oral toxicity study results to the inhalation route of exposure (**Tier 2 Suggested Revision**).
Leung	This is not my area of expertise, and I defer to the other reviewers.
Lin	a. The selected 10 epidemiological studies for developmental effects used in deriving the RfD values (both lifetime and subchronic) for PFNA are scientifically justified. These 10 studies are of either high or medium confidence studies with consistent sampling timeframe (i.e., early pregnancy sampling with predominately trimester one or early trimester two), which is considered to be highly relevant and also homogenous meta-analytical result to do BMD modeling. There are other human epidemiological studies that were excluded from BMD modeling and RfD derivation, but the exclusion was properly justified. For example, the studies by Lind et al. 2017a, Wang et al. 2016, and Robledo et al. 2015 were not used in the dose response modeling because these studies reported mixed reports across sexes, which were complicated to interpret. The effect selected in these 10 human epidemiological studies for dose response modeling was decreased body weight. This effect is appropriate for use in deriving the lifetime RfD because it is a consistent effect that was observed in many human epidemiological studies and this endpoint also has clearly public health criteria regarding normal birth weight and low birth weight. The modeling and the meta-analysis for decreased birth weight based on 10 human epidemiological studies with biomarkers collected early pregnancy appropriate for use in derivation of the RfD. These 10 selected human epidemiological studies are all of high or medium confidence. The maternal sampling timeframe and the endpoint (decreased birth weight) are consistent across these studies. The data from these studies are suitable for dose response modeling. The selection of the benchmark response levels for developmental endpoints is also properly justified in Table 5-8. In brief, for common developmental endpoints like decreased birth weights, it is recommended to use

	5% extra risk or 5% relative deviation as the BMR based on EPA's Benchmark Dose Technical Guidance (EPA, 2012a). Also for the endpoint of increased offspring mortality, it is reasonable to use 1% extra risk as the BMR because of the severity of the effect. I only have some **Tier 2 – Suggested Revisions** for this charge question: (1) It is said that 10 epidemiological studies for developmental effects were used to derive the RfD values. However, based on Table D-9 and Table D-10, there are only dose-response analysis results from 5 epidemiological studies. Where are the dose-response analysis results for other epidemiological studies for developmental effects? Some clarification would be helpful here. (2) Regarding Benchmark dose-response analyses of the human epidemiological studies for developmental effects, the results are shown in Table D-9 and Table D-10. The plots showing the comparisons of observed versus model-predicted data are not provided. It is suggested to provide plots of the benchmark dose-response analyses so that readers can see how robust are the epidemiological data sources and how good is the goodness-of-fit of the dose-response model. **References:** • EPA, 2012a. Benchmark dose technical guidance. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. • Lind DV, Priskorn L, Lassen TH, Nielsen F, Kyhl HB, Kristensen DM, Christesen HT, Jørgensen JS, Grandjean P, Jensen TK. Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol. 2017 Mar;68:200-206. doi: 10.1016/j.reprotox.2016.08.019. Epub 2016 Aug 31. PMID: 27592399. • Wang Y, Adgent M, Su PH, Chen HY, Chen PC, Hsiung CA, Wang SL. Prenatal Exposure to Perfluorocarboxylic Acids (PFCAs) and Fetal and Postnatal Growth in the Taiwan Maternal and Infant Cohort Study. Environ Health Perspect. 2016 Nov;124(11):1794-1800. doi: 10.1289/ehp.1509998. Epub 2016 Feb 19. PMID: 26895313; PMCID: PMC5089898. • Robledo CA, Yeung E, Mendola P, Sundaram R, Maisog J, Sweeney AM, Barr DB, Louis GM. Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. Environ Health Perspect. 2015 Jan;123(1):88-94. doi: 10.1289/ehp.1308016. Epub 2014 Aug 5. PMID: 25095280; PMCID: PMC4286275. b. The modeling approaches for the liver effects, selection of cutoff for abnormal, selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for hepatic effects are scientifically justified and clearly described. The endpoint of serum ALT in human epidemiological studies was selected as the focus of dose-response modeling. This is well justified on Page 467 (i.e., Page 5-9). Specifically, serum ALT is highly abundant in the liver and liver damage leads to increase ALT levels in serum. Also,

	ALT is normally present at relatively low levels in non-hepatic tissues and increases in ALT levels in serum due to other non-liver diseases are uncommon. Overall, the endpoint of serum ALT is better than other endpoints, such as serum GGT and AST. The focus on the data from the epidemiological study by Kim et al. (2023) is appropriate. This study was considered as of medium confidence. This study was also considered to have a "good" rating in the confounding domain during the study review (Figure 3-40), which means that this study's conclusion is less likely to be confounded by other factors. The selection of cutoff for abnormal is also appropriate. Different cutoff values for abnormal were used for males (42 U/L) and females (30 U/L), respectively. This selection was based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The results of PODs from two different BMRs (10% and 5%) are provided in Table 5-13 and are justified in Appendix D (Page 97). This is appropriate because 10% BMR is commonly used for hepatic endpoints, but a BMR of 5% was also considered because of reported association between severe liver diseases with elevated ALT from the study by Park et al. (2019). **References:** • Park JH, Choi J, Jun DW, Han SW, Yeo YH, Nguyen MH. Low Alanine Aminotransferase Cut-Off for Predicting Liver Outcomes; A Nationwide Population-Based Longitudinal Cohort Study. J Clin Med. 2019 Sep 11;8(9):1445. doi: 10.3390/jcm8091445. PMID: 31514449; PMCID: PMC6780691. • Kim OJ, Kim S, Park EY, Oh JK, Jung SK, Park S, Hong S, Jeon HL, Kim HJ, Park B, Park B, Kim S, Kim B. Exposure to serum perfluoroalkyl substances and biomarkers of liver function: The Korean national environmental health survey 2015-2017. Chemosphere. 2023 May;322:138208. doi: 10.1016/j.chemosphere.2023.138208. Epub 2023 Feb 21. PMID: 36822523. c. The provided scientific rationale supports the decision to consider male reproductive effects only for the subchronic RfD. This is because human epidemiological studies investigating associations between PFAS exposure and male reproductive effects was inconsistent among studies and lack of precision for some studies, and thus not suitable for dose-response modeling. For animal studies, high confidence chronic exposure studies for PFNA on male reproductive effects are not available. However, the 28-day subchronic toxicity study from NTP is a high confidence study and is suitable for dose-response analysis to derive subchronic RfD for PFNA. The use of a BMR of 10% for male reproductive effects is appropriate based on the guidance from EPA on Benchmark Dose Response Modeling (EPA, 2012a). **Reference:** • EPA, 2012a. Benchmark dose technical guidance. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. d.i. For immune effects, the modeling approaches are appropriate and scientifically justified. This is because both the single-PFAS model and the multi-PFAS model

	controlling for PFOA and PFOS and the BMR values of both ½ SD and 1 SD were considered. However, the dose-response analyses did not show statistically significant effects of PFNA on antibody concentrations in children aged 5 and 7 years. Therefore, it is reasonable to not advance the dose response modeling results for derivation of reference values. d.ii. For thyroid effects, it is scientifically justified to do dose-response modeling for data on thyroid effects of PFNA. This is because of several reasons: (1) there is a high confidence study with data that are sufficient to do dose-response modeling for serum T4 in females; (2) the observed change on this endpoint was large, raising a concern on downstream effects on other endpoints, especially neurodevelopmental endpoints; (3) thyroid effects are commonly observed for other structurally similar PFAS compounds. The decision to not advance the reductions in serum total T4 in female rats for derivation of a subchronic reference value is supported by the dose-response analysis results in Table 5-16. Specifically, the results showed that the PODHED values for the reduction effect in serum total T4 in female rats was 1.0-1.3 X 10-3 mg/kg/day, which are approximately four orders of magnitude less sensitive than the developmental PODHED (1.5 X 10−7 mg/kg-day for reduced fetal birth weight). These results support the decision that there is no need to advance the reductions in serum total T4 in female rats for derivation of a subchronic reference value. e. It is justified to not derive a RfC on inhalational exposure to PFNA due to lack of relevant studies. However, this reviewer has **one Tier 3 suggestion** on this charge question. **Tier 3 – Future Considerations:** It is suggested that a PBPK model be developed for PFNA in rodents and humans following different routes of exposure, including oral and inhalational routes. Once this model is available, it will greatly help to derive RfC for inhalational exposure based on toxicity data from oral exposure by conducting route-to-route extrapolation of the target tissue dosimetry using the PBPK model.
Savitz	a.i. The issue of "adverse change" is not straightforward for small variation in birth weight as these studies suggest. As an outcome it has the advantage of being measured with precision and as a continuous measure, provides good statistical power. The usual argument is that small shifts across the population may result in harmful effects in a subset, and that may be true but is unproven. Some influences on birthweight have other adverse effects (e.g., smoking) and other influences do not (e.g., sex, altitude). A chemically-induced reduction in birthweight may be more likely to have other harmful effects that have not been detected and are not necessarily mediated by changes in birthweight. **Tier 3: Future Considerations** – Explaining the logic for interpreting small biological changes as endpoints of health concern could be strengthened by a more detailed discussion. A biological change is not always of pathological consequence, so the judgment to rely on a given endpoint is not just that the research is sufficiently

	convincing to do so but that the endpoint itself constitutes a meaningful indicator of harm. a.ii. Given the precision and consistency of these studies of decreased birthweight, they lend themselves to modeling despite the limitations associated with this endpoint, namely the uncertain clinical significance. The effort addresses the question "if the effect is causal and detrimental" then these are the benchmark doses. No revisions. b. Addressing a benchmark dose based on a totally different endpoint is advantageous, even if the liver effects have limitations of their own. Relying on a single study is always a concern given that any one study is fallible, but there are other supportive studies in the literature. Changes in liver enzymes are concerning, not a disease in their own right, but correlated with the development of disease so that they can be viewed as a sensitive marker of "increased risk." Overall, the evidence for liver effects from various forms of PFAS is strong so that this endpoint ties into a broader literature, which is a strength of this analysis. No revisions. c. No comment since this is outside my area of expertise. d. Ancillary analyses of other endpoints is a good approach, the serious limitations for immunosuppression and thyroid effects notwithstanding. Generating this information across a series of health endpoints with varying strengths and limitations provides a stronger basis for conclusions than arbitrarily choosing one alone, even if the one chosen is the best one. No revisions. d.i. This is a reasonable use of the information and supports the decision not to proceed with modeling for deriving reference values. **No revisions.** d.ii. No comment since this is based solely on the toxicology. e. That is the appropriate response given the lack of evidence.
Zoeller	a.i. First, the Agency was justified in not deriving a reference concentration for inhalation exposures due to the lack of data. The selection of Sagiv et al. (2018) was reasonable in part because of the measures of hemodynamic changes in pregnancy (measurement of plasma albumin to follow plasma volume expansion, and creatinine as a measure of glomerular filtration) and measures of various birth measures (birth weight, gestation duration, preterm birth) that are clear markers of adversity on a population scale. **No Recommendation.** a.ii. This is outside my areas of expertise. b. These modeling approaches are outside my area of expertise. c. BMD models are outside my area of expertise. d.i. These modeling approaches are outside my area of expertise.

	d.ii. The identification of a POD for thyroid effects with emphasis on females is reasonable and scientifically supported. The decision not to advance these measures for derivation of a reference value is also supported. The identification of a POD value of 0.837 mg/kg-day meant that this POD would not drive the establishment of an RfD but that this endpoint would be protected by an RfD based on developmental endpoints. However, see above for further comments. **No Recommendation.** e. This is a reasonable decision. **No Recommendation.**

3.4 In addition, for PFNA, an RfD for less-than-lifetime ("subchronic") exposures is derived. No subchronic RfC was derived. The same studies and outcome used in deriving the lifetime RfD for developmental effects were chosen for use in deriving the developmental subchronic RfD.

a. Please comment on whether the selection of these studies and these effects for the derivation of the subchronic RfD for PFNA is scientifically justified.

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 effect selected is 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. Please comment on the other subchronic osRfDs (i.e., for liver and male reproductive effects).

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

Noncancer Toxicity Value Data Selection and Modeling	
Reviewer	**Comments**
Carignan	a. I agree that the selection of the same studies and outcome used in deriving the lifetime RfD for developmental effects is scientifically justified and that the modeling approaches are appropriate. b. Not applicable. c. The effect selected is appropriate for use in deriving the subchronic RfD, including considerations regarding adversity and the scientific support for their selection. d. The other subchronic osRfDs appear appropriate.

	e. I agree that a subchronic RfC cannot be derived due to the lack of studies. However, note that inhalation exposures can be important, particularly for workers in certain industries. Derivation of a subchronic RfC should be a priority, particularly for PFNA and other PFAS with historic and current occupational, product-use, and/or indoor exposures. (**Tier 3**)
Ducatman	*No response provided.*
Faustman	a. This reviewer believes that these approaches are scientifically justified in the absence of actual subchronic testing or studies available. Of course, this absence is regrettable but USEPA did use current practice and their own guidelines to proceed in this manner. b. This reviewer did not have an alternative approach to use to address this data gap. c. This reviewer supports USEPA and their identification of endpoints for the subchronic RfD. Please see my comments above (related to Chapter 4 and 5) and in the hazard identification chapter**.** Also Table 5-8 very clearly describes the choice of BMR for the various studies. It is consistent and well justified by the methods and references presented in this Table. For each endpoint within the three selected endpoint areas, developmental effects, hepatic effects and male reproductive endpoints the document details which response levels and studies were chosen for the BMR. These choices are made with listings of critical studies supportive of those choices for response. Responses chosen were presented with information on which approaches were used by effect and organ system. This is presented as a well-done concise summary. For Developmental Effects there were sufficient medium-high confidence studies on decreased birth wight for human epi studies to develop a human based osRfD. These studies were examined used a meta-analysis of this endpoint for determining the PFNA RfD. See Table 5-20 for this listing and identification of the osRfD. Related animal studies were supportive but were not as sensitive as the human epi literature and thus were not used to derive the osRfD. d. Section 5.2.2 specifically examines the subchronic oral reference dose derivations and provides excellent context for the assessment and decisions regarding use of these specific effects. For example, this section discusses the decision to not proceed with use of Human Liver ALT for subchronic derivations due to justified pharmacodynamic decisions. The USEPA did proceed with use of the adult female rat relative liver weights (NTP, 2018) as this study examined the relative sensitivity of this endpoint with supportive histopathological evidence. This reviewer agreed with this choice. USEPA appropriately justified their decision to use high confidence endpoints from the NTP, 2018 for the male reproductive effects. The most sensitive POD supported a BMDL at 1 std deviation of 2 x 10-3 mg/kg-day for decreased whole epididymis weight and this reviewer agreed with this choice. e. At the present time this is the only default option available that is consistent with both the biological as well as regulatory guidelines and best practices. This reviewer supports the USEPA in these actions. The rationale given on page 5-1 summary for not proceeding with modeling of the one acute, single dose

	inhalation study is rationale and illustrates the need for further consideration of inhalation as a potential concern for PFAS related compounds**. Tier 3 Future Considerations** USEPA should include placement of comments on the lack of inhalation studies and implications for developing RFDs in Footnote to summary Table 4-1 which highlights cross PFAS compound Hazards.
Georgopoulos	a. The selection of the same studies and outcomes for the derivation of the subchronic Reference Dose (RfD) for PFNA as those used for the lifetime RfD is scientifically justified. These studies provide direct evidence of adverse developmental effects associated with PFNA exposure. Using the same studies ensures consistency and coherence in assessment across different exposure durations. This approach is appropriate given that the adverse effects observed are relevant for both lifetime and subchronic exposures, and reflects appropriate use of justifiable scientific methods. b. Not applicable; see Response 4A. c. As mentioned in Response 4a, using the same outcomes and studies ensures consistency and coherence in assessment across different exposure durations. The adverse effects observed are relevant for both lifetime and subchronic exposures, and the approach reflects appropriate use of justifiable scientific methods to protect public health. d. The derivation of subchronic osRfDs for liver and for male reproductive effects is appropriate and scientifically justifiable. e. The decision not to derive a subchronic Reference Concentration (RfC) for inhalation exposure to PFNA, due to a lack of studies, is appropriate and justifiable.
Haney	a. My comments under Response 3a also apply here. Again, both ATSDR (2021) and FSANZ (2021) have found the epidemiology literature inadequate for use as the basis of deriving toxicity factors. The PFNA assessment should specifically outline why ATSDR, a federal public health agency of the U.S. HSS/CDC charged with protecting communities from the harmful health effects related to exposure to hazardous substances, is wrong about the epidemiology literature being inadequate for use as the basis for toxicity factors (**Tier 1 necessary revision**). b. To reiterate a portion of my comments under Response 3a, confounding of the effect of PFNA exposure by other PFAS seems a certainty in epidemiology mixture studies, and these studies are deficient under EPA guidelines because the potential for bias to explain some of the results is high based on an inability to rule out residual confounding by key confounders of the exposure-outcome relationship (e.g., see p. 4-21 of the *IRIS Handbook*; USEPA 2022). Based on this type of rationale, epidemiology studies should not be used as the basis for quantitative dose-response assessment and/or toxicity factor derivation, but

	rather sufficient animal studies[15] not plagued by the same types of significant limitations and uncertainties as the epidemiology studies should be relied on for RfD derivation, subchronic or lifetime (**Tier 1 necessary revision**). This would not only be consistent with the *IRIS Handbook* (USEPA 2022), but also more consistent with fairly recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021). c. Again, to reiterate a portion of my comments under Response 3a, generally, while clearly adverse reduced birth weight and hepatic effects can obviously be appropriately utilized as endpoints for toxicity factor derivation and Table 5-8 contains reasoned justifications for decreased birth weight and hepatic effect benchmark response levels (critical effect size), the epidemiology studies of PFAS are confounded by significant limitations (e.g., correlated co-exposures to other PFAS, both quantified and unquantified). Moreover, a confounder(s) *not completely explaining associations* is not a scientific standard making the confounded study reliable for providing an accurate POD for quantitative dose-response assessment and derivation of a toxicity factor, and certainly not a gold scientific standard to be used in chemical assessments held out as such. Correlated co-exposures, both quantified and unquantified (e.g., PFAS both measured and unmeasured in serum), to multiple other chemicals with the same or similar MOAs and endpoints should be considered among study "attributes that would be likely to have a large effect, compared to a small effect, on confidence in the study results" (Section 4.2.1 of the *IRIS Handbook*; USEPA 2022), positively biasing study results and producing mixture effects where the contributions from even the few PFAS analyzed for in serum cannot be accurately adjusted for.[16] The *IRIS handbook* (USEPA 2022) cites concerns about *confounding co-exposures to PFAS specifically*. Such issues make the selected effects in the selected epidemiology studies inappropriate for use in deriving the subchronic or lifetime RfD. d. The other subchronic (os) RfDs are based on more reliable laboratory animal toxicity studies. While the total combined UFs are higher than that based on decreased birth weight in humans (Table 5-22), the significant uncertainties associated with that derivation are inherent to the PFAS study type itself and not captured by the total combined UF that only seems to obscure them. Confounding of the effect of PFNA exposure by other PFAS seems a certainty in these epidemiology mixture studies, which should be considered deficient under EPA guidelines because the potential for bias to explain some of the results is high based on an inability to rule out residual confounding by key confounders of the exposure-outcome relationship (e.g., see p. 4-21 of the *IRIS Handbook*; USEPA 2022). Correlated co-exposures, both quantified and unquantified (e.g., PFAS both

[15] Wherein laboratory animals were exposed to PFNA alone, not PFAS mixtures, demonstrating a PFNA-specific dose-response for an adverse effect (as opposed to a mixture effect) from which a reasonably accurate POD can be derived (e.g., from BMD modeling) for PFNA and that is amongst the most sensitive demonstrated in laboratory animals.

[16] For example: (1) Grandjean et al. (2017a) states [*emphasis added*], "*Owing to the intercorrelations* between the serum PFAS concentrations, further analysis of *the possible role of individual PFASs was not pursued*, and *the observed associations may reflect the effects of the PFAS mixtures.*"; (2) Grandjean et al. (2017b) state, "The close correlations prevented meaningful adjustment for concomitant PFAS exposures."; and (3) Budtz-Jorgensen and Grandjean (2018) acknowledge that "an important weakness of epidemiological studies is the mixed exposures" and more than one PFAS may contribute to the lowering of antibody responses.

	measured and unmeasured in serum), to multiple other chemicals with the same or similar MOAs and endpoints should be considered among study "attributes that would be likely to have a large effect, compared to a small effect, on confidence in the study results" (Section 4.2.1 of the *IRIS Handbook*; USEPA 2022), positively biasing study results and producing mixture effects where the contributions from even the few PFAS analyzed for in serum cannot be accurately adjusted for.[17] The *IRIS handbook* (USEPA 2022) cites concerns about *confounding co-exposures to PFAS specifically*, and FSANZ (2021) and ATSDR (2021) found the epidemiology literature inadequate for use as the basis of deriving toxicity factors. e. Section 5.2.3 (p. 5-57) states that one acute, single-dose inhalation exposure study was identified (Kinney et al., 1989), but it was considered low confidence and provided inadequate evidence to draw conclusions regarding any potential health effects. Although there is a lengthy section in the draft on potential PK approaches (Section 3.1.6), further justification for not deriving a subchronic RfC would be provided by a statement (e.g., in Section 5.2.3) to the effect that a validated PBPK model (or acceptable alternative approach) is not available for route-to-route extrapolation of oral toxicity study results to the inhalation route of exposure (**Tier 2 Suggested Revision**).
Leung	This is not my area of expertise, and I defer to the other reviewers.
Lin	a. The selection of the same studies and outcome used in deriving the lifetime RfD for developmental effects and these same effects (i.e., fetal growth restriction) for the derivation of the subchronic RfD for PFNA is scientifically justified. This is because the endpoint of fetal growth restriction or decreased birth weight is mainly attributed to PFNA exposure during gestation (i.e., ≤40 weeks). This exposure duration meets the definition of subchronic exposure in humans. Also, PFAS exposure for these developmental effects occurs during sensitive life stages, i.e., gestational and fetal periods. Therefore, these effects are sensitive enough to be chosen to derive a subchronic RfD. b. Not applicable. c. The developmental effect selected (i.e., fetal growth restriction or more specifically decreased birth weight) is appropriate for use in deriving the subchronic RfD. This developmental endpoint is a high-confidence endpoint as it has been observed in more than 10 human epidemiological studies. There is a clear public health definition on normal versus abnormal birth weight, thus the criterion of adversity is clear. This effect occurs when PFNA exposure is during sensitive life stages, i.e., gestational and fetal periods. This effect is more sensitive than other selected toxicity endpoints, such as hepatic and male reproductive effects.

[17] For example: (1) Grandjean et al. (2017a) states [*emphasis added*], "*Owing to the intercorrelations* between the serum PFAS concentrations, further analysis of *the possible role of individual PFASs was not pursued*, and *the observed associations may reflect the effects of the PFAS mixtures.*"; (2) Grandjean et al. (2017b) state, "The close correlations prevented meaningful adjustment for concomitant PFAS exposures."; and (3) Budtz-Jorgensen and Grandjean (2018) acknowledge that "an important weakness of epidemiological studies is the mixed exposures" and more than one PFAS may contribute to the lowering of antibody responses.

	d. It is appropriate to use the 28-day short-term exposure study from NTP to derive subchronic RfD for hepatic and male reproductive toxicity endpoints. For hepatic endpoints, the observed hepatic toxicities in human epidemiological studies are mainly in adults who have been exposed to PFNA chronically. It is unknown whether the same hepatic toxicities would have occurred in humans at shorter exposure durations. Therefore, the human epidemiological evidence base of hepatic toxicities after chronic exposure cannot be reliably used to derive subchronic RfD. On the other hand, the 28-day exposure study from NTP contains dose-dependent data for hepatic and male reproductive endpoints, and these effects are also supported by other studies in the literature. Therefore, the selection of the NTP study to derive RfD for hepatic and male reproductive endpoints is appropriate. e. The decision to not derive a subchronic RfC on inhalational exposure to PFNA is appropriate due to lack of relevant studies on inhalational exposure to PFNA. Similar to Response 3e, this reviewer has **one Tier 3 suggestion** on this charge question. **Tier 3 – Future Considerations:** It is suggested that a PBPK model be developed for PFNA in rodents and humans following different routes of exposure, including oral and inhalational routes. Once this model is available, it will greatly help to derive RfC for inhalational exposure based on toxicity data from oral exposure by conducting route-to-route extrapolation of the target tissue dosimetry using the PBPK model.
Savitz	No comments since this is outside my area of expertise.
Zoeller	a. The same studies used for lifetime and subchronic RfDs represent the best available evidence currently and should be employed for both. The Review tabulated the various endpoints, study use and model approach to derive these values and clearly justified the use of those studies for both lifetime and less-than-lifetime RfDs. **No Recommendation.** c. The identification of reduced birth weight as an endpoint appropriate for representing an adverse effect of PFNA is well justified scientifically and well-described in the Review. In addition, PFNA exposure was associated with the risk of preterm birth. These endpoints are well-known to be predictive of adversity. **No Recommendation.** d. Please see responses to question 2. e. Yes, this is reasonable.

3.5 Section 3.1 evaluates and synthesizes the PK data in relevant species and sexes, and among human lifestages, up to the derivation of key PK parameters used in the subsequent analysis. Appendix E.1 provides a statistical analysis of PK parameters in male and female rats and mice while differences in clearance between male and female humans as a

function of lifestage are evaluated in Section 3.1.4 (subsection Excretion in Humans). However, the evaluation of existing physiologically based pharmacokinetic (PBPK) models and a classic PK model described in Appendix E.4 found that these options were not sufficiently reliable for use.

For PODs derived from laboratory animal studies, given the information available on potential interspecies differences in PFNA PK and the results of comparing PK model predictions to bioassay data (E.4.1), EPA concluded that a hybrid approach for extrapolation of POD values in animals to estimate corresponding human equivalent doses (HEDs) was the best option in the derivation of the respective RfDs. Specifically, distinct approaches are proposed for estimation of internal doses in male and female rats from the NTP bioassay vs. estimation for mice examined in developmental studies:

- **PFNA serum concentrations measured at the end of the NTP bioassay were algebraically interpolated to estimate internal dose POD (PODint) values for the applied dose PODs identified from that study. The interpolation for male rats assumed a linear increase in serum concentration over the 28-day study, whereas that for female rats assumed the average concentration is close to the end-of-study value.**
- **For endpoints from mouse developmental studies (including results in nonpregnant females from those studies), the PK model was used to estimate the PODint values. Specifically, the average serum concentration calculated from the time of mating until the day of observation for each endpoint was used to provide metrics consistent with the dosing regimen (gestation only) and endpoint evaluation at late gestation vs. multiple postnatal times.**
- **The estimated human clearance (CLH) was used to convert the PODint values from these animal experiments to PODHED values. Likewise, for PODint values that are human serum concentrations identified from epidemiological analyses, CLH was used to calculate the corresponding PODHED.**

Please comment on the following:

a. **Are the methods for calculating PODint values for PFNA for endpoints in rats (adult animals) vs. mice (adult females and pups) scientifically justified for conversion of PODs from animal toxicity studies to HEDs? If not, please provide an explanation and detail on a more appropriate approach.**

b. **Is the use of maternal clearance (in women of reproductive age) to calculate HED values for gestational and early postnatal endpoints appropriate and scientifically justified? If not, please provide specific alternatives for extrapolation of these endpoints.**

c. **Are the selected values of CLH¬, specifically the 95% lower CI of the geometric mean from Chiu et al. (2022), 0.090 mL/kg-day for males of all ages and females below 12.4 and above 40 years of age, and 0.124 mL/kg-day for women 12.4-40 years of age (Subsection: Total clearance in humans), appropriate and scientifically justified?**

d. **Is application of CLH to estimate PODHED values from PODint values (from animal or epidemiological studies as summarized above) scientifically justified? If not, please provide an explanation and detail on a more appropriate approach.**

e. **Have the uncertainties in the PODint estimates for animal studies and CLH been adequately evaluated and clearly described?**

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors	
Reviewer	**Comments**
Carignan	a. The methods for calculating POD_{int} values for PFNA for endpoints in rats (adult animals) vs. mice (adult females and pups) are scientifically justified for conversion of PODs from animal toxicity studies to HEDs. b. It remains unclear the degree to which higher clearance observed among women of reproductive age can be attributed to hormonal differences vs elimination pathways via pregnancy, childbirth and breastfeeding. Therefore the use of maternal clearance to calculate HED values is not sufficiently protective, and application of male clearance rates would assure protection of nulliparous women and their offspring. I urge IRIS consider nulliparous non-menstruating women in this assessment, as women who have menstruated little or not at all can conceive **(Tier 2)**. Examples include the many instances of teen pregnancy and the many women on hormonal birth control (which can limit or stop menstruation) who conceive shortly after birth control cessation. c. The 95% lower CI of the GM protects more of the population than application of a GM or upper 95% CI, however protection of the full population requires selection of the lowest observed human clearance value **(Tier 2)**. Additionally, see response 5b. d. I agree that application of CL_H to estimate POD_{HED} values from POD_{int} values is an appropriate and scientifically justified approach. e. The uncertainties are adequately evaluated and clearly described. I agree with NJDEP suggestions for improvement in clarifying the more prominent sex difference in excretion for rats compared to mice and the notable short-term elevation among infants **(Tier 2)**.
Ducatman	This reviewer is not an expert in derivation of POD. The approach seems mostly reasonable. **Discussion about a detail.** The reviewer did experience some uncertainty concerning the rationale for the reference to PFDA in the introduction to these 3 questions. (Calculations concerning PFDA in human populations have to account for nondetects and instability in detects at consistently low levels above detection thresholds. The survey cycle changes in national contaminant reporting for PFDA illustrate the instability in my view. The reviewer is not expert in POD derivation, but does question the relationship of PFDA data to more useable PFNA data in any circumstance where human data are also part of the question. The actual decisions made by EPA appear to this reviewer to not be based on assumptions concerning female kidney clearance as potentially implied by the reference to ages 12.4-40 in the quotation above.

Tier 1: EPA has already been advised that general details of its comparisons are not defensible (such as male-female differences limited to ages 12.4-40 as stated in the question).

Since I do not think EPA ultimately made any important decisions based on these problematic assumptions (the relevant points of departure in the document are not based on liver, and the liver), I think conclusions are likely justified and only the scientific assertions need to be addressed. However, I am not a risk assessor so I leave for EPA what they need to correct for the application of risk assessment. I am reasonably sure that parts of the underlying science are wrong.

Additional Reviewer Response: Please see the previous discussion about male-female differences in PFAS and PFNA as depicted in the literature vs in this document. The same topic returns here.

Quotiong from the section "excretion in humans"

> *"However**, empirical data on serum concentrations of multiple PFAS in women of reproductive age versus men do not indicate a consistent difference that would be expected for menstrual clearance, which should be independent of the specific PFAS**." (p 3-20 of the draft)*
>
> *While Jain and Ducatman (2022) observed lower PFNA levels in women versus men 20–48 years of age, **the difference was only 25%–30%, much less than would be predicted based on these urinary clearance values.** (p.3-21 of the draft)*

Tier 1: The bolded information is not correct.

The reviewer is sympathetic to and interested in the search for effects that might be other than menstruation, pregnancy, and lactation. (I have provided some data to the literature that do support a possible role for estrogen which is independent of menstruation, pregnancy, and lactation (hypothesized to be on renal clearance) (Jain & Ducatman, 2023). However, multiple statements in the EPA text – to the degree that they are intended to reflect on the role of menstruation - are inconsistent with EPA statements in the text and instead consistent with a role for menstruation. Nor are they supported by the additional logic presented by EPA, nor is it high probability to hypothesize that menstrual sections might not excrete PFAS given what we know about protein binding as well as the actual age-year comparisons. Unless EPA has data not presented, the language can be reconsidered. In the quotation above, there is an unsupported statement that says or implies that menstruation is not part of the picture because the differences should be larger based on renal data. It makes no sense. Please see the earlier discussion.

Tier 1: In the two quotations above, the second statement is partially incorrect and fully confusing. The ages of PFNA differences between males and females are already visible at age 12, not 20 (see text of (Jain & Ducatman, 2022) and pasted figure below), just as expected for a role of menstruation The reason for skepticism is unclear. **Tier 1 suggestion:** What can EPA say about this that is both clear and consistent with the literature?

- Furthermore, the text as quoted implies uses a logic which appears to rely on a perceived difference in the sex comparisons for PFNA vs the other legacy compounds which is not fully justified. (At least, it is not fully justified by the cited

	reference which does compare men and women year by year with sizable numbers for PFNA (Jain & Ducatman, 2022). Taking into account the a priori differences in serum concentrations, PFNA is much like PFOA and PFOS and PFHxS. **Tier 1**: Please remove (or better clarify with data so far not presented or language that is clearer about intent and justification) the inference that PFNA is different from other PFAS for sex-by-age comparisons. It is similar. Additional discussion. In the year-on-year comparison of males vs females (from age 12-75) (Jain & Ducatman, 2022), trajectories of PFOA, PFNA, PFHxS, ns PFOS were not identical, in particular they had different starting points, but they were sufficiently similar (especially given the serum concentration differences which also exist among PFAS species in humans), that the quoted statements seems unjustified. Further, the male-female differences are dynamic by age-year and not static, of course. At peak, the serum differences between men and women in the cited document are **very close to 2-fold, and not "only" 20-30%, as EPA has stated**. The peak age and the entire picture is about what one might expect from the trajectories of menstruation, breast feeding and lactation on a low concentration contaminant with a long half-life. **Tier 1**: please alter the "only" 20-30% reference. The adjective is unwarranted even if the comparison were correct (it isn't). The actual difference is nearly 2x at the expected peak age of difference. (The differences seen cold be explained wholly or at least mostly on menstruation, pregnancy, and lactation and their respective trajectories over the reproductive lifespan). That does not mean we should not search for other contributors. It does mean that EPA should be careful about what it is trying to say. **Tier 1**: Is EPA sure it wants to say that a role for menstruation in the serum concentrations differences is uncertain? If it is sure, perhaps it could do a better job explaining why it feels that way. (My thought is that it is not defensible. . It does not look to me that PFNA is different, and EPA explanations for downgrading a role of menstruation do not stand up to scrutiny). To avoid adjectives, the comparison figure follows: Please note that among the four PFAS compared, PFNA is the only PFAS with a starting serum concentration <1.0 ng/mL, and the author thinks all four compounds show about the same thing when differences in starting points are considered.

GRAPHICAL ABSTRACT

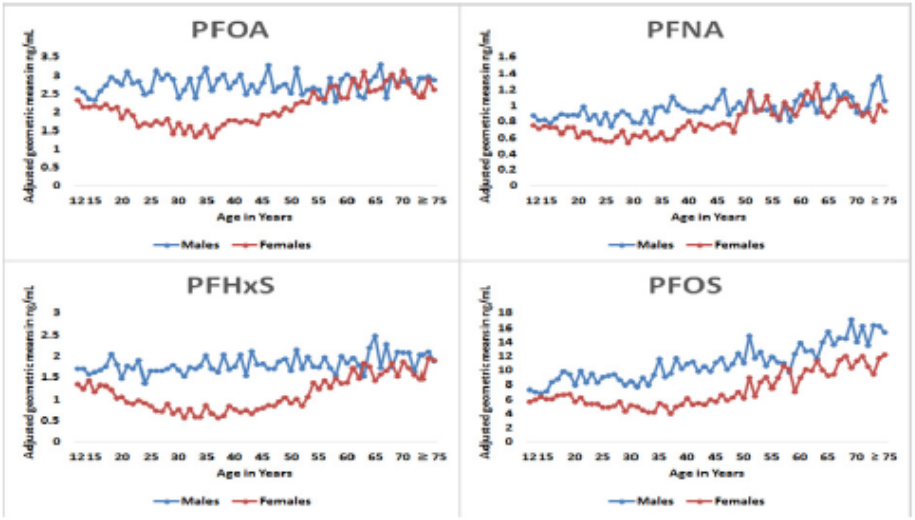

Image from (Jain & Ducatman, 2022). More detailed images available in the reference's text.

Quoting from the document

> *"A consistent, meaningful difference in serum levels in men versus women was not found for **PFDA** although the differences reported by Jain and Ducatman (2022) for PFHxS (not shown) and for PFNA (see Figure 3-1) were effectively replicated." (from p. 3-24)*

Reviewer comment: This statement is confusing. How do PFDA data contradict visible (and now replicated, thank you!) PFNA data? The weak argument by analogy to an unstable comparison (PFDA) does not have a justifiable basis and just as easily applies to all PFAS, and appears wrong in all cases. The four compounds addressed in Jain and Ducatman (2022) deliberately did not include PFDA, and there were specific reasons for PFDA exclusion. We could not say much given the rates of non-detection and mostly low serum concentrations (not a broad range, many values barely above detection). However, In a small study that did consider PFDA, the expected male-female differences were seen in a Polish population, (Goralczyk et al., 2015). Thus, it is at least possible that the difference is still detectable when sought, yet the larger point is that the numbers do not really permit the comparison on which EPA has laid so much weight. **Tier 1**: Omit the quoted statement or else align it with data which more reliably address its content.

Quoting from the document

> *"Hence, while the differences between men and women reported by Jain and Ducatman (2022) for some PFAS indicates a sex- and age-dependent factor, **it appears unlikely that menstrual clearance is the mechanism" p.3-25***

	"Koponen et al. (2018) performed a longitudinal study in children between 1 and 10 years of age and estimated the body burden of PFNA and other PFAS based on the measured serum concentration and body mass at 1, 6, and 10–11 years of age. A noteworthy finding is that the body burden of females over this age range appeared to remain fairly constant while that in males increased steadily, although the body burden of the males and females at 1 year of age were quite similar. ***This result indicates that a sex-related factor other than menstruation, which applies even before menarche, leads to differences in the body burden of males and females.*** *"* P.3-27 *"When estimating biological half-lives Zhang et al. (2013c) included a rate of menstrual CL = 0.029 mL/kg-day in younger women, which is* ***only qualitatively supported*** *by observations of* ***slightly*** *lower serum PFNA levels in women of childbearing age versus men (Jain and Ducatman, 2022)."* **Reviewer comment:** The bolded parts of the quotations are not justified. I am supportive of the search for alternative explanation. I even published a paper on the topic and proposed a hypothesis for an estrogen effect most visible in post-menopausal estrogen takers. However, that in no way suggests that we cannot see a clear role for menstruation, and it is frankly physiologically unlikely that there would not be such a role. The very visible corroborative data in NHANES (apparently replicated by EPA) are not consistent with the bolded statements. Other larger data sets similarly **do** support a difference in PFOA and PFOS before age 12 in boys vs girls in serum concentrations (Frisbee et al., 2010) (see Table 1), and that is based on thousands of children, just as anticipated for a role of menstruation, which has a range of onset. The distribution of age of menarche, which averages at around age 12 (more precise estimates provided elsewhere in this review) is a likely contributing cause. Obviously, there is an age range of menarche. (This difference in the age of menarche is famously presented at a personal level in a coming of age novel which has been adapted for film.) All young women do not begin menstruating on the median age date. (There may be other causes than the very well-accepted causes of sex differences attributable to menstruation, pregnancy, and lactation, and I too am really interested in this, but it is really far out on a limb to dismiss menstruation as a contributing cause based on the data EPA has presented.) Further, EPA menstruation conclusions drawn by EPA from Koponen et al are overstated and not defensible in my view. Their measures are relevant to events before the age of menarche, and do not do a good job of addressing the role of menstruation, and in no sense rule out a role for menstruation with its variable onset. Please review the actual data presented. If EPA thinks the Kaponen data present an opening for some additional role of sex differences, that can be posed as a research question. But the data do not address menstruation. The range of PFDA concentrations (with nondetects) do not say much about PFNA (and possibly PFOA and PFOS and PFHxS?). This is a very slender branch on which to hang a lot of concern, and the other publication on the same topic for the same unstable data somehow did find a sex difference. The quoted statement is currently problematic (except to the degree that pregnancy and lactation also explain the differences). It is indeed

	possible that estrogen or something else plays a role, yet dismissing menstruation and stating that differences are "small and qualitative" in this way is surprising and not consistent with the actual data. They are not "small" as percents, we do not expect the differences to appear full-fledged at the commencement of menarche nor to end precisely on the date of menopause, and the only way they can be characterized as small is based on the original small internal dose. They are impressive as a % delta from that dose, and they are fully consistent with menarche to menopause effects. . **Tier 1 revision:** The bolded language in the quotation can be reconsidered or better defended. Quoting from the EPA Document concerning a confusing/potentially misleading sequence: *"When estimating biological half-lives Zhang et al. (2013c) included a rate of menstrual CL = 0.029 mL/kg-day in younger women, which is only qualitatively supported by observations of slightly lower serum PFNA levels in women of childbearing age versus men (Jain and Ducatman, 2022). However, EPA's analysis did not find a meaningful, consistent difference in serum levels of **PFDA** in men versus women, **although a difference was confirmed for PFNA** (see Figure 3-1), which one would predict if menstrual clearance were a nonspecific mechanism. **Hence EPA does not consider that specific route of excretion to be supported by the overall empirical data for PFAS**. Therefore, the qualitatively best estimate of total CL in humans appears to be the result of Chiu et al. (2022), as it does not rely on uncertain estimates of fecal excretion that in turn rely to some degree on extrapolation from laboratory animals (and from other PFAS)."* (from EPA p. 3-28) **Reviewer comment: It is not clear what route of excretion is not supported. If it is meant to indicate that menstruation (or pregnancy or lactation) is not supported, that is neither consistent with the data nor with the logic presented.** **Tier 1:** There are confusing statements here, and they require either clarification of different, better, defensible evidence. In the cited actual evidence in humans, the difference between men and women at the peak age of difference (age 37, as one would expect if menstruation were an important contributing factor) **is a calculated 1.11 ng/ml in men vs 0.59 ng/mL in women for in the cited document which contains this information (and has apparently been replicated by EPA in its own work.) T**hat difference is greater than but consistent with differences in surrounding age/years. So, that is either "slight" as cited by EPA (since any difference when the baseline is 1.11 ng/mL is going to be < 1.11 ng/mL), or else more realistically **at this peak age of sex difference age women have about 53% (just over half) of the serum PFAS as age-matched male counterparts**. (It is just data. EPA is entitled to call a nearly 2x difference "slight" if that is its standard, but then it should be explicit that an almost 2x peak difference is what is meant by "slight" in its view, and it should explain what is "qualitative" about the replicated year-on-year differences.) **Tier 1**: Revise the language to communicate whatever point EPA is trying to make without the exaggerated and insupportable language that accompanies the point. • Further discussion: In the several citations, it is also unclear to the reviewer why estimates about **fecal excretion** have anything to do with direct comparative data

	between men and women in NHANES (or any other similar reliable dataset if such work can be found in other data sets). Rather, the direct comparisons between sexes are agnostic about excretion routes (and all the data from this and other sources are consistent with vaginal menstrual effluent). They do not measure anything in menstrual fluid or in stool, but they are fully compatible with menstruation as a source of the differences and inexplicable if the differences are about stool concentrations unless there are parallel postulates about sex differences in stool concentrations that happen to go along with the reproductive cycle of menstruation. PFAS travel brilliantly in blood/blood components and are bound to protein. Phlebotomy decreases serum PFAS. Menstruation is probably a smaller protein source than phlebotomy, but it is a great source of excreted proteins (Yang, Zhou, Prinz, & Siegel, 2012) over a longer time period and far more frequent. **Tier 2**: EPA should want to clarify why it has discussed fecal excretion more clearly. **Tier 1:** EPA should provide a rationale for why the discussion reflects on menstruation, or edit revise the quoted language so its point can more easily be followed by the reader. Personally, I think it could indeed ultimately be found that some non-mechanical difference such as an estrogen effect on renal excretion may modulate (and likely dampen) the sex differences. I have provided a paper which supports that possibility (Jain & Ducatman, 2023), However, the hypothesis is as yet not substantiated and in no sense contradicts a (much larger) role for menstruation in sex differences. The advice to EPA is that staking out this turf detracts from the document, unless it can be better explained/defended. Quoting from the document *For children, results in Figure 3-1 indicate that girls at age 12 have CL similar to boys the same age*. (p. 3-47) **Reviewer comment and suggestion:** For the cited paper The statement is defensible for the data points at age 1 and 6 and is only marginally defensible age 10) **and not for age 12** where there are no data presented (in the paper cited, but there is a difference in NHANES at age 12 and in the C8 Health population). In NHANES data, the sex divergence is visible at or before age 12, as one would expect. (The divergence widens until around age 37. Excretion by menstruation is iterative, and in general amounts increase and then decrease during reproductive years.) The actual data in NHANES showing divergence at or around the average age of menarche and persisting at or past the average age of menopause. These data are fully consistent with menstruation, and unfortunately, with lactation, and pregnancy as contributing causes, and do not rule out other causes. **Tier 1**: Revise or remove an inaccurate statement. Serum PFAS are already beginning to show sex differences at age 12. The cited paper includes data at age 1 and 6 which are compatible with the statement, and by age 10 it is already a question. In other work, there is a clear difference at age 12. If EPA wants to provide the precise comparison, for example as a ng/mL with corresponding %- or fold-difference at any age, that is doable and would represent an improvement.

	Quoting from the document about the science, and trying to understand EPA's perspective on a related topic. *Zhang et al (2013c) reported blood or serum and morning urine concentrations of PFNA for two demographic groups: (1) women ≤50 years of age (n = 16); and (2) men (all ages) combined with women >50 years of age (n = 50). This grouping arises from a presumption that urinary clearance in men is not age dependent, while it is in women, and that urinary clearance in older women is similar to men*." Quoted from P.3-45) Discussion: Is EPA really endorsing that we know that there are sex dependent changes in urine excretion that are independent of renal function? This is possible, but is there a reference that has sufficient data to support the statement? The Zhang reference does not have information to sufficiently support this innovative statement. One assumes, consistent with Zhang et al. (2013) that urinary clearance is not strictly first order, and thus will reflect some differences in internal doses that are incompletely amenable to simple adjustments. In Zhang (2013) et al., the differences, while hard to interpret (based on an insufficient number of women in the very broad age group of <50) do not provide additional clarity about sex differences in the lifespan related to menstruation, pregnancy, and lactation). (What can be seen in this small 'n' and brad age-range comparison is compatible with sex differences due to menstruation, lactation, and pregnancy.) Further, there are far more transparent comparisons between men and women, many done more recently than 2013, in far bigger data sets. If EPA has evidence from any of these studies in which the comparison is possible that supports rejecting/minimizing a role of menstruation in sex differences, it should present it.
Faustman	a. As mentioned in the background for this question and in text in Section 3.1, text entitled "Approach for pharmacokinetic modeling and extrapolating PFNA between rats, mice and humans" (starting page 5-26) and in Appendix E Detailed Pharmacokinetic analyses, the USEPA could not identify a validated human PBPK model. In addition, based on their evaluation of clearance of PFNA across species the USEPA reported observations of underprediction of human clearance using allometric scaling and the BW3/4 power. In support of their decision to look at alternatives USEPA reviewed both one and two compartment models and used Bayesian based models for Inference. The lack of an acceptable PBPK model for PFNA has also been a common issue for other PFAS compounds. USEPA looked at other approaches to calculate HEDs and they defined a "hybrid" approach. This reviewer felt this decision was well justified and clear (albeit the reader had to synthesize this information from two different chapters and from Appendix E). A **Tier 3 Future Consideration** would be for EPA to provide a synthesis of these findings across the various sections. For example, some of the conclusions from Appendix E could be shown in a Table that is also shown in the section for linking the POD to HEC in chapter 5. b. This reviewer felt that USEPA provided a very thorough discussion of the various factors in women across the lifestage that would impact clearance. This included examples for menstruation, kinetics throughout gestation and breast milk concentrations as factors that resulted in clearance impacts. By considering these

	various factors that contributed to lifestage differences in clearance they conservatively examine where their assumption in clearance would over or underestimate their estimations of population averages. Their approach was well documented and justified in application. c. The USEPA provided analysis that showed that "..distributional estimates form Chiu et al 2022 indicate[d] that CL values for some individuals may be 0.545mL/kg-day, 6 times higher than 0.09mL/kg-day, and by extrapolation CL values for young women may be as high as 0.744 mL/kg-day, the risk to high CL individuals will be less than estimated using the 0.09mL/kg-day." Thus a conservative, protective approach was presented that to this reviewer was consistent with USEPA guidelines and was aligned with overall goals of the pharmacokinetic investigations. (Note that quote was taken from conclusions on Page 3-47, summary of human clearance). d. Yes, this reviewer reviewed the overall document and specifically the sections specified in Section 5 that address these estimations and accepts USEPA's approaches. USEPA describes in great detail how the animal-to-human extrapolation of internal dose PODs to HEDs was conducted using hybrid PK approaches for all endpoints and specify on Page 5-27 the conditions of this application. They used internal doses (ie average serum concentrations) for endpoints obtained in both male and female mice using the PK model, internal doses in rats from "measured end-of -study concentrations" (see Appendix 4.1) and calculated HEDs from internal dose PODs using "lifestage appropriate values of human CL." Equation 5-1 on Page 5-27 describes the equation for calculating HEDcl. e. This reviewer feels that the uncertainties in the PODint estimates have been described in the overall document however the separation of the PK discussions in Section 3 and 5 and in Appendix E make it challenging for the reader of the document to ensure tracking and clarity for all steps and staging of the modeling. Tables 5-11 to 5-14 were useful in this portion of the review. This reviewer agrees with USEPA and their decisions in these portion of the report however has **a Tier 3 future considerations** to consider how to improve and integrate summaries from all these sections into a more easy and trackable format.
Georgopoulos	a. The methods for calculating POD_{int} values for PFNA for endpoints in rats (adult animals) versus mice (adult females and pups) for conversion of PODs from animal toxicity studies to HEDs appear reasonable and scientifically justified. The Draft Toxicological Review presents a thorough evaluation and assessment of PFNA pharmacokinetics (PK) in Section 3.1 and Appendix E. Since the evaluation of existing Physiologically-Based Pharmacokinetic (PBPK) models and a classic PK model, presented in Appendix E.4, concluded that these options were not sufficiently reliable for use, the Toxicological Review adopted a hybrid approach for extrapolation of animal POD values to estimate corresponding human equivalent doses (HEDs) in the derivation of the respective RfDs. For rats, PFNA serum concentrations measured at the end of the 28-day NTP study were interpolated to estimate internal dose POD (POD_{int}) values for the applied dose PODs identified from that study. The interpolation for male rats assumed a linear increase in serum concentration over the course of the study, and for female rats assumed that the average concentration is close to the end-of-study value. For mouse developmental studies a single-compartment PK

	model was used to estimate the POD_{int} values. Estimated human clearance (CL_H) values were used to convert the POD_{int} values from the rodent experiments to POD_{HED} values. For POD_{int} values that are human serum concentrations from epidemiological analyses, CL_H was used to calculate the corresponding POD_{HED} values. b. The use of maternal clearance in women of reproductive age to calculate HED values for gestational and early postnatal endpoints is appropriate and scientifically justified. c. The selected values of CL_H are appropriate and scientifically justified: the information provided in Section 3.1.4 of the Draft Toxicological Review adequately supports this selection. d. The application of CL_H to estimate POD_{HED} values from POD_{int} values (from animal or epidemiological studies) is scientifically justified since a valid and reliable PBPK model is not currently available for PFNA. e. Sections 3.1 and 5.2 of the Draft Toxicological Review (as well as Appendix E) provide adequate information on the uncertainties in the POD_{int} estimates for animal studies and in CL_H. **Note:** As mentioned above, the Draft Toxicological Review presents a thorough evaluation and assessment of PFNA pharmacokinetics (PK) in Section 3.1 and in Appendix E. Understanding and quantifying PFNA pharmacokinetics is a very challenging enterprise, as there are multiple uncertainties associated with processes involving protein binding, species/sex-dependent expression of renal transporters, tissue distribution of bound versus free PFNA, etc. PFAS typically display a biphasic elimination pattern, that is prominent in humans and other primates, with a rapid decline in an initial (alpha) phase and a slower decline in a second (beta) phase, associated with two half-lives, that, however, do not imply that there is a "universal" set of two values for their biological half-lives for a given species/gender. Furthermore, PFAS kinetics can involve saturable processes, with high interspecies and interindividual variabilities, that may induce nonlinearities in situations of high exposures (or significant co-exposures with other PFAS that have common molecular targets). Though classical 1-compartment and 2-compartment PK models can typically fit data from specific studies, robust interindividual and interspecies extrapolation requires a valid Physiologically-Based Pharmacokinetic (PBPK) model. The Draft Toxicological Review correctly concludes that there is currently no reliable PFNA PBPK model that can be used for this purpose; in fact, specific errors in existing PBPK models are identified and discussed in Section 3. However, the assessment provided for the model of Kim et al. (2019) and summarized on page 3-36 of the Review, appears to oversimplify the issue. Specifically, on page 3-36 (lines 23-35) of the Draft Toxicological Review it is stated: *"The conclusion of this analysis is that a key assumption on which the PBPK model of Kim et al. (2019) and most other existing PBPK models for PFAS are based, that distribution to tissues and clearance are strictly limited to the fraction unbound in blood (fub, as measured in vitro) is incorrect. While it is possible to relax this condition and fit specific parameters to obtain a rate of distribution to match the empirical data, EPA considers such an approach to effectively undermine a*

	fundamental mechanistic assumption of these models in the absence of independent data to demonstrate the cause of the discrepancy. For example, if an independent experiment showed that the original estimate of fub was too low and is in fact close to that obtained by empirical fitting, the assumed model structure would be corroborated. But otherwise, the result of empirical fitting distribution parameters is considered by EPA to be the equivalent of using a classical PK model for which the volume of distribution (and rate of distribution to a "tissue" compartment) is empirically fitted, which is the option evaluated further below and described in detail in Appendix E.1" However, estimating the value of a particular unknown parameter in a PBPK model from time-course biomarker data (typically via a statistical optimization method) is not "the equivalent of using a classical PK model for which the volume of distribution (and rate of distribution to a "tissue" compartment) is empirically fitted." Parameters in PBPK models have specific physical/biological meanings, and their plausible values are constrained by physics and biology. If there are data supporting the claim that the "unbound fraction assumption" used by existing PBPK models for PFAS, including the (very useful) "model template" developed by EPA (Bernstein et al., 2021), is "incorrect" (in the sense that it is not a reasonable approximation), these data should be included in the Toxicological Review. If such data are not available, efforts to develop them for multiple PFAS should be a high priority in the field. Another series of statements that seem to contradict each other appears in the discussion of menstruation as a clearance mechanism in humans. The Draft Toxicological Review reasonably decides that (page 3-25, lines 13-14): "menstrual clearance as a specific mechanism will not be evaluated further as a clearance pathway for PFNA." Indeed, the uncertainties involved in quantifying this clearance pathway, in conjunction with the relative magnitude of its contribution to total clearance, support EPA's decision. However, various other statements in the Review are phrased in a manner that appears to contradict this decision. For example, on page 3-20, lines 12-14, it is stated that: "Excretion in menstrual fluid has also been estimated to be a significant route for women because urinary excretion is extremely low." As another example, on page 3-24, lines 11-12, it is stated that the results of Jain and Ducatman (2022) "might indicate that menstrual fluid loss contributes to PFAS clearance in females of childbearing age." Though the discussion that follows the latter statement in the Draft Toxicological Review (pages 3-24 to 3-27) brings up the multiple uncertainties associated with assessing the contribution of menstruation to PFNA clearance, the overall rationale for not attempting to quantify this pathway should be stated more clearly. **Suggested Revisions and Future Considerations** ***Tier 1 Necessary Revision:*** EPA should clarify the statement made on page on page 3-36 (lines 23-35) regarding the validity of assumption of "most existing PBPK models" that PFAS distribution to tissues and clearance are strictly limited to the fraction unbound in blood. ***Tier 1 Necessary Revision:*** EPA should thoroughly edit Section 3.1 as well as Appendix E, to eliminate statements that are too "strong" or contradict each other. For example, the

	rationale underlying the decision to not include menstruation as a pathway in the derivation of PFNA clearance metrics should be explained in a more coherent manner. *Note:* As part of the editing recommended above, EPA could avoid the use of terms such as "human children" (page -3-12, line 11; page 3-32, line 26) and "human women" (Appendix E, page E32, line 10). ***Tier 2 Suggested Revision:*** EPA should evaluate and discuss the consistency of human pharmacokinetic parameters derived in the Draft Toxicological Review with the time-course human data and pharmacokinetic parameters reported in the study by Yu aet al. (2022) • Yu, C. H., Weisel, C. P., Alimokhtari, S., Georgopoulos, P. G., & Fan, Z. T. (2021). Biomonitoring: a tool to assess PFNA body burdens and evaluate the effectiveness of drinking water intervention for communities in New Jersey. International Journal of Hygiene and Environmental Health, 235, 113757.
Haney	a. Section 3.1.6 of the draft walks the reader through EPA's various efforts to evaluate the models/methods available for PK extrapolation, also referring to Appendix E. The draft assessment documents EPA taking a reasoned approach, doing the best job they can with the data/methods that are available and selecting the best supported options amongst those available for a given extrapolation (as cited in the charge question above). As such, the draft appears to document the justification needed for these PK extrapolations. However, I ultimately defer to reviewers with more expertise in this area (e.g., PBPK modelers) to better evaluate and answer this question, which also applies to subsections b-d below. b. This appears to be a reasoned approach. For example, the draft indicates that the human maternal clearance for women of reproductive age was used for the corresponding endpoints since their blood concentrations will be determined by that clearance level (p. 5-28, lines 11-14). c. The draft provides scientific reasoning to support these selected values (pp. 3-28 to 3-39). d. As indicated on p. 5-27 of the draft, HEDs are calculated from the corresponding internal dose PODs (i.e., POD_{int} values) using lifestage appropriate values of human CL (CL_H). As such, this appears scientifically justified. e. Uncertainty in the estimated clearance in experimental animals and humans is discussed in detail in Sections 3.1.7 and 3.1.8 of the draft, respectively, and discussed further on pp. 5-28 to 5-33. This includes uncertainty in the estimation of CL_H (e.g., for humans the uncertainty in the sex- and lifestage-specific clearance values is judged to be less than a factor of 3; p. 5-28, lines 32-33) and uncertainties in the POD_{int} estimates for animal studies (e.g., see the summary on p. 5-30, lines 22-31).
Leung	This is not my area of expertise, and I defer to the other reviewers.

Lin	a. The methods for calculating internal dose POD (POD_{int}) values for PFNA for endpoints in rats (adult animals) vs. mice (adult females and pups) are, overall, scientifically justified for conversion of PODs from animal toxicity studies to HEDs. This is mainly because better approaches, such as a well-validated PBPK model, are not available. Existing PBPK models for PFNA in rodents are of high uncertainty and have significant weaknesses as discussed in Section 3.1.6. Also, the use of distinct approaches for different species and different sexes enables to utilize the most confident data for each species and each sex to derive POD_{int} to calculate HED. To be more specific, in male rats, the PFNA serum concentrations measured at the end of the NTP bioassay were algebraically interpolated to estimate POD_{int} for the applied dose PODs from that study based on the assumption of a linear increase in serum concentration over the 28-day study period. This assumption is OK because the lower doses used in the NTP study (i.e., at doses where corresponding PODs were identified and hence from which HEDs needed to be estimated) were only 0.625 and 1.25 mg/kg/day, which likely fall into the linear range of PFNA PK profile and have not reached saturable PK range yet based on the measured plasma and liver concentration data in male rats from the NTP study (NTP, 2018). However, it should be noted that at higher doses, it is possible that the PK of PFNA may reach saturation, where linear algebraical interpolation from study doses to higher doses is not reasonable. In female rats, the assumption that the average concentration is close to the end-of-study value by the end of the 28-day period is also OK because the average half-life of PFNA in female rats is 2.77 days (Table 3-2), and after 28 days of repeated daily exposure, it is equivalent to repeatedly dose the animals for 10 half-lives, and the serum concentration of PFNA should have reached a steady-state level. It is appropriate to use the PK model developed by EPA to estimate the POD_{int} values in mice. The PK model for PFNA in rats and mice developed by EPA was described in detail in Appendix E.4.1. The results showed that the model adequately predicted the measured serum concentrations of PFNA in pregnant mouse dams, lactating mouse dams, fetuses/pups, and non-pregnant females. The differences between observed versus model-predicted values were generally within 2-fold, which is considered acceptable accuracy. It is appropriate to use POD_{int} values that are human serum concentrations identified from epidemiological analyses to multiply CL_H to calculate the corresponding POD_{HED}. It is also OK to use this approach to convert the POD_{int} values from animal experiments to POD_{HED} values. However, it is important to note that by using the same approach for both animal POD_{int} and human POD_{int}, it is assumed that the same internal dose in animals and humans would result in the same toxicity response. This assumption has an inherent uncertainty due to potential differences in pharmacodynamics between animals and humans. This assumption should be clarified when describing this approach (**Tier 2 – Suggested Revision**; Page 91).

In addition, I have the following general comments on the pharmacokinetics and target tissue dosimetry for EPA to consider in the future (**Tier 3: Future Considerations**).

1 One of the scientific gaps of PFNA is a lack of a well-validated PBPK model for PFNA in rodents and humans following oral and inhalational exposures. EPA has a team of excellent scientists who have experience in PBPK modeling research. It is suggested that EPA scientists develop a PBPK model for PFNA to support risk assessment of this chemical in the future.

2 In this risk assessment document, the pharmacokinetic extrapolation is based on plasma concentration data and plasma clearance. However, target organ dosimetry may be more accurate than plasma data in risk assessment because the target organ is the site where a toxicant exerts an adverse effect. In the literature, there are studies that measure the plasma and liver concentrations of PFNA after oral exposure (e.g., NTP, 2018). However, there are few studies that investigate the distribution and accumulation of PFNA in other target organs, such as thyroid and reproductive organs. The lack of time-dependent concentration data of PFNA in individual target organs, such as thyroid and reproductive organs represents a significant data gap for PFNA. It is suggested the EPA conduct a tissue distribution study of PFNA in rodents of both sexes to investigate the sex-dependent TK and distribution of PFNA to multiple target organs to full characterize the whole-body distribution profiles of PFNA.

Reference:

- NTP (National Toxicology Program). (2018). 28-day evaluation of the toxicity (C04049) of perfluorononaoic acid (PFNA) (375-95-1) on Harlan Sprague-Dawley rats exposed via gavage [NTP]. http://dx.doi.org/10.22427/NTP-DATA-002-02655-0003-0000-3.

b. It is appropriate to use a maternal clearance (in women of reproductive age) to calculate HED values for gestational and early postnatal endpoints. However, the description of the human maternal clearance for women of reproductive age is not clear. This reviewer only sees one paragraph describing the human maternal clearance (Page 486, Lines 9-18). Is this human maternal clearance equivalent to the clearance for females of age 12.4-40 years, which is 0.124 mL/kg/day (Page 77)? Probably not based on the description on Page 80. Further clarification is needed to clarify this point.

Tier 2: *Suggested Revision*: *please clarify the calculation and the final value of the human maternal clearance.*

In addition, menstruation is an important factor in the total clearance of several other PFAS (e.g., PFHxS), but this document concluded that menstruation is not an important mechanism of the total clearance of PFNA. Please further clarify why menstruation is an important factor of the total clearance for several other PFAS, but not for PFNA (**Tier 2: Suggested Revision**).

Tier 3: Future Consideration: To determine the potential impact of menstruation on the total clearance, is it possible to calculate the total clearance by assuming

	menstruation plays an important role in the total clearance, and then compare the value with that when assuming menstruation does not play a significant role? c. The selected value of 0.090 mL/kg/day for CL_H in males of all ages and females below 12.4 and above 40 years of age and the other selected value of 0.124 mL/kg/day for women 12.4-40 years of age are appropriate. These two values were both justified based on existing studies. Specifically, the value of 0.090 mL/kg/day for males of all ages and females below 12.4 and above 40 years of age was based on a rigorous population PK study from Chiu et al. (2022). This value was based on the lower 95% CI estimate of the geometric mean. This is somewhat conservative, but it is OK because the goal of this risk assessment is to derive a human health-protective value. The value of 0.124 mL/kg/day for women 12.4-40 years of age was based on EPA's analysis of serum PFNA data from NHANES. Physiologically, it is OK to use two clearance values for these two different subpopulations since women 12.4-40 years of age have distinct hormonal regulation and adolescent growth. However, one suggested revision here: **Tier 2: *Suggested Revision***: *One Page 77, it is said that the value of 0.124* mL/kg/day was based on EPA's analysis of serum PFNA data from NHANES, but in Table 3-3, the reference for this value is Chiu et al. (2022). *Please clarify this or correct it if needed.* **Reference**: • Chiu WA, Lynch MT, Lay CR, Antezana A, Malek P, Sokolinski S, Rogers RD. Bayesian Estimation of Human Population Toxicokinetics of PFOA, PFOS, PFHxS, and PFNA from Studies of Contaminated Drinking Water. Environ Health Perspect. 2022 Dec;130(12):127001. doi: 10.1289/EHP10103. Epub 2022 Dec 1. PMID: 36454223; PMCID: PMC9714558. d. It is appropriate to use POD_{int} values that are human serum concentrations identified from epidemiological analyses to multiply CL_H to calculate the corresponding POD_{HED}. It is also OK to use this approach to convert the POD_{int} values from animal experiments to POD_{HED} values. However, it is important to note that by using the same approach for both animal POD_{int} and human POD_{int}, it is assumed that the same internal dose in animals and humans would result in the same toxicity response. This assumption has an inherent uncertainty due to potential differences in pharmacodynamics between animals and humans. This assumption should be clarified when describing this approach (**Tier 2 – Suggested Revision**; Page 91). e. The uncertainties in the POD_{int} estimates for animal studies and CL_H have been extensively discussed and adequately evaluated. However, the description could be further improved with a suggested revision below. **Tier 2: *Suggested Revision***: *The ranges of PK parameters, including clearance, are clearly provided for each sex of rats and mice in Table 3-2. However, for humans, only a fixed value is provided for each subpopulation in Table 3-3. It would be better to provide the range of clearance values for different subpopulations of humans in Table 3-3 (Page 83).* *I also have the following suggested revisions for Section 3.1.*

Tier 2: *Suggested Revisions:*

1 On Page 84, it is said that "The conclusion of this analysis is that a key assumption on which the PBPK model of Kim et al. (2019) and most other existing PBPK models for PFAS are based, that distribution to tissues and clearance are strictly limited to the fraction unbound in blood (fub, as measured in vitro) is incorrect." Can EPA clarify why this is incorrect in this risk assessment document?

2 On Page 58, the sentence "In contrast to these previous results, Taibl et al. (2023) report higher serum levels of PFNA in first (GM = 0.37 ng/mL) and third trimester (GM = 0.41 ng/mL) women than first trimester women (GM = 0.26 ng/mL), …". Is there a typo in this sentence? Perhaps "in first (GM = 0.37 ng/mL)" should be "in second (GM = 0.37 ng/mL)"?

3 In Table 3-2 (Page 64), why some of the reported mean values are not within the range calculated from the Bayesian analysis? For example, in male rats, from Tatum-Gibbs et al. (2011) at 3 mg/kg, the reported half-life was 23.6 (20.0-27.8), but this is outside the Bayesian range of 36.34 (29.08-43.41) day.

4 Page 92, Line 18, there is a typo in this line.

5 Page 95, Line 18, "0.0.9" should be "0.09".

6 The PK model developed by EPA (Schlosser, 2024) is important in this risk assessment. The reference information is provided in HERO (https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/11374400). It would be better if the author also provides the model code in the HERO site. This will enhance transparency and reproducibility.

References:

- Schlosser, P. 2024. Draft Model Code for the Classical Pharmacokinetic Modeling and Alternate Dosimetric Analyses of PFNA in Support of the IRIS Toxicological Review. US EPA. HERO ID: 11374400.
- Kim SJ, Choi EJ, Choi GW, Lee YB, Cho HY. Exploring sex differences in human health risk assessment for PFNA and PFDA using a PBPK model. Arch Toxicol. 2019 Feb;93(2):311-330. doi: 10.1007/s00204-018-2365-y. Epub 2018 Nov 27. PMID: 30483840.
- Tatum-Gibbs K, Wambaugh JF, Das KP, Zehr RD, Strynar MJ, Lindstrom AB, Delinsky A, Lau C. Comparative pharmacokinetics of perfluorononanoic acid in rat and mouse. Toxicology. 2011 Mar 15;281(1-3):48-55. doi: 10.1016/j.tox.2011.01.003. Epub 2011 Jan 13. PMID: 21237237.
- Taibl KR, Liang D, Dunlop AL, Barr DB, Smith MR, Steenland K, Tan Y, Ryan PB, Panuwet P, Everson T, Marsit CJ, Kannan K, Jones DP, Eick SM. Pregnancy-related hemodynamic biomarkers in relation to trimester-specific maternal per - and polyfluoroalkyl substances exposures and adverse birth outcomes. Environ Pollut. 2023 Apr 15;323:121331. doi: 10.1016/j.envpol.2023.121331. Epub 2023 Feb 20. PMID: 36813097; PMCID: PMC10023492.

Savitz	a. No comment since this is outside my area of expertise. b. To the extent that there is evidence that clearance differs among reproductive age women or particularly, during pregnancy, use of those specific values is preferable to general population averages. No revisions. c. This seems like a reasonable choice. d. This seems like a reasonable choice. e. I'm not able to comment on this due to lack of expertise.
Zoeller	This is outside my area of expertise.

3.6 EPA has evaluated and applied, where appropriate, UFs to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL (lowest-observed-adverse-effect level to no-observed-adverse-effect level) extrapolation (UFL) for PFNA. For a–f below, please comment on whether the uncertainty in the derivation of the candidate and selected toxicity values is scientifically justified and clearly described.

a. Please comment specifically on whether the methods used to derive toxicity values for PFNA appropriately account for uncertainties in pharmacokinetics, including accounting for differences between the experimental animal data and humans.

b. For developmental effects, a UFA of 1 was used since the value was based on human data. A UFS 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). Uncertainties with regard to additional susceptible lifestages (e.g., other early-life developmental stages) are addressed as part of the UFD. Does the provided scientific rationale support this decision? If not, please explain.

c. For liver effects and derivation of the lifetime osRfD using human studies, a UFA of 1 was applied as the liver effects were reported in epidemiological studies and the value was based on human adult data. Does the provided scientific rationale support this decision? If not, please explain.

d. For liver effects and derivation of the subchronic osRfD using animal studies, a value of 3 is applied to extrapolate between effects in laboratory animals and in humans during the derivation of the subchronic RfD. Although PPARα dependence might support a value of UFA = 1 for hepatotoxicity if that were the sole pathway leading to these effects, evidence for the involvement of non-PPARα pathways is available in the PFNA database. Thus, uncertainty remains regarding the potential differences in sensitivity across species because of the involvement of both PPARα-dependent and PPARα-independent mechanisms. As such, the Toxicological Review concludes the available data are not adequate to determine whether humans are likely to be equally or less sensitive compared to laboratory animals with respect to the observed liver 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 and Derivation of Toxicity Values is clearly documented.

e. **For liver and male reproductive effects, a value of 10 is applied for the UFS when extrapolating from 28-day animal data to a subchronic exposure. Considering the potential for some health effects (decreased reproductive organ weights and sperm measures, liver enlargement and concurrent effects) to worsen with increasing duration and the large uncertainty associated with the lack of existing or reliable chemical-specific data to evaluate the effects of subchronic exposure on liver and male reproductive outcomes, respectively, the Toxicological Review concludes that application of a UFS of 10 is supported for the purpose of deriving the subchronic RfD from the 28-day toxicity data. Does the provided scientific rationale support this decision? If not, please explain.**

f. **Are the provided rationales for the remaining UF values (UFL, UFD, UFH) scientifically justified and clearly described (to inform the UFH, the assessment evaluates and considers the available evidence on potential susceptibility to PFNA within different populations or lifestages, including any potential impacts from early-life exposure to PFNA on lifelong health, although few studies on susceptibility were available)? If not, please explain.**

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors	
Reviewer	**Comments**
Carignan	a. The applied UFs appropriately account for uncertainty in pharmacokinetics, including differences between the experimental data and humans. b. The provided scientific rationale supports UF decisions for developmental effects. c. The provided scientific rationale support this decision to apply a UF_A of 1. d. The available animal and mechanistic studies support the conclusion to apply a UF_A of 3 and the analysis presented in the Toxicological Review and Derivation of Toxicity Values is clearly documented. e. The provided scientific rationale support the decision to apply a value of 10 for the UF_S. f. Rationales for the remaining UF values are scientifically justified and clearly described.
Ducatman	a. The work is straightforward and strong in the areas where a clinician can follow it, with the exception of the consistent and unsupported dismissal of a role for menstruation. I do not think this ultimately affected calculations, but am not expert. In the sections leading up to the Classical PK modeling section (begins on p.3-40) and in this section, EPA has done an excellent job presenting a difficult topic.

	Discussion: There is one reviewer suggestion for additional clarity. In the section on page 3-33 concerning urinary clearance vs glomerular filtration (also very well done), please consider this **the things that affect excretion that are not well-accounted by the PBPK model, but which are important to health studies nevertheless. As follows:** • **Tier 2:** Consider a brief description about the inverted U-shaped curve that describes the relationship between eGFR in humans and serum PFAS as well as its likely explanation **Discussion:** the explanation is hypothesized to be: gradually impaired excretion at healthy eGFR to early stages of impairment, a smaller association that affects a larger population, and then as state 3a kidney disease is reached, we see a failure of reabsorption at more impaired eGFR, leading to decreased reabsorption. The document states that there is no known explanation of the inverted U-shaped curve seen by multiple investigators (and it does cite some of them), citing the work of Conway et al (2018, see p. 3-385) for the absence of explanation. The document is misleading for this citation because other investigators have provided a probable explanation that is compatible with both human and experimental evidence (a small effect on excretion in most of the population at stage 3a or better, a larger effect on failure to reabsorb thereafter, affecting a smaller but critical population since those with kidney disease are often the participants of concern). In addition, the inconsistency noted by EPA is less inconsistent than the document states. Once the inverted U-shaped curve and the effect of albuminuria are understood, the studies with different outcomes are mostly explained by whether they were designed to consider these now known reverse-causal associations. The cited study and other studies by Conway et al is an example. The data as presented in the Conway studies are not explained in the studies, but are easily explained in whole or in large part by the inverted U-shaped excretion curve and the large presence of albuminuria in diabetics. Why consider this? There are two reasons, and they affect different types of studies. In general, this is potentially important because of overestimation sources of bias in the large part of the population that has relatively normal eGFR and the corresponding need to adjust when pursuing certain health outcomes. Equally as important or perhaps more important, is the potential for underestimation bias in the downward slope of serum PFAS for eGFR at-or-worse-than Stage 3b kidney disease. The increasing failure of renal reabsorption of PFAS and the corresponding lower serum PFAS in this circumstance of moderate to severe kidney failure (most often caused by diabetes, hypertension, and less common causes such as lupus nephritis) can lead to underestimation of outcomes for cross-sectional associations to these and their commonly associated diseases. The subpopulations with these

	outcomes are a smaller population than the larger population with more normal eGFR, but understanding this problem of failure of reabsorption/enhanced excretion/lower serum PFAS is critical for understanding disease outcomes that travel with adversely altered eGFR such as diabetes, hypertension, and lupus. Any albuminuria has the same effect, to a greater degree, leading to lower serum PFAS and underestimation bias for outcomes. (NCI included these considerations in its approach to PFAS and kidney cancer. They are relevant to outcomes of the diseased kidney in general) • **Tier 2.** Please be cautious with language such as on p 3-385 and anywhere else that the document implies that the excretion curve is not understood. There is always more to learn yet there is an emerged consensus that is probably (not certainly but probably) explained by slight decreased excretion effects in a large population with normal kidney function or mild kidney failure as eGFR worsens, followed by enhanced PFAS excretion as eGFR progresses to moderate kidney disease or includes any albuminuria. • **Tier 3:** Please consider discussing eGFR considerations and albuminuria considerations as a source of bias, variability, and potentially misleading inferences. **Discussion**: Adjustment alone will not solve this problem for some types of studies. The inverted U-shaped curve is not particularly amenable to simple adjustment. The presence of albuminuria may mean we are adjusting for the outcomes we care most about. Addressing these complex but probably understood topics requires innovative designs including stratification and especially premorbid longitudinal data. Long-term longitudinal data are important because proteinuria will be associated with lower serum PFAS and also be strongly associated with disease outcomes of interest such as diabetes, hypertension, autoimmune diseases, and the various complications/diseases associated with these outcomes. This is a very direct cause of underestimation bias in specific disease settings – anything that has kidney disease as an outcome or any disease that is a common cause of kidney disease. If EPA elevates **the Tier 3 suggestion** to the current document, the likely place to add this information is in section 3.1.8 (current p. 3-45), under uncertainty and in the section about urinary (3.2.1.0). These complex excretion issues do indeed add uncertainty to human studies in very specific situations. Further, ignoring the physiology can lead to erroneous conclusions. (An example among authors cited in this document by EPA is a series of papers proposing that PFAS are good for the kidney because those with kidney disease had lower serum PFAS. Reverse causation was not appreciated.) The discussion of "urinary" outcomes beginning p. 3-383 could be more completely informed by the modern understanding of the effect of renal disease on PFAS excretion (inverted U-shape and implications) and albuminuria causing lower serum PFAS. The existing discussion overestimates inconsistency in studies and underestimates the potential for causal associations to kidney

	disease. (Simple adjustment, present in many of the studies discussed, will underestimate associations to important disease states such as diabetes and diabetes complications, or hypertension and hypertension complications. This is likely part of the explanation for some of the variability noted by EPA.) d. The reviewer is not qualified to discuss points of departure and other aspects of Risk assessment. No important problems were seen in terms of foundation in health data. In addition, **concur** with EPA that PPAR-alpha is not the only relevant pathway. A minor question. If it is a good question, it can be considered a **Tier 2** consideration. I am not expert in applications of risk assessment but do have a question about downgrading PPAR-alpha for PFAS human liver noncancer outcomes. The reason to ask is this - Downgrading PPAR-alpha is <u>not</u> necessary to consider other mechanisms as equally plausible or more plausible mechanisms. PPAR-alpha response pertains to human liver and is considered relevant to noncancer outcomes (Kersten & Stienstra, 2017). **A Tier 2** question posed to EPA is if they think that some of the findings appear to also be consistent with a different PPAR, PPAR-gamma upregulation, as suggested by several articles (for example in (Chen et al., 2023; Evans et al., 2022)). (Why ask this question? On p 3-183 EPA expressed uncertainty about a role of PPAR-gamma for PFNA hepatic effects. Is the data really that inconsistent? This includes in accompanying tables.) e. **This topic is best addressed by risk assessment experts on the panel. I did not see health based problems. BTW:** the discussion of cutoff variability on p 5-42 is succinct and excellent as a basis of uncertainty. (it recurs on p. 5-44. It is on target in both places.) f. **This is left to risk assessment experts on the panel. I did not see health based problems**.
Faustman	a. The document describes the methods and decision review in Section 5 starting with Derivation of Candidate Toxicity Values for the Oral Reference Dose (RfD) page 5-39. Included in this section are tables like Table 5-17 that describes the uncertainty factors and justification for application that aligns with USEPA guidelines. The details and rationale in these tables were excellent and provide easily accessible, summarized review of these decisions. b. Table 5-18 provides justification for the uncertainty factors for lifetime RfD value development. This reviewer agreed with the use of UFa of 1 for UFa as a "susceptible lifestage" and with the selection of UFd of3 for developmental effects due to the lack of specific studies for many important effects in fetuses (like thyroid toxicity, neurobehavioral toxicity, mammary gland development and mutigenerational effects, etc) were missing. c. Yes, this reviewer believes that this use of the UFa as appropriate and in-line with agency guidance.

	d. This reviewer looked at the text and in particular information provided by Tables 5-22 and 5-23. Yes, this reviewer agrees with these choices. Evidence is described on page 5-51 lines 17 to 38 that suggests that both PPARa dependent and independent pathways are mechanistically involved in the hepatic toxicity observed after PFNA exposures. This is consistent with other PFAS compounds and hint at more human relevant pathway involvement. e. This reviewer is in complete agreement with the use of the UFs of 10 for this application. It is scientifically justifiable, consistent with agency policy guidelines and compatible with a protective context. f. This reviewer does not have any modifications to suggest. Use of these uncertainty factors is well done, consistent with agency guidelines.
Georgopoulos	a. The methods used in the Draft Toxicological Review to derive toxicity values for PFNA appear to appropriately account for uncertainties and interspecies differences in pharmacokinetics. b. The scientific rationale supporting the decision to use a UFA of 1 for developmental effects due to PFNA exposure is appropriate, since the analysis is based on human data. Since uncertainties associated with additional susceptible life stages are addressed as part of the UFD, this approach reflects an informed balancing of available scientific evidence with the need to protect public health. c. The scientific rationale supporting the decision to use a UFA of 1 for derivation of the lifetime osRfD for liver effects from PFNA exposures is appropriate, since the analysis is based on human data. d. The available animal and mechanistic studies presented in the Draft Toxicological Review support the selection of an interspecies uncertainty factor UFA=3 for the derivation of the subchronic RfD for hepatotoxicity, reflecting involvement of both PPARα-dependent and PPARα-independent mechanisms. The Review reasonably concludes that available data are not adequate to determine whether humans are likely to be equally or less sensitive compared to laboratory animals with respect to the observed liver effects and that a value of UFA = 3 is warranted to account for the residual uncertainty in toxicodynamic differences across species. e. The application of a UFS of 10 for the purpose of deriving the subchronic RfD from extrapolating 28-day toxicity data is scientifically justifiable. The UF accounts for the fact that multiple health effects (decreased reproductive organ weights and sperm measures, liver enlargement and concurrent effects) are expected to worsen with increasing exposure duration; furthermore, there are large uncertainties regarding the effects of subchronic exposures on hepatic and on male reproductive outcomes. f. The rationale provided in the Draft Toxicological Review for the selection of UF values (summarized in Table 5-17, on page 5-41, for lifetime RfD estimates and in Table 5-21, on page 5-50 for subchronic RfD estimates) appears to appropriately

	account for uncertainties associated with LOAEL-to-NOAEL extrapolation, deficiencies in databases, and intraspecies variability.
Haney	a. Yes, it appears that the methods used to derive toxicity values for PFNA appropriately account for uncertainties in pharmacokinetics (e.g., see the discussion in *Analysis of uncertainty in the pharmacokinetic extrapolation for PFNA*, as well as Sections 3.1.7 and 3.1.8). In regard to human interindividual variability specifically, as EPA points out (p. 5-29), this is a matter of uncertainty in the application of the estimated HEDs when predicting risk for the most sensitive individuals, and is addressed by application of the UF_H. EPA applies a full UF_H of 10 for interindividual variability in humans in the absence of quantitative information on potential differences in pharmacokinetics and pharmacodynamics of PFNA exposures in humans (e.g., see Table 5-17). b. Yes, for this derivation, a UF_A of 1 and UF_S of 1 are consistent with EPA practice/ guidance and reason. As stated in Table 5-17, a UF_D of 3 was selected for developmental effects due to deficiencies in the developmental database, but a value of 10 was not selected given the availability of data from well-conducted studies across a range of health outcomes in multiple species, including sensitive evaluations of developmental endpoints in humans per EPA (see Table 5-17 and the associated text for more details). Thus, the UF_D value is justified in the draft and appears consistent with EPA practice/guidance. c. This is entirely appropriate, consistent with EPA practice/guidance, and needs no further justification than, "the developmental and liver effects are reported in epidemiological studies" (Table 5-17). d. Table 5-21 indicates that, "A UF_A of 3 is applied to the experimental animal evidence to account for uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between mice or rats and humans following oral PFNA exposures. Some aspects of the cross-species extrapolation of pharmacokinetic and pharmacodynamic processes have been accounted for by using pharmacokinetic data, modeling and DDEF approaches to extrapolate internal doses in rodents to serum levels in humans. However, residual uncertainty related to potential pharmacokinetic and pharmacodynamic differences remains." While future mode of action/mechanistic data may reveal a UF_A of 3 to be conservative for these effects, EPA justifies this value based on the currently available data and it is not overly conservative to account for the cited associated uncertainties (see Table 5-21 and associated text). e. Given the lack of data to better inform selection of a UF_S value (see p. 5-52), this selection appears reasonable and consistent with EPA practice/guidance. f. Justifications for these UF values are provided in Table 5-17 and 5-21 and associated text, and appear consistent with EPA practice/guidance.
Leung	This is not my area of expertise and I defer to the other reviewers.

Lin	a. The methods used to derive toxicity values for PFNA appropriately account for uncertainties in pharmacokinetics, including accounting for differences between the experimental animal data and humans. These methods are described in Section 5. The toxicity values that were derived include point of departure (POD), human equivalent dose (HED), and reference dose (RfD). The POD values may refer to the lower end of the benchmark dose, and may also include no-observed-adverse-effect-level (NOAEL) or lowest-observed-adverse-effect-level (LOAEL) if the data are not sufficient for benchmark dose-response modeling. The methods and results of the benchmark dose-response modeling are presented in Appendix D. The POD results from human epidemiological studies and from animal studies are presented in different tables for each endpoint clearly. Next, the PODs were used to calculate HEDs using lifestage appropriate values of human clearance. There is some uncertainty in the pharmacokinetic extrapolation for PFNA. This is discussed in Section 5 (Pages 486-499). This uncertainty in pharmacokinetics will be addressed via the interspecies uncertain factor (UF_A) and the intraspecies uncertainty factor (UF_H). Overall, the methods used to derive toxicity values for PFNA are appropriate. b. Yes, the provided scientific rationale well supports this decision. This is clearly described and justified in Table 5-17 (Page 499). c. Yes, the provided scientific rationale supports this decision. It is appropriate to use a UF_A of 1 because the liver effects are based on human data, so there is not animal-to-human extrapolation and additional uncertainty is not needed. d. The available animal and mechanistic studies support this conclusion. The analysis presented in the Toxicological Review and Derivation of Toxicity Values is clearly documented in Section 5.2.2 (Pages 5-48 to 5-53). The default value of UF_A is 10, which includes 3 for uncertainty in pharmacokinetics and 3 for uncertainty in pharmacodynamics between rodents and humans. However, the UF_A of 3 has already been mostly accounted for by using species-specific and sex-specific PK data, PK modeling, and DDEF approaches. It is acknowledged that these approaches are not PBPK modeling, so some residual uncertainty in PK across species is possible, but this residual uncertainty is small. Therefore, it is appropriate to use an UF_A of 3 to account for the residual small uncertainty in PK and mainly the uncertainty for PD between rodents and humans. e. The application of a UF_S of 10 is supported for the purpose of deriving the subchronic RfD from the 28-day toxicity data. This is because a 28-day exposure is technically considered as a subacute exposure in rodents, and a subchronic exposure in rodents typically lasts from 3 months up to 12 months. It is a default approach to use a UF_S of 10 to account for uncertainty to extrapolate results from shorter exposure to longer exposure duration. f. The provided rationales for the remaining UF values (UF_L, UF_D, UF_H) are scientifically justified and clearly described in Table 5-17 and Table 5-21. UF_L is mainly used to account for uncertainty when extrapolating LOAEL to NOAEL, so it is OK to use a value of 1 when the POD is a BMDL or a NOAEL. A UF_H value of 10 is also a default value to account for interindividual variability in humans, especially

	considering the absence of quantitative information on potential differences in pharmacokinetics and pharmacodynamics relating to PFNA exposure within the human population. The use of a value of 3 for UF_D is also appropriate considering there are multiple high/medium confidence human epidemiological studies and animal toxicity studies on developmental and hepatic endpoints, but the data are not sufficient to conclude with 100% certainty.
Savitz	a. Although I am not an expert in quantitative risk assessment, the framework and approach seem reasonable. f. This is outside my scope of expertise. As always, these seem arbitrary but reasonable arguments can be made to support the choices. **No revisions.**
Zoeller	a. Pharmacokinetics are outside my area of expertise. b. The UF_D of 3 may be considered inadequate. The evidence base for development effects lacked studies examining effects in fetuses, infants, and children (e.g., thyroid toxicity, neurotoxicity, mammary gland development, and multigenerational effects). The evidence for neurotoxicity and thyroid toxicity *suggested* but were considered insufficient to infer a causal relationship between PFNA and these health domains. One of the reasons the evidence base is considered insufficient is because the endpoints measured (cognitive, ADHD, Autism-related, or hormone levels) represent imprecise measures that bias these studies toward the null. Thus, in the absence of a clear understanding (i.e., database sufficiency) of the mechanism by which PFNA exposure is related to observed developmental effects of birthweight, gestation duration, there is additional uncertainty relevant to the ability of PCNA to affect thyroid hormone during gestation or to have direct neurotoxic effect. A UF_D of 10 is warranted. **Tier 1 Recommendation:** Increase the UF_D from 3 to 10. c. Yes, the scientific rationale for a UF_A of 1 is supported. d. The Review thoroughly describes the evidence demonstrating PPARa-independence of the relationship between PFNA exposure and liver effects. This evidence supports a UF_A of 3. **No Recommendation.** e. The Review supports a UF_S of 10 in extrapolating from a 28-day study to a subchronic exposure. f. The argument that a UF_D of 3 is insufficient for developmental effects is not relevant to Hepatic and Male Reproductive effects. Thus, the remaining UFs (L, D, H) appear sufficient and well-justified.

3.7 The Toxicological Review concludes there is inadequate information to assess carcinogenic potential for PFNA and that this descriptor applies to oral and inhalation routes of human exposure. Please comment on whether the available human, animal, and

mechanistic studies, as well as the analysis presented in the Toxicological Review, are scientifically justified and clearly described.

Carcinogenicity Hazard Identification and Toxicity Value Derivation	
Reviewer	**Comments**
Carignan	I agree that there is inadequate information to assess carcinogenic potential for PFNA at this time. The available studies and analysis presented are scientifically justified and clearly described. It would be helpful to include a summary Table or Figure, similar to in the other sections (**Tier 3**). I appreciate that IRIS has noted in the Evidence Integration that there is some evidence of carcinogenicity for PFOA and PFOS, which should help readers avoid confusing 'inadequate information' with 'not carcinogenic'. High quality research on carcinogenicity of PFNA and other (historic and current use) PFAS, and their mixtures, is needed for adult and pediatric populations (**Tier 3**).
Ducatman	**The decision is reasonable.** There is also more information available. More information about the critical outcome of kidney cancer is particularly relevant. The following may be of interest to EPA. • **Tier 3:** A cross-sectional exploratory study in NHANES data revealed a PFNA association to uterine cancer (Cathey et al., 2023). The authors considered behavioral changes as a source of reverse causation, but it is less clear if the role of the uterus in excreting PFAS was considered, or if age at the time of treatment could have affected values. • **Tier 2**: Other PFAS but **not** PFNA were associated with **incident** breast cancer in a nested case-control (n=226 cases, 990 controls) in China (Feng, Bai, et al., 2022) • **Tier 3**: PFNA may instigate growth in prostate cancer cells in vivo (Wei et al., 2023) • **Tier 2**: PFNA data actually were investigated in the very important study by (Shearer et al., 2020) (results were interesting but equivocal for PFNA in my view). • **Tier 1** (if permitted by September 2023 acceptance date). Quoting from the multiethnic cohort study: "*Concentrations of several PFAS differed by race and ethnicity (e.g., higher PFOS and PFNA in African American vs. White participants)." "We found a strong positive PFNA-RCC association among African American participants"* (Rhee et al., 2023). **Discussion**: To the degree that dates permit, cited research could be added as relevant to the discussion (without expectation that it will affect selection of point of departure).

	• **Tier 2**: Trout have been selected as models for carcinogenesis when a goal is to avoid PPAR-alpha effects. Relatively high dose dietary PFNA in trout increased tumor multiplicity and size. In addition, a custom trout microarray detected altered gene expression (Benninghoff et al., 2012). Quoting from the discussion: *"We determined that chronic exposure to three different PFAAs via the diet, including PFOA, PFNA, and PFDA, markedly increased hepatocarcinogenesis in trout in a manner similar to the prototypical estrogen, E2*" (Benninghoff et al., 2012).
Faustman	This reviewer also concludes that there is "inadequate information" for the development of toxicity values for a carcinogenic impact. Mechanistic data from the USEPA review did not identify clear evident of cancer from a mechanistic view and the lack of any in vivo generated data, nor chronic studies would enable a different approach.
Georgopoulos	The available human, animal, and mechanistic studies relevant to potential carcinogenicity of PFNA are clearly described in the Toxicological Review. The information presented in the Review supports the conclusion that there is inadequate information to assess carcinogenic potential for PFNA.
Haney	Considering the limitations in the PFNA evidence base on cancer and in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA concluded that based on the available evidence, a classification of "Inadequate Information to Assess Carcinogenic Potential" of PFNA. For example, as indicated in Section 3.3 in regard to animal studies, no chronic/carcinogenicity studies are available for PFNA. The only animal testing was the NTP 28-day study that evaluated incidences of neoplastic lesions in male and female rats exposed orally to PFNA at doses ranging from 0 to 10 and 0 to 25 mg/kg-day, respectively (NTP, 2018). No neoplasms were reported. However, due to the short exposure duration, this study was considered low confidence for evaluation of carcinogenicity potential. To summarize human studies, EPA states that the available epidemiological evidence on PFNA and the risks of any type of cancer is limited, and that the studies are generally subject to serious sources of bias and results are inconsistent. This limited evidence amounts to inadequate information to confidently assess the carcinogenic potential of PFNA for any route of exposure. Accordingly, consistent with EPA guidance (EPA 2005) to apply a standard descriptor as part of the hazard narrative and to express a conclusion regarding the weight of evidence for the carcinogenic hazard potential, a descriptor of *inadequate information to assess carcinogenic potential* was applied by the EPA for PFNA and is clearly scientifically justified based on the information presented.
Leung	The available data on carcinogenicity have been clearly and appropriately synthesized. I agree with the conclusion that there is inadequate information to assess this effect, given the biases and unadjusted confounders of the included human studies, the very short 28-day duration of the single available animal study, and the inconsistent findings of the genotoxicity studies that also included some mutagenic endpoints of questionable relevance.

Lin	Regarding carcinogenicity, the available human, animal, and mechanistic studies, as well as the analysis presented in the Toxicological Review, are scientifically justified and clearly described in Section 3.3. Specifically, 10 available human epidemiological studies are discussed in Section 3.3.1. The confidence of each of these 10 studies are illustrated on Figure 3-109. The rationale of why these 10 studies were considered as uninformative or of low confidence and thus not suitable to evaluate carcinogenic potential is clearly described. Regarding animal studies, there are no chronic/carcinogenicity studies in the literature. The 28-day NTP evaluated the incidences of neoplastic lesions in rats, but since the exposure duration was short (i.e., 28 days), it is justified to consider this study as of low confidence to evaluate carcinogenicity. For mechanistic studies, the results on genotoxicity endpoints from existing studies are mixed. Some of the results from in vitro assays are difficult to interpret due to confounding by cytotoxicity. Since the evidence of genotoxicity is not consistent and not strong enough, it is reasonable to not advance to dose response analyses based on the genotoxicity end points.
Savitz	That is a valid conclusion based on the lack of studies.
Zoeller	The Review clearly documents that the database for the carcinogenicity of PFNA is inadequate.

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

Carcinogenicity Hazard Identification and Toxicity Value Derivation	
Reviewer	**Comments**
Carignan	I agree that quantitative estimates for cancer effects of oral and inhalation exposures could not be derived. This decision is scientifically justified and clearly described.
Ducatman	**Tier 2:** the discussion of cancer might be more nuanced after additional literature (see above) is assessed, and I do not assert that the main conclusion will necessarily change. The assessment of additional literature would nevertheless build confidence.
Faustman	This reviewer also supports the decision by USEPA to not develop either an oral or inhalation toxicity value.
Georgopoulos	Given the conclusion that there is inadequate information to assess carcinogenic potential for PFNA, the only reasonable option was to avoid deriving quantitative estimates for cancer effects for oral or inhalation exposures. This decision is scientifically justified and clearly described in the Draft PFNA Toxicological Review.

Haney	Yes, the lack of adequate carcinogenicity data for PFNA precludes the derivation of quantitative estimates of cancer for either oral (SFo) or inhalation (IUR) PFNA exposure (or any other exposure route). Furthermore, no robust scientific foundation has been laid, critically reviewed and broadly accepted by the scientific community for the use of any surrogate PFAS with carcinogenicity data (e.g., PFOA) for this purpose. Thus, the decision not to derive quantitative estimates for cancer effects for oral or inhalation PFNA exposures is both reasonable and scientifically justified.
Leung	The draft's conclusions support the inability to derive quantitative cancer estimates for PFNA oral and inhalation exposures.
Lin	This decision is scientifically justified and clearly described. As described in Section 3.3, there are 10 human epidemiological studies that evaluated the risks of cancer associated with exposures to PFNA. However, these studies were either uninformative or of low confidence, thus they are not sufficient to assess carcinogenic potential for PFNA. For animal studies, there are no chronic/carcinogenicity studies of PFNA in rodents. The 28-day NTP study evaluated incidences of neoplastic lesions in rats, but since the exposure duration was short (i.e., 28 days), this study was not sufficient to evaluate carcinogenic potential for PFNA. Therefore, it is appropriate to make the conclusion that there was inadequate information to assess carcinogenic potential for PFNA.
Savitz	Yes, that is the appropriate decision.
Zoeller	It is reasonable that the Agency does not derive quantitative estimates for cancer effects given the lack of data to develop these estimates.

4.0 ADDITIONAL COMMENTS

Reviewer	Comments
Carignan	• Recommend updating the *Military and Industrial Sites* section on Page 1-6, Table 1-2, and throughout the document to consistently use ppt (ng/L) as these are the typical units for reporting PFNA in water. (**Tier 2**) • The paragraph on occupational exposures on Page 1-9 should note the potential for exposure for workers in PFAS manufacturing, historically and currently, as well as in other occupations that use PFNA in production processes. If studies are lacking that should be noted. (**Tier 2**) • Studies on historic and current occupational exposure to PFNA in chemical and product manufacturing are needed. (**Tier 3**) • A justification and citation is needed for this statement on Page 1-10, "Human placental and breast milk transfer efficiencies may depend on PFAS chain length and binding affinity to serum- and breast milk-protein complexes." (**Tier 2**)

Ducatman	**Summary Statement** The PFNA document shows the maturity and increasing excellence of the process and the product. I appreciate the various ways it shows attention to quality improvement. I still have Tier 1, Tier 2, and Tier 3 recommendations for EPA authors. I do not think any of the recommendations address topics that are critical the document's mission of creating an evidence- based value. However, I have made the recommendations because I think the science can improve. There is no need to reiterate Tier levels in a summary. This is simply a summary for EPA consideration. 1. PFAS and menstruation. I have provided literally pages of evaluation and recommendations concerning a topic that is, in my opinion, tangential to the document. If EPA did not have a number of innovative (and in my opinion poorly supported and seriously overstated) concepts that can be read to mean that EPA knows that there is no appreciable PFNA in menstrual excretion, my reviewer comments would be much shorter. I would happily not send these comments at all if I were sure that EPA understands from the discussion that these statements are not well supported. Since I am unsure, I have retained the comments in my report. EPA has the option of omitting this discussion (it has little bearing on anything), explaining it better if EPA is sure (and certainly doing a much better job reporting what is actually in references, revising it so it says something more defensible), or reframing it as a research need while removing the iterated strong statements of no menstrual excretion. While these statements are theoretically possible, they are unlikely. (EPA could partner with CDC to measure PFAS in menstrual excretions for a year or more in a small population while following the serum PFAS, if methods permit. That kind of data would be convincing.) By the way, I was interested in and impressed with the work concerning serum PFAS in women who have and have not been pregnant. Good work. I believe that type of innovative investigation in extant data is a very effective way to consider new hypotheses. However, unless there is more, the data presented at most raises hypotheses about other factors that are of interest, and does not begin to support the assertions made in the document. 2. From a previous document, EPA is aware that I recommend considering an outcome to the organ(s) of metabolism and not to organ(s)/outcomes of downstream effects. EPA has not changed its organization much, yet the PFNA document suggests to me that EPA is more sensitive to thinking about the organ of action. IF so, that is good. Less clear to me is if EPA understands how difficult it will be to actually find some of the downstream effects they seek as proof. This problem is manifest for cardiovascular outcomes. When I was an intern (the Rocky Horror Picture Show

	was playing for the first time In the local movie house), we did not have good drugs that dealt with the cholesterol side of hyperlipidemia. High lipids had reasonably high impact. That changed by the 1980s. Now we contend with side effects of lipid lowering drugs, yet populations taking those drugs have their lipid related cardiovascular risks remarkably lowered. (These drugs work so well to limit morbidity and mortality from CV disease that some cardiologists advocate them for everyone. However, that misses some of the non-CV side effects.) Censoring the population taking the drugs defeats the purpose of the quest. Not censoring the population taking the effective drugs changes the nature of the question. Is there some large, seriously disadvantaged, enrolled population with banked serum in the right containers that can be ethically followed past the time they should be treated but don't get treated, with a comparison population? Maybe there is some more likely study design. Articulating the idea describes the limits. In my view, the atomization of organ effects to downstream outcomes works only when we are sure that we can see the outcome. A related topic is catch-all classifications. I know there are experts who like them, but they lead to unscientific work. I can understand a classification as broad as "genitourinary." It can be stratified to organs if needed. It is hard to know what to do with "metabolic." 3. Confusing use of the word "small" when the same "small" adverse changes have predictable, undesirable, robust adverse influence on abnormal values. The predictability of this "small" causes appreciable, important adverse changes in abnormal values is because of the distribution of the biomarker in the population. Neither ALT nor AST nor cholesterol have many examples of people at the lower end of the ostensible normal range. 4. Missing pieces. Again, I think this could be an outcome of the lumping organization. It can for points of departure because the lumping can be limited to things not considered. It works less well for the science. I think there is a lot of hope (not limited to me) that these important documents are science documents. • Bone health/bone mineral density or other measures - a fair amount of literature. • Uric acid – enough literature to discuss, and also likely a liver effect. Uric acid is one of our antioxidant defenses against disrupted liver metabolism. Uric acid is of course also a product of failed excretion. That management by two organ systems makes it harder to study but it can and has been done, and the higher uric acid of PFAS does not appear to be caused by kidney disease in well-designed studies. Neither of these recommended topics is making assumptions about what EPA will decide about PFNA specifically. The goal is to point out that there is a relevant PFNA literature and a larger PFAS context for these two topics.

	Miscellaneous reviewer comments: Section 1.1.2. and 1.1.3. The review of uses and sources and fate of PFNA in the environment is clear, succinct, and useful. If PFNA migrates in rainwater specifically, as I suspect from other PFAS data and also from the strong description in section 1.1.3, the text could be clearer about rainwater as a source of global transport. **Tier 2**: "PFNA can travel from the source in rainwater" or some such wording could be helpful (if EPA thinks that is the case). **Tier 2**: In addition, the reviewer wonders about the assertion that PFNA does not migrate from wet soil to the atmosphere (section 1.1.3). There are no arguments with the coefficients, yet the adjacent atmosphere is a very big place. (From experience, low vapor pressure transfers to vast spaces are distressingly common. They occur frequently in indoor workplaces and the outdoors is bigger. In my experience, statements such as this usually fall in the face of practical atmospheric work.) EPA has presented two hypothetical reasons why it shouldn't (poor migration in soil, hydroxyl radical destruction in the atmosphere). It is reasonable to present the theories so long as reality intervenes. (I am not qualified to dispute the science point, only asking if EPA wants to be clearer that both statements are theoretical and the compound moves around the world we live in very well, and the reasons necessarily include soil and atmosphere, not just water.) The polar bears (or name the species and remote place) maybe shouldn't have PFNA in mothers and cubs based on the theories, they just do (Bytingsvik et al., 2012). **Tier 2**: The discussion of the hydroxyl radical degradation hypothesis (p. 1-4) could better express uncertainty. **Tier 3**: I agree with the several external reviewers who support more clear health communications concerning terms used that designate different levels of certainty concerning information. A glossary of definitions is a reasonable suggestion. **Tier 2**; In the draft document, the citation manager occasionally did not fully update a reference. An example is the fourth and fifth Kim reference in the paragraph at the bottom of page 5-40. **References in Support of Reviewer Comments.** 1. Abbott, B. D. (2009). Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR alpha), beta (PPAR beta), and gamma (PPAR gamma) in rodent and human development. *Reprod Toxicol, 27*(3-4), 246-257. doi:10.1016/j.reprotox.2008.10.00110.1016/j.reprotox.2008.10.001. Epub 2008 Oct 18. 2. Abbott, B. D., Wood, C. R., Watkins, A. M., Das, K. P., & Lau, C. S. (2010). Peroxisome proliferator-activated receptors alpha, Beta, and gamma mRNA and protein expression in human fetal tissues. *PPAR Res, 2010*. doi:10.1155/2010/690907 3. Banjabi, A. A., Li, A. J., Kumosani, T. A., Yousef, J. M., & Kannan, K. (2020). Serum concentrations of perfluoroalkyl substances and their association with

osteoporosis in a population in Jeddah, Saudi Arabia. *Environ Res, 187*, 109676. doi:10.1016/j.envres.2020.109676

4. Beglarian, E., Costello, E., Walker, D. I., Wang, H., Alderete, T. L., Chen, Z., . . . Chatzi, L. (2023). Exposure to perfluoroalkyl substances and longitudinal changes in bone mineral density in adolescents and young adults: A multi-cohort study. *Environ Res, 244*, 117611. doi:10.1016/j.envres.2023.117611
5. Benninghoff, A. D., Orner, G. A., Buchner, C. H., Hendricks, J. D., Duffy, A. M., & Williams, D. E. (2012). Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. *Toxicol Sci, 125*(1), 69-78. doi:10.1093/toxsci/kfr267
6. Blomberg, A., Mortensen, J., Weihe, P., & Grandjean, P. (2022). Bone mass density following developmental exposures to perfluoroalkyl substances (PFAS): a longitudinal cohort study. *Environ Health, 21*(1), 113. doi:10.1186/s12940-022-00929-w
7. Buckley, J. P., Kuiper, J. R., Lanphear, B. P., Calafat, A. M., Cecil, K. M., Chen, A., . . . Braun, J. M. (2021). Associations of Maternal Serum Perfluoroalkyl Substances Concentrations with Early Adolescent Bone Mineral Content and Density: The Health Outcomes and Measures of the Environment (HOME) Study. *Environ Health Perspect, 129*(9), 97011. doi:10.1289/EHP9424
8. Buckley, J. P., Zhou, J., Marquess, K. M., Lanphear, B. P., Cecil, K. M., Chen, A., . . . Kuiper, J. R. (2024). Per- and polyfluoroalkyl substances and bone mineral content in early adolescence: Modification by diet and physical activity. *Environ Res, 252*(Pt 1), 118872. doi:10.1016/j.envres.2024.118872
9. Bytingsvik, J., van Leeuwen, S. P., Hamers, T., Swart, K., Aars, J., Lie, E., . . . Jenssen, B. M. (2012). Perfluoroalkyl substances in polar bear mother-cub pairs: a comparative study based on plasma levels from 1998 and 2008. *Environ Int, 49*, 92-99. doi:10.1016/j.envint.2012.08.004
10. Carrizosa, C., Murcia, M., Ballesteros, V., Costa, O., Manzano-Salgado, C. B., Ibarluzea, J., . . . Lopez-Espinosa, M. J. (2021). Prenatal perfluoroalkyl substance exposure and neuropsychological development throughout childhood: The INMA Project. *J Hazard Mater, 416*, 125185. doi:10.1016/j.jhazmat.2021.125185
11. Carwile, J. L., Seshasayee, S. M., Ahrens, K. A., Hauser, R., Driban, J. B., Rosen, C. J., . . . Fleisch, A. F. (2022). Serum PFAS and Urinary Phthalate Biomarker Concentrations and Bone Mineral Density in 12-19 Year Olds: 2011-2016 NHANES. *J Clin Endocrinol Metab, 107*(8), e3343-e3352. doi:10.1210/clinem/dgac228
12. Cathey, A. L., Nguyen, V. K., Colacino, J. A., Woodruff, T. J., Reynolds, P., & Aung, M. T. (2023). Exploratory profiles of phenols, parabens, and per- and poly-fluoroalkyl substances among NHANES study participants in association with previous cancer diagnoses. *J Expo Sci Environ Epidemiol, 33*(5), 687-698. doi:10.1038/s41370-023-00601-6

13. Chen, Y., Lv, J., Fu, L., Wu, Y., Zhou, S., Liu, S., . . . Zhang, L. (2023). Metabolome-wide association study of four groups of persistent organic pollutants and abnormal blood lipids. *Environ Int, 173*, 107817. doi:10.1016/j.envint.2023.107817

14. Choi, J. W., Oh, J., Bennett, D. H., Calafat, A. M., Schmidt, R. J., & Shin, H. M. (2024). Prenatal exposure to per- and polyfluoroalkyl substances and child behavioral problems. *Environ Res, 251*(Pt 1), 118511. doi:10.1016/j.envres.2024.118511

15. Cluett, R., Seshasayee, S. M., Rokoff, L. B., Rifas-Shiman, S. L., Ye, X., Calafat, A. M., . . . Fleisch, A. F. (2019). Per- and Polyfluoroalkyl Substance Plasma Concentrations and Bone Mineral Density in Midchildhood: A Cross-Sectional Study (Project Viva, United States). *Environ Health Perspect, 127*(8), 87006. doi:10.1289/EHP4918

16. Darrow, L. A., Groth, A. C., Winquist, A., Shin, H. M., Bartell, S. M., & Steenland, K. (2016). Modeled Perfluorooctanoic Acid (PFOA) Exposure and Liver Function in a Mid-Ohio Valley Community. *Environ Health Perspect, 124*(8), 1227-1233. doi:10.1289/ehp.151039110.1289/ehp.1510391. Epub 2016 Mar 15.

17. Ducatman, A., Tan, Y., Nadeau, B., & Steenland, K. (2023). Perfluorooctanoic Acid (PFOA) Exposure and Abnormal Alanine Aminotransferase: Using Clinical Consensus Cutoffs Compared to Statistical Cutoffs for Abnormal Values. *Toxics, 11*(5). doi:10.3390/toxics11050449

18. Enright, E. A., Eick, S. M., Morello-Frosch, R., Aguiar, A., Woodbury, M. L., Sprowles, J. L. N., . . . Schantz, S. L. (2023). Associations of prenatal exposure to per- and polyfluoroalkyl substances (PFAS) with measures of cognition in 7.5-month-old infants: An exploratory study. *Neurotoxicol Teratol, 98*, 107182. doi:10.1016/j.ntt.2023.107182

19. Evans, N., Conley, J. M., Cardon, M., Hartig, P., Medlock-Kakaley, E., & Gray, L. E., Jr. (2022). In vitro activity of a panel of per- and polyfluoroalkyl substances (PFAS), fatty acids, and pharmaceuticals in peroxisome proliferator-activated receptor (PPAR) alpha, PPAR gamma, and estrogen receptor assays. *Toxicol Appl Pharmacol, 449*, 116136. doi:10.1016/j.taap.2022.116136

20. Fan, S., Wu, Y., Bloom, M. S., Lv, J., Chen, L., Wang, W., . . . Feng, W. (2023). Associations of per- and polyfluoroalkyl substances and their alternatives with bone mineral density levels and osteoporosis prevalence: A community-based population study in Guangzhou, Southern China. *Sci Total Environ, 862*, 160617. doi:10.1016/j.scitotenv.2022.160617

21. Feng, Y., Bai, Y., Lu, Y., Chen, M., Fu, M., Guan, X., . . . Guo, H. (2022). Plasma perfluoroalkyl substance exposure and incidence risk of breast cancer: A case-cohort study in the Dongfeng-Tongji cohort. *Environ Pollut, 306*, 119345. doi:10.1016/j.envpol.2022.119345

22. Feng, Y., Fu, M., Guan, X., Wang, C., Meng, H., Zhou, Y., . . . Guo, H. (2022). Associations of exposure to perfluoroalkyl substances with serum uric acid

change and hyperuricemia among Chinese women: Results from a longitudinal study. *Chemosphere, 308*(Pt 3), 136438. doi:10.1016/j.chemosphere.2022.136438

23. Frisbee, S. J., Shankar, A., Knox, S. S., Steenland, K., Savitz, D. A., Fletcher, T., & Ducatman, A. M. (2010). Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. *Arch Pediatr Adolesc Med, 164*(9), 860-869. doi:10.1001/archpediatrics.2010.163

24. Goralczyk, K., Pachocki, K. A., Hernik, A., Strucinski, P., Czaja, K., Lindh, C. H., . . . Ludwicki, J. K. (2015). Perfluorinated chemicals in blood serum of inhabitants in central Poland in relation to gender and age. *Sci Total Environ, 532*, 548-555. doi:10.1016/j.scitotenv.2015.06.050

25. Grey, A., Bolland, M., Gamble, G., Wattie, D., Horne, A., Davidson, J., & Reid, I. R. (2007). The peroxisome proliferator-activated receptor-gamma agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial. *J Clin Endocrinol Metab, 92*(4), 1305-1310. doi:10.1210/jc.2006-2646

26. Hojsager, F. D., Andersen, M., Juul, A., Nielsen, F., Moller, S., Christensen, H. T., . . . Jensen, T. K. (2022). Prenatal and early postnatal exposure to perfluoroalkyl substances and bone mineral content and density in the Odense child cohort. *Environ Int, 167*, 107417. doi:10.1016/j.envint.2022.107417

27. Hu, Y., Liu, G., Rood, J., Liang, L., Bray, G. A., de Jonge, L., . . . Sun, Q. (2019). Perfluoroalkyl substances and changes in bone mineral density: A prospective analysis in the POUNDS-LOST study. *Environ Res, 179*(Pt A), 108775. doi:10.1016/j.envres.2019.108775

28. Jain, R. B., & Ducatman, A. (2022). Serum concentrations of selected perfluoroalkyl substances for US females compared to males as they age. *Sci Total Environ, 842*, 156891. doi:10.1016/j.scitotenv.2022.156891

29. Jain, R. B., & Ducatman, A. (2023). Factors affecting serum PFAS concentrations among US females with surgically and naturally induced menopause: data from NHANES 2003-2018. *Environ Sci Pollut Res Int, 30*(35), 84705-84724. doi:10.1007/s11356-023-28395-y

30. Jeddy, Z., Tobias, J. H., Taylor, E. V., Northstone, K., Flanders, W. D., & Hartman, T. J. (2018). Prenatal concentrations of perfluoroalkyl substances and bone health in British girls at age 17. *Arch Osteoporos, 13*(1), 84. doi:10.1007/s11657-018-0498-5

31. Kasarala, G., & Tillmann, H. L. (2016). Standard liver tests. *Clin Liver Dis (Hoboken), 8*(1), 13-18. doi:10.1002/cld.562

32. Kersten, S., & Stienstra, R. (2017). The role and regulation of the peroxisome proliferator activated receptor alpha in human liver. *Biochimie, 136*, 75-84. doi:10.1016/j.biochi.2016.12.019

33. Khalil, N., Chen, A., Lee, M., Czerwinski, S. A., Ebert, J. R., DeWitt, J. C., & Kannan, K. (2016). Association of Perfluoroalkyl Substances, Bone Mineral Density, and Osteoporosis in the U.S. Population in NHANES 2009-2010. *Environ Health Perspect, 124*(1), 81-87. doi:10.1289/ehp.130790910.1289/ehp.1307909. Epub 2015 Jun 9.

34. Khalil, N., Ebert, J. R., Honda, M., Lee, M., Nahhas, R. W., Koskela, A., . . . Kannan, K. (2018). Perfluoroalkyl substances, bone density, and cardio-metabolic risk factors in obese 8-12 year old children: A pilot study. *Environ Res, 160*, 314-321. doi:10.1016/j.envres.2017.10.014

35. Kirk, A. B., DeStefano, A., Martin, A., Kirk, K. C., & Martin, C. F. (2023). A New Interpretation of Relative Importance on an Analysis of Per and Polyfluorinated Alkyl Substances (PFAS) Exposures on Bone Mineral Density. *Int J Environ Res Public Health, 20*(5). doi:10.3390/ijerph20054539

36. Marques, P., Madeira, T., & Gama, A. (2022). Menstrual cycle among adolescents: girls' awareness and influence of age at menarche and overweight. *Rev Paul Pediatr, 40*, e2020494. doi:10.1590/1984-0462/2022/40/2020494

37. Mertens, J. J., Sved, D. W., Marit, G. B., Myers, N. R., Stetson, P. L., Murphy, S. R., . . . Farr, C. H. (2010). Subchronic toxicity of S-111-S-WB in Sprague Dawley rats. *Int J Toxicol, 29*(4), 358-371. doi:10.1177/1091581810370372

38. Rhee, J., Chang, V. C., Cheng, I., Calafat, A. M., Botelho, J. C., Shearer, J. J., . . . Hofmann, J. N. (2023). Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma in the Multiethnic Cohort Study. *Environ Int, 180*, 108197. doi:10.1016/j.envint.2023.108197

39. Romano, M. E., Gallagher, L. G., Price, G., Crawford, K. A., Criswell, R., Baker, E., . . . Karagas, M. R. (2024). Plasma per- and polyfluoroalkyl substance mixtures during pregnancy and duration of breastfeeding in the New Hampshire birth cohort study. *Int J Hyg Environ Health, 258*, 114359. doi:10.1016/j.ijheh.2024.114359

40. Rosen, E. M., Brantsaeter, A. L., Carroll, R., Haug, L., Singer, A. B., Zhao, S., & Ferguson, K. K. (2018). Maternal Plasma Concentrations of Per- and polyfluoroalkyl Substances and Breastfeeding Duration in the Norwegian Mother and Child Cohort. *Environ Epidemiol, 2*(3). doi:10.1097/EE9.0000000000000027

41. Save-Soderbergh, M., Gyllenhammar, I., Schillemans, T., Lindfeldt, E., Vogs, C., Donat-Vargas, C., . . . Akesson, A. (2024). Per- and polyfluoroalkyl substances (PFAS) and fetal growth: A nation-wide register-based study on PFAS in drinking water. *Environ Int, 187*, 108727. doi:10.1016/j.envint.2024.108727

42. Shearer, J. J., Callahan, C. L., Calafat, A. M., Huang, W. Y., Jones, R. R., Sabbisetti, V. S., . . . Hofmann, J. N. (2020). Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. *J Natl Cancer Inst*. doi:10.1093/jnci/djaa143

	43. Song, X., Ye, T., Jing, D., Wei, K., Ge, Y., Bei, X., . . . Zhang, Y. (2024). Association between exposure to per- and polyfluoroalkyl substances and levels of lipid profile based on human studies. *Rev Environ Health*. doi:10.1515/reveh-2023-0146 44. Stump, D. G., Holson, J. F., Murphy, S. R., Farr, C. H., Schmit, B., & Shinohara, M. (2008). An oral two-generation reproductive toxicity study of S-111-S-WB in rats. *Reprod Toxicol, 25*(1), 7-20. doi:10.1016/j.reprotox.2007.10.002 45. Timmermann, A., Avenbuan, O. N., Romano, M. E., Braun, J. M., Tolstrup, J. S., Vandenberg, L. N., & Fenton, S. E. (2023). Per- and Polyfluoroalkyl Substances and Breastfeeding as a Vulnerable Function: A Systematic Review of Epidemiological Studies. *Toxics, 11*(4). doi:10.3390/toxics11040325 46. Wang, Y., Zhang, W., Qian, T., Sun, H., Xu, Q., Hou, X., . . . Yang, G. (2021). Reduced renal function may explain the higher prevalence of hyperuricemia in older people. *Sci Rep, 11*(1), 1302. doi:10.1038/s41598-020-80250-z 47. Wang, Z., Asokan, G., Onnela, J. P., Baird, D. D., Jukic, A. M. Z., Wilcox, A. J., . . . Mahalingaiah, S. (2024). Menarche and Time to Cycle Regularity Among Individuals Born Between 1950 and 2005 in the US. *JAMA Netw Open, 7*(5), e2412854. doi:10.1001/jamanetworkopen.2024.12854 48. Wei, Y., He, H., Han, T., Wang, B., Ji, P., Wu, X., . . . Shao, P. (2023). Environmental explanation of prostate cancer progression based on the comprehensive analysis of perfluorinated compounds. *Ecotoxicol Environ Saf, 263*, 115267. doi:10.1016/j.ecoenv.2023.115267 49. Xiong, X., Chen, B., Wang, Z., Ma, L., Li, S., & Gao, Y. (2022). Association between perfluoroalkyl substances concentration and bone mineral density in the US adolescents aged 12-19 years in NHANES 2005-2010. *Front Endocrinol (Lausanne), 13*, 980608. doi:10.3389/fendo.2022.980608 50. Xu, Y., Hansson, E., Andersson, E. M., Jakobsson, K., & Li, H. (2023). High exposure to perfluoroalkyl substances in drinking water is associated with increased risk of osteoporotic fractures - A cohort study from Ronneby, Sweden. *Environ Res, 217*, 114796. doi:10.1016/j.envres.2022.114796 51. Yang, H., Zhou, B., Prinz, M., & Siegel, D. (2012). Proteomic analysis of menstrual blood. *Mol Cell Proteomics, 11*(10), 1024-1035. doi:10.1074/mcp.M112.018390 52. Zhao, X., Lin, J. Y., Dong, W. W., Tang, M. L., & Yan, S. G. (2023). Per- and polyfluoroalkyl substances exposure and bone mineral density in the U.S. population from NHANES 2005-2014. *J Expo Sci Environ Epidemiol, 33*(1), 69-75. doi:10.1038/s41370-022-00452-7
Georgopoulos	**Recommendations for Revisions and Future Considerations**

	Tier 3 Future Consideration: Future efforts and revisions of the assessment for PFNA (and other PFAS) should consider cumulative risks in the context of real-world population exposures to PFAS mixtures Individuals and communities experiencing high exposures to PFNA are reasonably expected to also have potentially high exposures to other PFAS, including the major legacy PFAS. Since it is widely recognized that different PFAS share multiple common Adverse Outcome Pathways, it is critical for EPA to focus on PFAS mixtures. Also, though the IRIS Program does not aim to address the exposure component of risk assessment, it is still important to provide context for the metrics that are developed in the Toxicological Reviews for PFAS. This requires a concise characterization of the distributions and of the geographical and temporal trends of real-world exposures to PFAS mixtures that include PFNA. Again, it is fully recognized that evaluation and assessment of environmental fate and transport and even ecological and human exposure assessments are not within the scope of the IRIS program; however, a concise characterization of "real world" exposures in the form of tables summarizing environmental data (e.g., in addition to the data presented in Table 1-2, on page 1-7, that are limited to military installations) and biomarker data (e.g., in addition to Table 1-3 on page 1-8, that summarizes NHANES data), representative of the range of on-going environmental and biomonitoring studies across the world. Finally, we must acknowledge that the authors of the draft PFNA Toxicological Review have successfully completed an extremely challenging and demanding task. Despite the wide range of issues considered, the gaps and "noise" in the available data, and the multiple endpoints of concern, they have produced a readable, thorough, and balanced document. They deserve our thanks and congratulations. Notably, the authors appear to have incorporated many suggestions from earlier reviews of other PFAS, resulting in a more transparent document. They should be commended for this achievement.
Haney	Similar to other recent draft IRIS PFAS assessments, this assessment cites Weisskopf et al. (2018) as an example of when amplification bias can occur with adjustment for highly correlated PFAS (e.g., p. 1-15, Appendix C). Weisskopf et al. (2018) indicates: (1) sometimes, depending on causal structure, the inclusion of multiple exposure variables in a model can amplify the amount of bias in a regression estimate compared to analyzing single exposures; and (2) this potential amplification of biases increases with stronger correlations between mixture components. To demonstrate that this can occur in some cases, the study authors used “highly correlated exposures” (e.g., r^2=0.9), whereas the correlation coefficients between PFAS considered in various studies cited within this and other drafts (e.g., Grandjean et al. 2012) have been appreciably lower (generally <0.6). Thus, as the relatively few PFAS being considered in such studies are not “highly correlated”, the *potential* for amplification bias “following adjustment of *highly correlated* PFAS (Weisskopf et al., 2018)” (pp. 1-14 to 1-15) is inapplicable to the study circumstances being considered in these draft assessments. Circumstance-specific results of limited studies (e.g., Weisskopf et al. 2018, Weisskopf and Webster 2017) do not constitute reasonable doubt that as a general matter, the confounding from not adjusting for moderately correlated co-exposures to similarly acting compounds is significantly greater than the potential amplification of biases that remains undemonstrated under circumstances applicable to these IRIS drafts, particularly when

	data for other correlated PFAS have been cited in several drafts as evidence that while the data for the specific PFAS under consideration may be inadequate to demonstrate a certain type of adverse effect, it might be expected to have such an effect due to similarity to another/other PFAS with sufficient endpoint data and for which co-exposure is occurring. Studies such as Weisskopf et al. (2018) should not be cited under inapplicable circumstances, and as such this citation should be removed (**Tier 1 necessary revision**). In conclusion, it is obvious that the EPA has put a great deal of time and work into the draft PFNA assessment. Similarly, the external scientific peer review panel has no doubt spent significant time and effort in reviewing and providing thoughtful comments on the draft assessment. At the same time, a great deal of scientific expertise and resources (e.g., subject area experts, total review time available) exist outside of any peer review panel or environmental regulatory agency. Additionally, the public is obviously an important stakeholder in chemical dose-response assessments such as this that will ultimately be utilized in the protection of public health. Consequently, in addition to careful review of comments from the peer review panel, the EPA should duly consider the scientific credibility of all public comments submitted so that the draft assessment for PFNA is the beneficiary of EPA staff having considered the most diverse set of scientifically credible perspectives possible. Regulatory decisions should be based on the best available evidence and knowledge, which includes due consideration of legitimate scientific opinion that may differ (e.g., from the draft opinion of EPA) but is needed for both advancing science and informing sound decisions (De Melo-Martín and Intemann 2013). Thank you for the opportunity to have peer reviewed this important draft assessment. **Comment-Specific References** • ATSDR (Agency for Toxic Substances and Disease Registry). 2021. Toxicological Profile for Perfluoroalkyls. U.S. Department of Health and Human Services. Released May 2021. Available at: https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf • De Melo-Martín, I, Intemann, K. 2013. Scientific dissent and public policy. Is targeting dissent a reasonable way to protect sound policy decisions? EMBO Rep. 14(3): 231-235. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3589084/ • FSANZ (Food Standards Australia and New Zealand). 2021. PFAS and Immunomodulation Review and Update. Available at: www.foodstandards.gov.au/publications/Documents/PFAS%20and%20Immunomodulatory%20Review%20and%20Update%202021.pdf • Maisonet M, Terrell ML, McGeehin MA, et al. 2012. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environ Health Perspect 120(10): 1432-1437. • United States Environmental Protection Agency (USEPA). 2022. ORD Staff Handbook for Developing IRIS Assessments. U.S. United States Environmental

	Protection Agency, Office of Research and Development, Washington, DC, EPA/600/R-22/268. • World Health Organization (WHO). 2017. Tetanus Vaccines: WHO Position paper – February 2017. Available at: www.who.int/publications/i/item/tetanus-vaccines-who-position-paper-february-2017 • World Health Organization (WHO). 2018. The Immunological Basis for Immunization Series Module 3: Tetanus (Update 2018). Available at: www.who.int/publications/i/item/9789241513616
Zoeller	The Agency should consider being more specific in the use of terms such as "uncertainty" and "(im)precision". It seems clear that the Agency knows what they mean, and that these terms are justified when used. But in the main body of the text, it produces a great deal of ambiguity. For example, when there is "uncertainty" surrounding the impact of PFNA on thyroid hormone measures, is it "uncertain" that the data are "real" and generalizable or is it "uncertain" the mechanism by which the PFNA effect occurs (e.g., PFNA-induced decrease in both T4 and TSH in male rats). It would not add much to the complexity of the document to simply define where the uncertainty lies. The same is true for "precision". Is precision compromised because of technical issues or biological issues. For example, the use of analogue approaches to measure "free" T4 can be compromised by serum factors during life stages like pregnancy. But, intra-individual variability in the set-point around which T4 (and fT4 and TSH) is regulated also produces "noise" that can be viewed as "imprecision" in a single point-estimate. Thus, it seems important to define these terms as they are used, because it would be mean something different for each use.

APPENDIX A

LIST OF REVIEWERS

External Peer Review of the EPA's "Draft IRIS Toxicological Review of Perfluorononanoic Acid (PFNA) and Related Salts"

Tuesday, July 30, 2024: 10:00 AM - 4:00 PM EST
Wednesday, July 31, 2024: 10:00 AM - 4:00 PM EST
Thursday, August 1, 2024: 11:00 AM - 2:00 PM EST
Virtual Meeting via Zoom.gov

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APPENDIX B

CHARGE TO REVIEWERS

Technical Charge to External Peer Reviewers

Contract No. EP-C-17-017
Task Order 68HERH20F0407 (ERG Task 44)
June 2024

External Peer Review of EPA's Draft IRIS Toxicological Review of Perfluorononanoic Acid [PFNA, CASRN 375-95-1] and Related Salts

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of the draft *IRIS Toxicological Review of Perfluorononanoic Acid (PFNA) and Related Salts*. IRIS assessments are developed by 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 PFNA. The draft Toxicological Review of PFNA 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 PFNA or salts of PFNA. The systematic review protocol for PFNA and appendices for dose-response modeling, mechanistic evaluations, and pharmacokinetic information and other supporting materials are provided as Supplemental Information (see Appendices A to F) to the draft Toxicological Review.

CHARGE QUESTIONS

Please respond to all charge questions that apply to your areas of expertise. For other questions, simply indicate no or insufficient expertise in your response.

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 PFNA 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 PFNA Toxicological Review, they could inform future reviews or research efforts.

Literature Search Methods and Documentation

1. The Toxicological Review for PFNA 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 PFNA are appropriate and clearly described.

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

        i. EPA fully synthesized the literature published through April 2022 in the external review draft and has been monitoring newly identified studies (i.e., studies identified by EPA or the public that meet the PECO (population, exposure, comparator, and outcome) criteria or otherwise inform key assessment conclusions but that were not addressed in the external review draft—for example, due to publication after April 2022). EPA characterizes these studies in a tabular format in Appendix B.2 (studies identified in the most recent literature search update or public comments on PFDA or PFHxS) and in a second table provided to peer reviewers (studies identified from the PFHxS peer review or PFNA public comment). In both tables, the characterization focuses 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. Studies that were classified as having a possible material impact on the conclusions (e.g., epidemiological studies of hepatic effects and breastfeeding duration; absorption, distribution, metabolism, and excretion/pharmacokinetic [ADME/PK] studies that informed clearance values or otherwise were helpful in the interpretation of the available ADME/PK data) were incorporated into the evidence synthesis. Please review EPA's characterizations 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 the following:

    - Are the available data 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.

    - Are the study confidence conclusions for the PFNA studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes.[1] Please identify any study confidence conclusions that are not justified and explain any

[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. 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 online resource.

alternative study evaluation decisions.

- Are the weight-of-evidence decisions for hazard identification clearly described and scientifically justified.
- Are there any studies not considered in the assessment that would be expected to materially impact the weight-of-evidence decisions. Please describe the scientific rationale for any recommended inclusions.

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

a. For <u>developmental effects</u>, the Toxicological Review concludes that the available ***evidence demonstrates*** that PFNA exposure causes developmental effects in humans given sufficient exposure conditions, based primarily on growth impairments observed in epidemiological studies. It was determined that there was *robust* evidence of decreased birth weight in studies of exposed humans, with support from generally coherent epidemiological findings for other fetal and postnatal growth restriction endpoints (e.g., birth length, postnatal weight and height). In further support, cross-stream coherence is provided by *moderate* animal evidence for PFNA-induced developmental effects in gestationally exposed rodent offspring that included reduced postnatal survival and body weights, and delays in attaining developmental milestones.

    i. The evidence synthesis and integration for potential PFNA-induced developmental effects included a meta-analysis (see Appendix C.1) conducted by EPA (Wright et al., 2023) that considered the findings of birth weight deficit to be statistically robust across all sampling periods and study confidence levels, indicating there are demonstrated birth weight deficits as PFNA exposure levels increase. Although the epidemiological data were ultimately judged as *robust*, there is residual uncertainty regarding some potential for confounding by other per- and polyfluoroalkyl substances (PFAS) and sample timing; however, these factors were not interpreted by EPA to substantially reduce confidence in the evidence base. Please comment on whether the determination that the epidemiological evidence is *robust* is scientifically justified.

b. For <u>liver effects</u>, the Toxicological Review concludes that the available ***evidence indicates*** PFNA exposure is likely to cause liver effects in humans given sufficient exposure conditions, based on consistent and coherent evidence from human, animal, and mechanistic studies. There is *moderate* evidence in human studies that PFNA is associated with liver injury based on increased ALT, AST GGT, and bilirubin. In animals, there was *robust* evidence from a series of short-term studies in rats and mice demonstrating consistent and coherent effects on liver weight, clinical pathology, and histopathology that included hepatocellular necrosis, cholestasis, and triglyceride accumulation. The liver findings for PFNA were similar to those for other structurally related long-chain PFAS and were determined to be adverse.

    i. The judgment that there is *moderate* evidence in human studies was based primarily on cross-sectional studies in general population adults. For nearly all epidemiological studies of PFNA exposure, there is potential that exposure to other highly correlated PFAS could contribute to the observed effects. The

evidence synthesis for potential PFNA-induced hepatic effects included evaluation of the adequacy of studies with exposure and outcome measured concurrently as well as the likelihood of confounding across PFAS. It was concluded that these sources of uncertainty were unlikely to explain the observed effects. Please comment on whether these conclusions are scientifically justified.

ii. Additional considerations influenced the liver effects hazard identification decisions. Appendix A (*Systematic Review Protocol for the PFAS 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. For PFNA, there is evidence of both PPARα-dependent and -independent (e.g., CAR/PXR) pathways contributing to hepatotoxic effects, consistent with the judgment drawn for several other PFAS. The Toxicological Review evaluates the evidence relevant to the potential involvement of PPARα and non-PPARα pathways with respect to the reported liver effects. The Toxicological Review ultimately concludes that evidence from in vivo and in vitro studies supports a potential role for multiple pathways operant in the induction of hepatic effects from PFNA exposure and that the effects are potentially relevant to humans. Detailed information is provided in the Mechanistic and Supplemental Information of Section 3.2.4, Hepatic Effects. Please comment on the basis for the judgment of human relevance of the liver effects and whether it is scientifically justified.

iii. In judging that the animal evidence for hepatic effects is *robust*, the Toxicological Review concludes that the hepatic effects in animals were adverse (vs. adaptive), based in part on consideration of criteria from Hall et al. (2012). The liver enlargement from short-term testing in rats and mice was accompanied by histopathological lesions, including adverse lesions such as necrosis. However, the lack of longer-duration exposures was a substantial source of uncertainty. Therefore, although the linkage between liver hypertrophy and histological evidence of necrotic changes was found to support adversity, the short-term data were further evaluated based on additional criteria set forth in Hall et al. (2012) that considers dose-dependent and biologically significant changes in at least two clinical pathology parameters (see Hall et al., 2012) as confirmatory indicators of hepatocellular damage. The PFNA database was found to meet at least two of the additional criteria set forth by Hall et al. (2012), including large increases in ALT and AST in mice (effects in rats were mild); large increases in bile acids and bilirubin in male rats considered by the National Toxicology Program (NTP) to be indicators of intrahepatic cholestasis; in addition to reductions in blood proteins, increasing triglyceride accumulations and disrupted lipid homeostasis. Please comment on the basis for determination under the criteria set forth in Hall et al. (2012) and others (e.g., U.S. EPA, 2002; EMEA, 2008; Thoolen et al., 2010; Boone et al., 2005) that the hepatoxic effects observed in rodents are considered adverse.

c. For <u>male reproductive effects</u>, the Toxicological Review concludes that the available ***evidence indicates*** PFNA exposure is likely to cause male reproductive effects in humans given sufficient exposure conditions. This conclusion is based primarily on a *high* confidence

28-day oral toxicity study in adult rats that reported a consistent and coherent pattern of adverse male reproductive effects, with additional support from *medium* confidence, short-term studies in adult rats and prepubertal mice observing effects at similar doses.

d. For immune effects, the Toxicological Review concludes that the available ***evidence suggests***, but is not sufficient to infer, that PFNA exposure has the potential to cause immunosuppression in humans. This conclusion is primarily based on epidemiological studies (see Table 3-22) providing evidence of reduced antibody response with PFNA exposure, and possible evidence for effects on asthma and asthma-related outcomes, but with concerns regarding imprecision and potential residual confounding by other PFAS. The human evidence was considered *slight* and the animal evidence *indeterminate*.

    i. The evidence for immune effects for PFNA differs from that of other long-chain PFAS (e.g., perfluorodecanoic acid [PFDA] and perfluorohexanesulfonic acid [PFHxS]), which found stronger evidence of immunosuppression. Please comment specifically on whether the weight-of-evidence decisions for immunosuppression have been clearly described and are scientifically justified.

e. For thyroid effects, the Toxicological Review concludes that the available ***evidence suggests***, but is not sufficient to infer, that PFNA exposure may have the potential to cause effects on the thyroid in humans. This was a complex evidence base to interpret, and the judgment was based primarily on *moderate* animal evidence from a *high* confidence 28-day study in adult rats that showed large, dose-dependent reductions in serum free and total T4 in females and in serum free T4 in males. Although this study provided evidence of effects on T4 homeostasis, there were uncertainties surrounding the reliability of methods used for measuring free T4 in both sexes. There were also body weight losses in males at higher doses that challenged interpretation of the T4 reductions, as well as additional responses in males that were difficult to decipher (i.e., decrease in thyroid-stimulating hormone [TSH], including at doses absent substantial body weight loss). The epidemiological database was *slight* and did not demonstrate coherence with the animal evidence, with the strongest evidence showing positive associations with T4 in children/adolescents, although effect sizes were small. However, there was considerable uncertainty in the human evidence because of inconsistent directions of association and concerns related to study sensitivity.

f. For cardiometabolic effects, the Toxicological Review concludes that the available ***evidence suggests***, but is not sufficient to infer, that PFNA exposure may have the potential to cause cardiometabolic effects in humans. This conclusion was based on studies in humans that showed generally increased serum lipids and some potentially supportive but mixed results for other increased risk factors for cardiovascular disease. However, the evidence has unexplained inconsistencies within and across studies and concerns for imprecision, which add considerable uncertainty. Evidence in experimental animals was *indeterminate*.

g. For neurodevelopmental effects, the Toxicological Review concludes that the available ***evidence suggests***, but is not sufficient to infer, that PFNA exposure may have the potential to cause neurobehavioral effects in humans, based on associations between PFNA and outcomes related to attention and behavior in epidemiological studies. However, there is considerable uncertainty in this association, including imprecision in all the estimates from the three studies evaluating attention-deficit/hyperactivity disorder (ADHD) diagnosis, the most specific outcome, and some unexplained inconsistency. There

was no relevant evidence in experimental animals to inform this outcome.

h. For female reproductive, urinary, adrenal, and other noncancer effects (i.e., hematological, respiratory, digestive, dermal, and musculoskeletal), the Toxicological Review concludes there is ***inadequate evidence*** to determine whether PFNA exposure has the potential to cause these effects in humans based on the sparsity and/or uncertainties of available evidence.

Noncancer Toxicity Value Data Selection and Modeling

3. For PFNA, no reference concentration (RfC) was derived for inhalation exposures. A reference dose (RfD) was derived based on a meta-analysis (Wright, 2023, 10699259) examining reduced birth weight in humans from 10 studies with biomarkers collected early in pregnancy. Note that the selected RfD based on developmental effects is further supported by the lifetime oral hepatic organ-specific osRfD, based on Kim et al. (2023).

   a. Are the selected 10 epidemiological studies for developmental effects used in deriving the RfD values (both lifetime and subchronic) for PFNA scientifically justified? If so, please provide an explanation. If not, 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.

      i. As part of the recommendations in "a" above, please comment on whether the effect selected is appropriate for use in deriving the lifetime RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for its selection. Please also see charge questions 2a and 2a(i).

      ii. EPA used benchmark dose (BMD) modeling (U.S. EPA, 2012) to identify points of departure (PODs) for PFNA-induced developmental effects. In addition, different meta-analyses were performed to evaluate the relationship between PFNA and mean birth weight differences in humans (Wright, 2023, 10699259). Are the modeling and the meta-analysis for decreased birth weight based on 10 studies with biomarkers collected early in pregnancy appropriate for use in derivation of the RfD? Are the selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for developmental effects scientifically justified and clearly described?

   b. For liver effects, an osRfD was derived based on the epidemiological study by Kim et al. (2023) examining biomarkers of liver functions in humans. Are the modeling approaches for the liver effects, selection of cutoff for abnormal, selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for hepatic effects scientifically justified and clearly described?

   c. For male reproductive effects, quantitative information was limited to studies in animals exposed to PFNA for 28 days, and little to no information was available to evaluate the effects of chronic exposure on these health hazards. Therefore, the derivation of lifetime osRfD values was not attempted for male reproductive effects. However, this endpoint was considered for the derivation of a subchronic osRfD (see Question 4). Please comment on whether the provided scientific rationale supports the decision to consider only these

effects for the subchronic RfD? Are the selection and justification of benchmark response levels, selection of the BMD models used to identify each POD for toxicity value derivation, and the POD selected for deriving the candidate value for male reproductive effects scientifically justified and clearly described?

d. For immune and thyroid effects, no reference values were derived given uncertainties in the databases that were judged to indicate *suggestive* evidence of effects. However, while a dose-response assessment is typically not conducted for health effect judgments of "evidence suggests," when the database includes at least one well-conducted study, quantitative analysis may still be useful for some purposes, such as providing a sense of the magnitude and uncertainty of estimates for health effects of concern, informing responses in potentially susceptible populations, or setting research priorities (U.S. EPA, 2005; U.S. EPA, 2020). For this assessment, immunosuppression in children and reduced serum T4 in adult female rats were advanced for dose-response modeling to facilitate comparisons with other PODs and to inform uncertainty factor (UF) selection given that effects have been observed for several other PFAS.

    i. For immune effects, the BMD modeling of the selected *medium* confidence epidemiological studies by Grandjean et al. (2012) using untransformed PFNA concentrations by Budtz-Jørgensen et al. (2018) was null and did not show effects of PFNA on antibody concentrations in children aged five and seven years in both the single-PFAS model and in the multi-PFAS model of PFNA controlling for PFOS and PFOA. Thus, BMDs and BMDLs (benchmark dose [lower confidence limits]) for the effects of PFNA on childhood antibody concentrations to diphtheria and tetanus are provided to compare to other PODs but are not advanced further for RfD derivations. Are the modeling approaches for immune endpoints appropriate and scientifically justified, and is the decision to not advance the modeling for derivation of reference values supported?

    ii. For thyroid effects, with emphasis on results observed in females (results in males were uncertain), the 28-day study in adult rats indicates reductions in serum T4 that are suggestive of an effect but were found insufficient to infer a hazard (see Question 2e). Despite the uncertainties, there is concern for effects given that the T4 reductions in rats from a *high* confidence study were large in magnitude, and there are concerns for downstream effects on neurodevelopment, which is generally a data gap for this chemical. These concerns were further informed by delays in eye opening observed in developmental toxicity studies in two strains of mice, which is a well-characterized effect of T4 insufficiency although thyroid effects were not evaluated in these studies. Given these results and observations of thyroid effects for other PFAS, PODs were derived for total T4 in adult females for comparative purposes and to inform uncertainty. Is the approach taken for thyroid effects appropriate and scientifically justified, and is the decision to not advance the reductions in serum total T4 in female rats for derivation of a subchronic reference value supported?

e. Given the lack of studies on inhalation exposure to PFNA, no RfC is derived. Please comment on this decision.

4. In addition, for PFNA, an RfD for less-than-lifetime ("subchronic") exposures is derived. No subchronic RfC was derived. The same studies and outcome used in deriving the lifetime RfD for developmental effects were chosen for use in deriving the developmental subchronic RfD.

   a. Please comment on whether the selection of these studies and these effects for the derivation of the subchronic RfD for PFNA is scientifically justified.

   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 effect selected is 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. Please comment on the other subchronic osRfDs (i.e., for liver and male reproductive effects).

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

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors

5. Section 3.1 evaluates and synthesizes the PK data in relevant species and sexes, and among human lifestages, up to the derivation of key PK parameters used in the subsequent analysis. Appendix E.1 provides a statistical analysis of PK parameters in male and female rats and mice while differences in clearance between male and female humans as a function of lifestage are evaluated in Section 3.1.4 (subsection Excretion in Humans). However, the evaluation of existing physiologically based pharmacokinetic (PBPK) models and a classic PK model described in Appendix E.4 found that these options were not sufficiently reliable for use.

   For PODs derived from laboratory animal studies, given the information available on potential interspecies differences in PFNA PK and the results of comparing PK model predictions to bioassay data (E.4.1), EPA concluded that a hybrid approach for extrapolation of POD values in animals to estimate corresponding human equivalent doses (HEDs) was the best option in the derivation of the respective RfDs. Specifically, distinct approaches are proposed for estimation of internal doses in male and female rats from the NTP bioassay vs. estimation for mice examined in developmental studies:

   - PFNA serum concentrations measured at the end of the NTP bioassay were algebraically interpolated to estimate internal dose POD (POD_{int}) values for the applied dose PODs identified from that study. The interpolation for male rats assumed a linear increase in serum concentration over the 28-day study, whereas that for female rats assumed the average concentration is close to the end-of-study value.

   - For endpoints from mouse developmental studies (including results in nonpregnant females from those studies), the PK model was used to estimate the POD_{int} values. Specifically, the average serum concentration calculated from the time of mating until the day of observation for each endpoint was used to provide metrics consistent with the dosing regimen (gestation only) and endpoint evaluation at late gestation vs. multiple postnatal times.

   - The estimated human clearance (CL_H) was used to convert the POD_{int} values from these animal

experiments to POD_{HED} values. Likewise, for POD_{int} values that are human serum concentrations identified from epidemiological analyses, CL_H was used to calculate the corresponding POD_{HED}.

Please comment on the following:

a. Are the methods for calculating POD_{int} values for PFNA for endpoints in rats (adult animals) vs. mice (adult females and pups) scientifically justified for conversion of PODs from animal toxicity studies to HEDs? If not, please provide an explanation and detail on a more appropriate approach.

b. Is the use of maternal clearance (in women of reproductive age) to calculate HED values for gestational and early postnatal endpoints appropriate and scientifically justified? If not, please provide specific alternatives for extrapolation of these endpoints.

c. Are the selected values of CL_H, specifically the 95% lower CI of the geometric mean from Chiu et al. (2022), 0.090 mL/kg-day for males of all ages and females below 12.4 and above 40 years of age, and 0.124 mL/kg-day for women 12.4-40 years of age (Subsection: *Total clearance in humans)*, appropriate and scientifically justified?

d. Is application of CL_H to estimate POD_{HED} values from POD_{int} values (from animal or epidemiological studies as summarized above) scientifically justified? If not, please provide an explanation and detail on a more appropriate approach.

e. Have the uncertainties in the POD_{int} estimates for animal studies and CL_H been adequately evaluated and clearly described?

6. EPA has evaluated and applied, where appropriate, UFs to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), duration (UF_S), and LOAEL-to-NOAEL (lowest-observed-adverse-effect level to no-observed-adverse-effect level) extrapolation (UF_L) for PFNA. For a–f below, please comment on whether the uncertainty in the derivation of the candidate and selected toxicity values is scientifically justified and clearly described.

   a. Please comment specifically on whether the methods used to derive toxicity values for PFNA appropriately account for uncertainties in pharmacokinetics, including accounting for differences between the experimental animal data and humans.

   b. For <u>developmental effects</u>, a UF_A of 1 was used since the value was based on human data. 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). Uncertainties with regard to additional susceptible lifestages (e.g., other early-life developmental stages) are addressed as part of the UF_D. Does the provided scientific rationale support this decision? If not, please explain.

   c. For <u>liver effects and derivation of the lifetime os</u>RfD using human studies, a UF_A of 1 was applied as the liver effects were reported in epidemiological studies and the value was based on human adult data. Does the provided scientific rationale support this decision? If not, please explain.

   d. For <u>liver effects and derivation of the subchronic os</u>RfD using animal studies, a value of 3 is applied to extrapolate between effects in laboratory animals and in humans during the derivation of the subchronic RfD. Although PPARα dependence might support a value of

UF_A = 1 for hepatotoxicity if that were the sole pathway leading to these effects, evidence for the involvement of non-PPARα pathways is available in the PFNA database. Thus, uncertainty remains regarding the potential differences in sensitivity across species because of the involvement of both PPARα-dependent and PPARα-independent mechanisms. As such, the Toxicological Review concludes the available data are not adequate to determine whether humans are likely to be equally or less sensitive compared to laboratory animals with respect to the observed liver 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 and Derivation of Toxicity Values is clearly documented.

e. For liver and male reproductive effects, a value of 10 is applied for the UF_S when extrapolating from 28-day animal data to a subchronic exposure. Considering the potential for some health effects (decreased reproductive organ weights and sperm measures, liver enlargement and concurrent effects) to worsen with increasing duration and the large uncertainty associated with the lack of existing or reliable chemical-specific data to evaluate the effects of subchronic exposure on liver and male reproductive outcomes, respectively, the Toxicological Review concludes that application of a UF_S of 10 is supported for the purpose of deriving the subchronic RfD from the 28-day toxicity data. Does the provided scientific rationale support this decision? If not, please explain.

f. Are the provided rationales for the remaining UF values (UF_L, UF_D, UF_H) scientifically justified and clearly described (to inform the UF_H, the assessment evaluates and considers the available evidence on potential susceptibility to PFNA within different populations or lifestages, including any potential impacts from early-life exposure to PFNA on lifelong health, although few studies on susceptibility were available)? 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 PFNA and that this descriptor applies to oral and inhalation routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies, as well as the analysis presented in the Toxicological Review, are scientifically justified and clearly described.

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

APPENDIX C

MEETING AGENDA

External Peer Review of EPA's "Draft IRIS Toxicological Review of Perfluorononanoic Acid (PFNA) and Related Salts"

Virtual Meeting via Zoom.gov:

Tuesday, July 30, 2024: 10:00 AM - 4:00 PM EDT
Wednesday, July 31, 2024: 10:00 AM - 4:00 PM EDT
Thursday, August 1, 2024: 11:00 AM - 2:00 PM EDT

Note: Daily meeting start times are fixed; discussion and break times may be adjusted by reviewers.

Agenda

DAY 1: Tuesday, July 30

10:00 AM	**Meeting Purpose, Peer Review Process, and Intros**	*Cheryl Keenan, ERG (Facilitator)*
10:20 AM	**U.S. EPA Office of Research and Development (ORD) Background Presentation**	*EPA*
11:15 AM	**Public Comments**	*Facilitator, ERG*
11:35 AM	**Reviewer Discussion Agenda and Process**	*Facilitator, ERG*
11:45 AM	BREAK	
12:05 PM	**Chair Opening Remarks to Panel**	*Peer Review Chair*
12:10 PM	**Reviewer Discussions:**	
	Literature Search Methods and Documentation	
	Charge Question 1: Literature Search Methods and Documentation	*Peer Review Panel*
	Noncancer Hazard Identification	
12:40 PM	**Charge Question 2(a): Developmental Effects**	*Peer Review Panel*
1:30 PM	**Charge Question 2(d): Immune Effects**	*Peer Review Panel*
2:00 PM	BREAK	
2:30 PM	**Charge Question 2(b): Liver Effects**	*Peer Review Panel*
3:20 PM	**Charge Question 2(f): Cardiometabolic Effects**	*Peer Review Panel*
3:40 PM	**Charge Question 2(g): Neurodevelopmental Effects**	*Peer Review Panel*
4:00 PM	**ADJOURN DAY 1**	

Agenda (continued)

DAY 2: Wednesday, July 31

10:00 AM	**Day 1 Recap**, **Day 2 Agenda and Process**	*Facilitator, ERG*
10:05 AM	**Reviewer Discussions:**	
	Noncancer Hazard Identification (cont.)	
	Charge Question 2(c): Male Reproductive Effects	*Peer Review Panel*
10:35 AM	**Charge Question 2(e): Thyroid Effects**	*Peer Review Panel*
11:15 AM	**Charge Question 2(h):**	
	Female Reproductive, Urinary, Adrenal & Other Non-Cancer Effects	*Peer Review Panel*
11:45 AM	BREAK	
12:10 PM	**Reviewer Discussions (cont.):**	
	Noncancer Toxicity Value Derivation	
	Charge Question 5: Pharmacokinetic Extrapolation	*Peer Review Panel*
1:25 PM	**Charge Question 6: Uncertainty Factors**	*Peer Review Panel*
1:55 PM	BREAK	
2:25 PM	**Charge Question 3: Derivation of RfD Values**	*Peer Review Panel*
3:40 PM	**Charge Question 4: Derivation of Subchronic RfD**	*Peer Review Panel*
4:00 PM	**ADJOURN DAY 2**	

DAY 3: Thursday, August 1

11:00 AM	**Day 2 Recap**, **Day 3 Agenda and Process**	*Facilitator, ERG*
11:05 AM	**Reviewer Discussions:**	
	Carcinogenicity Hazard Identification and Toxicity Value Derivation	
	Charge Question 7: Carcinogenicity Conclusion	*Peer Review Panel*
11:20 AM	**Charge Question 8: Carcinogenic Quantification Justification**	*Peer Review Panel*
	General Discussion and Closing	
11:35 AM	**Reviewer Integrative Comments and Discussion**	*Peer Review Panel*
12:15 PM	BREAK	
12:45 PM	**Individual Reviewer Key Comments**	*Peer Review Panel*
1:55 PM	**Closing Remarks**	*EPA, ERG*
2:00 PM	**ADJOURN DAY 3**	