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

EXTERNAL PANEL PEER REVIEW OF EPA'S DRAFT "IRIS TOXICOLOGICAL REVIEW OF PERFLUORODECANOIC ACID [PFDA, CASRN 335-76-2] AND RELATED SALTS"

FINAL PEER REVIEW REPORT

October 2023

Submitted to:
U.S. Environmental Protection Agency
Office of Research and Development
Center for Public Health and Environmental Assessment
Attn: Vicki Soto
Soto.Vicki@EPA.gov

Submitted by:
ERG
561 Virginia Road
Building 4 (Suite 300)
Concord, MA 01741

ERG
www.erg.com

CONTENTS

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 Perfluorodecanoic Acid [PFDA, CASRN 335-76-2] 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 PFDA 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). 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 2023, ERG organized and managed an external peer review of EPA's draft "IRIS Toxicological Review of Perfluorodecanoic Acid [PFDA, CASRN 335-76-2] 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 PFDA. EPA's draft Toxicological Review of PFDA 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 PFDA 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 PFDA review, ERG selected the following nine experts after confirming they had no conflict of interest for this review:

- John L. Adgate, Ph.D., MSPH
- Courtney C. Carignan, Ph.D.
- Elaine M. Faustman, Ph.D., DABT (Panel Chair)
- Jeffrey W. Fisher, Ph.D.
- Panagiotis G. Georgopoulos, Ph.D.
- Joseph T. Haney, Jr., M.S.
- Alan M. Hoberman, Ph.D., DABT

- Angela M. Leung, M.D.
- R. Thomas Zoeller, Ph.D.

See Appendix A for a detailed list of reviewers.

ERG provided reviewers with the draft PFDA 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 PFDA 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 PFDA 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 PFDA 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, 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 10, 11 and 13, 2023. 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 PFDA 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 Section 3.0) and ERG prepared a high-level summary (Section 2.0) of reviewer comments (Appendix B).

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, EPA's charge questions are shown in italic font.[1] Note that:

- The summary 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. 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 italicized text does not include charge question text that provides background or summarizes EPA's rationale. The italicized text does include charge question text that poses a question or otherwise requests comment. For the full text of EPA's charge to reviewers, see Appendix B.

- Occasionally, the summary includes a key comment a reviewer made during the peer review meeting but did not include in his or her final written comments. In such cases, the summary clarifies that the reviewer made the comment during the meeting.

Please see Section 3 for the full text of all reviewer post-meeting comments, including comments not included in this summary and the original and compete text of comments that are included.

Literature Search Methods and Documentation

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

- While several reviewers provided Tier 1 comments with suggested revisions, all nine reviewers agreed that the literature search strategy and screening criteria are clearly described and the process is well documented (Adgate, Carignan, Faustman, Fisher, Georgopoulos, Haney, Hoberman, Leung, Zoeller).

- Four reviewers commented that the description of the exclusion criteria should be expanded, particularly around populations, exposures, comparators, and outcomes (PECO) exclusion and Figure 2-1 (Faustman [Tier 2], Carignan [Tier 1], Georgopoulos, Leung [Tier 1]).

- Three reviewers commented that the exposure section should be updated (Adgate [Tier 3], Carignan [Tier 1], Georgopoulos [Tier 1]).

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

- Leung, Zoeller, Fisher, Haney, and Hoberman did not identify any additional studies of PFDA for EPA to consider prior to finalizing the assessment.

- Carignan and Georgopoulos each provided a few studies for EPA to consider, and Faustman identified several review articles.

- Carignan noted a lack of literature on mammary gland development and identified several peer-reviewed studies on the subject (Tier 1).

- Two reviewers (Fisher [Tier 2] and Georgopoulos [Tier 3]) noted that a list of ongoing studies on PFDA should be recorded and that EPA should describe how these studies would be considered as results become available.

1c. Review EPA's characterization and provide tiered recommendations regarding which 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.

- Adgate, Carignan, Fisher, Georgopoulos, Haney, Hoberman, Leung, and Zoeller did not note any material impact of the new studies on the draft's conclusions.

- Faustman commented that EPA should clarify how the updated literature review will be incorporated into the document (Tier 1). Adgate similarly suggested that EPA should enumerate additional studies and describe how they fit into the larger picture of the assessment.

- Georgopoulos commented that new studies may help identify data gaps for future research (this comment was not tiered) and recommended developing a plan for the systematic and regular updating of database that tracking information relevant to the Toxicological Reviews (Tier 3). Fisher (Tier 2) also noted that tracking ongoing studies may be beneficial. Faustman recommended clarifying how additional studies marked for inclusion would be included in the draft (Tier 2).

Noncancer Hazard Identification

2. For each health effect considered in the assessment and outlined below, please comment on whether the available data have been clearly and appropriately synthesized to describe the strengths and limitations, including whether the presentation and analysis of study results are clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. Please comment on whether the study confidence conclusions for the PFDA studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes. Please specify any study confidence conclusions that are not justified and explain any alternative study evaluation decisions. For each, please also comment on whether the weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified.

2a. Liver effects.

- Seven reviewers (Leung, Carignan, Faustman, Fisher, Georgopoulos, Hoberman, Zoeller) commented that available data are clearly and appropriately synthesized and study confidence conclusions are scientifically justified and appropriate, including with regard to characterizing the PPPRα.

- Adgate disagreed with the characterization of evidence of liver effects in humans as "slight" and noted that evidence is between "slight" and "moderate." He thought that additional discussion of the coherence between animal and human studies would be helpful (Tier 2).

- While Haney generally noted agreement with EPA's text on liver effects, he suggested that EPA make revisions to clarify the selection of species deemed to be the most biologically representative of humans such that the same or similar effects are expected in humans (Tier 1). Haney also commented that EPA should explicitly show that the Hall et al. (2012) criteria for adversity are met (Tier 2).

2b. Immune effects.

- Faustman and Leung agreed with the EPA's overall conclusions and had no Tier 1 or 2 recommendations.

- Five reviewers (Adgate, Carignan, Fisher, Georgopoulos, and Hoberman) agreed with EPA's overall conclusions but also made some suggestions for improvement.

- Zoeller commented that EPA needs to explain its reasoning more fully in this section, including adding further discussion of confounding by other PFAS and the use of diphtheria and tetanus antibodies as an index of immune suppression (Tier 2).

- Adgate noted a need for further discussion of the sensitivity and uncertainty inherent in the cited

human epidemiology studies and for further comment on the generalizability of these studies to U.S. populations (Tier 1).

- Carignan provided several comments on the number, quality, and presentation of studies in this section of the review document:
    - She recommended clarifying the number and quality of studies on immunostimulation and autoimmunity to explain why these outcomes were excluded (Tier 1).
    - She recommended including the dichotomous titer outcome from Timmerman et al. (2021) in Table 3-12 (Tier 1).
    - She commented that it was confusing that the final data set used for developing a reference dose is not included in the document. This, along with a prominent clarification about units, should be added to Table 3-12 (Tier 1).
- During the peer review meeting, multiple reviewers (Adgate, Fisher [Tier 2], Zoeller [Tier 2], Haney [Tier 1], and Hoberman) noted that discussion of mixtures and confounding needed to be further developed in the document. Georgopoulos noted that confounding effects from other exposures could impact estimates used to derive RfDs (Tier 1), and Haney argued that issues of mixtures and confounding in the epidemiology studies, combined with the low effect estimates, precluded the use of these studies for quantitative risk assessment and derivation of toxicity factors (Tier 1).
- Haney disagreed with EPA's conclusion on the strength of evidence for human immunosuppression from the epidemiological studies, citing concerns about the strength of unadjusted associations and the necessity of considering confounding from correlated PFAS exposures, which further reduced the strength and significance of associations (Tier 1). In addition, Haney did not agree that the measures of immunosuppression (titers) were clinically significant, particularly for rare diseases such as diphtheria and tetanus (Tier 1).
- Carignan agreed with the use of immune titers as an endpoint for the epidemiology studies and suggested that EPA prominently state their rationale (Tier 1).

2c. Developmental effects.

- Fisher, Georgopoulos, and Faustman agreed with what EPA had written in this section and had no suggested Tier 1 or 2 revisions.
- Adgate agreed with the strength of evidence and noted that the structure of the evidence integration section should be revised to increase the section's coherence (Tier 2).
- Carignan, Hoberman, and Leung agreed with the conclusions made by the EPA and thought that study data were clearly and appropriately synthesized and conclusions were scientifically justified. They also provided several suggested revisions.
    - Carignan disagreed with the confidence rating for Bach et al. (2016), recommended the inclusion of a new study (Padula et al., 2023), and recommended that EPA include an

indicator for studies that used gestational age-adjusted birth weight (Tier 1).

- Hoberman suggested adding discussion of pubertal development as a developmental endpoint (Tier 1).
- Leung suggested identifying the possible exposure windows for each study (Tier 1).

- Zoeller commented that the argument that uncertainty around the relationship between sample timing differences and reported fetal growth restriction deficits needs to be strengthened, particularly in the areas of weight-of-evidence designation (Tier 1).
- Haney noted that since there is considerable uncertainty and only 'slight' evidence provided by the epidemiology studies, he recommends using the Harris and Birnbaum (1989) mouse study to develop RfDs rather than a single epidemiology study as is currently done by the EPA (Tier 1).

2d. Male reproductive effects.

- Adgate, Carignan, Georgopoulos, Haney, Hoberman, Leung, and Zoeller agreed with EPA's conclusions and would have liked to have longer-term data.
- Carignan recommended adding a discussion of the non-monotonicity of testosterone effects and how this may explain inconsistency among male reproductive endpoints in human studies (Tier 2).
- Haney noted that characterization of the body weight reduction for rats as "moderate" was inconsistent with typical characterization of body weight reduction and should be further explained (Tier 1).
- Fisher did not comment on this topic.

2e. Female reproductive effects.

- Adgate, Carignan, Faustman, Fisher, Georgopoulos, Hoberman, Zoeller, and Haney agreed with EPA's conclusions and would have liked longer-term data and more mechanistic studies.
- Carignan noted that lactation duration should be included as an outcome as the pregnancy and postpartum periods are a sensitive developmental window for mammary gland development (Tier 1).
- Leung commented that the report's conclusion that there is a likely association between PFDA exposure and female reproductive toxicity may be too strong given the sparse data of the single rat study (Tier 2).
- Haney commented that characterization of the body weight reduction for rats as "moderate" was inconsistent with typical characterization of body weight reduction and should be further explained (Tier 1).

2f. Cardiometabolic effects.

- All nine reviewers agreed with EPA's conclusions.
- Adgate added that the justification would be more coherent if there were a logic tree to walk the reader through the pathways by which PFDA affects each contributor to overall cardiometabolic health (Tier 2).
- Similarly, Leung suggested that EPA add lipid subfractions, where available, in addition to total cholesterol levels, as some components are protective while others are associated with adverse outcomes (Tier 2).

2g. Neurodevelopmental effects.

- All reviewers agreed with EPA's conclusions.
- Adgate commented that adding ages or age categories and gender, when feasible and clinically relevant, to Table 3-38 would increase the utility of this table in clearly synthesizing the studies (Tier 2).

2h. Endocrine, urinary, and other noncancer effects.

- Adgate, Georgopoulos, Hoberman, Haney, and Carignan agreed with EPA's conclusions.
- Faustman and Leung noted agreement with EPA's conclusions on urinary and other non-cancer endpoints.
- Faustman commented that designation of thyroid studies as "deficient" because of non-fasted measurements or variation in time-of-day was inappropriate; Leung and Zoeller concurred (Tier 1).
- Faustman also commented that the interpretation of studies regarding thyroid pathway responses was different from other recent PFAS IRIS documents and other current PFAS research (Tier 1). In the peer review meeting, Fisher seconded this and noted that he thought that EPA had interpreted the thyroid data incorrectly (Tier 1). Zoeller concurred. These three reviewers disagreed with EPA's conclusion that the thyroid data was "incoherent" (Tier 1).
- Leung commented that studies of thyroid hormones in pregnant people and non-pregnant people should be considered separately; exposure period should also be stated, particularly for studies concerning maternal and/or neonatal exposure (Tier 1).

Noncancer Toxicity Value Data Selection and Modeling

3. Are the selection of the studies for the immune (Budtz-Jorgensen and Grandjean, 2018) and developmental (Wikstrom, 2020) effects for use in deriving the RfD values for PFDA scientifically justified? Are the modeling approaches appropriate?

- Seven reviewers (Adgate, Carignan, Faustman, Fisher, Georgopolous, Hoberman, and Zoeller) agreed that Butdtz-Jorgensen and Grandjean (2018) and Wikstrom (2020) were

scientifically justified and that the modeling approaches were appropriate for deriving the RfD values for PFDA for immune and developmental effects, respectively.

- Leung did not comment in response to this question.
- Haney commented that the selection of the studies was not scientifically justified and appropriate for modeling RfDs for immune and developmental effects of PFDA.
- Carignan added that future research should consider mixtures and that EPA should acknowledge dermal exposure routes; Adgate and Georgopoulos agreed (Tier 3).

3a. If so, please provide an explanation.

- Adgate commented that these studies were reliable sources given the underlying data, but that EPA should present the data more clearly, including comment on clinical relevance, uncertainties, and justification of the choices made in the derivation of the RfDs (Tier 2). Faustman, Fisher, and Leung concurred on this point during the meeting.
- Carignan added that selection of co-critical effects is reasonable given the proximity of the points of departure for the effects and that they are both observed during the developmental period.
- Georgopoulos recommended that EPA add additional discussion of confounding in the selected studies into the document (Tier 1). Fisher agreed; he recommended that EPA acknowledge potential confounding by PFAS mixtures in the immune studies and explore the possibility of assigning relative potency for each PFAS molecule or leaning into computational toxicology methods (Tier 3).

3b. If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the lifetime RfD and detail the rationale for use of such an alternative.

- Haney did not agree with the use of these studies to develop RfDs for immune and developmental effects. He noted that for immune effects, Budtz-Jorgensen and Grandjean (2018) reported inconsistent and/or insignificant associations between antibody concentrations and PFDA, did not adjust for confounding from associated other PFAS, defined the "clinically protective" level of serum antibodies as a higher concentration than typical for the assay, and also noted no evidence of increased incidence of tetanus or diphtheria, despite NHANES data indicating that the U.S. population geometric mean serum level of PFDA is higher than the POD. Further, Haney commented that EPA did not adequately consider other studies with null findings. Haney suggested sorting study data extracted by NOAEL/LOAEL, BMD, or a similar criterion to identify the most sensitive effects based on "less problematic" studies (Tier 1). For birthweight, Haney commented that EPA should use a metanalysis or data from Harris and Birnbaum (1989) rather than Wikstrom (2020), given the weight of evidence designation for the epidemiological literature as "slight." Further, confounding from correlated PFAS is of concern when using Wickstrom (2020) to calculate RfDs (Tier 1).

3c. As part of the recommendations in "a" or "b" above, please comment on whether the effects selected are appropriate for use in deriving the lifetime RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection. Please also see charge questions 2b and 2c.

- Carignan, Hoberman, and Faustman agreed that the selected effects are appropriate for use in deriving the lifetime RfD; Faustman suggested adding an acknowledgment of the broader range of pharmacokinetic differences associated with pregnancy that could affect the interpretation of the epidemiology studies beyond what is currently discussed for the birth weight endpoint (Tier 2).

3d. Are the benchmark dose modeling (BMD) modeling approaches, selection and justification of benchmark response levels, and selection of the BMD models used to identify each POD for toxicity value derivation scientifically justified and clearly described?

- Carignan, Adgate, Faustman, Hoberman and Zoeller agreed that the BMD modeling approaches, selection and justification of BMRs, and selection of BMD models used to identify each POD for toxicity value derivation are scientifically justified and clearly described.

- Fisher commented that while some aspects are clearly described, the issue of mixtures and confounding from other associated PFAS is not adequately addressed. EPA should add text on the strengths and weaknesses of the assumptions and methodology using non-epidemiological language. Georgopoulos expressed a similar concern about not adjusting for confounding from correlated PFAS, including PFNA (Tier 1).

- Haney opined that the modeling approaches, model selection process, and BMRs used to derive PODs are scientifically justified; however, the draft assessment is not using the demonstrated severity/adversity of the effect itself (decreased antibodies) as support for the lower BMR for immune effects and is using an internally inconsistent definition of an adverse effect level to justify the BMR for immune effects. Haney disagreed with the justification EPA provided for the use of the lower BMR for immune effects and recommended removing the justification (Tier 1). Haney also suggested using Harris and Birnbaum (1989) as the basis of an osRfD based on decreases in fetal bodyweight(Tier 1).

3e. For liver, male reproductive and female reproductive effects, quantitative information was limited to studies in animals exposed to PFDA 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 organ-specific (os) RfD values was not attempted for liver, male reproductive and female reproductive effects. However, these endpoints were considered for the derivation of subchronic osRfDs. Does the provided scientific rationale support this decision? Please explain.

- Six reviewers (Carignan, Faustman, Georgopoulos, Haney, Hoberman, and Zoeller) agreed with the scientific rationale to consider deriving subchronic osRfDs but not lifetime osRfDs due to the limited quantitative information.

- Carignan commented that future research should involve longer-term studies appropriate for deriving lifetime osRfDs (Tier 3).

- Haney suggested increasing the uncertainty factor range to estimate chronic osRfDs (Tier 2).
- Fisher commented that the Kim et al. (2019) PBPK model should have been modified for PFDA to derive subchronic osRfDs, as a PBPK model includes organs and tissue groups in a physiologically relevant manner and can predict internal organ dosimetry (Tier 1).

3f. Given the lack of studies on inhalation exposure to PFDA, no reference concentration (RfC) is derived. Please comment on this decision.

- All nine reviewers agreed with EPA's decision not to derive an RfC for inhalation exposure to PFDA due to the lack of data. However, several reviewers suggested exploring methods of gaining information about inhalation exposure to PFDA:
    - Adgate suggested an analysis of the physical properties of PFDA and the potential resulting air concentrations in indoor environments (Tier 3).
    - Carignan suggested that information on inhalation as an exposure route should be gleaned from other PFAS with similar properties (Tier 3).
    - Haney suggested that EPA clearly state that they have not identified a reliable PBPK/PK model for route-to-route extrapolation to support the decision not to derive a RfC (Tier 2).
    - Fisher suggested that EPA consider using multi-path dosimetry software with a PBPK model to simulate inhalation uptake of particles containing PFDA (Tier 3).

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

- Carignan, Faustman, Georgopoulos, Hoberman, and Zoeller agreed with the selection of the studies and effects for the derivation of the subchronic RfD for PFDA.
- Adgate commented that the derivation of a subchronic RfD is not strictly necessary considering that the exposure will be chronic since the chemical is highly persistent (Tier 3).
- Citing the same concerns as in his response to charge question 3, Haney disagreed with the appropriateness of these studies to derive subchronic RfDs for immune and developmental endpoints.
- Leung refrained from comment, explaining that toxicological modeling is not her area of expertise.
- All reviewers noted similarities in their responses to charge question 3 with their responses to this question.

4a. If so, please provide an explanation.

- In general, reviewers referred to their explanations provided for charge question 3.

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 suggested the use of Harris and Birnbaum (1989) as the basis of a subchronic osRfD based on decreases in fetal body weight (Tier 1). Haney also recommended conducting a more complete evaluation of alternative studies and effects, with one option for such an evaluation being to sort study data by duration, NOAEL/LOAEL, and BMD or a similar criterion that would allow EPA to identify the next most sensitive effects based on more robust studies that are adequate for subchronic RfD derivation (Tier 1).

4c. As part of the recommendations in 4a or 4b, please comment on whether the effects selected are appropriate for use in deriving the subchronic RfD, including considerations regarding adversity (or appropriateness in representing an adverse change) and the scientific support for their selection.

- Fisher suggested adding any existing vitro studies to Table 5-16 for organ-specific endpoints (Tier 2).

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

- Faustman, Georgopoulos, and Hoberman noted agreement with EPA's selected endpoints and with the choice of studies used to derive the subchronic osRfDs.

- Haney found the rationale provided by the EPA for these subchronic osRfDs to be reasonable; he further suggested that the uncertainty factor could be increased from 1,000 to 3,000 to estimate chronic osRfDs using these studies (Tier 2).

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

- Adgate, Carignan, Faustman, Fisher, Georgopoulos, Hoberman, and Zoeller agreed with the EPA's decision not to calculate a subchronic RfC due to the lack of data.

- Haney suggested that EPA clearly state in the document that they have not identified a reliable PFDA PBPK/PK model from which they could perform route-to-route extrapolation (Tier 2).

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors

5a. Is applying the estimated DDEF values for PFDA 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.

- Adgate, Faustman, Haney, and Hoberman commented that the approach is scientifically justified, given the shortcomings of the underlying data.

- Carignan and Georgopoulos agreed that the approach is scientifically justified, but the presentation in the document is complicated and the justification is not readily apparent in the document text (Tier 1).

- Fisher disagreed with the use of DDEFs and encouraged EPA to pursue a PBPK model beyond what has been published by EA authors (such as Bernstein et al., 2021) and to consider harmonization or read across from other PFAS (Tier 1).

- Georgopoulos and Haney commented on the uncertainty introduced because serum PFDA levels would not reach steady-state by the end of the 28-day dosing period in the animal studies. Georgopoulos suggested using average measured PFDA serum levels over the course of the study or the maximum PFDA levels at the end of the dosing period to determine the POD for benchmark dose modeling (Tier 1).

- Leung and Zoeller refrained from commenting on charge question 5, explaining that toxicological modeling is not within their areas of expertise.

5b. Is application of the human CL to estimate HEDs from PODint values scientifically justified? If not, please provide an explanation and detail on a more appropriate approach.

- Faustman and Hoberman commented that the application of the human CL to estimate HEDs from PODint values is scientifically justified.

- Georgopoulos commented that the approach is justified, assuming that a reliable value of human clearance can be estimated. In the document, EPA assumes that the ratio of fecal to urinary clearance is the same in humans and rats; this has not been established and requires further discussion in the document (Tier 1).

- Carignan recommended that EPA indicate where assumptions are based on animal data and discuss the associated uncertainties in the document (Tier 1).

5c. Have the uncertainties in the DDEFs and human CL been adequately evaluated and described? In answering this question, please provide an explicit recommendation on whether or not EPA should expand its adjustment for menstrual fluid loss as outlined in (U.S. EPA, 2023, HERO ID 11181055) prior to finalizing the assessment. As these newer data are from other PFAS, note that such an expansion would be based on the assumption that the pharmacokinetic effect of pregnancy and lactation on PFDA is similar to that of the other PFAS (i.e., a read-across based interpretation).

- Fisher and Haney commented that the uncertainties had been adequately evaluated and described.

- Adgate, Carignan, and Georgopoulos recommended that the document discuss uncertainty further (Tier 1), including a section on evidence integration and the magnitude and directionality of uncertainty.

- Faustman commented that EPA should not expand its adjustment for menstrual fluid loss as the adjustment would rely on many assumptions about menstrual flow. At the meeting, Carignan concurred with the notion that the adjustment relies on many assumptions, and in her written

comments, Carignan noted that if EPA does adjust for menstrual fluid loss, the agency will need to consider that not all women menstruate, birth children, or breastfeed (Tier 1).

- Fisher, Haney (Tier 1), Hoberman (Tier 3), and Georgopoulos commented that EPA should expand its adjustment for menstrual fluid loss.
- Faustman noted that the document lacks a discussion of PFDA transfer via breast milk (Tier 1).

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

- Leung refrained from commenting, explaining that this topic was not her area of expertise.

6a. Is uncertainty in the derivation of the toxicity values scientifically justified and clearly described? Please describe and provide comments, if needed.

- Adgate, Faustman, Haney, and Hoberman agreed that uncertainty in the derivation of the toxicity values was scientifically justified and clearly described.
- Georgopoulos noted several inconsistencies regarding uncertainty factors for intraspecies variability, interspecies differences, database limitations, duration, and LOAEL-to-NOAEL extrapolation within the main document and appendices. Georgopoulos recommended ensuring that the values of pharmacokinetic properties listed in the document match those reported in their cited sources (Tier 1).
- Carignan and Zoeller refrained from commenting, explaining that this question was not within their areas of expertise.

6ai. Please comment specifically on whether the methods used to derive toxicity values for PFDA appropriately account for uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans.

- Fisher noted that the lack of fit to pharmacokinetic data for repeated exposures suggests that acute dosing CL values do not predict repeat exposures; this will need to be further studied, perhaps using computational toxicology methods (Tier 3).
- Georgopoulos noted that the Bernstein et al. (2021) methods of reparametrizing the Kim et al. (2019) PBPK model should be included in the document (Tier 1).
- Haney commented that there may not be an acceptable way for EPA to further quantitatively account for uncertainty surrounding interspecies differences in pharmacokinetics based on currently available data and methods.

6b. For immune effects, a UFS of 1 and 3 were considered to account for extrapolation from less than lifetime human data; ultimately a UFS of 1 was selected. Does the provided scientific rationale support this decision?

- Carignan, Faustman, Georgopoulos, Haney, and Hoberman agreed that the scientific rationale supports the decision on UFs.
- Zoeller opined that the justification of a UF of 1 for sensitive life stage was not clear (Tier 2).

6c. For liver effects, a value of 3 is applied to extrapolate between effects in laboratory animals and in humans during the derivation of the subchronic RfD. Please comment on whether the available animal and mechanistic studies support this conclusion and whether the analysis presented in the Toxicological Review is clearly documented.

- Adgate, Carignan, Fisher, Georgopoulos, Haney, Hoberman, and Zoeller agreed that there was adequate support for an uncertainty value of 3 to extrapolate between the effects in laboratory animals and humans during the derivation of the subchronic RfD.
- Faustman did not support the use of a value of 3. She commented that an uncertainty factor of 10 would be more appropriate to extrapolate the available data for chronic considerations due to the lack of underlying data. Faustman did support a UFA greater than 1 to account for the complexities of PPARα and non-PPARα receptor involvement (Tier 1).

6d. For liver, male reproductive, and female reproductive effects, a default value of 10 is applied for the UFs when extrapolating from 28-day animal data to a subchronic exposure. Does the provided scientific rationale support this decision? Please explain.

- Adgate, Carignan, Faustman, Georgopoulos, Hoberman, and Zoeller agreed that the scientific rationale provides clear support for this decision.
- Fisher suggested considering the use of an animal/human PBPK model to gain insights into subchronic and chronic exposures and accumulation in the body over time (Tier 2).
- Haney noted that EPA should consider whether data from structurally related PFAS could help to further inform the UFs values. Hoberman seconded this (Tier 2).

6e. Are the provided rationales for the remaining uncertainty factors (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 PFDA within different populations or lifestages, including any potential impacts from early life exposure to PFDA on children's health or health later in life, although few studies on susceptibility were available)? If not, please explain.

- Adgate, Carignan, Faustman, Georgopoulos, Haney, Hoberman, and Zoeller agreed that the rationales for the remaining uncertainty factors were scientifically justified and clearly described.

- Fisher noted that EPA could, using language accessible to audiences without a background in epidemiology, explain the epidemiologic tools used to tease out the health effects of PFDA when PFDA is part of a mixture of exposures (Tier 2).

Carcinogenicity Hazard Identification and Toxicity Value Derivation

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

- Adgate, Carignan, Faustman, Georgopoulos, Haney, Hoberman, Leung, and Zoeller agreed that there currently is inadequate information to assess the carcinogenetic potential of PFDA.

- However, Faustman also commented on observations of chromosomal abnormalities and genotoxicity; these endpoints should not be ignored. Faustman encouraged EPA to clearly record where DNA impacts are reviewed in the document and note where these topics were discussed in multiple sections (e.g., if a study outcome was discussed in the male reproductive section as well as the carcinogenicity hazard identification section). Leung agreed with Faustman on these points (Tier 1).

- Fisher refrained from commenting.

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

- All nine reviewers agreed that the decision not to derive quantitative estimates for cancer effects for oral or inhalation exposures was scientifically justified and clearly described.

3.0 REVIEWER RESPONSE TO CHARGE QUESTIONS

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

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

EPA 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 criteria or otherwise inform key assessment conclusions, but which were not addressed in the external review draft, for example due to publication after April 2022). EPA will characterize these studies in a document that will be provided to the peer review panel and the public and, following the review, included as an Appendix

to the assessment prior to finalization. The characterization will focus on EPA's judgment of whether the studies would have a material impact on the conclusions (i.e., identified hazards or toxicity values) in the external review draft. Following receipt of this additional document after the review is underway, please:

c. Review EPA's characterization and provide tiered recommendations regarding which 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**
Adgate	a. **Tier 3:** In general, the literature search strategy and screening criteria used are clearly described and the process is well documented and therefore transparent due to a comprehensive protocol. Links to Appendix A in the "Supplemental Information" document did not work, and it is confusing that there's are two Addenda to the main document (i.e., "Tox Profile") which the Agency should rename as that is easily confused with Appendix. Some of the systematic review methods can be clarified, particularly the studies that were put in the potentially useful for future consideration category. An improved version of the document will clarify the process for deciding what's important and the goals of future analyses and describing the main uncertainties that these. Per Dr Georgopoulos's comment, what would be useful in the document is a better summary of the exposure information, particularly as relevant to the epidemiology studies and animal studies, i.e., some summary of NHANES serum levels to put the IRIS analysis in context. c. No additional comment as the rationale provided by EPA is reasonable but enumerating the additional studies and how they fit into the larger picture, even if not strongly supporting the assessment (example below), should be done as feasible. *Bailey JM, Wang L, McDonald JM, Gray JS, Petrie JG, Martin ET, Savitz DA, Karrer TA, Fisher KA, Geiger MJ, Wasilevich EA. Immune response to COVID-19 vaccination in a population with a history of elevated exposure to per- and polyfluoroalkyl substances (PFAS) through drinking water. J Expo Sci Environ Epidemiol. 2023 Jun 19. doi: 10.1038/s41370-023-00564-8. Epub ahead of print. PMID: 37337047.*
Carignan	a. The literature search strategy and screening criteria for PFDA appear appropriate and clearly described with the exception of the **Tier 1** comments noted below. Section 1.2, Page 28, Line 25: "*are provided in Appendix A*." Recommend updating to 'Appendix A and B,' checking that the link for Appendix A works and that the dates in Appendix B are current. [**Tier 1**] Section 1.1.4, Page 24 Line 36: Correction needed, first says 2002-2001 then 2000-2001.

	Section 1.2, Page 28, Line 28: Literature appears to be misspelled. [**Tier 2**] Section 1.2, Page 29, Line 18: Check that 'SEMs' is previously defined. [**Tier 2**] Section 1.2, Page 29, Table 1-4: In the Evidence column for Populations, Human: Recommend adding text similar to for Animal, "*(including preconception, in utero, lactation, peripubertal, and adult stages)*" or otherwise assure inclusion of sensitive developmental windows including those relevant to mammary gland development (e.g., duration of lactation as an outcome), fertility, infant exposure, etc. [**Tier 1**] Should consider alteration in mammary gland function (including lactation duration) as the mammary gland is a sensitive endpoint for other PFAS (e.g., PFOA). Mammary gland development has at least two sensitive exposure windows: early life and pregnancy/postnatal. In the Evidence column for Outcomes, *"(Note: Other than genotoxicity studies, studies including only molecular endpoints [e.g., gene or protein changes; receptor binding or activation] or other nonphenotypic endpoints addressing the potential biological or chemical progression of events contributing towards toxic effects will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol])*." Recommend clarifying here or elsewhere how vaccine immune titer is a functional measure of adaptive immune response and therefore a phenotypic outcome. [**Tier 1**] Sections 1.2.2. (Evaluation of Individual Studies) and 1.2.3. (Additional Epidemiology Considerations) are clear and highlight important points regarding bias and confounding. Section 1.2.4., Page 33, Line 34: '*all health effects for animal studies and <u>some health effects</u> for epidemiologic studies*.' Since details are in Appendix A, suggest highlighting here the health effects included or excluded for epidemiologic studies. [**Tier 1**] Figure 2-1: More explanation needed for the 595 studies excluded as 'not PICO relevant'. [**Tier 1**] The exposure assessment sections (Sections 1.1.3: Environmental Fate and Transport and 1.1.4: Potential for Human Exposure, including Populations and Lifestages with Potentially Greater Exposure) should be expanded and updated to provide important and relevant context. Specific recommendations are provided: Section 1.1.3, Page 24 Line 11: *"Yoo et al. (2011) estimated a grass-soil accumulation factor (grass concentration divided by soil concentration) of 0.10 for PFDA, based on samples collected from a site with bio-solids-amended soil."* Should update with results from Blaine et al. 2013, which calculated a BAF of 0.52 for lettuce grown in industrially impacted soil. [**Tier 1**] Andrea C. Blaine, Courtney D. Rich, Lakhwinder S. Hundal, Christopher Lau, Marc A. Mills, Kimberly M. Harris, and Christopher P. Higgins. Uptake of

	Perfluoroalkyl Acids into Edible Crops via Land Applied Biosolids: Field and Greenhouse Studies. Environ. Sci. Technol. 2013, 47, 14062−14069 Section 1.1.4, Page 24 Line 25: "*Gebbink et al. (2015) modelled exposure to PFDA among the adult general population. 'Intermediate' exposure (i.e., based on median inputs for all exposure parameters) from direct and indirect (i.e., precursor) sources was estimated to be 67 pg/kg-day. Of the pathways evaluated (i.e., ingestion of dust, food, water; inhalation of air), direct intake of PFDA in the diet accounted for the largest portion of exposure for the intermediate scenario.*" Should note that this estimate is for the general population. [**Tier 1**] Here are two additional references that also support this statement. Haug LS, Huber S, Becher G, Thomsen C. 2011. Characterization of human exposure pathways to perfluorinated compounds--comparing exposure estimates with biomarkers of exposure. Environ Int 37:687–693. Tittlemier SA, Pepper K, Seymour C, Moisey J, Bronson R, Cao XL, et al. 2007. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. J Agric Food Chem 55:3203–3210. Section 1.1.4, Page 25 Line 2: "*For example, Lankova et al. (2013) detected PFDA in 10% of human milk samples collected from 50 Czech women at concentrations ranging from <6 to 12 pg/mL indicating that breastmilk is a potential route of exposure for infants*." More recently, Zheng et al (2021) detected PFDA in 94% of breast milk samples collected in 2019 from 50 U.S. mothers at median concentration of 7.4 and maximum concentration of 697 pg/mL. A meta analysis by LaKind et al. (2023) found that breast milk concentrations of some PFAS often exceed children's drinking water screening values, regardless of geographic location. A time trend analysis of breast milk samples collected from women in Sweden identified an increasing trend for a subset of women in Stockholm (n= 50) over the past 17 years. [**Tier 1**] Guomao Zheng, Erika Schreder, Jennifer C. Dempsey, Nancy Uding, Valerie Chu, Gabriel Andres, Sheela Sathyanarayana, and Amina Salamova. Per- and Polyfluoroalkyl Substances (PFAS) in Breast Milk: Concerning Trends for Current-Use PFAS. Environmental Science & Technology 2021 55 (11), 7510-7520. DOI: 10.1021/acs.est.0c06978 LaKind JS, Naiman J, Verner MA, Lévêque L, Fenton S. Per- and polyfluoroalkyl substances (PFAS) in breast milk and infant formula: A global issue. Environ Res. 2023 Feb 15;219:115042. doi: 10.1016/j.envres.2022.115042. Epub 2022 Dec 16. PMID: 36529330; PMCID: PMC9872587. Nyberg E , Awad R , Bignert A , Ek C , Sallsten G , Benskin JP . Inter-individual, inter-city, and temporal trends of per- and polyfluoroalkyl substances in human milk from Swedish mothers between 1972 and 2016. Environ Sci Process Impacts. 2018 Aug 16;20(8):1136-1147. doi: 10.1039/c8em00174j. PMID: 29987291. Section 1.1.4, Page 25 Line 4: "*Exposure can also occur through hand-to-mouth*

	transfer of materials containing these compounds (ATSDR, 2018b) or in infants through ingestion of formula reconstituted with contaminated drinking water." Should note limited data on formula itself. Lakind et al (2023) reviewed the limited data on PFAS in formula and noted, "Regarding infant formula PFAS levels, there is a general dearth of information. At present, in any geographic location, it is not possible to state with any certainty that infant formula will have lower PFAS levels compared to breast milk. The same is true for drinking water used to reconstitute infant formula…" [**Tier 1**] LaKind JS, Naiman J, Verner MA, Lévêque L, Fenton S. Per- and polyfluoroalkyl substances (PFAS) in breast milk and infant formula: A global issue. Environ Res. 2023 Feb 15;219:115042. doi: 10.1016/j.envres.2022.115042. Epub 2022 Dec 16. PMID: 36529330; PMCID: PMC9872587. Section 1.1.4, Page 25 Line 9: "*…such as firefighters or individuals who install and treat carpets."* Should also note workers in the PFAS manufacturing and other PFAS using industries (e.g., textile, leather, plastics, paper). [**Tier 1**] Franziska Heydebreck, Jianhui Tang, Zhiyong Xie, and Ralf Ebinghaus.Emissions of Per- and Polyfluoroalkyl Substances in a Textile Manufacturing Plant in China and Their Relevance for Workers' Exposure. Environmental Science & Technology 2016 50 (19), 10386-10396. DOI: 10.1021/acs.est.6b03213 Håkon A. Langberg, Hans Peter H. Arp, Gijs D. Breedveld, Gøril A. Slinde, Åse Høiseter, Hege M. Grønning, Morten Jartun, Thomas Rundberget, Bjørn M. Jenssen, Sarah E. Hale. Paper product production identified as the main source of per- and polyfluoroalkyl substances (PFAS) in a Norwegian lake: Source and historic emission tracking. Environmental Pollution. 2021. Volume 273. https://doi.org/10.1016/j.envpol.2020.116259. Section 1.1.4, Page 25 Line 11: "…*professional ski waxing may be more highly exposed because PFAS in dust may become airborne and inhaled during this process*." Recommend removing 'in dust' as ski waxing often involves heating and therefore there is also the potential for inhalation of volatile PFASs. [**Tier 2**] Section 1.1.4, Page 25 Line 14: "*Populations living near fluorochemical facilities where environmental contamination has occurred may also be more highly exposed (ATSDR, 2018a). Yamada et al. (2014) estimated exposure to PFDA and other PFAS among high seafood consumers and high freshwater fish consumers in France."* Recommend describing important fate and transport mechanisms: direct discharge, WWTP effluent to DW and fish, WWTP biosolids to agriculture. Dry deposition to crops and to ground/drinking/irrigation water. [**Tier 1**] It would be extremely useful to include a figure that shows exposure pathways for PFAS. [**Tier 1**] Section 1.1.4, Page 25, Table 1-2: Should note that PFDA blood levels were estimated to increase 18% per month among breastfeeding infants. [**Tier 1**] Mogensen UB, Grandjean P, Nielsen F, Weihe P, Budtz-Jørgensen E. Breastfeeding as an Exposure Pathway for Perfluorinated Alkylates. Environ Sci Technol. 2015

	Sep 1;49(17):10466-73. doi: 10.1021/acs.est.5b02237. Epub 2015 Aug 20. PMID: 26291735; PMCID: PMC6190571. Section 1.1.4, Page 26, Line 9: "…*PFAS-treated carpets or other textiles*." Recommend editing to say 'and textiles' since carpets aren't really textiles. [**Tier 2**] Section 1.1.4, Page 26: Paragraph starting at Line 8: A table would be helpful showing location, year, detection limits, frequencies, percentiles and maximum for each study. Comparisons and overall takeaway are difficult as currently presented. [**Tier 2**] Section 1.1.4, Page 26, Line 24: Should also note results of water monitoring for the ATSDR multi-site study and other locations with considerable point-source PFAS contamination. [**Tier 1**] Section 1.1.4, Page 27, Line 25: "*in Decatur, AL where wastewater treatment sludge had been applied*." Suggested rephrase to provide useful context, "…where sludge had been applied from a wastewater treatment plant receiving effluent from PFAS manufacturing." [**Tier 2**] Section 1.1.4, Page 27: Recommend adding a section on foods that notes pathways (irrigation water, biosolids and dry deposition), highlights examples, and notes challenges as well as recent advancements including analytical chemistry methods. [**Tier 1**] Section 1.1.4, Page 27, Sections on AFFF and Military: Should note impacts on surrounding community drinking water systems, recreational water bodies, fish, wildlife and agriculture. [**Tier 2**] Should note PFAS accumulation on the surface microlayer of waterbodies and in surface water foam. [**Tier 1**] Schwichtenberg T, Bogdan D, Carignan CC, Reardon P, Rewerts J, Wanzek T, Field JA. PFAS and Dissolved Organic Carbon Enrichment in Surface Water Foams on a Northern U.S. Freshwater Lake. Environ Sci Technol. 2020 Nov 17;54(22):14455-14464. doi: 10.1021/acs.est.0c05697. Epub 2020 Nov 8. PMID: 33164508. Section 1.1.4, Page 27, Table 1-3: Should update to include more recent data on PFDA in environmental media at PFAS impacted military installations. [**Tier 1**] Section 1.1.4, Page 28, Line 9: Should specify whether this is a *mean or median* concentration of 0.014 ng/g. [**Tier 2**] Section 1.1.4, Page 28, Line 19: Should also note findings from the recent review of EPA fish data. [**Tier 1**] Barbo N, Stoiber T, Naidenko O, Andrews D. Locally caught freshwater fish across the United States are likely a significant source of exposure to PFOS and other perfluorinated compounds. 2023. Environmental Research. 220. https://doi.org/10.1016/j.envres.2022.115165 b. A recent high quality study investigating birth outcomes (e.g., birth weight) should

	be included: Padula et al. Birth outcomes in relation to prenatal exposure to per- and polyfluoroalkyl substances and stress in the environmental influences on child health outcomes (ECHO) program. March 2023. Environmental Health Perspectives. 131(3). https://ehp.niehs.nih.gov/doi/10.1289/EHP10723 Studies of lactation duration (an indicator of adverse mammary gland development) should be included. Timmermann CAG, et al. Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. 2017. Reprod Toxicol 68:164-170. doi:10.1016/j.reprotox.2016.07.010. Romano ME et al. Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. 2016. Environ Res. 149:239-246. DOI: 10.1016/j.envres.2016.04.034 For additional context: https://ehp.niehs.nih.gov/doi/full/10.1289/ehp.125-A17 c. This reviewer did not identify any studies that would have a material impact on the draft's conclusions.
Faustman	a. This reviewer found the literature search strategy and screening criteria for PFDA as appropriate and clearly described and in alignment with IRIS methodologies. The methodology used was also aligned with the methods used for many if not all of the PFAS related compounds and that provides a good comparison across these IRIS reviews. Figure 2-1 presents an important summary of this process including listing of the source for the searches (PubMed, Toxline, etc.) and the disposition of all the identified items. This figure closely follows the text which explains how many were unique (not duplicates) and how many were excluded at each stage. Of those texts included for full screening rationale for 443 texts were given. Thirty five were excluded as not relevant to PECO , were reviews, were abstracts only and or without full text. This information was important in order to track the process as was the breakdown of the 262 texts that met the PECO criteria as well as 374 which were tagged as supplemental. **For this reviewer, the first exclusion of 595 texts as not relevant for PECO remains a mystery and this reviewer would like to see a summary breakdown for the rationale for exclusion similar to that provided later in the full text screening materials**. Again, this rationale could be at a very high level but it provides at least two things to the reviewer, how effective the search and search terms are at identifying relevant literature but also provides an insight as to what types of literature, endpoints, and information is in general in this larger information population and can sometimes provide hints at what new areas are forthcoming**. This request is a Tier 2 Suggested Revision.** b. This reviewer did not separately identify critical missing literature specific for PFDA for this review. However, I will be attaching several recent papers that are reviews

	on the impact of PFAS related compounds for male reproductive toxicity and for cancer (including testicular cancer). **Clarification**: Please see some of the references that are cited in these reviews regarding sperm effects. The reviewer recognizes that these references include review type articles but also references to other PFAS male reproductive effects that should at least be discussed to provide context and to be responsive to public comments. • Front Endocrinol (Lausanne). 2023 Feb 20;14:1114463. doi: 10.3389/fendo.2023.1114463. eCollection 2023. Toxic effects of per- and polyfluoroalkyl substances on sperm: Epidemiological and experimental evidence; Zhangbei Sun, Yiqian Wen, Binhui Wang, Shiyi Deng, Fan Zhang, Zhendong Fu, Yangyang Yuan, Dalei Zhang **Abstract** As emerging organic contaminants, per- and polyfluoroalkyl substances (PFASs) have aroused worldwide concern due to their environmental persistence, ubiquitous presence, bioaccumulation, and potential toxicity. It has been demonstrated that PFASs can accumulate in human body and cause multiple adverse health outcomes. Notably, PFASs have been detected in the semen of human, posing a potential hazard to male fecundity. This article reviews the evidence about the toxic effects of exposure to PFASs on male reproduction, focusing on the sperm quality. Epidemiological studies showed that PFASs, such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), were adversely associated with the semen parameters in humans, including sperm count, morphology and motility. Experimental results also confirmed that PFAS exposure led to testicular and epididymal damage, therefore impairing spermatogenesis and sperm quality. The mechanisms of reproductive toxicity of PFASs may be involved in blood-testosterone barrier destruction, testicular apoptosis, testosterone synthesis disorder, and membrane lipid composition alteration, oxidative stress and Ca2+ influx in sperm. In conclusion, this review highlighted the potential threat of exposure to PFASs to human spermatozoa. Keywords: male fecundity; per- and polyfluoroalkyl substances; reproductive toxicity; sperm; testosterone. • Int J Environ Res Public Health; 2021 Apr 5;18(7):3794. doi: 10.3390/ijerph18073794. Perfluoroalkyl Chemicals and Male Reproductive Health: Do PFOA and PFOS Increase Risk for Male Infertility? Pheruza Tarapore, Bin Ouyang **Abstract** Poly- and perfluoroalkyl substances (PFAS) are manmade synthetic chemicals which have been in existence for over 70 years. Though they are currently being phased out, their persistence in the environment is widespread. There is increasing evidence linking PFAS exposure to health effects, an issue of concern since PFAS such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) bioaccumulate in humans, with a half-life of years. Many epidemiological studies suggest that, worldwide, semen quality has decreased over the past several decades. One of the

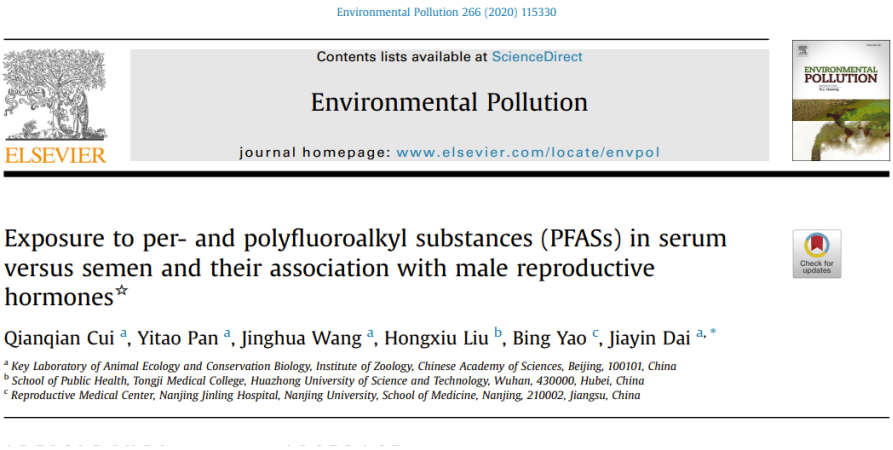
	most worrying effects of PFOS and PFOA is their associations with lower testosterone levels, similar to clinical observations in infertile men. This review thus focuses on PFOS/PFOA-associated effects on male reproductive health. The sources of PFAS in drinking water are listed. The current epidemiological studies linking increased exposure to PFAS with lowered testosterone and semen quality, and evidence from rodent studies supporting their function as endocrine disruptors on the reproductive system, exhibiting non-monotonic dose responses, are noted. Finally, their mechanisms of action and possible toxic effects on the Leydig, Sertoli, and germ cells are discussed. Future research efforts must consider utilizing better human model systems for exposure, using more accurate PFAS exposure susceptibility windows, and improvements in statistical modeling of data to account for the endocrine disruptor properties of PFAS. **Reviews that could be used to support consistence of findings across the PFAS compounds and also PFDA but not directly studies. Can address the uncertainty in observations only with PFDA.** • Front. Endocrinol., 09 March 2022; Sec. Reproduction, Volume 12 - 2021 https://doi.org/10.3389/fendo.2021.799043. **Assessment of the Emerging Threat Posed by Perfluoroalkyl and Polyfluoroalkyl Substances to Male Reproduction in Humans.** Leah Calvert, Mark P. Green, Geoffry N. De Iuliis, Matthew D. Dun, Brett D. Turner, Bradley O. Clarke, Andrew L. Eamens, Shaun D. Roman and Brett Nixon Per-fluoroalkyl and polyfluoroalkyl substances (PFAS) are a diverse group of synthetic fluorinated chemicals used widely in industry and consumer products. Due to their extensive use and chemical stability, PFAS are ubiquitous environmental contaminants and as such, form an emerging risk factor for male reproductive health. The long half-lives of PFAS is of particular concern as the propensity to accumulate in biological systems prolong the time taken for excretion, taking years in many cases. Accordingly, there is mounting evidence supporting a negative association between PFAS exposure and an array of human health conditions. However, inconsistencies among epidemiological and experimental findings have hindered the ability to definitively link negative reproductive outcomes to specific PFAS exposure. This situation highlights the requirement for further investigation and the identification of reliable biological models that can inform health risks, allowing sensitive assessment of the spectrum of effects of PFAS exposure on humans. Here, we review the literature on the biological effects of PFAS exposure, with a specific focus on male reproduction, owing to its utility as a sentinel marker of general health. Indeed, male infertility has increasingly been shown to serve as an early indicator of a range of co-morbidities such as coronary, inflammatory, and metabolic diseases. It follows that adverse associations have been established between PFAS exposure and the incidence of testicular dysfunction, including pathologies such as testicular cancer and a reduction in semen quality. We also give consideration to the mechanisms that render the male reproductive tract vulnerable to PFAS mediated damage, and discuss novel remediation strategies to mitigate the negative impact of PFAS contamination and/or to ameliorate the PFAS load of exposed individuals.

Papers addressing the carcinogenic potential of other PFAS related compounds

- IARC has classified PFOA as "possibly carcinogenic to humans" (Group 2B), based on limited evidence in humans that it can cause testicular and kidney cancer, and limited evidence that it can cause cancer in lab animals. Mar 21, 2023
- Perfluorooctanoic Acid (PFOA), Perfluorooctane Sulfonate ... American Cancer Society https://www.cancer.org/
- PFAS Exposure and Risk of Cancer – NCI – National Cancer Institute (.gov). https://dceg.cancer.gov/research/waht-we-study/pfas Perfluorooctanoic acid (PFOA), the most well-studied PFAS, has been classified as a possible human carcinogen based in part on limited epidemiologic ...See: Kidney Cancer, Testicular Cancer, Prostate Cancer

References that emphasize differences in the appropriate measure to use for evaluating Testicular effects from PFAS compounds.

- The Pan et al references do suggest that semen versus serum levels of PFAS compounds are a better comparison to use for such pharmacokinetic and sperm effect studies and may provide an explanation for variable comparisons observed in the literature.



Environmental Pollution 266 (2020) 115330

Contents lists available at ScienceDirect

Environmental Pollution

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Exposure to per- and polyfluoroalkyl substances (PFASs) in serum versus semen and their association with male reproductive hormones☆

Qianqian Cui [a], Yitao Pan [a], Jinghua Wang [a], Hongxiu Liu [b], Bing Yao [c], Jiayin Dai [a,*]

[a] Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China
[b] School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430000, Hubei, China
[c] Reproductive Medical Center, Nanjing Jinling Hospital, Nanjing University, School of Medicine, Nanjing, 210002, Jiangsu, China

REVIEWER comments on these references. Please note some of these citations are reviews however because of the observation of testicular cancer from other PFAS related comments the references that discuss sperm quality cited in the above references suggest that more use of these references is needed as noted in my comments on male reproductive endpoints and in the comments regarding cancer risks. Inclusion would also help to address some of the public comments that were raised.

c. This reviewer appreciated the detailed information characterizing the updated literature searches. Figure 1 was useful in looking at where the new studies mapped on the HAWC visual. In general, this reviewer agreed with the agency regarding what endpoints the additional studies might impact. The impacts of the additional data on the supplemental evidence is of significance for the ADME considerations. For example, Louisse et al 2022, where the determination of OAT4 transporter

	involvement was determined as potentially providing insight on the reduced human urinary clearance of PFDA was important. Likewise, the paper by Yao et al 2023 which estimated infant urinary CL of PFDA from matched blood and urine samples. The literature review says that this helps to address the potential for lifestage CL difference and this reviewer would say yes, it is a start however because the commentary on EPA use of this reference does not provide a detailed review of this paper and the number of matched samples available nor the range of levels of PFDA in the samples analyzed it is difficult to fully interpret. Clarification on how this will be incorporated in the review, as an addendum? Foot note on the relevant page of the revised IRIS report, etc.? **Tier 1 recommendation is to clarify overall how the recent updated literature review will be incorporated into the current draft, Tier 2 recommendation deals with clarification of how those papers identified as YES, will be incorporated into the specific relevant sections of the report?**
Fisher	a. It appears that the search strategy was adequate and there are plans to update the search. Listing any major research activities that are ongoing would be useful (**Tier 2**) because of the number of people contributing to the literature. b. The updated EPA studies covered the few papers that I found relevant to PFDA.
Georgopoulos	a. The search strategy and screening criteria for PFDA literature related to pharmacokinetics and health effects are clearly described in Section 1.2 (pp. 1-7 to 1-16) and Appendix B of the EPA Review document, as well as in the updated Protocol available at https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=345065 The methods used for the PFDA Toxicological Review were appropriate and consistent with scientific standards and practices. **Note:** It should be recognized that the process for identifying and screening pertinent studies for the PFDA Toxicological Review (as well as for the Toxicological Reviews for other PFAS) is inherently challenging. The fact that multiple health effects are associated (or are potentially associated) with exposures to individual PFAS and PFAS mixtures has led to numerous studies worldwide, other completed and many on-going, with multiple related publications appearing at an increasing rate. This makes it necessary to develop a process of efficiently "aligning" the information presented in peer-reviewed documents, such as the present Toxicological Review, with the content of regularly updated online resources, such as the Health & Environmental Research Online portal (https://heronet.epa.gov/heronet/), the Health Assessment Workspace Collaborative (HAWC) portal (https://hawc.epa.gov/assessment/100500073), or 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. Even without accounting for inconsistencies with information appearing on the online resources (e.g. Table 1-1 on page 1-1 and corresponding information available on the CompTox Chemicals Dashboard), the current PFDA Toxicological Review document should address inconsistencies in the information presented in

	Figure 2-1 "Literature search for perfluorodecanoic acid and related salts" (page 2-2): in that figure it is stated that 1,057 records were identified (after duplicate removal) for "Title & Abstract Screening" and 595 of those were excluded as "Not relevant to PECO." That results in 462 (=1,057-595) records, while the "Full Text Screening" box in the Figure lists 443 records, leaving 19 (=462-443) records "missing". From the 443 "Full Text Screening" records 35 are excluded as "not relevant to PECO," resulting in 408 (443-35) records: however, the "Studies Meeting PECO" box lists only 262 records, leaving 146 (=408-262) records "missing." Furthermore, the "Tagged as Supplemental" box states in its header that the number of records identified is n=374, but if one adds the records listed in individual categories within that box [mechanistic or MOA (n = 142), ADME 31), exposure assessment or qualitative exposure only (n = 125), mixture-only (n = 6), non-PECO route of exposure (n = 88), case report or case study (n = 0), other (n =112)] gets a total of 504 records, creating a discrepancy of 130 (=504-374) records. Another point to be addressed here is that though the Technical Review states that 1,057 records were identified (after duplicate removal) for PFDA, the HERO portal PFDA page (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2614) lists currently (June 2023) over 2,100 references. On page 1-10 (lines 11-13) of PFDA Toxicological Review it is stated that "Literature inventories for studies meeting PECO criteria and studies tagged as "potentially relevant supplemental material" during screening were created to facilitate subsequent review of individual studies or sets of studies by topic-specific experts." It would be very useful to identify and systematically organize and screening Supplemental PFDA-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 PFDA (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., and (b) the characterization of PFDA binding affinities with serum proteins presented in Allendorf et al. (2019) and Allendorf (2021), Chen et al. (2020), etc. Finally, it would be useful if exposure and risk assessment activities of regulatory agencies, both in the US and internationally, collecting and/or organizing information relevant to PFDA, were mentioned and cited in the Toxicological Review: representative examples are • the European Chemicals Agency document on PFDA (ECHA, 2016) • 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 (ITRC, 2022) • the CDC/ATSDR PFAS Multi-site Study (MSS) (https://www.atsdr.cdc.gov/pfas/activities/studies/multi-site.html) • the (13) PFAS studies listed at the HHEAR (Human Health Exposure Analysis Resource) data Center (https://hheardatacenter.mssm.edu) • etc. b. A representative selection of peer-reviewed studies for potential consideration by EPA is provided after my responses to the charge questions. c. EPA evaluated a substantial number of additional studies, that were not included in the draft of the Toxicological Review initially provided to the panel, for incorporation in the Toxicological Review and characterization of impact on specific conclusions (i.e., identified hazards or toxicity values). According to the information provided by the EPA, "186 studies were submitted by the State of New Jersey Department of Environmental Protection and the Natural Resources Defense Council (NRDC)" and of these 186 studies "119 studies had been previously identified and can be found in the HERO database" while the "remaining 67 new studies were screened for PECO criteria and evaluated for potential incorporation and impact on the assessment's conclusions". EPA further clarified that "only studies that would notably impact the primary EPA draft judgments (i.e., the health effects identified as human health hazards and the final reference values) in the Step 4 draft will be added to the Toxicological Review by EPA prior to finalization." Studies meeting assessment PECO criteria were: 6 studies on immune effects, 10 studies on developmental effects, 6 studies on cancer, 6 studies on neurodevelopment, 5 studies on male reproductive effects, 11 studies on female reproductive effects, 6 studies on urinary system effects, 26 studies on cardiometabolic effects, 7 studies on endocrine effects, and 11 studies on other effects. Studies meeting select categories of supplemental evidence were 7 ADME studies and 20 mechanistic studies, including non-PECO routes of exposure. EPA indicated that it plans to incorporate only 4 of these additional studies in the revision of the Technical Review: all 4 are supplemental evidence studies: 3 are ADME studies (Louisse et al., 2022; Wang et al., 2018; Yao et al., 2023) and 1 is a mechanistic study (Wang et al., 2022). EPA's rationale for incorporating these 4 studies in the final Technical Review, is that, though they do not affect draft judgments, they provide useful information and address important data gaps. This rationale is reasonable and appropriate. EPA indicated that all other additional studies will not be included in the final Review for reasons ranging from presenting inconsistent, indeterminate, imprecise or unreliable results, to not providing significant new data that would affect the draft assessments. Again, EPA's rationale for this decision is in general reasonable and appropriate; however, though this may not be a primary objective of the Toxicological Review, there is value in identifying areas where studies and associated data are inconsistent or inconclusive, potentially facilitating the development of new studies and application

	of alternative methodologies that could address these inconsistencies. **Suggested Revisions and Future Considerations** • ***Tier 1 Necessary Revision:*** *Update the subsection on Potential for Human Exposure (subsection 1.1.4) with up-to-date references (as currently the most recent reference cited in the subsection is from 2018) and include basic summary information on distributions of population serum PFDA levels (available from NHANES for the US) and on temporal trends for these distributions.* • ***Tier 1 Necessary Revision:*** *Check Table 1-1 for consistency with information currently available on online (on portals such as EPA's CompTox Chemicals Dashboard); evaluate the feasibility of expanding Table 1-1 by including values of PFDA 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 PFDA risk characterization that have been developed by US and international regulatory agencies; compile a summary (e.g., in the form of a table or a brief Appendix) 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 of databases (such as HERO and HAWC) tracking information relevant to the Toxicological Review(s); furthermore, specify criteria for new information that would require re-evaluation and updating of the contents and conclusions of the Toxicological Review(s).*
Haney	a. The literature search strategy and screening criteria for PFDA appear appropriate and clearly described. As indicated in Section 4.1 of Appendix A, the initial literature search strategy performed in July 2017 was designed to identify a broad range of topics relevant to PFAS, including studies on physicochemical properties, environmental fate and occurrences, human exposures, and biological effects representative of all types of evidence (i.e., human, animal, in vitro, in silico) and health outcomes. In February 2018, the literature search was updated and included all PFAS nomenclature from the initial search as well as a broader non-date-limited search of several new PFAS synonyms that were identified after the original search. Full details of the July 2017 and February 2018 search strategies are presented in Addendum B to Appendix A of the draft assessment. Additional relevant literature not found through database searching was identified through other means (see p. 4-2 of Appendix A), and literature searches have been updated throughout the assessment's development to identify newly published literature. As indicated in Section 4.2 of Appendix A, PECO (populations, exposures, comparators, and outcomes) criteria or predefined inclusion and exclusion criteria (i.e., the latter were used for the initial search; see p. 4-5 of Appendix A for exclusion criteria in addition to PECO) were used by two independent reviewers to screen and inventory studies at the title and abstract level. For those studies considered relevant at the title and abstract level, these criteria were then used to

	determine inclusion or exclusion of a reference based on the full text. In addition to including studies that meet PECO criteria, other studies containing material potentially relevant to the assessments objectives and specific aims were tracked during the screening process as "potentially relevant supplemental material" for potential incorporation into the assessment to address key science issues, etc. (see pp. 4-5 and 4-6 of Appendix A). See Appendix A of the draft assessment for additional information. Again, the literature search strategy and screening criteria for PFDA appear overall to be appropriate and clearly described. b. This reviewer personally knew of no additional peer-reviewed studies of PFDA that the assessment should have incorporated. However, some public comments had recommendations for the EPA's consideration (e.g., see the *Literature Search and Screening* section of compiled public comments). If not already considered under subsection "c" below, EPA should consider (**Tier 1 necessary revision**) whether or not the results of the studies recommended by public commenters should be incorporated into the assessment utilizing relevant practical criteria (e.g., based on their potential impact (if any) on data gaps, assessment conclusions, and/or toxicity values). However, it appears that EPA has already done so (see *EPA characterization of studies identified after public release of the draft IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA, CASRN 335-76-2) and Related Salts*). Additionally, panel member Dr. Carignan identified a newly published study, Padula et al. (2013). c. In regard to studies published after release of the public comment draft assessment, the EPA should apply reasonable and practical rationales regarding whether study results should be included in the final assessment based on their potential impact (if any) on data gaps, assessment conclusions, and/or toxicity values (**Tier 1 necessary revision**). In fact, it appears that EPA has already done so (see *EPA characterization of studies identified after public release of the draft IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA, CASRN 335-76-2) and Related Salts*). However, as mentioned above, panel member Dr. Carignan identified a newly published study, Padula et al. (2013).
Hoberman	The literature search strategy and screening criteria for PFDA was found to be appropriate and clearly described. As noted in the review, all literature was tracked in the U.S. EPA Health and Environmental Research Online (HERO). The database includes: • PubMed (National Library of Medicine) • Web of Science (Thomson Reuters) • Toxline (National Library of Medicine) • TSCATS (Toxic Substances Control Act Test Submissions) In addition, relevant literature not found through database searching was identified by: • Review of studies cited in any PECO-relevant studies and published journal reviews; finalized or draft U.S. state, U.S. federal, and international assessments (e.g., the draft 15 Agency for Toxic Substances and Disease Registry [ATSDR] assessment released publicly in 16 2018).

	• In addition, studies included in ongoing IRIS PFAS assessments (PFHxA, PFHxS, 17 PFNA, PFDA) were also scanned for any studies that met PFBA PECO criteria. Searches of published PFAS SEMs (Carlson et al., 2022; Pelch et al., 2022) starting in 2021. Review of studies submitted to federal regulatory agencies and brought to the attention of EPA. For example, studies submitted to EPA by the manufacturers in support of requirements under the Toxic Substances Control Act (TSCA). Identification of studies during screening for other PFAS. • For example, epidemiology studies relevant to PFDA were sometimes identified by searches focused on one of the other four PFAS currently being assessed by the Integrated Risk Information System (IRIS) Program. Other gray literature (e.g., primary studies not indexed in typical databases, such as technical reports from government agencies or scientific research groups; unpublished laboratory studies conducted by industry; or working reports/white papers from research groups or committees) brought to the attention of EPA. **This reviewer could find no additional peer-reviewed studies of PFDA that EPA should consider incorporating prior to finalizing the assessment.** • **Tier 1: *Necessary Revisions*** –None - An additional literature update has been conducted and was provided as supplemental information. • **Tier 2: *Suggested Revisions*** – With the limited number of studies on PFDA some mention of the location of summary charts with all PFAS effects showing a comparison across all PFAS would be useful. • **Tier 3: *Future Considerations*** – Continual updating of database as new relevant articles are published. The need for an updated literature search based on the time between the searches used for the Toxicological Review of PFDA, CASRN 335- 76-2.
Leung	a. The report is clear in describing the PECO literature search and screening process for papers related to PFDA exposure. The initial search was conducted in 2017 and has been updated yearly since; the last full update was conducted in June 2023. The output was screened by two independent reviewers and the entire process was captured in DistillerSR. Although the PECO search was rigorous, includes both the appropriate library reference data sources and other complementary mechanisms, with search results tracked in the HERO database, I appreciate the public comments noting that some human and animal studies as listed in the PFAS-Tox Database were not included, which appears mostly due to the more broad PECO criteria in the latter. I agree with the comments made in the reviewer panel public meeting to add the reasons for why any studies may have been excluded to Figure 2-1 (**Tier 1 Recommendation**). It is also noted that select studies of interest, but which did not fulfill PECO criteria, were inventoried as potentially relevant supplemental material; the listed reasons for assigning them as such are appropriate.

	b. The search process appears appropriate. I do not have any additional studies to recommend. c. I agree with the EPA's disposition of these additional new studies and of studies received from the open public comment period; none of the studies would substantially change the current drafts' judgement for each of the health effect categories. As noted, some of the studies regarded as supplemental evidence, particularly for the ADME of PFDA, are worth noting for their possible mechanistic data, but would not change the draft's assessment.
Zoeller	a. The literature search strategy and screening criteria for PFDA is described in section 1.2. The Agency strategy appears appropriate and clearly described. This search strategy ended in June of 2022. The Agency has updated this search and identified a number of new publications related to the PECO criteria for inclusion. However, the Agency has not deemed that these papers will substantially alter the risk analysis. b. The Agency's update and analysis appears well-justified and thorough. c. No Recommendation.

3.2. For each health effect considered in the assessment and outlined below, please comment on whether the available data have been clearly and appropriately synthesized to describe the strengths and limitations, including whether the presentation and analysis of study results are clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. Please comment on whether the study confidence conclusions for the PFDA studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes[2]. Please specify any study confidence conclusions that are not justified and explain any alternative study evaluation decisions. For each, please also comment on whether the weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified. Note that the data from studies considered informative to the assessment are synthesized in the relevant health effect-specific sections and available in the Health Assessment Workspace Collaborative (HAWC).

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

i. 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 PPARα receptors as a key science issue. To the extent supported by the PFDA literature (and to a lesser extent, literature for other PFAS), the Toxicological Review

[2] The Toxicological Review provides an overview of individual study evaluations within each evidence synthesis section, and the results of those outcome-specific evaluations are made available in the Health Assessment Workplace Collaborative linked here HAWC. Note that a "HAWC FAQ for assessment readers" document, linked here (scroll to the bottom of the page, and the document is available for download under "attachments"), is intended to help the reviewer navigate this on-line resource.

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 evidence from *in vivo* and *in vitro* studies support a potential role for multiple pathways operant in the induction of hepatic effects from PFDA exposure, although how those pathways interact within a mode of action (MOA) cannot be specifically determined.

b. For immune effects, the Toxicological Review concludes that the available *evidence indicates* PFDA exposure is likely to cause immunosuppression in humans given sufficient exposure conditions, primarily on the basis of consistent evidence of reduced antibody responses from two epidemiological studies in children and one study in adults. Although some evidence for coherent immunomodulatory responses consistent with immunosuppression was identified in short-term animal studies, the animal evidence overall is uncertain. The Toxicological Review concludes the immune effects are considered relevant to humans as the judgment is based on studies in humans.

i. For nearly all epidemiology studies of PFDA, there is potential that exposure to other highly correlated PFAS could contribute to the observed effects. The evidence synthesis for potential PFDA-induced immune effects included evaluation of the potential for confounding across PFAS as well as other sources of confounding and, based on the available data, determined that residual confounding could explain part of the observed effect, but concern was minimal, and it was unlikely to fully explain the associations seen in the literature.

c. For developmental effects, the Toxicological Review concludes that the available *evidence indicates* PFDA exposure is likely to cause developmental effects in humans given sufficient exposure conditions, based primarily on consistent findings of dose-dependent decreases in fetal weight in mice gestationally exposed to PFDA supported by some coherent evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy, although uncertainties in the available epidemiological evidence reduced the impact of these latter findings. The Toxicological Review concludes the developmental effects in mice are considered relevant to humans given similar findings of fetal growth restriction in mice and humans.

i. As described in question 3.c and footnote to 3.c, the evidence synthesis for potential PFDA-induced developmental effects considered potential confounding factors and concluded that confounding across PFAS or from other potential sources of bias (e.g., pregnancy hemodynamics in studies where PFDA was measured during or after pregnancy) introduce significant uncertainty. These sources of uncertainty ultimately reduce the strength of the available human evidence to *slight* for an evidence base that might otherwise be interpreted as *moderate*.

d. For male reproductive effects, the Toxicological Review concludes that the available *evidence indicates* PFDA exposure is likely to cause male reproductive effects in humans given sufficient exposure conditions, based on coherent evidence in adult male rats exposed to PFDA for 28 days. Although no direct information on the human relevance of the animal evidence is available, the findings in animals are presumed to be relevant based on the conserved role of androgen-dependent pathways in male productive functions across species.

e. For female reproductive effects, the Toxicological Review concludes that the available *evidence indicates* PFDA exposure is likely to cause female reproductive effects in humans given sufficient exposure conditions, based primarily on coherent evidence

from a 28-day study in adult female rats. Although human studies are available examining associations between PFDA and female reproductive toxicity (e.g., fecundity), the results were mostly null, possibly due to their low sensitivity for observing effects. The Toxicological Review concludes the female reproductive effects are considered relevant to humans given that mechanisms of female reproduction are similar between rats and humans.

f. For cardiometabolic effects, the Toxicological Review concludes that the available *evidence suggests* but is not sufficient to infer that PFDA exposure may have the potential to cause cardiometabolic effects in humans given sufficient exposure conditions, based on associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis in a few epidemiological studies. However, the evidence is largely inconsistent across studies, which adds 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 PFDA exposure may have the potential to cause neurobehavioral effects in humans given sufficient exposure conditions, based on associations between PFDA and outcomes related to attention and behavior in epidemiological studies. However, the evidence is largely inconsistent across studies, which adds considerable uncertainty. No evidence was found in experimental animals to inform this outcome (*indeterminate)*.

h. For endocrine, urinary, and other noncancer effects (i.e., hematological, respiratory, digestive, dermal, musculoskeletal, and nervous systems), the Toxicological Review concludes there is *inadequate evidence* to determine whether PFDA exposure has the potential to cause these effects in humans on the basis of the sparsity of available evidence.

Noncancer Hazard Identification	
Reviewer	**Comments**
Adgate	a. Liver effects: **Tier 2**: Page 3.-49, line 4: meets the "criteria for adversity" is an odd way of saying the evidence supports the finding of "adverse effects in the liver" in animals. **Tier 2**: Page 3-50 and surrounding text/evidence Integration. The overall determination that "slight evidence" of liver effects in humans (exposure duration not mentioned) is inconsistent given the positive effects observed 4 of 5 studies for PFDA and ALT. The finding of an association is largely consistent with the extensive literature on other longer chain legacy PFAS and liver effects. The subsequent sentence saying that the results lack coherence and mentions other (unnamed) biomarkers in humans is therefore unclear. Overall, this section would be clearer if it clearly separated the determinations from the human and the animal data, then discussed integration and consistency (or lack thereof) between the two data types. A key point on this issue would be to clearly state the relevance of a 28-day animal study for the overall determination and its relevance in humans given the time needed to reach steady-state.

	Authors should also note that given the extensive evidence from related PFAS I would say the determination lies somewhere in between slight and moderate. I agree that the mechanistic evidence supports the observed effects, which increases confidence in the conclusions and thus the writing on weight of evidence determination (WOE) needs to be clearer on what a scientifically justified finding is for this endpoint. b. Immune Effects: **Tier 2**: Table 3-12. Address header issues as noted in general comments above. **Clarification:** Table 3-12. Look at the document. If any table breaks across pages (and there are several) you can easily search "Table" and find them) the headers need to be there. **Tier 1**: Page 3-90 and surrounding text. The overall determination that evidence indicates that the association between immunosuppression and PFDA exposure is known with moderate confidence is a reasonable synthesis, though this needs to be more clearly linked to several caveats. While I concur with the finding of increased likelihood of immunosuppression in humans, the clarity can be improved as sensitivity to detect effects and uncertainties inherent in these studies should be more clearly acknowledged and integrated into the write up. Noting that this is consistent with other PFAS is also an important point that cannot be understated. What is also missing from this write up is a clear statement on why the findings from the Faroe Islands cohorts, which have several unique characteristics and dietary habits, are generalizable to the United States. While the evidence integration and related tables address the potential for confounding and note that effects are significant despite relatively low sensitivity to detect them (thus increasing confidence that the findings are real), a clearer characterization of the effect of multiple correlated PFAS on the immunosuppression attributed to PFDA is needed. There are several highly correlated PFAS (and other persistent contaminants) in the serum of most any environmental epidemiology study, including those in the Faroe Islands, so confounding is a legitimate concern, but there are limits to both the data and adjustment process that are well known. The pros (and cons) of multiple confounder adjustment/potential for overfitting are all issues that should be explicitly addressed in the write up as part of the summary judgment. A more explicit discussion of the strengths, for example, of looking at titers of multiple vaccine endpoints in more than one cohort are needed as well as a discussion of how relatively small changes in titer levels at the measurement timepoints are justifiable as key endpoints. The justification for using the response level is not completely clear, and should be compared to other options, e.g., a 10% BMR, so the authors can discuss and illustrate the significance of this choice. I agree that the short-term animal studies and mechanistic evidence are coherent and generally support the findings, and that this increases confidence in the conclusions for this section. c. Developmental effects: **Tier 2**: The characterization of an association between PFDA and a wide range of developmental effects as slight in human and moderate in animals is an reasonable synthesis of a large and varied dataset. The evidence

	integration section lacks coherence as its sequencing of topics and determinations could be clearer: it is easier to understand the findings by consulting Table 3-24. Table 3-24 is a reasonable synthesis of the major strengths and limitations and the determinations about WOE and are, for the most part, clear and scientifically justified. **Tier 1**: Page 156, line 24: The sentence starting with "Although" discussing potential bias needs to be rewritten for clarity as it is unclear what it means. **Tier 2**: Page 156, ~line 30-35: The text starting at line 30 seems like a more appropriate place to start the evidence integration section as it identifies the main endpoints and overall confidence. d. Male reproductive effects: **Tier 3**: The finding of indeterminacy for the human data on male reproductive effects is appropriate given the limitations of the few available studies and short duration of exposure. The finding that PFDA likely causes reproductive effects in male humans is a reasonable synthesis and extrapolation from single short-term animal study available and thus the scientific justification clear, though 28 days is likely too short a duration to meaningfully observe all possible effects for this endpoint. e. Female reproductive effects: **Tier 3**: The finding of indeterminacy for the human data on female reproductive effects is appropriate given the scientific limitations of the available epidemiology studies and the short-term exposure study in animals. While there are many epidemiology studies, general lack of data in this area is striking and there are no studies of key endpoints (e.g., fecundity) in animals. The finding that PFDA likely causes reproductive effects in female humans is a reasonable synthesis and extrapolation from a single short-term animal study available and thus the scientific justification clear, though 28 days is likely too short a duration to meaningfully observe all possible effects for these endpoints. f. Cardiometabolic effects: **Tier 2**: The finding of slight evidence for an association between PFDA and cardiometabolic effects in humans is a reasonable conclusion based on a synthesis of the available studies, as is the finding of indeterminate results based on the evidence from a study in animals. The justification of would be more coherent if there was a logic tree that walked the reader through the various pathways by which PFDA affects each examined contributor to overall cardiometabolic health. The finding that evidence suggests that PFDA can potentially affect cardiometabolic health is reasonable and thus the scientific justification for these findings is as clear as feasible given the limited and mixed evidence base. g. Neurodevelopmental effects: **Tier 2:** The finding of slight evidence for an association between PFDA and neurodevelopmental effects in humans is a reasonable conclusion based on a synthesis of the available studies, as is the finding of indeterminate results in animals. The finding that evidence suggests that PFDA can potentially affect neurodevelopment is reasonable and thus the scientific justification for these findings is as clear as feasible given the limited and mixed evidence base. Table 3-38 can be strengthened if it has more information on

	participant ages/age categories and breaking out results by gender as feasible when clinically relevant (e.g., ADHD tends to be more often diagnosed in boys vs girls). h. Endocrine, urinary, and other noncancer effects: **Tier 3**: The finding of indeterminacy for an association between PFDA and endocrine, urinary, and other noncancer effects in humans is a reasonable conclusion based on the analysis of the available human and animal studies. The finding that there is inadequate evidence across human animal and other data is clear and scientifically justified.
Carignan	a. Liver effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is likely to cause liver effects in humans and that evidence from *in vivo* and *in vitro* studies support a potential role for multiple pathways operant in the induction of hepatic effects from PFDA exposure. Study data are clearly and appropriately synthesized to describe strengths and limitations. Study findings are clearly, appropriately and effectively detailed to allow for scientifically supported syntheses of the findings across sets of studies. Animal study comparisons for PFDA across dose and by sex were presented and supported by the limited information available from human studies. Confidence and conclusions for the studies are scientifically justified, giving appropriate consideration to potential involvement of PPARα and non-PPARα pathways with respect to the reported liver effects. Weight-of-evidence decisions for liver effects are clearly described and scientifically justified. This reviewer agrees with the overall conclusion that hepatic effects of PFDA in rodent studies should be considered adverse and relevant to humans. Table 3-5: Should note units of the median exposure levels (e.g., ppb). [**Tier 2**] Stratification by sex and menopause status would be useful for future studies. [**Tier 3**] Exposure reduction, medical monitoring and other public health interventions are needed for communities exposed to PFDA to better protect against and monitor for liver related health outcomes. Future priorities should simultaneously focus on legacy (e.g., PFDA) and current use PFASs for such public health interventions. [**Tier 3**] b. Immune effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is likely to cause immunosuppression in humans and that residual confounding is unlikely to fully explain the associations seen in the literature. Study data are clearly and appropriately synthesized to describe strengths and limitations. Most of the study findings are clearly, appropriately and effectively detailed to allow for scientifically supported syntheses of the findings across sets of studies. Recommendations for improvement are detailed below. Confidence and conclusions for the studies are scientifically justified, giving appropriate consideration to the timing of the exposure and outcome measurements. Weight-of-evidence decisions for immune effects are clearly described and scientifically justified. P 95, Line 18: Should clarify the number and quality of studies on

	immunostimulation and autoimmunity to explain why these outcomes were excluded. [**Tier 1**] Page 96, Line 15: The first comma should be deleted. "*The analyses, in Grandjean*…" Page 99, Line 6: This seems reasonably rigorous. Perhaps note that mixtures of PFAS can have cumulative effects. [**Tier 2**] Page 99, Line 12: Effects should be plural "*examining effects in children*". [**Tier 2**] Table 3-12: Should include the dichotomous titer outcome from Timmerman et al. (2021). [**Tier 1**] Table 3-12: Recommend also including a figure showing effect estimates and 95% confidence intervals, ranked by confidence level. [**Tier 2**] Table 3-12: It is confusing that the final data set used for developing a reference dose is not included. I understand that it is because the units are different, but should find a way to prominently clarify in this section and table, and clearly show those results, as it is confusing for the reader. [**Tier 1**] Immune titer section: Should prominently state that vaccine immune titer is a functional measure of adaptive immune response and therefore an important indicator of clinically relevent immunotoxicity. [**Tier 1**] Page 102, Line 2: 'in' is grammatically incorrect. "*Another prospective birth cohort ~~in~~ examined…"* [**Tier 2**] Page 102, Line 18: Conversely, those with chronic health concerns may be more susceptible and thus a reasonable model for investigating such effects. [**Tier 2**] Future research on PFAS exposure and effects on adaptive immune response in humans (e.g., vaccine immune titer) should utilize a birth cohort study design, consider exposure across early life, include participants with a wide range of exposure levels, investigate both legacy and current use PFASs, utilize the most appropriate exposure measures (e.g., serum for legacy PFAS), measure titer at consistent time points across subjects, and consider sex differences. [**Tier 3**] Given the sensitivity of humans to immune effects of PFAS, indication of health effects using any toxicological model (computational, in vitro, animal or human) for current use PFASs should be acted on promptly for the protection of public health. [**Tier 3**] c. Developmental effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is likely to cause developmental effects in humans, and that pregnancy hemodynamics are a potential source of bias that reduces confidence in studies where exposure is measured after the first trimester. Study data are clearly and appropriately synthesized to describe strengths and limitations. Most of the study findings are clearly, appropriately and effectively detailed to allow for scientifically supported syntheses of the findings across sets of studies. Recommendations for improvement are detailed below. Confidence and

	conclusions for the studies are scientifically justified, with the exception of the continuous measure reported by Bach et al. (2016) as discussed below. Appropriate consideration was given to potential bias of pregnancy hemodynamics. Weight-of-evidence decisions for developmental effects are clearly described and scientifically justified. [**Tier 1**] Should include robust new study from combined U.S. birth cohorts, which reported a significant negative association for PFDA with birthweight in the combined analysis (Table 2, PFDA measured in any trimester), a sensitivity analysis for PFDA in first trimester maternal serum (Table S7), and stratified by infant sex (Table 6). Padula et al. Birth outcomes in relation to prenatal exposure to per- and polyfluoroalkyl substances and stress in the environmental influences on child health outcomes (ECHO) program. March 2023. Environmental Health Perspectives. 131(3). https://ehp.niehs.nih.gov/doi/10.1289/EHP10723 [**Tier 1**] Bach et al. (2016) did not log transform maternal PFDA serum levels in the continuous analysis, therefore the confidence rating in that analysis should be reduced or excluded. Should note that gestational age adjusted birth weight is the preferred measure for the birth weight outcome, and indicate which studies utilized that approach. [**Tier 1**] d. Male reproductive effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is likely to cause male reproductive effects in humans based on evidence in adult male rats. Study data are clearly and appropriately synthesized to describe strengths and limitations. Most of the study findings are clearly, appropriately and effectively detailed to allow for scientifically supported syntheses of the findings across sets of studies. Confidence and conclusions for the studies are scientifically justified given the similarity of the mechanism of male reproduction between rats and humans. Weight-of-evidence decisions for female reproductive effects are clearly described and scientifically justified. Figure 3-64: The clear and substantial non-monotonic dose response for testosterone (NTP 2018) is interesting and notable, with an increase in sperm concentration at the lower dose followed by a significant linear decline at subsequent increasing doses. Human studies should be discussed in this context for all male reproductive endpoints. [**Tier 2**] Evidence integration, Page 225, Line 2 and Table 3-26. Non-monotonicity of testosterone effects should be noted as a possible explanation for inconsistency among male reproductive endpoints in the human studies, which rarely considered such a dose response. [**Tier 1**] Recommend integrating evaluation of non-monotonicity of dose response into the literature review and evidence integration. [**Tier 2**] Future studies should consider possible non-monotonicity of associations with all male reproductive endpoints. Previous studies could re-analyze

data with this consideration and consider combining cohorts to improve sample size and exposure range. [**Tier 3**]

e. Female reproductive effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is likely to cause female reproductive effects in humans, and that existing human studies have low sensitivity. Study data are clearly and appropriately synthesized to describe strengths and limitations. Most of the study findings are clearly, appropriately and effectively detailed to allow for scientifically supported syntheses of the findings across sets of studies. Recommendations for improvement are detailed below. Confidence and conclusions for the studies are scientifically justified given the similarity of the mechanism of female reproduction between rats and humans. Weight-of-evidence decisions for female reproductive effects are clearly described and scientifically justified. Recommendations for improvement are detailed below.

Figure 3-53: It is unclear which are male and which are female, these should be labeled. [**Tier 1**]

Should note that while the histopathology study found no effects on the mammary glands, timing of the exposure and outcome was not in a sensitive developmental window (in utero or pregnancy). [**Tier 2**]

The lactation duration outcome should be included in this section, as the pregnancy and postpartum period is a sensitive developmental window for mammary gland development (as is in utero/early life). It should also be noted that mammary gland development is a sensitive outcome for other PFAS (e.g., PFOA). [**Tier 1**]

Timmermann CAG, et al. Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. 2017. Reprod Toxicol 68:164-170. doi:10.1016/j.reprotox.2016.07.010.

Romano ME et al. Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. 2016. Environ Res. 149:239-246. DOI: 10.1016/j.envres.2016.04.034

For additional relevant references: https://ehp.niehs.nih.gov/doi/full/10.1289/ehp.125-A17

The discussion and evidence stream (Table 3-29) should be revised to reflect the following:

The statement of 'unclear biological relevance of increases' in testosterone in the single (high confidence) animal study (NTP 2018) should be revised to note coherence with the finding of continuous diestrous as high levels of androgens in women causes infrequent, irregular or non-existent menstruation. [**Tier 1**]

The finding of high testosterone and a continuous phase of diesterous in the NTP (2018) study is also consistent with the finding of decreased progesterone in Leydig tumor cells (Zhao et al. 2017). High levels of testosterone in women are associated with levels of progesterone, irregular menstruation and decreased

	fertility. [**Tier 1**] f. Cardiometabolic effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is suggestive of cardiometabolic effects in humans, and of clear associations with serum lipids. Study data are clearly and appropriately synthesized to describe strengths and limitations. Most of the study findings are clearly, appropriately and effectively detailed to allow for scientifically supported syntheses of the findings across sets of studies. Confidence and conclusions for the studies are scientifically justified. Weight-of-evidence decisions for cardiometabolic effects are clearly described and scientifically justified. g. Neurodevelopmental effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is suggestive of neurodevelopmental effects in humans, but not sufficient to infer causation given inconsistent findings in epidemiologic studies and an absence of animal studies. Study data are clearly and appropriately synthesized to describe strengths and limitations. Most of the study findings are clearly, appropriately and effectively detailed to allow for scientifically supported syntheses of the findings across sets of studies. Recommendations for improvement are detailed below. Confidence and conclusions for the studies are scientifically justified. Weight-of-evidence decisions for neurodevelopmental effects are clearly described and scientifically justified. High quality research is needed on neurodevelopmental effects in animal studies. [**Tier 3**] Future research should also investigate neurodegenerative outcomes. [**Tier 3**] h. Endocrine, urinary, and other noncancer effects: This reviewer agrees with the conclusions made by USEPA that PFDA exposure is currently inadequate for endocrine, urinary and other noncancer effects. It should be made clear that an exception to this is conclusion is effects on reproductive hormones, which are also part of the endocrine system and for which effects were previously discussed (male and female reproduction). [**Tier 1**] The thyroid hormone section would benefit from a table and/or figure grouped by confidence level and noting limitations. [**Tier 2**] **Clarification:** Human evidence, but this comment could apply to animal as well. Established determinations of thyroid effects for other PFASs (e.g., PFOA and PFOS) should be noted. [**Tier 2**]
Faustman	a. Liver effects: This reviewer is in agreement with the "available data synthesis" of data input for this non-cancer endpoint. The human epi and animal in vivo data in the report is reviewed and the strengths and weaknesses of each study is presented and those reviews support the study confidence score as well as the strengths and limitations described for each study. Information on cross species consistency as well as dose response and effect level discussion support Section 3 Non Cancer Hazard Identification processes for hepatic endpoints (and subsequent non cancer

	endpoint evaluations). The presentation style and thoroughness of the hepatic endpoint evaluations contributes to the clarity and transparence in all aspects of this organ system review. Dose response information for the individual studies is presented methodically in Tables such as in Figures 3-5, 3-7 and 3-9 (examples). In addition, information on specifics of the severity and incidence of various hepatocyte lesions in tested species is given in Tables such as 3-6. This reviewer did support basic assumptions of relevance statements such as assumed relevance of animal studies unless mechanistically proven to be non relevant for humans. The overall synthesis of these data across platforms and species and hepatic relevant endpoints are then presented in a weight of evidence table (Table 3-11) that provides a very clear evidence path for excellent reviews of available data and provide both in text explanation as well as in Table 3-3 an excellent study by study evaluation. The section 3.2.1 Hepatic effects provide a robust discussion of what serum enzyme biomarkers are assumed to be indicative of a potential adverse outcome versus adaptive response providing dose response information, cross species consistency and mechanistic discussions to support the assumption of adversity for human responses. Likewise for the mechanistic evaluations of PPAR a versus non PPAR a dependent responses following PFDA, this reviewer supports the initial discussion of and support of using both in vivo and in vitro data and Computational Toxicological data to support the decision for not being able to discount the non PPAR a dependent PFDA response pathways. As mentioned in this PFDA report this decision is also supported by the decision for other longer chain PFAS compounds. b. <u>Immune effects</u>: The IRIS team have very carefully detailed the complexity of potential immune system impacts of PFDA in Section 3.2.2. Due to this complexity of immunotoxicity responses, the report lays out the definitions and response types that will be reviewed for this IRIS report. Consistent with WHO/IPCS definitions and with the availability of data, this hazard identification reviewed available data for both immunosuppression and sensitization and allergic response. This reviewer agrees with their decision to not examine immunostimulation and or autoimmune disease response based on lack of references for these responses. Overall, this reviewer agrees with the assessments of the available data and the study confidence conclusions. I also agree with the lists of potential strengths and weaknesses of the studies available and noted in particular that most to many of the human epidemiology studies for immunosuppression and infectious disease are reported from country cohorts outside of the US with an emphasis of detailed findings from the Faroe Islands. This reviewer also noted and agreed that “cross-stream coherence” existed with evidence of immunosuppression in both animals and humans (Table 3-19). It was noted that children and in utero fetuses maybe susceptible populations. Minimal information is available on MOA but both animal and human data points to possible NFkB pathway involvement as well as modification of cytokine response. Many questions remain for this endpoint but this

	reviewer is **not requesting any Tier 1 or 2 recommendations** at this time and agrees with both the study confidence conclusions and weight of evidence tables. **A Tier 3 recommendation** is for further clarification of how the WHO guidelines differ from some of the other texts on immunotoxicity as this reviewer believes that this will be an important area of expanding research on response across the PRAS universe of chemicals. Also following COVID it is understandable that we are looking more comprehensibly about the immune system responses. c. Developmental effects: The draft IRIS report identifies 46 epidemiological studies of relevance for assessing developmental impacts. The report notes that many of these have some information on fetal growth decreases and that in general many of these observations are suggestive of fetal growth restriction. In addition, several rodent studies support these observations. Again, USEPA has presented a thoughtful and critical evaluation of these studies. I concur with the observations that the Human Epidemiology studies are suggestive for associations of fetal and postnatal growth restrictions however these are appropriately labeled as slight. Observations of fetal growth reduction findings in rodent studies provides cross-stream coherence which I agree with. This evidence for growth reduction is observed as dose related changes in the rodent models. The report discusses the evidence for this reduction at non-toxic versus maternally toxic levels of exposure and Figures 3-56 and 3-57 and Table 3-23 show evidence for these reductions occurring at less than maternally toxic doses. This reviewer agreed with these findings and concurred with the evidence stream summary for rodent growth retardation during development as moderate and together agreed with the statements that the overall inferences and summary judgment as likely evidence for growth retardation in humans. Evidence for other endpoints following PFAS exposure such as spontaneous abortion, gestational duration, etc did not support there use in the development of toxicology values. These details were presented in Table 3-24 and these are detailed and rationale is presented in a clear manor. The authors discuss reasons for variability in response between the human and rodent models and speculate that there maybe hemodynamic changes across gestation. The evidence and magnitude of these hemodynamic changes are consistent with pregnancy related changes however this reviewer remained skeptical for these suggestions and look forward to additional research that USEPA had will provide that could resolve this issue. Minimal information was obtained by the zebra fish study other than to acknowledge that growth restriction will manifest its self under high exposures esp in sensitive populations such as children. Interpreting the complexity of the human epidemiological studies considering PFDA association with developmental endpoints has been challenging and the USEPA discussions on gestational duration and pre term birth illustrate their methodical evaluation (see Figures 3-50, 3-51and 3-52 as examples). In many cases the available animal studies helped to support when dose response relationships were present and also when these relationships were complicated by significant maternal toxicity. This reviewer agrees with the USEPA conclusions that complications of when PFAS compound exposures were taken across varied lifecourse times and variations in the ability to associate impacts with specific compounds such as PFDA remain unresolved for these endpoints in particular.

	This reviewer will make a **Tier 3 recommendation** that in the future when the USEPA does a cumulative risk assessment across the multiple PFAS related compounds that they are reviewing, these developmental endpoints in particular are re-considered for mixture effects. d. Male reproductive effects: This reviewer agrees with the approach that USEPA used in their review of male reproductive effects. Their evidence profile for PFDA exposure and male reproductive effects is given as Table 3-26 and that table identified the availability of both human, animal and some mechanistic evidence. The human evidence overall was labeled with an evidence stream judgment of Indeterminate for semen evaluation, pubertal development and reproductive hormonal changes and this reviewer agreed with this designation and with the reasons provided for that judgement. Despite having observed decreased motility with increased PFDA exposure in one study, 3 others did not show a similar relationship. In addition, decreased testosterone was seen in one adult study and one study of adolescents however other studies of both medium and low confidence resulted in "unexplained inconsistencies" in the overall studies available for assessment. The availability of a 28 day animal study, albeit inadequate for evaluating the full spermatogenesis cycle, provided moderate evidence for related effects in a dose dependent manner for sperm impacts, testicular histopathology, reproductive hormones and organ weights. When combined with mechanistic data on altered Leydig cell androgen function and Tox Cast observations following PFDA exposure, this assessment provided plausible mechanistic evidence for how these effects were occurring. (Please see notes regarding the sperm DNA fragmentation study discussed under the cancer endpoint. There should be cross referencing with those finding under this toxicity endpoint. **Tier 1 recommendation** is to ensure that this cross- referencing is done and sited as mechanistic evidence supportive of the current conclusion in this IRIS review.) e. Female reproductive effects: This reviewer agrees with the USEPA assessment of this endpoint. Despite the presence of various human studies looking at endpoints(such as reproductive hormones, fecundity, puberal development, menstrual cycle and endometriosis) relevant to female reproductive function, these studies were identified by the evidence stream summary as "indeterminate" and this reviewer agreed with this designation since these studies had unexplained inconsistencies and limited sensitivity to determine dose response relationships and associations. The availability of a 28 day rodent study, albeit limited in the lifecourse that it examined, provided moderate evidence for dose response for estrous cycle, organ weight and altered hormone levels and provided context for the assumed relevance of animal studies for human and designation of evidence integration of likely. Mechanistic studies that showed the ability of PFDA to inhibit progesterone production provided supportive context for the in vivo animal studies. It is striking to this reviewer the lack of adequate toxicological studies to cover important life

	stages such as that covered in normal development and reproductive assessments. Given the widespread exposure to the PFAS family of compounds and consistent impacts on important reproductive endpoints, that these compounds have not been prioritized for additional in vivo characterization. This reviewer supports and adds as a **Tier 3 recommendation** the need for future studies on "the effect of PFDA on female fertility and pregnancy outcomes in exposed animals from sub-chronic, chronic, development, or multigenerational studies as well as in vivo or cell culture mechanistic studies." (see page 3-204, lines 19-25). f. Cardiometabolic effects: The USEPA presents a review of five of 6 medium confidence human epidemiological studies that showed an increase in serum total cholesterol with higher PFDA exposures. Other serum lipid changes were not this consistent and lack of coherence was noted across many of the serum lipid assessments. The evidence stream judgement was labeled as slight due to the positive associations noted above. However most other observations were inconsistent or lacked coherence. The animal studies were indeterminate due to species differences and lack of coherence. This reviewer was supportive of the USEPA's conclusions. g. Neurodevelopmental effects: USEPA identified 13 human studies of neurodevelopmental outcomes in humans and five out of six studies that looked at behavioral issues and/ or attention problems were positively associated (note that some studies were from the same cohort and USEPA detailed when separate cohorts were evaluated). USEPA stated that the ADHD diagnosis studies were the most clinically relevant but these had "inverse findings" for association with PFDA. Figure 3-76 and Table 3-38 (medium confidence studies only) summarize the details of these studies and look at specific outcomes such as diagnosed ADHA, behavioral regulation, externalizing problems and various behavioral summary scores. Note that only Niu et al, 2019 was identified by USEPA as having sensitivity while the other studies were deficient due to "limited exposure contrast". In summary this evidence stream judgment was slight and the evidence integration summary judgement was suggestive. There was not a neurodevelopmental study in animals available nor were any references cited for in vitro or Comp Tox data. Also, no mention of consistency or inconsistency of neurodevelopmental outcomes from other structurally related PFAS compounds cited**. Tier 2 recommendation** is made by this reviewer to clarify (ie add one or two sentences) that state or modify the availability of any supportive evidence such as cross coherence with animal or mechanistic data and a reference to consistency with other PFAS related long chain compounds. h. Endocrine, urinary, and other noncancer effects: Consistent with studies of other PFAS compounds, the USEPA focused on thyroid function and response. The document reports 23 human studies that have examined thyroid hormones and PFDA exposure and Figure 3-77 evaluates these studies. The text describes several factors that resulted in a designation of "deficient" for outcome ascertainment and several of these are consistent with other sections of this report such as a study being under powered and unable to ascertain association with exposure with needed sensitivity (7 of the studies listed in Figure 3-77 as

	listed as having adequate sensitivity). Several other factors that were used to identify "deficient" studies are concerning. These include factors such as only studies with fasting thyroid measurements being deemed non deficient and also considering studies that did not take thyroid measures at the same time of day for all measurements deficient. It is good that these studies were not excluded but designation as "deficient" definitely affected interpretation during the epidemiology study evaluation. Other factors that are more concerning regard the fact that interpretation of the studies of this PFAS compound, PFDA was not consistent with the interpretation of other recent PFAS IRIS documents as well as not clearly supported by more recent references in the literature. For example, prior PFAS compounds have been reported as having consistent effects on thyroid hormones and yet these changes were not always associated with concurrent or "anticipated" changes in TSH. Lines 27-32 on page 3-250 refer to these differences and this is listed as "inconsistent" and the resulting impact of this designation has resulted in listing as "indeterminate" on Table 3-41 whereas this reviewer would have designated this evidence stream as "slight" or higher. **Tier 1 recommendation from this reviewer is to re-examine this study evaluation of the epidemiology studies for thyroid effects given the comments listed above and the comments received from the public on this topic as well for consistency in interpretation of the pathway responses from related recent IRIS documents on other related PFAS compounds such as PRHxA, etc.** Similar concerns are raised by this reviewer for the interpretation of the animal studies and for the interpretation of the mechanistic studies. For example, Table 3-41 lists a high confidence study available for evaluation and this study showed consistency for decreased fT4 in both male and female rats and showed a dose-response for deceased TSH in males and decreased fT4 in males and females at levels (of 1.25 mg/kg-d. No change in tT4 was observed. These observations were designated as "lack of expected coherence across thyroid measures with any currently available understanding of adverse thyroid-related changes" and this designation is inconsistent with previously observed and discussed PFAS associated and observed changes. **Tier 1 recommendation from this reviewer is to re-examine the in vivo animal studies as this evidence stream would suggest an evidence steam judgement as moderate.** Mechanistic data cited in Table 3-41 include information from studies that "PFDA may impair" HPT axis response, PFDA may impair binding of thyroid hormones, and PFDA may alter thyroid activity in rats. This supplemental data is limited due to route of exposure ip and high doses but does illustrate a potential for receptor binding. See comments in the preceding paragraph**.** Note that the animal study discussion and summary in Table 3-41 notes that no histopathology was observed but frequently such a suggestion continues to have a comment about the length of time that observations were made ie a 28 day study or longer as such pathologies would not necessarily be expected in the shorter studies. **Tier 1 recommendation is to clarify this in the table and the text on lines 1-11 on page 3-255.** Lines 32 to 38 (page 3-263) and lines 1-4 (page 3-264) are important to note

	regarding the potential impact of such thyroid effects during sensitive life-stages. This reviewer applauds the inclusion of such information. **Tier 1 recommendation** to USEPA is to reevaluate the designation of "inadequate evidence" in Table 3-41 for the evidence integration summary judgement given the recommendations and discussions above and by other reviewers and public commentors. It is the opinion of this reviewer given the suggested changes above that the overall designation would be "suggestive" of higher. Urinary and non cancer endpoints: This reviewer did not have significant comments about the summary of potential Kidney toxicity or general toxicity associated with PFDA studies. High doses used in the general toxicity studies diminished the use of these studies and this reviewer agrees with the decision not to use these in the evidence integration process. This reviewer noted that the duration of the animal studies would not be long enough to definitely diagnosis presence of histopathological changes in the kidney or most other organs.
Fisher	a. Liver effects: Yes, liver effects are relevant for human exposures. An extensive effort by EPA. MOA discussion is important. a.i. Yes, the PPARα and non-PPARα issues are relevant for humans. Good review. Clear. b. Immune effects: Yes, these studies may be relevant to humans. b.i. I think this is a huge issue that is not adequately addressed, mixtures. A more rigorous methodology is required to examine the potencies of PFAS for specific endpoints. Statistics should be supported by biological plausibility (**Tier 2**). **Clarification:** An example of a rigorous method would be direct experimentation with a similar mixture, either in vitro or in vivo which would support the approach used. I do not know if this data is available. I suspect in vitro methods would be the best. c. Developmental effects: Yes, the evidence does indicate that developmental effects may be possible given sufficient exposures to PFDA. c.i. Slight evidence in humans seems adequate. d. Male reproductive effects: I am not qualified to answer this question. e. Female reproductive effects: Yes, given sufficient exposure conditions (if they exist), may cause female reproductive effects in humans. f. Cardiometabolic effects: I agree with this statement. It would be useful to include some background on why this was evaluated based on MOA. (**Tier 2).** g. Neurodevelopmental effects: I agree with this this statement.

	h. Endocrine, urinary, and other noncancer effects: I agree with this conclusion of inadequate evidence. The endocrine evidence (thyroid) discussion seems dated when talking about classical thyroid biomarkers (serum TSH and thyroid hormones). There are many examples in the literature where serum free T4 in humans and total and/or free T4 in animals decrease in response to a chemical or drug and TSH appears unchanged or normal. I recommend that EPA form a focus group to address the expectations for thyroid hormone perturbations and related adverse toxicity. This is an important topic in light of high-throughput efforts looking at thyroid molecular initiating events. I think the EPA interpreted the thyroid data incorrectly (the data are ok) given the growing body of evidence about the thyroid system. **Clarification:** Animal studies and even human studies with compounds that target the thyroid system result in outcomes that are not classic (decrease in serum T4 and increase in serum TSH). The results are often not considered or understood. It would be very useful for the EPA offices to have the same view about thyroid endpoints (**Tier 2**).
Georgopoulos	a. For liver effects, the Toxicological Review appropriately concludes that the available evidence indicates PFDA is likely to cause liver effects in humans given sufficient exposure conditions, based on short-term studies in rats and mice demonstrating consistent and coherent effects with a clear biological gradient. The liver findings for PFDA were similar to those for other structurally related long-chain PFAS and determined to be adverse and relevant to humans. The Toxicological Review also appropriately evaluated evidence relevant to the potential involvement of PPARα and non-PPARα pathways with respect to the reported liver effects to conclude that available data support a potential role for multiple pathways operant in the induction of hepatic effects from PFDA exposure, although the interaction of those pathways within a mode of action (MOA) cannot be specifically determined. b. For immune effects, the Toxicological Review appropriately concludes that the available evidence indicates PFDA is likely to cause immunosuppression in humans given sufficient exposure conditions. EPA reached this decision primarily based on consistent evidence of reduced antibody responses from two epidemiological studies in children and one study in adults. The document also presents literature evidence from short-term animal studies for coherent immunomodulatory responses consistent with immunosuppression, but the animal evidence overall is uncertain. As the assessment is based on studies in humans, the Toxicological Review appropriately concludes that the immune effects are considered relevant to humans. The Toxicological Review document also presented and discussed concerns that potentially exposures to other co-occurring ("highly correlated") PFAS (and other immunomodulating agents) could contribute to observed immune effects and concluded that confounding by other factors, including PFAS co-exposures, was unlikely to fully explain the associations of immune effects with PFDA that are reported in the literature. As a qualitative conclusion this can be considered appropriate, but confounding effects can impact estimates of PFDA levels used in deriving RfDs. • **Note:** I am discussing the issue of confounding by other PFAS exposures in

	my response to Charge Question 3, as confounding may impact the calculated values of metrics involved in the derivation of the RfD for immune and developmental effects. c. For developmental effects, the Toxicological Review appropriately concludes that the available evidence indicates PFDA is likely to cause developmental effects in humans given sufficient exposure conditions, based primarily on consistent findings of dose-dependent decreases in fetal weight in mice gestationally exposed to PFDA, supported by some coherent evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy, although uncertainties in the available epidemiological evidence reduced the strength of the available human evidence to slight for an evidence base that might otherwise be interpreted as moderate. The Toxicological Review reasonably concludes that the developmental effects in mice are considered relevant to humans given similar findings of fetal growth restriction in mice and humans. d. For male reproductive effects, the Toxicological Review appropriately concludes that the available evidence indicates that PFDA is likely to cause male reproductive effects in humans given sufficient exposure conditions, based on coherent evidence from a study that exposed adult male rats to PFDA for 28 days. The findings from the animal study were reasonably presumed to be relevant based on the conserved role of androgen-dependent pathways in male productive functions across species. e. For female reproductive effects, the Toxicological Review appropriately concludes that the available evidence indicates that PFDA exposure is likely to cause female reproductive effects in humans given sufficient exposure conditions, based on coherent evidence from a study that exposed adult female rats to PFDA for 28 days. The document discussed available human data showing mostly null associations between PFDA and adverse female reproductive outcomes but concluded that this was probably due to low study sensitivity for observing effects. The Toxicological Review reasonably concludes the female reproductive effects are considered relevant to humans given that mechanisms of female reproduction are similar between rats and humans. f. For cardiometabolic effects, the Toxicological Review appropriately concludes that the available evidence suggests but is not sufficient to infer that PFDA exposures may have the potential to cause cardiometabolic effects in humans given sufficient exposure conditions, based on associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis, as there is considerable uncertainty and the evidence is largely inconsistent across available epidemiological studies. EPA also appropriately concluded that evidence in experimental animals was indeterminate. g. For neurodevelopmental effects, the Toxicological Review appropriately concludes that the available evidence suggests but is not sufficient to infer that PFDA exposure may have the potential to cause neurobehavioral effects in humans given sufficient exposure conditions, based on associations between PFDA and outcomes related to attention and behavior in epidemiological studies.

	However, the evidence is largely inconsistent across studies, which adds considerable uncertainty. As no information was found for relevant experimental animal studies, EPA appropriately concluded that neurodevelopmental effects evidence in animals was indeterminate. h. For endocrine, urinary, and other noncancer effects (i.e., hematological, respiratory, digestive, dermal, musculoskeletal, and nervous systems), the Toxicological Review appropriately concludes that there is inadequate evidence to determine whether PFDA exposure has the potential to cause these effects in humans.
Haney	a. Liver effects: The study confidence conclusions appear scientifically justified, giving appropriate consideration to important study attributes. For each study in the relevant figures of Section 3.2.1 (e.g., Figure 3-8), the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level rating. Accordingly, the study confidence conclusions appear scientifically justified, with EPA having given appropriate consideration to important study attributes. This question (under "2") also concerns whether the presentation and analysis of study results is clear, appropriate, and effective, a step just upstream of synthesis that if well done, then allows for appropriate syntheses of the endpoint-specific findings. In this reviewer's opinion, the overall presentation and analysis of study results appears clear, appropriate, and effective. Additionally, it appears that by and large, the available data on hepatic effects are clearly and appropriately synthesized to describe the strengths and limitations. P. 3-50, lines 18-19 state that, "Taken together, the available ***evidence indicates*** that PFDA exposure is likely to cause hepatotoxicity in humans given sufficient exposure conditions (see Table 3-11)." Table 3-11 is the evidence profile table for hepatic effects, which among other information contains both factors that increase certainty and factors that decrease certainty along with evidence stream (i.e., human, animal, mechanistic/ supplemental) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.1) also contains information relevant to and supporting the weight-of-evidence (WOE) for hepatic effects. Importantly, recommendations of the Hall et al. (2012) paper were considered by the EPA in assessing the adversity of observed hepatic effects (e.g., in discussing sufficiently supporting histological evidence). However, while p. 3-49 (lines 1-4) state that, "Overall, application of the recommendations from Hall et al. (2012) clearly supports the conclusion that PFDA exposure has multiple and coherent effects on liver histopathology, serum biomarkers and liver weights in exposed animals (primarily rats) that meet the criteria for adversity", this is not clear from the preceding text. As a **Tier 2 suggested revision**, EPA should explicitly show how the Hall et al. (2012) criteria for adversity are met. For example, while fold increases in biomarkers of liver dysfunction (e.g., bilirubin, bile acids/salts) are explicitly discussed on p. 3-48 (lines 36-38), the fold increases needed to fulfill a second criterion (e.g., in ALT or ALP) are not explicitly discussed on lines 34-35 (e.g., a 2- to 3-fold increase in ALT or biomarkers of hepatobiliary damage such as AST, ALP and γ-glutamyltranspeptidase [γGT]; Tables 3-7 and 3-8 are relevant). Additionally, as a

	Tier 2 suggested revision, the EPA should consider additional tables and/or figures that would help readers visualize important EPA conclusions, such as "coherent changes in serum biomarkers, histopathology, and liver weights" cited in Table 3-11. This being said, taken together, the weight of the available scientific information presented (e.g., evidence of PPARα-independent pathways) provides support that assuming sufficiently high exposure over a sufficiently long duration (i.e., "given relevant exposure circumstances"), PFDA exposure is likely to cause hepatic toxicity in the general human population, which includes potentially susceptible subpopulations (e.g., those with pre-existing liver disease). Though there is some room for improvement (e.g., additional tables/figures), the bases for this WOE decision are clearly described in the text (*Evidence Integration*) and Table 3-11 of the document. Last but not least, in regard to PFDA being likely to cause hepatic effects in humans given sufficient exposure conditions, footnote 9 (p. 3-50) states that, "The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5." For such dose-response analyses conducted on animal data, this begs questions such as… "What laboratory animal species, if any, has a dose-response for hepatic effects that is predictive (after dosimetric adjustments) of the same or similar effects in humans?" While the document states that the "likely to cause" conclusion is "based primarily on coherent liver effects in rats (and, to a lesser extent, mice)" (p. 3-50, lines 19-21), no attempt is made to establish rodents as good laboratory animal models for PFDA-induced liver effects in humans, much less identify one species as the better animal model for predicting the same or similar effects in humans. Scientific justification should be provided for why the species (i.e., the rat) ultimately providing the basis of the organ-specific toxicity factor (i.e., osRfD) is expected to have a dose-response more predictive of that in humans than other species (i.e., the mouse) for which data are available (**Tier 1 necessary revision**). It is not scientifically robust to simply state "that without evidence to the contrary, the human relevance of animal findings is assumed" (p. 1-14, lines 20-21), and this statement could be equally applied to negative findings in a given laboratory animal species. When not addressed scientifically, use of data from a given animal species for dose-response assessment applied to humans can be a large and key area of uncertainty (e.g., where significant interspecies differences in sensitivity exist in the absence of data to inform identification of the most human-relevant laboratory animal species) that pertains directly to the meaningfulness of the resultant toxicity factor itself and for credibly informing risk/hazard management decisions. The National Research Council (NRC) has advised that proper characterization of uncertainty is essential in risk assessment since an assessment that omits or underestimates uncertainty can leave decisionmakers with a false sense of confidence in estimates of risk (NRC 1983,1994,1996,2002). Use of an animal model as a surrogate for humans is an aspect of uncertainty that should be adequately addressed and characterized in an assessment (USEPA 2005a). However, the document does not address the uncertainty regarding the most appropriate animal model and whether a similar dose-response may or may not be expected in humans (after appropriate dosimetric adjustments). As a **Tier 1 necessary revision**, EPA should: (1) attempt to scientifically justify whether the mouse or rat is likely most

	biologically representative of humans such that the same or similar effects (hepatic, etc.) are expected in humans at similar doses when converted to human equivalent doses (HEDs); and (2) in the event (1) cannot be established with sufficient scientific confidence, acknowledge within the assessment that the choice of the most appropriate laboratory animal model for prediction of PFDA-induced adverse effects in humans has not been scientifically established (i.e., is not "settled science") but rather species selection is based on policy. This is a major area of uncertainty, but it is not without precedent that it be explicitly acknowledged in the associated uncertainty section (e.g., USEPA 2021).[3] it is imperative for EPA to acknowledge whether or not the dose-response data for a species selected for osRfD derivation (most often the most sensitive species) is known or likely to be (e.g., based on greater relevant biological similarities) the most predictive available for similar effects in humans or if dose-response data from another species might be equally relevant to humans and result in a significantly different (e.g., higher) osRfD. Such evaluations and statements are **Tier 1 necessary revisions** that would increase transparency and thereby improve influential EPA dose-response assessments, significantly improve uncertainty sections, and provide a more complete picture of the extent to which important/key aspects of an assessment are "settled science." These comments on laboratory animal species also apply as **Tier 1 necessary revisions** to other effects where animal data form the basis of an osRfD (e.g., male and female reproductive effects for subchronic osRfDs). a.i. In regard to rodent hepatic effects involving PPARα, the bottom line from Appendix A seems to be that "human relevance is the default, and mechanistic evidence will need to be compelling and strong to conclude otherwise (i.e., to conclude that findings in animals are not relevant to humans)" (p. 9-14, lines 25-27). As opposed to WOE for establishing the unlikely relevance of an effect to humans, this default appears to establish a scientific burden of proof of being able to confidently rule out the potential of non-PPARα pathways as possible contributors to hepatic effects. Accordingly, it makes the following comment almost moot. Section 2.4.2 of Appendix A states [*emphasis added*] that, "Activation of the peroxisome proliferator-activated receptor alpha (PPARα) by PFAS has been reported, with in vitro evidence that the potency of human and mouse PPARα activation is positively correlated with increasing PFCA chain length up to C9 (*no human receptor activation was noted for PFDA*…" (p. 2-25, lines 1-4). This seems to contradict the draft assessment statement, "PFDA can activate the human PPARα in vitro" (p. 3-50, line 5). EPA should resolve this apparent discrepancy (**Tier 1 necessary revision**). b. <u>Immune effects</u>: EPA has given consideration to important study attributes. For each study in the relevant figures of Section 3.2.2 (e.g., many of the figures from Figures 3-10 through 3-23), 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. However, I do have comments below regarding whether EPA has given full consideration to the epidemiological

[3] For example, in Section 2.2.8 (p. 2-20), the IRIS assessment for tert-butyl alcohol (USEPA 2021) acknowledges that, "Most importantly, which animal species or sexes might be more comparable to humans is unknown."

	study weaknesses/limitations and whether the associated results should be used for quantitative risk assessment and toxicity factor (e.g., RfD) derivation. Additionally, this question (under "2") concerns whether the presentation and analysis of study results is clear, appropriate, and effective, a step just upstream of synthesis that if well done, then allows for appropriate syntheses of the endpoint-specific findings. The overall presentation of study results appears clear and various strengths and limitations are described. Table 3-19 is the evidence profile table for immune effects, which among other information contains some factors that increase and decrease certainty along with evidence stream (i.e., human, animal, mechanistic/supplemental) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.2) also contains information relevant to the WOE for immune effects. As a **Tier 2 suggestion**, the EPA should consider additional tables and/or figures that would help readers visualize important EPA conclusions, such as "coherent evidence of potential immunosuppression in rats and mice at doses ≥0.089 mg/kg-d across two high/medium confidence studies" cited in Table 3-19. P. 3-92, lines 35-37 state that, "Altogether, considering the available evidence from human, animal and mechanistic studies, the ***evidence indicates*** that PFDA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans, given sufficient exposure conditions (see Table 3-19)." EPA indicates that this hazard judgment is driven primarily by consistent evidence of reduced antibody response from human epidemiological studies (mostly from two birth cohort studies)…" (pp. 3-92 and 3-93, lines 37-1). *However, it is not clear that EPA has sufficiently considered adversity for the primary basis of this hazard judgment, fully considered the weaknesses/limitations of this evidence, or fully considered the lack of supporting evidence from studies on potentially increased incidences of disease*. For example, even without considering potential confounding, 3/4 odds ratios (ORs) for PFDA and antibody concentrations falling below the generally considered protective level of 0.1 IU/mL for tetanus and diphtheria in children ages 5 years (n=510) or 7 years (n=386) contain 1, indicating that the WOE from this key study cohort is for no statistically significant associations with less-than-protective serum antibody concentrations in children (see eTable 4 of Grandjean et al. 2012).[4] Consistent with this, the more recent Grandjean et al. (2017a) study states [*emphasis added*] that, "With many antibody concentrations being close to the assumed clinically protective level of 0.1 IU/mL, *logistic regression showed only weak tendencies for antibody levels below the limit to be associated with serum PFAS concentrations*."[5] Also consistent with a WOE for no statistically significant associations (much less being able to say "effects" as there are problems with causal attribution to PFDA), 6 of 8 confidence intervals (95% CIs) for both tetanus

[4] The confidence interval (CI) for the one statistically significant OR of 1.36 (age 5, diphtheria) is (1.04, 1.77), with the lower end of the CI practically equal to 1 (eTable 4 of Grandjean et al. 2012).

[5] Also, while Grandjean et al. (2017b) state that, "At age 5, 152 (44%) children had antibody concentrations lower than the protective level of 0.1 IU/mL for diphtheria and 126 (36%) for tetanus", this appears inconsistent with Table 1 of that study, which shows that the 25th percentiles for diphtheria and tetanus serum antibody concentrations were 0.1 IU/mL.

	and diphtheria serum antibodies included 0% change per 2-fold increase in maternal and age 5 serum PFDA (see Table 3 of the study). Additionally, in Grandjean et al. (2012), PFDA was correlated with other PFAS (e.g., PFOS, PFOA, PFNA) that had some associations with antibody concentrations falling below the protective level of 0.1 IU/mL (see Table 2 and eTable 4 of Grandjean et al. 2012).[6] Moreover, the level of serum antibodies corresponding to a clinically protective level appears to be assay specific. For the toxin binding inhibition (ToBI) assay apparently used in the Faroe Islands studies, ≥0.01 IU/mL is considered to be the clinically protective level, not the value of ≥0.1 IU/mL indicated by study authors. Considering the comments above, this means that the reported decreases in serum antibodies are even less likely to be biologically/clinically significant. This is not surprising given the rarity of tetanus/diphtheria cases, particularly in those fully vaccinated, and the WOE for PFDA not being associated with statistically significant increases in the incidences of diseases based on the epidemiological literature (all discussed below). To say the least, all this brings into serious question the validity of the EPA's 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 the 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.[7] 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

[6] A 2-fold increase in PFOS and PFOA concentrations at age 5 years was associated with odds ratios between 2.38 (95% CI, 0.89 to 6.35) and 4.20 (95% CI, 1.54 to 11.44) for falling below a clinically protective level of 0.1 IU/mL for tetanus and diphtheria antibodies at age 7 years.

[7] 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."

	level of 0.01 IU/mL.[8] 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), even further calling into question the biological/clinical significance and adversity of the reported results. *In regard to confounding and the ability to causally attribute associated effects to PFDA*: • 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*." • Similarly, Grandjean et al. (2017b) state [*emphasis added*], "The close correlations *prevented meaningful adjustment* for concomitant PFAS exposures." *Thus, it appears that effects may neither rise to the level of adversity nor be attributable specifically to PFDA.* Co-exposures to 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 PFDA and PFNA had a correlation coefficient of 0.78 in the blood sera of 5-year olds and IQR differences in blood sera concentrations of less than 1.9-fold each (see Table 2 of the study). Despite Grandjean et al. (2017b) stating that the close correlations prevented

[8] 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."

meaningful adjustment for concomitant PFAS exposures, Budtz-Jørgensen and Grandjean (2018a) attempts to do just that for benchmark dose, and the results appear to help demonstrate the effects of confounding co-exposures and/or the inability to properly adjust for them. Table 2 of Grandjean et al. (2017b) reports the change (in percent) of the pre-booster serum-antibody concentrations at age 5 years associated with a doubling of the serum concentration of PFDA. Results for cohorts 5, 3, and joint results are in the negative direction for tetanus at age 5 but none are statistically significant, which already points to the unreliability of an effect having been demonstrated by these results and the unreliability of any RfD based on these results or association. Table 2 of Budtz-Jørgensen and Grandjean (2018a) reports benchmark results for the five prenatal PFAS concentrations in regard to antibody concentrations at age 5 years (pre-booster) both unadjusted and adjusted for PFOS/PFOA co-exposures. For tetanus antibodies, while unadjusted benchmark doses for three models for PFDA (linear, piecewise, conservative) appear to show excellent agreement (BMDs of 0.11-0.25 ng/mL) with insignificant reliance on choice of model, when adjusted for PFOS/PFOA the three models' best estimates of the PFDA serum concentrations associated with a 5% change go to infinity (although BMDLs can still be estimated). For diphtheria, Table 2 of Grandjean et al. (2017b) reports statistically significant changes for cohort 3 and joint results for the pre-booster serum-antibody concentrations at age 5 years associated with a doubling of the serum concentration of PFDA. However, similar to results for tetanus, Table 2 of Budtz-Jørgensen and Grandjean (2018a) reports that when benchmark results are adjusted for PFOS/PFOA, two of the three models' best estimates of the PFDA serum concentrations associated with a 5% change go to infinity (although BMDLs can still be estimated). While Table 1 of Budtz-Jørgensen and Grandjean (2018a; benchmark results for the age-5 serum concentrations of five PFASs in regard to tetanus and diphtheria antibody concentrations at age 7 years) provides no benchmark doses for PFDA that go to infinity, Table 2 results point to the unreliability of this endpoint and should be considered along with the other issues raised above. For example, the odds ratios for PFDA and inadequate antibody concentrations for diphtheria and tetanus at 7 years was not statistically significant (see eTable 4 of Grandjean et al. 2012).

Key BMD results in the draft assessment itself demonstrate the importance of co-exposures to other PFAS. Tables C-1 and C-3 of the draft assessment (below) provide BMD results for the critical effects used for RfD derivation (e.g., see Table ES-1).

Table C-1. Results specific to the slope from the linear analyses of PFDA measured in serum at age 5 years and $\log_2$(tetanus antibody concentrations) measured at age 7 years in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b).

Exposure	Model shape	PFOS & PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/m Lin serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at Age 5	Linear	No	-1.55	0.602	$p = 0.01$	-2.55
PFDA at Age 5	Linear	Yes	-0.98	0.681	$p = 0.15$	-2.10

PFOS and PFOA had correlation coefficients of 0.39 and 0.35 with serum PFDA at age 5, respectively (Table 2 of Grandjean et al. 2012). Despite these relatively low correlation coefficients (Mukaka 2012), Table C-1 shows that just controlling for co-exposures from these two PFAS (PFOS, PFOA) resulted in significant impacts on slope (β) and slope fit. The slope estimate for PFDA was reduced 37% and *PFDA is no longer a significant predictor of tetanus antibody concentrations* (p=0.15). The most likely explanation (Occam's razor) is classic confounding as PFOS and PFOA are documented immunotoxicants (e.g., per Budtz-Jørgensen and Grandjean 2018a), and the existence of some chance that correction for these co-exposures could create some confounding is not a scientifically robust justification for dismissing the important implications of the results of adjustments for PFOS/PFOA that the study authors themselves (Budtz-Jørgensen and Grandjean 2018a) thought it important to adjust for, and with good reason. *These results demonstrate the statistical unreliability of serum PFDA predicting tetanus antibody concentrations when just two other PFAS are controlled for*. Table C-3 concerns the critical effect for the RfD based on diphtheria antibody concentrations.

Table C-3. Results specific to the slope from the linear analyses of PFDA in serum measured at age 5 years and $\log_2$(diphtheria antibodies) measured at age 7 years from Table 1 in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b).

Exposure	Model shape	PFOS & PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/mL in serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at Age 5	Linear	No	-0.894	0.561	p = 0.11	-1.82
PFDA at Age 5	Linear	Yes	-0.297	0.635	p = 0.64	-1.35

The implications of these results are worse. First, even when evaluated alone without accounting for co-exposures to relatively low correlated PFOS and PFOA, serum PFDA is not a significant predictor of diphtheria antibody concentrations (p=0.11). Table C-3 further shows that controlling for co-exposures from these two PFAS (PFOS, PFOA) resulted in significant impacts on slope (β) and slope fit. *The slope estimate for PFDA was reduced 67% and serum PFDA became a worse nonsignificant predictor of diphtheria antibody concentrations (p=0.64)*. The most likely explanation (Occam's razor) is classic confounding as PFOS and PFOA are documented immunotoxicants (e.g., per Budtz-Jørgensen and Grandjean 2018a), and the existence of some chance that correction for these co-exposures could create some confounding is not a scientifically robust justification for dismissing the important implications of the results of adjustments for PFOS/PFOA that the study authors themselves (Budtz-Jørgensen and Grandjean 2018a) thought it important to adjust for, and with good reason. *Thus, when co-exposures are taken into account for two modestly correlated PFAS (PFOS, PFOA), serum PFDA is not a significant (i.e., reliable) predictor of these critical effects serving as the basis of the RfD (i.e., decreases in serum tetanus and diphtheria antibody concentrations)*.[9]

[9] Knowing this, disparate results are not particularly surprising, such as the 18.7% increase in tetanus antibodies predicted for children (age 13) with a 2-fold increase in serum PFDA based on the same study and type of analysis used for the RfD critical effects (see Table 3-12, p. 3-59).

	Moreover, Budtz-Jørgensen and Grandjean (2018a) also controlled for PFOS or PFOA when deriving BMDs/BMDLs for the other, and EPA did use those co-exposure-adjusted results for RfD derivation (i.e., PFOA adjusted for PFOS, PFOS adjusted for PFOA; see Tables B-1 and B-2 in Sections B.1.1 and B.1.2 of the PFOA and PFOS draft assessments USEPA 2021a,b) without expressing any similar concerns about creating confounding by adjusting for these co-exposures (low/moderate correlation coefficient of 0.50 (Mukaka 2012); see Table 2 of Grandjean et al. 2012) or citing Weisskopf et al. (2018) and Weisskopf and Webster (2017). Rather, these concerns have been selectively cited for PFDA in an attempt to provide some rationale for dismissal of the co-exposure-controlled results (e.g., slope (β) values are reduced; serum PFDA is a nonsignificant predictor of tetanus and diphtheria antibody concentrations) and thus for selection of BMDLs uncontrolled for PFOS and PFOA co-exposures (see Tables C-2 and C-4 of the draft), but this is not a scientifically robust rationale and is furthermore inconsistent with EPA's draft assessments for PFOA and PFOS (USEPA 2021a,b). Lastly, it is further noted that 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 PFDA and PFOS/PFOA are low (0.35 and 0.39, respectively). The results of Weisskopf et al. (2018) do not constitute reasonable doubt that for these PFDA results, the confounding from not adjusting for co-exposures to documented immunotoxicants (PFOS, PFOA) is significantly greater than the potential amplification of biases that remains undemonstrated under the same or similar circumstances.EPA should reevaluate the issues raised above in regard to implications for their draft PFDA assessment (**Tier 1 necessary revision**). Regarding potential immunosuppressive effects by PFDA, effects that rise to the level of adversity would be expected to result in *increased incidences of disease*, reflecting lower immunity and lower resistance to disease in the real world. However, consistent with the WOE for no statistically significant associations with antibody concentrations falling below the generally cited protective level of 0.1 IU/mL based on results from Grandjean et al., almost all ORs in Table 3-13 of the draft assessment include 1, indicating that *the WOE from studies on PFDA and infectious disease in humans is for no statistically significant associations*. Consistent with this, host resistance was unaffected by PFDA based on the limited animal study data available (p. 3-73, lines 8-9), and host resistance assays are considered highly relevant to the evaluation of immunotoxicity in the context of human health assessment (p. 3-72, lines 15-18; IPCS 2012). Host. Again, EPA should reevaluate the adversity of these presumed antibody level effects, including the association with PFDA itself, and do so within the context of potential confounding, other limitations, and available human/animal data on disease incidence (**Tier 1 necessary revision**), as this has important implications for the hazard judgment and the strength of human evidence descriptor for immunosuppression (listed as "moderate" in Table 3-19).

	Moreover, it is noted that the PODs for immunosuppressive effects from these epidemiological studies range from 2.57E-04 mg PFDA/L blood serum to 7.02E-04 mg PFDA/L blood serum ($BMDL_{1/2\ SD}$ values from Table 5-8, pp. 5-16 to 5-17), and when intrahuman variability is considered (through application of a UF_H of 10), the resulting values range from 2.57E-05 to 7.02E-05 mg PFDA/L blood or 0.0257 to 0.0702 µg PFDA/L blood serum.[10] Data from NHANES show that geometric means (GMs) representative of the U.S. population are well above these blood serum levels (see Appendix A to these comments).[11] Most notably, 2005-2018 population GMs range from 0.154-0.355 µg/L, which are 2.2- to 13.8-fold higher than the PODs adjusted for intrahuman variability (cited above). Despite these exceedances, tetanus and diphtheria appear to be quite rare in the U.S. population. The average annual number of tetanus cases in the U.S. from 2009-2018 was 29, with the CDC attributing most cases to individuals who either have not been vaccinated or who are not current on their boosters (e.g., only 3% of the cases from 2001-2008 were in people who had received a complete tetanus toxoid series with the last dose within 10 years; Tiwari et al. 2021). Tetanus also appears rare in U.S. children specifically, occurring primarily in older adults. Per Liang et al. (2018): "During 2001-2016, three neonatal tetanus cases and 459 non-neonatal tetanus cases were reported to the National Notifiable Diseases Surveillance System (NNDSS). The median age for non-neonatal cases was 44.0 years (range: 2-95 years)… The risk for both tetanus disease and mortality was higher among persons aged ≥65 years than among persons aged <65 years. Tetanus occurs almost exclusively among persons who are unvaccinated or inadequately vaccinated or in those whose vaccination histories are unknown or uncertain." The incidence of U.S. diphtheria cases is even more rare. The CDC reported only 14 cases from 1996 through 2018 (Acosta et al. 2021). *Thus, consistent with the WOE for the lack of statistically significant associations from the epidemiology study data discussed above and despite the NHANES blood serum data showing exceedances of the draft assessment human PODs adjusted for intrahuman variability (e.g., toxicodynamic) for most of the U.S. population for a prolonged period of time (see the 50th percentile concentrations in Appendix A), U.S. surveillance disease incidence data are not supportive of adversity.* That is, U.S. surveillance disease incidence data do not support that serum PFDA (or any other serum PFAS) is suppressing tetanus and diphtheria vaccine responses and leaving people vulnerable to infection from these diseases.[12] Additionally, it appears that EPA has not fully considered *all the relevant evidence* or the weaknesses/limitations of the epidemiological evidence, which in turn are relevant for the hazard judgment. Table 3-19 indicates that human data provide

[10] Based on $BMD_{1/2\ SD}$ values (Table C-9, p. C-16), these values are 0.0385 to 0.226 µg PFDA/L blood serum.

[11] See NHANES Biomonitoring Data Tables at https://www.cdc.gov/exposurereport/data_tables.html. Budtz-Jørgensen and Grandjean (2018a) also acknowledge that, "Our BMDL results, both before and after adjustment are generally below current exposure levels…"

[12] The apparent lack of adversity/ consequence for the effects reported for tetanus and diphtheria certainly does not provide support for an expectation of adversity/consequence for other effects not measured/observed (e.g., for vaccines for other diseases and their incidences).

	"moderate" evidence and that "the inconsistent and low confidence evidence on infectious disease did not influence this judgment." However, this points to the fact that *EPA has not duly considered the implications of the null findings on human and laboratory animal infectious disease and other relevant considerations (e.g., some discussed above) for the scientific WOE, which is not a scientifically supportable approach* as it does not consider all relevant data, directly relevant human data in particular. EPA should consider such null findings (and other relevant considerations) in their WOE (**Tier 1 necessary revision**). Combined with the "slight" human data for sensitization and allergic response, the "slight" laboratory animal data for immunosuppression, and the "indeterminate" animal data for sensitization and allergic response (Table 3-19), *it does not appear that PFDA exposure is "likely to cause" adverse immune effects in humans is sufficiently supported*. EPA should reevaluate this determination (**Tier 1 necessary revision**) as *"may cause" might very well be the better supported hazard judgement*, and also reconsider their use of the serum antibody endpoints for quantitative risk assessment/derivation of toxicity factors (**Tier 1 necessary revision**). This would appear more consistent with the data discussed above and recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021). *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." *Based on the information in the draft assessment and reviewed elsewhere, this reviewer finds it difficult to disagree with the recent conclusions of the Australian government (FSANZ 2021) and ATSDR (2021) that the epidemiology literature* (e.g., on PFAS blood levels and impaired vaccine response) *is inadequate for quantitative risk assessment and use as the basis for deriving toxicity factors* (e.g., RfDs). 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals. All this is not to say that PFAS are incapable of causing immune effects, 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. A counter argument appears to be that real-world exposures are to mixtures, but nevertheless, it is not scientifically defensible or realistic to attribute the effects of a mixture of chemically-similar chemicals to a single component (i.e., co-exposures to other components of the mixture contributing to the observed effects would have to be able to be adequately adjusted for).[13] Again, EPA should reconsider their use of the serum antibody endpoints for quantitative risk assessment and derivation of toxicity factors (**Tier 1 necessary revision**). b.i. See the comments above, some of which concern confounding while others concern important issues such as adversity, the WOE against statistically significant associations with serum antibody concentrations below the generally cited protective level, the overall WOE against PFDA-associated immunosuppressive effects (i.e., increased disease incidence) in humans, and other important considerations. Here is simply noted that associations are not causation, and residual confounding being judged "unlikely to fully explain the associations" is not a scientific criterion for presuming that: (1) associated effects are in fact attributable to the one chemical exposure in question (i.e., confounding not being known to account for 100% of an association or effect is not scientifically-defensible grounds to then causally attribute the mixture effect of exposure to a group of similarly acting compounds to just one component); or for presuming that (2) the dose-response data, without causation being demonstrated and with residual confounding explaining some unknown part of the association(s), are suitable for quantitative dose-response assessment and derivation of toxicity factors (e.g., RfDs). The

[13] For example, just as there are thousands of PFAS, there are numerous hydrocarbon components of gasoline that people are exposed to as a mixture, and even though they number fewer than the number of PFAS, it still would not be scientifically defensible to derive a toxicity factor for just one component, toluene for example, attributing the totality of the mixture effects observed solely to toluene following exposure to gasoline (e.g., even if two co-exposures such as ethylbenzene and xylenes were adjusted for).

	Australian government (FSANZ 2021) and ATSDR (2021) would seem to concur (see the quotes provided above). As an additional example, following review of the relevant literature, FSANZ (2021) stated [*emphasis added*]: "While these studies provide limited evidence of statistical associations, *a causal relationship between increased PFAS blood levels and impaired vaccine response cannot be established with reasonable confidence*. *The evidence* for an association between increasing PFAS blood levels and impaired vaccine response *is insufficient for quantitative risk assessment* on the basis of *substantial uncertainties and limitations* including: • the small number of studies and participants, and mostly cross-sectional design of studies such that *conclusions around causality should be drawn with caution*. • limited dose-response information with *most studies investigating a narrow range of blood levels associated with background levels* of PFAS exposure. • *inconsistency* in antibody response to vaccines between different PFAS congeners *which cannot explained by study design*. • *potential for confounding* by other known environmental immunotoxicants *such as PCBs for which inverse associations* with blood serum antibody concentrations against tetanus and diphtheria *have previously been reported in the child populations living in the Faroe islands* (Heilmann et al. 2010). • *uncertainty about the clinical relevance, if any, of the observed statistical associations to susceptibility to infectious disease*." FSANZ (2021) adds that this conclusion is consistent with the recent decisions of the German Human Biomonitoring Commission (Hölzer et al. 2021; Schümann et al. 2021), ATSDR (2018, 2021), and a number of earlier opinions from national agencies and bodies such as Danish EPA (2016), Expert Health Panel for PFAS (2018), and Kirk et al. (2018). See FSANZ (2021) for references. Furthermore, *the importance of the fact that the study authors themselves acknowledge the inability to causally attribute associated effects to PFDA cannot be overstated*: • 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*."

- Similarly, Grandjean et al. (2017b) state [*emphasis added*], "The close correlations *prevented meaningful adjustment* for concomitant PFAS exposures."

It appears that EPA may not have fully considered the implications of these study author-cited limitations for use of the data for quantitative risk assessment and derivation of toxicity factors (e.g., RfDs).

Considering my comments under this subsection and "2.b" above, I seem to concur with the Australian government (FSANZ 2021) and ATSDR (2021) that the epidemiology literature on PFAS (e.g., PFDA) blood levels and impaired vaccine/antibody response is inadequate for dose-response assessment and deriving toxicity factors (e.g., RfDs). Part of this shared opinion is based on confounding considerations which are the subject of this question subsection (2.b.i). 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals. As indicated above, EPA should reconsider their use of the serum antibody endpoints for quantitative risk assessment/derivation of toxicity factors (**Tier 1 necessary revision**).

c. Developmental effects: For the most part, the available data on developmental effects are clearly and appropriately synthesized to describe the strengths and limitations. P. 3-158, lines 18-25 state that, "Taken together, the available ***evidence indicates*** that PFDA exposure is likely to cause developmental toxicity in humans given sufficient exposure conditions (see Table 3-24). This conclusion is based primarily on findings of dose-dependent decreases in fetal weight in the only available toxicology study, with mice gestationally exposed to PFDA doses ≥0.5 mg/kg-day and supported by evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy, primarily with median PFDA values ranging from 0.11 to 0.46 ng/mL. The conclusion is further supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth and birth length)." *There are comments below concerning the coherence or incoherence of key epidemiological data for birth weight* (e.g., Wikström et al. 2020, Bach et al. 2016, and other studies). Table 3-24 is the evidence profile table for developmental effects. As quoted above, EPA's hazard conclusion for developmental effects primarily relies on in vivo animal data (i.e., the mouse study of Harris and Birnbaum 1989), for which Table 3-24 contains factors that increase certainty for fetal growth but no factors that decrease certainty. *Based on over 45 different epidemiological studies included in the draft assessment, the evidence of an association between PFDA exposure and developmental effects in humans is considered only "slight"* (p. 3-156, lines 10-11). Despite the "slight" evidence in humans, Table ES-1 indicates that RfDs were nevertheless calculated based on decreased birth weight in male and female children (Wikström et al.

	2020). While data in humans, as the species of ultimate interest, are usually preferred as the basis for derivation of toxicity factors, in this case it appears that the mere "slight" totally of the evidence for developmental effects from the over 45 different epidemiological studies included in the draft assessment should not be considered sufficient for quantitative dose-response assessment (e.g., birth weight), but rather only for potentially supportive information for hazard identification. EPA should reconsider their use of these epidemiological data for quantitative risk assessment/derivation of toxicity factors (**Tier 1 necessary revision**). EPA Figure 3-28 (p. 3-108), reproduced below, helps illustrate the weakness and incoherence of the overall epidemiological database for demonstrating decreased birth weight, particularly statistically significantly decreased birth weight, associated with PFDA levels and how no one epidemiological study could possibly be representative of these inconsistent and disparate results or the results of a meta-analysis of relevant studies.

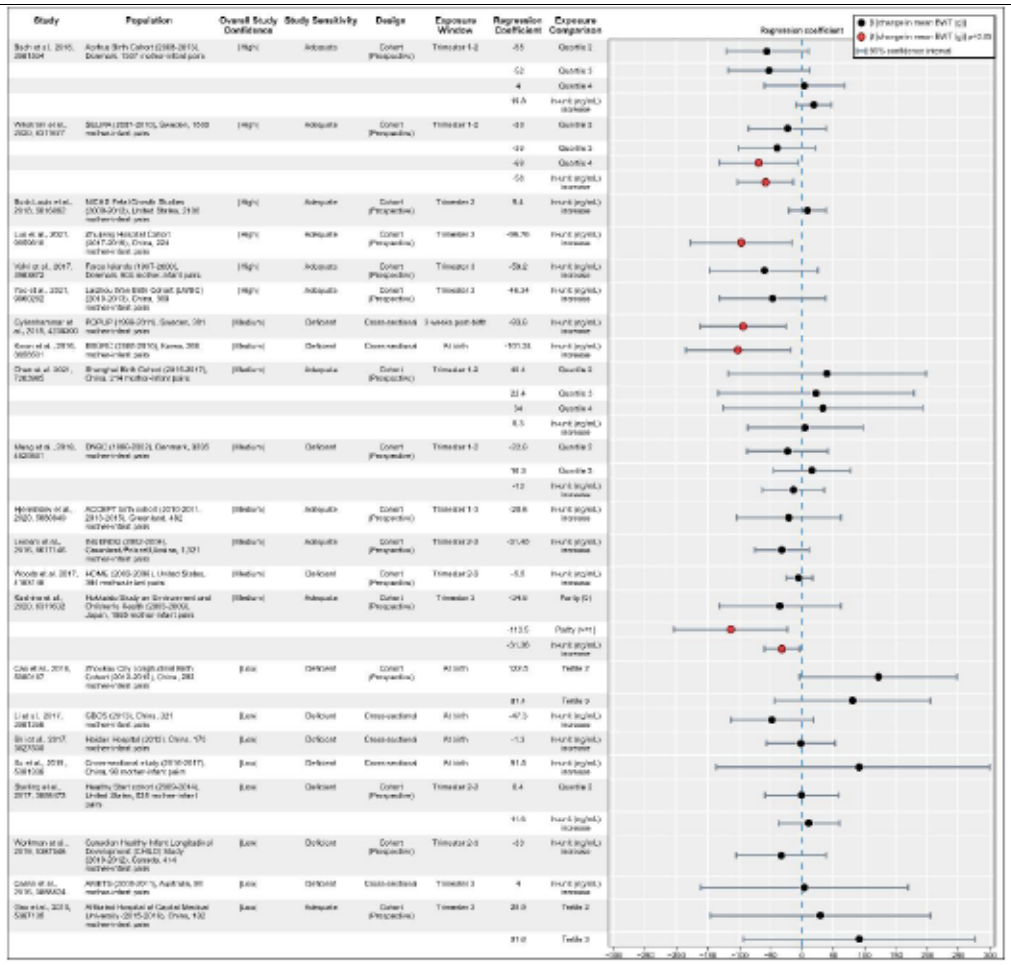

Figure 3-28. Overall study population mean birth weight results for 22 PFDA epidemiological studies[a-e]. (results can be viewed by clicking the HAWC link).

Abbreviation: BWT = Birth Weight

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b] Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during the first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c] If a study presented regression coefficients for continuous exposure with multiple exposure units, only one unit change is shown (e.g., (Bach et al., 2016), with the exception of (Li et al., 2017), which displays both IQR and ln-unit (ng/mL) values.

[d] The results displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared to a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[e] Xu et al. (2019a) results are truncated for the 210.7 gram increase; the complete 95% CI ranges from -314.3 to 735.8 grams.

Similarly, Figure 3-26 (p. 3-104) below illustrates how the results of Bach et al. (2016), the other high confidence prospective cohort for trimester 1-2 with adequate study sensitivity, had a nonmonotonic response, no statistically significant quartile results for the overall population, and a regression coefficient of +0.03 per each ln-unit (ng/mL) (compared to -0.147 per each ln-unit increase for Wikström et al. 2020), all of which does not support the Wikström et al. (2020) study results or their use in toxicity factor derivation. The Bach et al. (2016) and Wikström et al. (2020) studies are the first two entries in Figure 3-26, respectively.

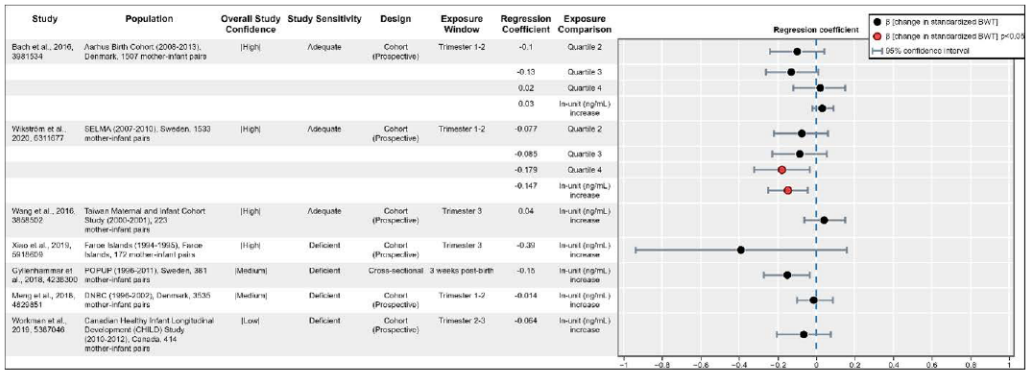

Figure 3-26.PFDA and birth weight z-scores (overall population)[a]. Refer to Birth Weight-Z for details on the individual study evaluation review.

Moreover, the BMD_{5RD} values for decreased birth weight based on Wikström et al. (2020) range from 0.54 to 0.71 ng/mL (note that BMD_{5RD} values are not treated like NOAELs for purposes of RfD derivation; Table C-10, p. C-22).[14] By comparison, PFDA blood serum concentrations in quartile 4 of the Bach et al. (2016) study range from 0.43 to 2.87 ng/mL (see Table 1 of the study). Despite these quartile 4 blood serum concentrations being up to 5.3-fold higher than the BMD_{5RD} values for decreased birth weight based on Wikström et al. (2020), birth weight and birth weight z-scores for quartile 4 in Bach et al. (2016) were actually increased (nonsignificantly) (see Table 3 of the study). Again, this is from the other high confidence, prospective cohort for trimester 1-2 with adequate study sensitivity. These results from Bach et al. (2016), obtained from the other high confidence study in the same PFDA blood serum concentration range (and higher) compared to EPA's BMD_{5RD} values, are inconsistent with and unsupportive of EPA's use of the PODs for adverse birth weight effects based on Wikström et al. (2020) for RfD derivation. Sex-specific results from Bach et al. (2016) do not provide strong support for use of Wikström et al. (2020) results for quantitative dose-response assessment either (e.g., Figure 3-29, p. 3-109 of the draft assessment). Other important comments on Wikström et al. (2020) appear under question "2.c.i" below (e.g., confounding).

Given the drastically different results across epidemiological studies and the mere "slight" evidence of developmental effects across more than 45 such studies, EPA should reconsider use of a single epidemiological study (Wikström et al. 2020) for dose-response assessment of birth weight and RfD derivation and consider a meta-analysis and/or using the more definitive dose-response data from the mouse study for dose-response assessment of birth weight and RfD derivation (**Tier 1 necessary revision**). It is remarkable that per EPA, the single mouse study by Harris and Birnbaum (1989) gives rise to a greater level of evidence for developmental effects ("moderate") than the results of over 45 epidemiological studies. This fact alone, in this case, justifies use of the animal data for dose-response assessment and RfD derivation. Based on EPA Figure 3-56 (p. 3-152),

[14] For comparison, the $BMDL_{5RD}$ values range from 0.31 to 0.37 ng/mL (Table C-10, p. C-22).

reproduced below, the dose-response data for fetal body weight (as an example) appear well suited for benchmark dose analysis.

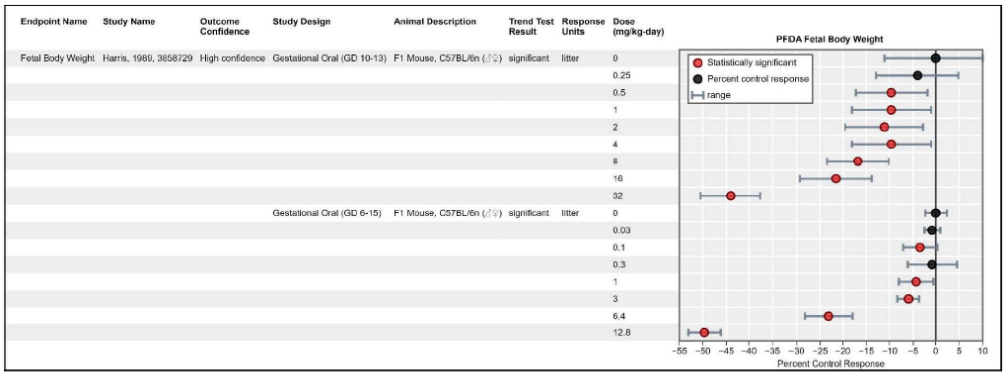

Figure 3-56. PFDA fetal body weight after gestational exposure (results can be viewed by clicking the HAWC link).

More specifically, Figure 3-56 shows statistically significant and progressive decreases/trends in fetal body weight with dose across a range that includes the >5% decrease historically used by regulatory agencies as the demarcation of adversity in dose-response assessment (i.e., as a >5% reduction in fetal body weight is usually considered adverse, a benchmark response of 5% is typically used in benchmark dose analysis), from -1% to -50% and including doses that resulted in decreases of -4% and -6% (Table 3-23, p. 3-153). Furthermore, the changes in fetal body weight were at doses not associated with maternal toxicity (p. 3-151, lines 5-6).

Harris and Birnbaum (1989) is a medium/high-confidence study (p. 3-150, lines 9-12), and EPA should consider mouse data from the Harris and Birnbaum (1989) study for use as the primary basis for osRfD development (**Tier 1 necessary revision**) based on developmental growth effects consistent with: (1) this mouse study providing the primary basis for EPA's developmental effects hazard conclusion; (2) the lack of factors that decrease certainty for fetal growth as evaluated in the mouse study (see Table 3-24); and (3) the extensive epidemiological database merely being able to provide support for this mouse study with what amounts to "slight" evidence for developmental effects across over 45 epidemiological studies. Again, per the draft assessment, it is the mouse data that primarily support that assuming sufficiently high exposure over a sufficiently long duration (i.e., "given sufficient exposure conditions"), PFDA exposure is likely to cause developmental toxicity in the general human population, which includes potentially susceptible subpopulations (e.g., developing fetuses of pregnant women). Moreover, 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals. So all this is not to say that PFAS are incapable of causing developmental effects, 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. A counter argument appears to be that real-world exposures are to mixtures, but nevertheless, it is not scientifically defensible or realistic to attribute the effects of a mixture of chemically-similar chemicals to a single component (i.e., co-exposures to other components of the mixture contributing to the observed effects would have to be able to be adequately adjusted for).[15] c.i. I wholly agree that the strength of the available human evidence is "slight", at least partly due to potential confounding. Given the slight human evidence, above I suggest use of the mouse data (Harris and Birnbaum 1989) instead of epidemiological study data (Wikström et al. 2020) for dose-response assessment and osRfD derivation based on developmental growth effects (**Tier 1 necessary revision**). To do so would make EPA's assessment somewhat more consistent with ATSDR (2021), who found the epidemiology literature inadequate for use as the basis of deriving 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." To be entirely consistent with this conclusion from ATSDR (2021) and my own comments and conclusions under question 2.b (above), the EPA assessment would further need to omit the highly uncertain RfD derivations based on PFDA associations with decreases in antibody levels/vaccine response (**Tier 1 necessary revision**) reported in some epidemiological study results that appear overall to be inconsistent, non-adverse, and uncertain in nature (e.g., causal attribution problems, unsupported by epidemiological disease incidence studies). Getting back to use of Wikström et al. (2020) for dose-response assessment of birth weight and RfD derivation, in addition to important comments above under "2.c" (e.g.., inconsistency of Bach et al. 2016 with Wikström et al. 2020

[15] For example, just as there are thousands of PFAS, there are numerous hydrocarbon components of gasoline that people are exposed to as a mixture, and even though they number fewer than the number of PFAS, it still would not be scientifically defensible to derive a toxicity factor for just one component, toluene for example, attributing the totality of the mixture effects observed solely to toluene following exposure to gasoline (e.g., even if two co-exposures such as ethylbenzene and xylenes were adjusted for).

	and the weakness/incoherence of the overall epidemiological database for demonstrating decreased birth weight, particularly statistically significant decreases), Tables 2 and 3 from that study appear below (unaltered; see license at https://creativecommons.org/licenses/by/4.0/) and demonstrate: (1) significant co-exposures to other PFAS (e.g., PFOS, PFOA) associated with birth weight deficits, an ATSDR concern and significant uncertainty; and (2) few statistically significant ORs for decreased body weight in the PFDA exposure quartiles; that is, only 2/9 of the ORs showed statistically significant birth weight decrements, which were for quartile 4 where both PFOS and PFOA were certainly co-exposures and also showed statistically significant decreases for birth weight. In regard to this latter point concerning potential confounding by co-exposure to other PFAS, which was cited by ATSDR as a significant uncertainty, Wikström et al. state [*emphasis added*]: "*Another limitation is the compound-by-compound approach*. Theoretically, *a health outcome is simultaneously influenced by multiple environmental factors*. Nevertheless, the exposure to several PFASs may be correlated with each other due to common sources. Our findings were consistent across different PFAS compounds, and we regard correction for multiple comparisons overly conservative to be suitable for the investigations on such interrelated compounds. If the single compound's level could represent the levels of several other compounds, *our findings based on single compound analyses may still shed some light on the joint effects of multiple PFAS compounds*. However, carefully designed statistical models, such as *mixture-based approaches* within the PFAS compound class, *should be explored in follow-up studies*." *Thus, the study authors are acknowledging confounding by other PFAS as an important limitation of their compound-by-compound approach* as joint effects of multiple PFAS compounds may be occurring such that mixture-based approaches should be explored for data analysis. *The compound-by-compound approach used in this study gives rise to significant uncertainty that precludes use of these data for dose-response assessment*. It cannot be confidently said scientifically that the presumed effects are due to PFDA exposure (e.g., PFOS and PFOA were certainly co-exposures and also showed statistically significant decreases for birth weight). Consistent with my opinion on the significant uncertainty and confounding associated with these Wikström et al. results and the weakness/limitations of the epidemiological evidence overall, Section F.3 of the draft assessment states that, "In the six studies using mutually adjusted PFAS approaches to address coexposures, there was not consistent evidence for birth weight deficits associated with increased exposure to PFDA." (p. F-27, lines 13-14), and acknowledges that "there is considerable uncertainty due to potential confounding by co-occurring PFAS in the existing literature." (p. F-27, lines 30-32). Indeed, significant co-exposure to multiple PFAS is not the exception but the rule, and is just the condition to result in significant bias

away from the null for adverse effects.[16] The high confidence study of Luo et al. (2021) is an example that serves as a cautionary tale on the obvious importance of PFAS co-exposures, which reported large statistically significant birth weight deficits (-97 g; -178, -16 per each ln-unit PFDA increase) in a single-pollutant PFDA model, but results were null and their direction reversed in the multipollutant model with a nonsignificant increase in birth weight associated with PFDA (Table F-2).[17]

As the data from epidemiological studies provide only "slight" evidence of developmental effects, including data from Wikström et al. (2020) that appears too uncertain and unsuitable for quantitative dose-response assessment, I suggest use of the mouse data (Harris and Birnbaum 1989) for dose-response assessment and osRfD derivation based on developmental growth effects (**Tier 1 necessary revision**). 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals.

Table 2. Prenatal exposure to eight PFAS, measured as maternal serum concentrations (ng/mL) during early pregnancy.

Compound	Geometric mean [95% CI]	Median (IQR)	95th %	LOD	Above LOD (%)
PFOS	5.35 [5.21–5.50]	5.38 (3.97–7.60)	10.34	0.06	100
PFOA	1.60 [1.56–1.65]	1.61 (1.11–2.30)	3.18	0.02	100
PFHxS	1.31 [1.28–1.35]	1.23 (0.86–1.99)	2.94	0.03	100
PFNA	0.54 [0.53–0.56]	0.53 (0.39–0.73)	1.02	0.01	100
PFDA	0.26 [0.26–0.27]	0.26 (0.19–0.34)	0.50	0.02	100
PFUnDA	0.21 [0.21–0.22]	0.23 (0.15–0.33)	0.44	0.02	99.5
PFHpA	0.018 [0.017–0.019]	0.02 (<LOD–0.04)	0.077	0.01	73.9
PFDoDA	0.027 [0.026–0.027]	<LOD (<LOD–0.05)	0.08	0.03	46.7

LOD limit of detection

[16] To be a confounder, the co-occurring PFAS would need to be associated with both the PFAS of interest and the outcome, but not an intermediate in the causal pathway; such PFAS would be considered positive confounders if their effect estimate with the endpoint of interest is in the same direction as the primary PFAS of interest. If positive confounders are not accounted for, the anticipation is that any resultant bias would be away from the null. (p. F-24, lines 16-21). The stronger the association between co-exposures, the larger the effect size for the co-exposure of interest (i.e., the greater the confounding) (p. F-25, lines 14-15).

[17] The other high confidence study in Table F-2 (Starling et al. 2017) showed that adjustment for PFAS co-exposures in the multipollutant model resulted in a large statistically significant increase in birth weight for PFDA (+97.5 g; 31.5, 163.6), which for some reason is not discussed in Section F.3 (*PFDA and PFAS Coexposure Study Results*) but serves as yet another example of the obvious, commonsensical importance of accounting for PFAS co-exposures.

Table 3. Associations between prenatal PFAS exposure[a] and birth weight[b], together with odds ratios for birth weight small for gestational age in 1533 children.

	All children		Girls		Boys	
	BW (g) β (95% CI)	SGA OR (95% CI)	BW (g) β (95% CI)	SGA OR (95% CI)	BW (g) β (95% CI)	SGA OR (95% CI)
PFOS						
Per ln-unit	−46 (−88; −3)	1.19 (0.87; 1.64)	−85 (−145; −25)	1.40 (0.83; 2.35)	−13 (−73; 47)	1.08 (0.72; 1.63)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−27 (−89; 35)	0.69 (0.43; 1.08)	−32 (−115; 52)	0.89 (0.39; 2.03)	−28 (−118; 63)	1.26 (0.67; 2.37)
Q3	−22 (−84; 41)	0.79 (0.53; 1.18)	−51 (−137; 34)	0.82 (0.36; 2.03)	5 (−86; 96)	0.86 (0.45; 1.67)
Q4	−80 (−144; −16)	1.56 (1.09; 2.22)	−142 (−231; −54)	2.05 (1.00; 4.21)	−28 (−119; 63)	1.30 (0.70; 2.40)
PFOA						
Per ln-unit	−68 (−112; −24)	1.43 (1.03, 1.99)	−86 (−145; −26)	1.96 (1.18; 3.28)	−49 (−113; 15)	1.16 (0.75; 1.78)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	27 (−35; 89)	0.77 (0.45; 1.32)	30 (−55; 115)	1.00 (0.40; 2.51)	26 (−66; 116)	0.67 (0.34; 1.31)
Q3	−41 (−106; 23)	0.96 (0.57; 1.61)	−36 (−124; 52)	1.64 (0.71; 3.83)	−44 (−139; 50)	0.66 (0.33; 1.29)
Q4	−90 (−159; −91)	1.44 (0.86; 2.40)	−136 (−231;−40)	2.33 (1.00; 5.43)	−47 (−147; 54)	1.04 (0.54; 2.01)
PFHxS						
Per ln-unit	−0.1 (−38; 38)	0.96 (0.72; 1.27)	−14 (−68; 39)	1.14 (0.73; 1.80)	−13 (−67; 41)	0.84 (0.58; 1.22)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−4 (−66; 58)	1.37 (0.86; 2.20)	30 (−56; 116)	1.77 (0.78; 3.99)	−39 (−129; 50)	1.24 (0.69; 2.23)
Q3	−15 (−78; 48)	0.89 (0.54; 1.47)	28 (−59; 115)	1.05 (0.44; 2.49)	−51 (−141; 39)	0.82 (0.44; 1.54)
Q4	−6 (−69; 57)	1.04 (0.63; 1.69)	−16 (−104; 71)	1.76 (0.79; 3.90)	1 (−90; 92)	0.73 (0.38; 1.41)
PFNA						
Per ln-unit	−46 (−89; −4)	1.38 (1.02, 1.87)	−52 (−117; −2)	1.34 (0.85; 2.11)	−50 (−113; 14)	1.42 (0.94; 2.17)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	7 (−55; 69)	0.83 (0.49; 1.38)	−2 (−86; 82)	0.66 (0.29; 1.52)	15 (76; 106)	0.97 (0.50; 1.89)
Q3	−39 (−102; 24)	1.14 (0.70; 1.85)	−49 (−137; 38)	1.39 (0.66; 2.90)	−28 (−119; 64)	0.95 (0.50; 1.82)
Q4	−33 (−96; 31)	1.23 (0.77; 1.99)	−66 (−153; 20)	1.22 (0.59; 2.53)	1 (−94; 95)	1.24 (0.66; 2.33)
PFDA						
Per ln-unit	−58 (−103; −13)	1.46 (1.06, 2.01)	−69 (−133; −6)	1.62 (0.98, 2.67)	−47 (−112;17)	1.36 (0.90; 2.07)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−23 (−85; 39)	1.03 (0.62; 1.69)	−42 (−126; 42)	0.86 (0.37; 2.00)	−8 (−99; 82)	1.18 (0.63; 2.23)
Q3	−39 (−101; 23)	1.07 (0.65; 1.76)	−74 (−160; 13)	1.20 (0.54; 2.67)	−8 (−98; 81)	0.99 (0.52; 1.89)
Q4	−69 (−132; −5)	1.50 (0.94; 2.38)	−116 (−204; −27)	1.95 (0.94; 4.06)	−27 (−118; 64)	1.21 (0.66; 2.23)
PFUnDA						
Per ln-unit	−13 (−49; 22)	1.21 (0.92; 1.58)	−24 (−75; 27)	1.08 (0.70; 1.67)	−6 (−55; 42)	1.29 (0.82; 1.83)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−67 (−153; 19)	1.24 (0.77; 2.01)	−67 (−153;19)	2.09 (0.94; 4.63)	41 (−48; 130)	0.90 (0.48; 1.68)
Q3	−42 (−128; 44)	0.85 (0.51; 1.43)	−42 (−128; 44)	1.01 (0.42; 2.44)	46 (−44; 136)	0.81 (0.43; 1.56)
Q4	−46 (−110; 17)	1.52 (0.95; 2.44)	−93 (−183; −3)	1.92 (0.86; 4.25)	−10 (−100; 80)	1.36 (0.76; 2.46)
PFHpA						
Per ln-unit	−1 (−24; 21)	1.06 (0.90; 1.25)	−4 (−34; 26)	1.06 (0.83, 1.36)	−0.03 (−33; 32)	1.07 (0.86; 1.37)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−0 (−62; 61)	0.92 (0.57; 1.48)	17 (−70; 103)	0.92 (0.44; 1.92)	−15 (−102; 73)	0.94 (0.50; 1.75)
Q3	3 (−59; 65)	0.78 (0.48; 1.27)	−21 (−106; 65)	0.75 (0.35; 1.65)	23 (−65; 112)	0.78 (0.42; 1.45)
Q4	31 (−31; 93)	1.25 (0.85; 1.84)	27 (−57; 112)	1.31 (0.66; 2.60)	33 (−58; 124)	1.15 (0.65; 2.04)

All analyses were adjusted for maternal weight, parity (three categories) and cotinine levels. Analyses including both boys and girls were in addition adjusted for sex and analyses of BW were adjusted for GA. SGA was defined as BW 10th percentile for sex and GA
[a]Associations with PFAS are presented per ln-unit and by quartiles of exposure, as related to
[b]Birth weight (g) and odds ratios (adjusted) for birth weight small for gestational age

d. Male reproductive effects: The study confidence conclusions appear scientifically justified, giving appropriate consideration to important study attributes. For each study in the relevant figures of Section 3.2.4 (e.g., Figures 3-58 to 3-61), the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level rating. Accordingly, the study confidence conclusions appear scientifically justified, with EPA having given appropriate consideration to important study attributes.

This question (under "2") also concerns whether the presentation and analysis of study results is clear, appropriate, and effective, a step just upstream of synthesis that if well done, then allows for appropriate syntheses of the endpoint-specific findings. Although I have two comments

	below, the presentation and analysis of study results appears clear, appropriate, and effective overall. Additionally, it appears that generally, the available data on male reproductive effects are clearly and appropriately synthesized to describe the strengths and limitations. P. 3-185, lines 17-18 state that, "Taken together, available ***evidence indicates*** that PFDA is likely to cause male reproductive effects in humans under sufficient exposure conditions (see Table 3-26). This conclusion is based primarily on a constellation of coherent evidence from a high confidence study in animals exposed to 0.625-2.5 mg/kg-day for 28 days, with some support for biological plausibility provided by mechanistic evidence from i.p. and cell culture models. Although no direct information on the human relevance of the animal evidence is available, many aspects of the male reproductive system are conserved across species, and the limited sensitivity in human studies may explain the lack of associations observed." Table 3-26 is the evidence profile table for male reproductive effects, which among other information contains both factors that increase certainty and factors that decrease certainty along with evidence stream (i.e., human, animal, mechanistic/supplemental) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.4) also contains information relevant to and supporting the WOE for male reproductive effects. My comment regarding presentation and analysis concerns p. 3-183, lines 19-22 that state, "The body weight reductions in male rats observed in the 28-day gavage study at 1.25-2.5 mg/kg-day are consistent with moderate body weight changes (21-38%) that are not associated with confounding effects from overt systemic toxicity in supplemental studies tailored to examine that potential linkage." While described as "moderate" body weight changes, reductions of 21-38% are well above those typically considered adverse (e.g., >10% in adult animals) and would be associated with doses above the maximum tolerated dose (MTD) and overt toxicity. As such, the draft assessment should further elaborate upon, explain, and/or clarify the statement cited above (**Tier 1 necessary revision**). Lastly, in regard to "no direct information on the human relevance of the animal evidence" being available, **Tier 1 necessary revision** comments under question "2.a" on the most human-relevant laboratory animal species also apply here for male reproductive effects. e. <u>Female reproductive effects</u>: The study confidence conclusions appear scientifically justified, giving appropriate consideration to important study attributes. For each study in the relevant figures of Section 3.2.5 (e.g., Figures 3-66 and 3-67), the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level rating. Accordingly, the study confidence conclusions appear scientifically justified, with EPA having given appropriate consideration to important study attributes. This question (under "2") also concerns whether the presentation and analysis of study results is clear, appropriate, and effective, a step just

	upstream of synthesis that if well done, then allows for appropriate syntheses of the endpoint-specific findings. Although I have three comments below, the presentation and analysis of study results appears clear, appropriate, and effective overall. Additionally, it appears that generally, the available data on female reproductive effects are clearly and appropriately synthesized to describe the strengths and limitations. P. 3-204, lines 12-19 state that, "Taken together, the available ***evidence indicates*** that PFDA is likely to cause female reproductive toxicity in humans under sufficient exposure conditions (see Table 3-29). This conclusion is based primarily on evidence from a high confidence study in rats exposed to doses ranging from 1.25-2.5 mg/kg-day PFDA for 28 days. The PFDA-induced disruption of estrous cyclicity observed in female rats from the NTP study (NTP, 2018) and its implications for infertility can be considered relevant to humans given that the mechanisms responsible for regulating female reproductivity (e.g., estrous cyclicity in rats and menstrual cycling in humans) are similar between rats and humans (Goldman et al., 2007; Bretveld et al., 2006)." Table 3-29 is the evidence profile table for female reproductive effects, which among other information contains both factors that increase certainty and factors that decrease certainty along with evidence stream (i.e., human, animal, mechanistic/supplemental) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.5) also contains information relevant to and supporting the WOE for female reproductive effects. My first comment regarding presentation and analysis concerns pp. 3-198 to 3-199, lines 17-5 that state, "Although decreased body weight in female rats was observed at the same doses (body weight decreases were 12-36% at ≥1.25 mg/kg-day; refer to Section 3.2.10 on General toxicity effects for more details) as effects on estrous cyclicity, it is unclear if these effects are related and the effect on female reproductive function is disproportionately more severe and concerning than the moderate changes in body weight." While described as "moderate" body weight changes, reductions of 12-36% are well above those typically considered adverse (e.g., >10% in adult animals) and would be associated with doses above the MTD and overt toxicity. As such, the draft assessment should further elaborate upon, explain, and/or clarify the statement cited above (**Tier 1 necessary revision**), especially how these effects may be related. My second comment concerns p. 3-199, lines 5-12 that state [*emphasis added*], "Although body weight has been shown to fluctuate during the different estrous stages and weight loss has been shown to correlate with disrupted estrous cyclicity in rats (Tropp and Markus, 2001), *it is not possible to determine if the decreases in body weight in female rats might be responsible for the effects on estrous cyclicity observed* in the NTP (2018) study. Furthermore, even though no changes were observed on other stages of the estrous cycle (i.e., proestrus and metestrus), *the effects of PFDA on estrus and diestrus are still considered biologically relevant* given the potential influence that the lack of cyclicity may have on fertility,

	regardless of whether the observed decrease in body weight may have partially contributed to these changes." EPA should clarify (**Tier 1 necessary revision**) how it was determined that decreases in body weight only "may have partially contributed" to estrous cycle effects given the statement [*emphasis added*] that "*it is not possible to determine if the decreases in body weight* in female rats *might be responsible for the effects* on estrous cyclicity observed." Additionally, it is important for EPA to transparently determine whether or not the effects on estrous cyclicity may be secondary to the observed decreases in body weight because if so, these effects should not be further considered for dose-response assessment (i.e., only the body weight effects) and this should be explicitly stated in the assessment (**Tier 1 necessary revision**). This is not to argue against the adverse nature of these effects (e.g., as supported on pp. 3-203 to 3-204), but rather to recognize the importance of whether these effects are a direct effect of PFDA (i.e., a specific MOA) or merely a secondary effect due to body weight decreases more generally. Lastly, **Tier 1 necessary revision** comments under question "2.a" on the most human-relevant laboratory animal species also apply here for female reproductive effects. f. Cardiometabolic effects: The study confidence conclusions appear scientifically justified, giving appropriate consideration to important study attributes. For each study in the relevant figures of Section 3.2.6 (e.g., Figures 3-69 to 3-74), the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level rating. Accordingly, the study confidence conclusions appear scientifically justified, with EPA having given appropriate consideration to important study attributes. This question (under "2") also concerns whether the presentation and analysis of study results is clear, appropriate, and effective, a step just upstream of synthesis that if well done, then allows for appropriate syntheses of the endpoint-specific findings. Overall, the presentation and analysis of study results appears clear, appropriate, and effective. Additionally, it appears that by and large, the available data on cardiometabolic effects are clearly and appropriately synthesized to describe the strengths and limitations. P. 3-235, lines 26-32 state that, "Overall, ***evidence suggests*** that PFDA exposure has the potential to cause cardiometabolic effects in humans under sufficient exposure conditions (see Table 3-38). This conclusion is based on evidence of an association between PFDA exposure and certain cardiometabolic outcomes (serum lipids, adiposity, cardiovascular disease, and atherosclerosis) in a small number of epidemiological studies with median exposure levels from 0.1-0.4 ng/mL; however, issues with inconsistency across studies raise considerable uncertainty. Moreover, evidence in animals is sparse and largely uninterpretable regarding its relevance to humans." Table 3-38 is the evidence profile table for cardiometabolic effects, which among other information frequently contains both factors that increase certainty and factors that decrease certainty along with evidence stream (i.e., human, animal) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.6) also contains information relevant to and supporting the WOE for cardiometabolic

	effects. g. Neurodevelopmental effects: The study confidence conclusions appear scientifically justified, giving appropriate consideration to important study attributes. For each study in the relevant figures of Section 3.2.7 (i.e., Figure 3-76), the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level rating. Accordingly, the study confidence conclusions appear scientifically justified, with EPA having given appropriate consideration to important study attributes. This question (under "2") also concerns whether the presentation and analysis of study results is clear, appropriate, and effective, a step just upstream of synthesis that if well done, then allows for appropriate syntheses of the endpoint-specific findings. Overall, the presentation and analysis of study results appears clear, appropriate, and effective. Additionally, it appears that by and large, the available data on neurodevelopmental effects are clearly and appropriately synthesized to describe the strengths and limitations. P. 3-245, lines 5-13 state [*emphasis added*] that, "The evidence for potential neurodevelopmental effects in humans is considered slight. Associations between PFDA exposure and outcomes related to attention and behavior were reported in multiple epidemiological studies, though there was inconsistency between these findings and the more clinically relevant measure of ADHD diagnosis. Results for other neurodevelopmental effects were largely inconsistent, though poor sensitivity due to limited exposure contrast may explain the lack of association in some studies. No animal toxicity studies are available. *Altogether, based on the available human studies, the* ***evidence suggests*** *that PFDA exposure might cause neurodevelopmental effects in humans under sufficient exposure conditions* (see Table 3-40)." Table 3-40 is the evidence profile table for neurodevelopmental effects, which contains both factors that increase certainty and factors that decrease certainty along with evidence stream (i.e., human) judgments/rationales and a summary judgment. Obviously, the text of the document (Section 3.2.7) also contains information relevant to and supporting the WOE for neurodevelopmental effects. h. Endocrine, urinary, and other noncancer effects: The study confidence conclusions appear scientifically justified, giving appropriate consideration to important study attributes. For each study in the relevant figures of Sections 3.2.8 (*Endocrine Effects*; Figures 3-77, 3-78, 3-80, 3-82), 3.2.9 (*Urinary Effects*; Figures 3-84 and 3-85), and 3.2.10 (*General Toxicity*; Figure 3-88), the consideration ratings appear overall consistent with (i.e., provide the scientific justification needed for) the overall study confidence level rating. Accordingly, the study confidence conclusions appear scientifically justified, with EPA having given appropriate consideration to important study attributes. Section 3.2.11 (*Other Health Effects*) had no such study evaluation heatmap figures. • This question (under "2") also concerns whether the presentation and analysis of study results is clear, appropriate, and effective, a step just upstream of synthesis that if well done, then allows for appropriate syntheses of the endpoint-specific findings. Overall, the presentation and analysis of study results appears clear, appropriate, and effective.

	Additionally, it appears that overall, the available data on these effects are clearly and appropriately synthesized to describe the strengths and limitations. Sections 3.2.8 through 3.2.11 contain the following statements [*emphasis added*]: • "Taken together, there is ***inadequate evidence*** across human, animal, and mechanistic data to determine whether PFDA exposure would cause *endocrine effects* in humans. This conclusion is based on inconsistent evidence from human studies and from a single high confidence rat study investigating PFDA doses 2.5 ≤mg/kg-day that reported largely incoherent effects on thyroid hormone homeostasis and thyroid structure (i.e., increased T3, decreased TSH and T4; increased thyroid weight; no histopathology) that cannot be interpreted based on the currently available evidence base." (Section 3.2.8, p. 3-264, lines 5-11) • "Altogether, based on the available human and animal studies, there is ***inadequate evidence*** to assess whether PFDA exposure can cause *urinary system effects* in humans (see Table 3-46)." (Section 3.2.9, p. 3-279, lines 23-24) • "The potential for PFDA exposure-induced *general toxicity* is specifically discussed given that PFDA has been shown to cause a "wasting syndrome" in rodents, which is characterized by decreased food intake and reduced body weight (Goecke-Flora et al., 1995). *In animals, decreased body weights can be indicative of non-specific overt toxicity and some effects that occur at doses associated with this and other frank effects should be interpreted cautiously when drawing conclusions about organ-/system-specific hazards*. Thus, this section informs judgments drawn for other potential health hazards, but *a specific evidence integration judgment is not drawn*."[18] (Section 3.2.10, p. 3-282, lines 1-7) • "Short-term oral exposure studies (high/medium confidence) in experimental animals evaluated potential health effects related to the *hematological, respiratory, digestive, dermal, musculoskeletal, and adult nervous system* (please see Section 3.2.7 for the synthesis of evidence on neurodevelopmental effects). The available evidence from these animal studies is briefly summarized below. Given the limitations of the evidence base and the lack of consistent or coherent effects of PFDA exposure, there is ***inadequate evidence*** to determine whether any of the evaluated outcomes below might represent potential human health hazards of PFDA exposure." (Section 3.2.11, p. 3-287, lines 3-10) Tables 3-41 and 3-45 are the evidence profile tables for endocrine and urinary effects, which generally contain both factors that increase certainty and factors

[18] It is noted that the question's statement [*emphasis added*] that "for endocrine, urinary, and *other noncancer effects* (i.e., hematological, respiratory, digestive, dermal, musculoskeletal, and nervous systems), the Toxicological Review concludes there is ***inadequate evidence*** to determine whether PFDA exposure has the potential to cause these effects in humans" apparently excludes general toxicity for which "a specific evidence integration judgment is not drawn" (Section 3.2.10, p. 3-282, lines 6-7).

	that decrease certainty along with evidence stream (i.e., human, animal, mechanistic/supplemental) judgments/rationales and a summary judgment. Sections 3.2.10 (*General Toxicity*) and 3.2.11 (*Other Health Effects*) contain no such tables. Obviously, the text of the document in Sections 3.2.8 through 3.2.11 also contains information relevant to and supporting the WOE for these effects.
Hoberman	a. Liver effects: For liver effects, the available data have been clearly and appropriately synthesized to describe the strengths and limitations. On a weight-of-evidence approach for hazard identification the conclusion that liver effects in rodents appears relevant to humans and supports the possibility of liver toxicity in humans at relevant exposures is clearly described and scientifically justified. The conclusion is supported by the data from rats and mice tested in short-term and subchronic studies, and rats in a developmental toxicity study. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies. This reviewer agrees that the available evidence indicates that PFDA exposure is likely to cause hepatotoxicity in humans given sufficient exposure conditions. This conclusion is based primarily on coherent liver effects in rats (and, to a lesser extent, mice) exposed to doses ≥0.156 mg/kg-day for 28 days. The available mechanistic information overall provides support for the biological plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings (page 3-50). (page 3-49) The evidence in humans based on liver biomarkers indicates slight evidence of an association between PFDA exposure and hepatic effects. Positive associations between exposure to PFDA and ALT were observed in four of five studies of adults. A lack of coherence across clinical markers in humans reduces the strength of the evidence. The evidence for PFDA-induced liver effects is the strongest based on the short-term animal studies that demonstrated coherent effects across serum biomarkers, histopathology, and organ weight. Increases in serum biomarkers of hepatocellular/hepatobiliary injury (ALT, AST, ALP, bile salts/acids and bilirubin) (NTP, 2018) and liver weights were reported in male and female rats at ≥0.156 mg/kg-day after 28-day gavage exposure (Frawley et al., 2018; NTP, 2018). The evidence for increased liver weights was consistent across several species (rats and mice), strains (S-D, Wistar, Fischer F344, C57BL/6N, C57BL/6J and B6C3F1/N) and exposure designs (gavage and dietary. At higher doses (≥0.5 mg/kg-day), a consistent pattern of hepatocellular lesions was observed in S-D rats that included cytoplastic alterations and vacuolization, hypertrophy, and necrosis (Frawley et al., 2018; NTP, 2018). The pattern of hepatocellular changes showed a progression in severity within and across lesions with an increase in exposure dose, which adds certainty to the interpretation of the evidence.

	In combination with the histopathological findings, alterations in serum biomarkers and liver weights support the development of adverse liver effects in rats after continuous PFDA exposure. The evidence base is limited in that there is an absence of studies via relevant exposure routes with durations longer than 28 days examining potential hepatic effects of PFDA exposure. Analysis of mechanistic and supplementary data from *in vivo* and *in vitro* rodent models provide experimental (e.g., liver weight changes after i.p. exposure) and biological support for the phenotypic effects reported in the short-term oral studies summarized above. Exposure to PFDA was associated with the activation of several molecular signaling pathways and altered cellular functions hypothesized to be involved in the MOA for liver toxicity of related perfluorinated compounds. Additionally, the evidence for PFDA-mediated liver effects implicates both PPARα-dependent and independent mechanisms. The activation of PPARα in the MOA for non-cancer liver effects in rodents has implications to human health assessment based on perceived differences in PPARα response between rats/mice versus humans. PFDA can activate the human PPARα in vitro but it exhibits less sensitivity towards the human isoform in comparison to other mammalian species. PFDA also interacts with other nuclear receptors and cell signaling pathways relevant to its potential mechanism of hepatotoxicity in both human and animal models. Some hepatic responses in animals occurred, at least in part, independent of PPARα or were found to be activated in human *in vitro* assays or animal models that are more relevant to humans with respect to PPARα sensitivity. These observations are consistent with studies in PPARα null and humanized animals for other long-chain PFAS such as PFOA, PFHxS and PFNA that suggest non-PPARα mechanisms of liver toxicity. Given that the precise role of PPARα in the non-cancer liver effects of PFDA remains largely unknown and the possible involvement of PPARα-dependent and independent pathways, the effects observed in animals are considered potentially relevant to humans (Soldatow et al., 2013). • **Tier 1: *Necessary Revisions*** –None • **Tier 2: *Suggested Revisions*** – None • **Tier 3: *Future Considerations*** – Longer term studies in rats would confirm trends observed in liver toxicity in the shorter-term studies used in for this assessment. b. <u>Immune effects</u>: For immune effects, the available data have been clearly and appropriately synthesized to describe the strengths and limitations. Two epidemiological studies in children and one study in adults demonstrated consistent evidence of reduced antibody responses while the evidence of immunosuppression in animal studies was not clear. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies. (Page 3-92) The available evidence from human, animal and mechanistic studies, indicates that PFDA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans, given sufficient exposure conditions. The hazard

	is driven primarily by consistent evidence of reduced antibody response from human Toxicological Review of Perfluorodecanoic Acid and Related Salts epidemiological studies (mostly from two birth cohort studies) at levels of 0.3 ng/mL (median exposure in studies observing an adverse effect). The evidence in animals showed coherent immunomodulatory responses at ≥0.089 mg/kg-day that Pare consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. A small number of studies conducted via i.p. injection and *in vitro* exposure in human and rodent cell culture models added some support for the biological plausibility of the observed phenotypic effects. While there is some evidence that PFDA exposure might also have the potential to affect sensitization and allergic responses, the human evidence underlying this possibility is uncertain and without support from animal or mechanistic studies. Based on the antibody response data in humans, children and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFDA. Studies in humans and animals exposed to PFDA are available for the evaluation of potential immunosuppression and sensitization or allergic responses. The evidence of an association between PFDA exposure and immunosuppressive effects in human studies is moderate. This is based on largely consistent decreases in antibody response following vaccination (against two different infectious agents) in two medium confidence studies describing results from two independent birth cohorts in the Faroe Islands with outcome measurement in childhood. Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease (IPCS, 2012). The antibody results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFDA were associated with suppression of at least one measure of the anti-vaccine antibody response to common vaccines in two well-conducted birth cohorts in the Faroe Islands and supported by a low confidence study in adults. An inverse association was observed in 21 of 26 evaluations, with a minimum of a 2% decrease in antibody concentration per doubling of PFDA concentration at levels consistent with the general population in NHANES; six of these evaluations were statistically significant and exhibited a large magnitude of effect (i.e., >18% decrease in response). These associations were observed despite poor study sensitivity, which increases confidence in the findings. There is some remaining uncertainty resulting from variability in the response, including positive associations in a few exposure-outcome combinations, differences in the responses by age of exposure and outcome measures as well as timing of vaccination (initial and boosters), from potential confounding across PFAS, and from inconsistency in two other medium confidence studies with outcome measurement in adults and cross-sectional exposure measurement in children.

	Overall, the evidence supports an association with immunosuppressive-type effects. These results are consistent with hazard identification conclusions from the NTP (2016) monograph on immunotoxicity associated with exposure to PFOS and PFOA, which concluded that PFOA and PFOS are presumed to be an immune hazard to humans based largely on evidence of suppression of antibody responses in both human and animal studies (NTP, 2016). Although no effects were observed in T-dependent antibody responses with PFDA in one rat and one mouse study (both high confidence), other immunomodulatory responses were observed in animals that indicate potential for immunosuppression (see summary of animal evidence below for more details). The database of animal studies examining PFDA-induced immunosuppressive responses consists of two high or medium confidence studies in B6C3F1/N mice (Frawley et al., 2018) and/or S-D rats (Frawley et al., 2018; NTP, 2018) exposed via gavage for 28 days. PFDA did not elicit a strong immunotoxic response in animals as evidenced by the absence of treatment-related effects in a host resistance assay and most immune function assays (NK cell activity and T-dependent antibody responses to SRBC, mixed leukocyte response and DTH response to C. albicans). Nevertheless, coherent responses that suggest potential immunosuppression by PFDA exposure were observed, which is consistent with the human evidence. The immunomodulatory responses included dose-related decreases in phagocytic activity of rat liver macrophages (MPS activity) at ≥ 0.25 mg/kg-day and in immune cell population counts in mouse spleen at ≥0.089 mg/kg-day (Frawley et al., 2018), but issues regarding overt organ toxicity (increased liver weight and hepatocyte necrosis and spleen atrophy, respectively) introduce significant uncertainty (Frawley et al., 2018). Morphological changes occurred in the bone marrow (hypocellularity) and thymus (atrophy and lymphocyte apoptosis) of rats at PFDA doses associated with systemic toxicity (i.e., decreased body weights at ≥1.25 mg/kg-day) (NTP, 2018); the changes are consistent with the wasting syndrome that PFDA elicits and could represent secondary effects of the accompanying systemic toxicity. The evidence for potential immunosuppression from short-term animal studies is considered slight. Mechanistic evidence from a high-dose, i.p. injection study is supportive of potential PFDA induced immunosuppression (i.e., decreased antibody and DTH responses) in rats at ≥20 mg/kg (Nelson et al., 1992). Furthermore, an *in vitro* study using stimulated human primary and cultured leukocytes suggests that PFDA is capable of inhibiting NF-κB transcription and suppressing cytokine production (Corsini et al., 2012), which may be relevant to its mechanisms of immunotoxicity. Limitations in the mechanistic information include issues interpreting the exposure context (i.e., acute, high-dose exposure) of the i.p. injection study and general lack of studies in animal and human models that can provide support for the biological plausibility of putative immunosuppression observed in human and animal studies. There is slight evidence for sensitization and allergic responses from studies in humans, but notable limitations and uncertainties in the evidence base remain. In

human studies, the available evidence for infectious disease and hypersensitivity was less consistent than the evidence on immunosuppression and had more uncertainties resulting from a limited number of studies, unexplained heterogeneity in outcome or results, variable exposure assessment approaches that considered exposure at different times in relation to outcomes, and in some cases self-reported outcomes.

For asthma, two of the three available studies reported no association with PFDA exposure. However, significant associations with asthma diagnosis and asthma-related outcomes, including an exposure response gradient, were observed in one well-conducted (medium confidence) study with adequate sensitivity (Dong et al., 2013). This study also had the most specific outcome definition, based on asthma incidence in the past year. These differences could account for the inconsistency with other asthma studies, including the other medium confidence study which examined "ever asthma". In addition, increases in biomarkers related to asthma were reported in this study, providing biological plausibility to the apical association. Still, the number of available studies is small, and poor sensitivity makes the null results difficult to interpret.

In animals, the single, short-term, low confidence study that examined endpoints relevant to sensitization and allergic responses reporting findings coherent with immediate-type hypersensitivity (i.e., exacerbation of hypothermia and markers of mast cell-mediated allergic inflammation in OVA-induced mice) (Lee and Kim, 2018); however, the high exposure dose used (21.4 mg/kg-day) raises significant concerns about potential confounding effects by indirect systemic toxicity and thus these coherent results were not interpreted to provide biological plausibility for the findings in humans and the animal evidence was considered indeterminate (Lee 34 and Kim, 2018).

- **Tier 1: *Necessary Revisions*** –None
- **Tier 2: *Suggested Revisions*** – Some indication if the suspected effects on the immune system were in line with other PFOA would be helpful.
- **Tier 3: *Future Considerations*** –The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental life stages represents a major source of uncertainty in the immunotoxicity database of PFDA.

c. Developmental effects: For developmental effects, the available data have been clearly and appropriately synthesized to describe the strengths and limitations. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies.

This reviewer agrees that based on the available evidence PFDA exposure is likely to cause developmental toxicity in humans given sufficient exposure conditions. This conclusion is based on dose-dependent decreases in fetal weight in the single toxicology study, that exposed mice during gestation to PFDA at doses ≥0.5

	mg/kg-day and evidence of decreased birth weight from studies of exposed humans with median PFDA values ranging from 0.11 to 0.46 ng/mL and epidemiological evidence for biologically related effects (e.g., decreased postnatal growth and birth length). (Page 3- 156) Based on over 45 different epidemiological studies included here the evidence of an association between PFDA exposure and developmental effects in humans is considered slight but was supported by the moderate evidence in animals. The epidemiological evidence was strongest 13 and most consistent for fetal growth restriction and in particular for birth-weight related measures, which were assessed by the most accurate growth restriction measures available. Out of 28 in total, 18 different studies showed some deficits for the overall population or for both/either sex across various birth weight measures. For example, 11 out of 22 PFDA studies in the overall population reported some birth weight deficits; this included 9 out of 14 medium and high confidence studies. Data for growth measurements were mixed, but there appeared to be some coherence across studies and other prenatal growth measures with different postnatal growth parameters. For example, there was some consistency across 2 (one high and one low confidence) of the 3 postnatal weight studies with a common examination window (~2 years of age). The evidence for other endpoints was not as strong or consistent, including 10 of 17 birth length studies that showed some associations. Consistency of results varied across the developmental endpoints, the dearth of birth weight and birth length results in the overall study populations based on early or pre-pregnancy measures might be indicative of potential bias due to the impact due to pregnancy hemodynamics on PFDA levels. Despite consistent evidence of an association between PFDA and different BWT-related measures, there is considerable uncertainty given that some sample timing differences may explain some of the reported fetal growth restriction deficits. Across the outcomes, this set of developmental studies was of good quality and generally had a low risk of bias, as out of the 45 studies across the six primary endpoints [fetal growth restriction (including both birth weight and length measures), gestational duration, postnatal growth, anogenital distance, birth defects, and spontaneous abortions] were either medium or high overall confidence. Several studies demonstrated sufficient sensitivity to detect associations in the overall population and across sub-groups. However, many studies lacked power to detect statistical interactions or differences across populations especially those based on stratified analyses. This often results from low exposure levels with limited contrasts in many of the study populations, which may have diminished the sensitivity of some studies to detect associations. As such, any null findings for studies with endpoints which lacked sensitivity should not be interpreted as supporting a lack of effect.

	Fetal growth restriction endpoints provided the strongest evidence for adverse developmental effects among the available studies. In considering the dose-dependence of the birth weight decreases, only one out of four medium or high confidence studies with categorical PFDA exposure data showed an exposure-response relationship. In addition, 9 of 14 medium or high confidence studies of the overall population as well as 9 of 14 sex-specific results showing adverse results based on continuous exposure also offer support for a biological gradient. Exposure-response relationships were less evident for other endpoints that were examined. There was considerable variability in BWT deficits (β range: -29 to -101 g per ln-unit increases) in the overall population, with seven studies ranging from 31 to 59 g deficits per each l unit increase. The clinical significance of these changes may not be immediately evident, but effects of this magnitude can increase the number of infants at higher risk for other co-morbidities and mortality especially during the first year of life. These population-level changes may have a large public health impact when these mean birth weight deficits shift the birth weight distribution to include more infants in the low-birth-weight category. Decreased birth weight has been associated with long-term adverse health outcomes (Osmond and Barker, 2000). Supporting the human evidence, the large and dose-dependent effects on fetal body weight observed across two independent experiments reported in the lone mouse study by Harris and 28 Birnbaum (1989) (medium confidence for this endpoint) are without evidence to the contrary and thus provide moderate evidence coherent with the findings in humans. Following gestational PFDA exposure, decreases in fetal body weight with a significant trend were consistently observed in both experiments at ≥0.5 mg/kg-day, including doses (0.5–4 mg/kg-day) well below those that produced maternal toxicity. The changes in fetal body weight were also large in magnitude with the percent changes of up to 10% at the lower doses and ranging as high as 44–50% at the highest doses tested in both experiments. The rodent data for decreased fetal body weight are coherent with data from the human studies in which the strongest and most consistent evidence was for fetal growth restriction. Although an increased fetal incidence of several skeletal variations (i.e., delayed braincase and phalanges ossification and absence of fifth sternebrae) was observed, the delays in brain ossification, which started at ≥0.03 mg/kg-day, well below doses eliciting maternal toxicity, were most notable. This change is potentially indicative of delayed development (which would be coherent with the PFDA-induced changes on fetal body weight); however, the significance of this variation (in terms of future adverse consequences), is unknown, and malformations, which are known to be adverse, were not observed. PFDA was reported to be teratogenic in embryonic zebrafish (Truong et al., 2022; Ulhaq et al., 2013). There were also statistically significant changes reported for fetal viability in mice (i.e., increased % of resorptions per litter and reduced number of live fetuses per litter) at the highest dose tested in the GD 6–15

	experiment (Harris and Birnbaum, 1989); however, effects on fetal viability were observed at the same doses as significant maternal toxicity, preventing the ability to draw conclusions at these doses. A notable data gap exists, as animal studies evaluating the effect of PFDA on postnatal development were not identified. Some effects of PFDA on postnatal growth were observed in humans. Additionally, effects on postnatal development (e.g., delayed eye opening; reduced postnatal growth) have been observed in rodents exposed to other PFAS such as PFOA, PFBS, PFBA. • **Tier 1: *Necessary Revisions*** –Pubertal development is one of the four endpoints of developmental toxicity and should be discussed in this section. • **Tier 2: *Suggested Revisions*** – None • **Tier 3: *Future Considerations*** – as noted in the review more investigation of the potential mechanism that causes reductions on fetal growth in animal and human studies would be useful. d. <u>Male reproductive effects</u>: For male reproductive effects, the available data have been clearly and appropriately synthesized to describe the strengths and limitations. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies. (Page 3-184) Evidence indicates that PFDA is likely to cause male reproductive effects in humans under sufficient exposure conditions. This conclusion is based primarily on a constellation of coherent evidence from a high confidence study in animals exposed to 0.625–2.5 mg/kg-day for 28 days, with some support for biological plausibility provided by mechanistic evidence from i.p. and cell culture models. Although no direct information on the human relevance of the animal evidence is available, many aspects of the male reproductive system are conserved across species, and the limited sensitivity in human studies may explain the lack of associations observed. Uncertainties in the database of PFDA-induced male reproductive toxicity includes the absence of subchronic, chronic, developmental, or multigenerational studies testing these outcomes in animals (which, overall, are anticipated to be more sensitive than the available short-term study design), and a general lack of adequate epidemiological or toxicological studies evaluating the potential for effects of early life PFDA exposure on male reproductive system development. The evidence of an association between PFDA exposure and male reproductive effects in humans is limited to two medium (Tian et al., 2019; Joensen et al., 2013) and one low confidence study (Zhou et al., 2016), with findings suggesting potential decreases in testosterone, decreased sperm motility, and anogenital distance with higher PFDA exposure. There are concerns over inconsistency and imprecision, thus, the evidence is considered indeterminate. The available evidence from a 28-day gavage study in rats and supportive data from i.p injection and cell culture studies

	in rodents provide moderate evidence of male reproductive toxicity in animals with PFDA exposure. The 28-day rat study showed coherent effects across several relevant endpoints, including sperm evaluations, histopathology, hormone levels and organ weights (NTP, 2018), with most effects observed at doses below those shown to cause overt toxicity. Adverse histopathological changes were observed at doses associated with body weight decrements of potential concern. The study methods were considered high confidence for all endpoints other than sperm evaluations, which were considered potentially insensitive due to an inadequate exposure duration (i.e., biased towards the null; confidence is reduced specifically in the interpreted reliability of null findings [i.e., sperm motility]). A consistent pattern of decreased testicular and epididymal sperm counts occurred at ≥0.625 mg/kg-day, but only the effects in the epididymis were dose related. Dose-related decreases in serum testosterone levels and testicular and epididymal weights were also reported in rats at ≥0.625 mg/kg-day. The reductions in sperm counts, serum testosterone levels and organ weights are coherent with the mild degenerative changes found in testes and epididymis at similar doses, particularly Leydig cell atrophy, which is associated with androgen deficiency and decreased spermatogenesis (Creasy et al., 2012). Consistent effects on serum androgen levels, male reproductive organ weights, and histopathology were observed in rodents exposed to high doses of PFDA (≥20 mg/kg) in, single, i.p. injection studies. The adverse effects observed in the *in vivo* oral and i.p. exposure studies are biologically consistent with a potential mechanism for PFDA-induced reproductive effects in which alterations in Leydig cell functions result in decreased steroidogenesis and androgen levels. Limitations of the animal evidence base include the availability of only a single, short-term oral exposure study in a single species, and uncertainties regarding the potential impact of systemic toxicity, particularly with regard to the observed histopathological effects. Significant reductions in body weight were reported in the highest dose groups in the 28-day gavage study (21% at 29 1.25 mg/kg-day and 38% at 2.5 mg/kg-day; (NTP, 2018). Concern for nonspecific effects on the male reproductive system is attenuated by the observed dose-related effects (i.e., sperm counts, testosterone levels and organ weights) at a lower PFDA dose, not associated with body weight changes (0.625 mg/kg-day). An i.p. injection study that examined potential effects of PFDA-induced "wasting syndrome" using pair-fed control rats observed androgenic deficiency and male reproductive toxicity at 20 and 40 mg/kg that were independent from severe body weight depression at the highest dose (72% at 80 mg/kg) (Bookstaff et al., 1990).

	in vitro models derived from the male reproductive system, and models restricted to rodents, limits the ability of the available evidence to inform potential pathways involved in PFDA- induced male reproductive toxicity and to elucidate conserved mechanisms across species, including humans. Nonetheless, the mechanistic information from acute i.p. and *in vitro* animal studies is both consistent and coherent with the oral exposure study evidence, and therefore, provides support for the biological plausibility of the phenotypic responses. In the absence of information to the contrary and given the conserved role of androgen-dependent pathways in male reproductive functions across species (including humans), the available evidence is relevant to humans. A potentially susceptible population for PFDA-induced male reproductive effects are young individuals exposed during critical developmental life stages (e.g. the masculinization programming, which occurs prior to the differentiation of androgen-sensitive tissues and determines penis size and anogenital distance (Dent et al., 2015), although no such studies were available in the current animal evidence base and few epidemiological studies examining pubertal development and anogenital distance were available. Androgens play a critical role in the normal development of the male reproductive system and disruptions caused by exposures to reproductive toxicants during gestation and early post-natal life stages can lead to agenesis of the male reproductive system and/or infertility (Foster and Gray, 2013; Sharpe, 2010; Scott et al., 2009). • **Tier 1: *Necessary Revisions*** –None • **Tier 2: *Suggested Revisions*** – None • **Tier 3: *Future Considerations*** – Additional long term studies would be useful to examine potential effects on reproductive organs. e. <u>Female reproductive effects</u>: For female reproductive effects, the available data have been clearly and appropriately synthesized to describe the strengths and limitations. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies. (Page 3-202) Evidence indicates that PFDA is likely to cause female reproductive toxicity in humans under sufficient exposure conditions. This conclusion is based primarily on evidence from a high confidence study in rats exposed to doses ranging from 1.25–2.5 mg/kg-day PFDA for 28 days. The PFDA-induced disruption of estrous cyclicity observed in female rats from the NTP study (NTP, 2018) and its implications for infertility can be considered relevant to humans given that the mechanisms responsible for regulating female reproductivity (e.g., estrous cyclicity in rats and menstrual cycling in humans) are similar between rats and humans (Goldman et al., 2007; Bretveld et al., 2006). There is indeterminate evidence of an association between PFDA exposure and female reproductive effects in human studies, though the low confidence studies

	that were available had concerns for study sensitivity which reduces the ability to interpret the observed null findings. A significant inverse association between PFDA and anogenital distance in girls was observed in one study (see Developmental Effects), which is relevant to female reproductive toxicity. The biological relevance of this effect on anogenital distance is unclear given that an increase in this measure is considered adverse in girls rather than a decrease per the U.S. EPA's Guidelines for Reproductive Toxicity Risk Assessment. The available reproductive hormone evidence for PFDA does not support an association. Previous studies have shown an association between increased testosterone and increased anogenital distance in women (Mira-Escolano et al., 2014), however the human evidence is inadequate for examining PFDA-induced effects on testosterone in women. Whereas increased testosterone was observed in female rats in the NTP (2018) study, the study authors did not measure anogenital distance given that there was no developmental exposure in the study. The increased testosterone observed in female rats is considered relevant to humans and given the known association between increased testosterone and anogenital distance in women, an increase in anogenital distance rather than a decrease would be expected in women exposed to PFDA. There is little biological understanding of how hormonal perturbation or other biological processes might result in a decrease in anogenital distance owing to PFDA exposure. In addition to the outcomes, there is potential for two of the outcomes described in the developmental section, preterm birth and spontaneous abortion, to be related to female reproductive toxicity. The evidence for these outcomes was inconsistent. Given that most of the evidence for female reproductive effects was null or inconsistent, there is little clear indication of an association. However, the exposure levels in most of the study populations were low, which resulted in low sensitivity to detect an effect, and thus these findings should not be interpreted as supporting a lack of effect. The available data from a 28-day gavage study in rats provide moderate evidence that PFDA exposure may cause female reproductive toxicity.. The evidence is sparse. The data are from a single animal study that did not evaluate fertility, pregnancy outcomes, multiple hormone levels (only testosterone was measured), or markers of reproductive development. PFDA was observed to cause effects on the following female reproductive parameters: organ weight (i.e., decreased uterine weights at ≥1.25 mg/kg-day), hormone levels (i.e., increased testosterone levels at ≥0.312 mg/kg-day), and estrous cycle (i.e., percentage of time spent in estrus and diestrus at ≥1.25 mg/kg-day). One factor increasing the strength of the evidence is the severity of the effect on estrous cyclicity; specifically, that PFDA induced a continuous state of diestrus in 100% of rats treated at the highest dose tested (2.5 mg/kg-day), which could be indicative of reductions or delays in fertility. However, some caution in the interpretation of the higher dose effects is warranted given the significant decreases in body weight, particularly at 2.5 mg/kg-day (36% decrease). Support for the adversity and concerning nature of prolonged diestrus and its association

with infertility is provided by the following text in the U.S. EPA's Guidelines for Reproductive Toxicity Risk Assessment:

- "Persistent diestrus indicates temporary or permanent cessation of follicular development and ovulation, and thus at least temporary infertility,"
- "Pseudopregnancy is another altered endocrine state reflected by persistent diestrus."
- "Significant evidence that the estrous cycle (or menstrual cycle in primates) has been disrupted should be considered an adverse effect."
- "The greatest confidence for identification of a reproductive hazard should be placed on significant adverse effects on sexual behavior, fertility or development, or other endpoints that are directly related to reproductive function such as menstrual (estrous) cycle normality, sperm evaluations, reproductive histopathology, reproductive organ weights, and reproductive endocrinology."

Prolonged diestrus is commonly reported in rodent models of impaired fertility (Li et al., 2017; Caldwell et al., 2014; Miller and Takahashi, 2014; Mayer and Boehm, 2011) and continuous diestrus is observed during reproductive senescence in aged female rats (Lefevre and Mcclintock, 1988).

There was also coherence between decreased uterus weight and increased percentage of time spent in diestrus at ≥1.25 mg/kg-day. Previous studies have shown that decreased uterus weight in rats is commonly observed during diestrus (Westwood, 2008; Vasilenko et al., 1981; Walaas, 1952; Boettiger, 1946). In addition to prolonged diestrus, PFDA decreased the percentage of time spent in estrus (NTP, 2018), which could indirectly cause infertility given that rodents are sexually receptive only during estrus (Goldman et al., 2007). The severe, PFDA-induced decreased time spent in estrus is expected to result in decreased opportunities for mating in the rats, and therefore reductions or delays in fertility.

Unfortunately, no multi-generational studies of PFDA were available to inform this hypothesis. In the 28 day study, PFDA did not cause histopathological changes in female reproductive tissues. Given the short-term duration of the lone animal study, it cannot be reasonably ruled out that detectable histopathological effects could have become apparent with a longer observation window. The short-term duration of the lone animal study does not reduce confidence in the database for PFDA-induced female reproductive effects given that biologically relevant effects (e.g., prolonged diestrus) were still observed.

- **Tier 1: *Necessary Revisions*** –None
- **Tier 2: *Suggested Revisions*** – None
- **Tier 3: *Future Considerations*** – As the only animal data for female reproductive effects is from a single short term toxicity study, there is a need to investigate the effects of longer exposures in study designs that include the effect of PFDA on female fertility and pregnancy outcomes and

possibly for more than one generations due to the continued presence of PFDA in the body.

f. Cardiometabolic effects: For cardiometabolic effects, the available data have been clearly and appropriately synthesized to describe the strengths and limitations. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies. (Page 3-235).

Evidence suggests that PFDA exposure has the potential to cause cardiometabolic effects in humans under sufficient exposure conditions. This conclusion is based on evidence of an association between PFDA exposure and certain cardiometabolic outcomes (serum lipids, adiposity, cardiovascular disease, and atherosclerosis) in a small number of epidemiological studies with median exposure levels from 0.1–0.4 ng/mL; however, issues with inconsistency across studies raise considerable uncertainty. Moreover, evidence in animals is sparse and largely uninterpretable regarding its relevance to humans

The evidence of an association between PFDA exposure and cardiometabolic effects in humans is slight, with an indication of higher serum lipids, adiposity, cardiovascular disease, and possible markers of atherosclerosis with higher PFDA exposure. While most results were imprecise and not statistically significant, exposure contrasts for PFDA in the study populations were relatively narrow, which is interpreted to result in low sensitivity to detect an effect.

There is inconsistency across studies for similar outcomes, so there is considerable uncertainty in the evidence. There is no evidence of an association with diabetes, insulin resistance, and metabolic syndrome, but the null results are difficult to interpret due to concerns for sensitivity.

The animal evidence is indeterminate given that the observed changes fail to establish a coherent pattern of adverse cardiometabolic effects in animals following short-term PFDA exposure.

The evidence in animals is limited to a high confidence study in rats exposed via gavage for 28 days that examined cardiovascular histopathology, serum lipids and heart weights (NTP, 2018). Dose-related decreases in triglyceride levels occurred in males and females and cholesterol also decreased dose-dependently in females. However, the biological significance of these responses is unclear. Absolute heart weights decreased dose-dependently in rats at the highest doses (≥1.25 mg/kg-day) but confidence in the results is reduced by potential confounding with decreased body weights and a lack of corroborative findings from histopathological evaluations or other organ weight measures (relative heart weight was unchanged).

- **Tier 1: *Necessary Revisions*** –None
- **Tier 2: *Suggested Revisions*** – None
- **Tier 3: *Future Considerations*** – A major limitation in the animal toxicity database of this chemical is the lack of studies examining prolonged or

	chronic oral exposures. In addition, for some cardiometabolic endpoints (i.e., serum lipids), it would be preferred if studies were available in models that are more physiologically relevant to humans given species differences in lipid metabolism between humans and rodents (Getz and Reardon, 2012; Davidson, 2010). g. Neurodevelopmental effects: For neurodevelopmental effects, available data have been clearly and appropriately synthesized to describe the strengths and limitations. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies. (Page 3- 245) Based on human studies, the evidence suggests that PFDA exposure might cause neurodevelopmental effects in humans under sufficient exposure conditions. The evidence for potential neurodevelopmental effects in humans is considered slight. Associations between PFDA exposure and outcomes related to attention and behavior were reported in multiple epidemiological studies, though there was inconsistency between these findings and the more clinically relevant measure of ADHD diagnosis. Results for other neurodevelopmental effects were largely inconsistent, though poor sensitivity due to limited 1exposure contrast may explain the lack of association in some studies. No animal toxicity studies are available. • **Tier 1: *Necessary Revisions*** –None • **Tier 2: *Suggested Revisions*** – None • **Tier 3: *Future Considerations*** – None h. Endocrine, urinary, and other noncancer effects: For endocrine, urinary and other non-cancer effects, available data have been clearly and appropriately synthesized to describe the strengths and limitations. The presentation and analysis of study results was found to be clear, appropriate and effective, allowing for scientifically supported syntheses of the findings across the various sets of studies. (Page 3- 262) There is inadequate evidence across human, animal, and mechanistic data to determine whether PFDA exposure would cause **endocrine effects** in humans. This conclusion is based on inconsistent evidence from human studies and from a single high confidence rat study investigating PFDA doses ≤2.5 mg/kg-day that reported largely incoherent effects on thyroid hormone homeostasis and thyroid structure (i.e., increased T3, decreased TSH and T4; increased thyroid weight; no histopathology) that cannot be interpreted based on the currently available evidence base. There is indeterminate evidence of an association between PFDA exposure and endocrine related effects in studies of exposed humans. The evidence is largely null, but there are concerns for study sensitivity.

	The observed associations are inconsistent across studies and not coherent across thyroid hormones. There is indeterminate animal evidence of endocrine toxicity- specifically, thyroid effects, with PFDA based on incoherent evidence from a single high confidence short term study in rats (a second short term study examined adrenal effects). PFDA was shown to cause changes in thyroid hormone levels, some of which may be interpreted as suggestive of secondary hypothyroidism, a phenotype characterized by decreased T4 and decreased or normal levels of TSH (Lewiński and Stasiak, 2017); however, the PFDA data are not entirely coherent with such a hypothesis. In the NTP (2018) study, significant trends were reported for decreased TSH and fT4 (but not tT4) in male rats at ≥0.312 mg/kg-day, while significant trends were also reported for increased T3 (the latter findings are not coherent with hypothyroidism). Likewise, in females, increased T3 and decreased fT4 was observed at ≥1.25 mg/kg-day. High dose PFDA exposure induced decreases in total T4 were consistently observed in multiple, high dose i.p. studies in rats. The cause of secondary hypothyroidism is thought to be due to impaired responsiveness of the hypothalamus-pituitary-thyroid axis (Lewiński and Stasiak, 2017). Consistent with this, PFDA was shown to impair the response of the hypothalamic-pituitary-thyroid axis to TRH stimulation in rats from a high dose i.p. study (Gutshall et al., 1989). These data provide mechanistic insight and biological plausibility for how PFDA could be decreasing serum levels of T4. Furthermore, th ≥1.25 mg/kg-day in male and female rats. A previous study observed increased relative thyroid weight in a rat model of methimazole-induced hypothyroidism (Soukup et al., 2001). Also, an enlarged thyroid is a symptom of hypothyroidism in humans (IQEHC, 2014). In support for PFDA induced changes on thyroid hormone homeostasis, structurally related PFAS compounds (e.g., PFNA; PFOA) have been shown to effect thyroid hormone levels in rodents. However, several aspects of the available animal data decrease the strength or certainty of the evidence informing thyroid effects, which was only available from a single oral exposure study. Whereas the NTP (2018) study reported changes in fT4 and TSH in rats that may indicate secondary hypothyroidism, there was an increase in T3 that cannot be explained. Furthermore, there are no mechanistic studies that determined the effect of PFDA on deiodinase activity that could offer insight on how PFDA decreased T4 and TSH while increasing T3. Additionally, while T4 was decreased in male and female rats from the NTP (2018) study, a consistent decrease in tT4 was not observed. However as noted above, fT4 not tT4 is the preferred measure in adult animals. Whereas there was potential coherence between decreased fT4 and increased thyroid weight in rats, it is unclear how thyroid weight and T3 were increased in the absence of increased TSH or histopathological changes. Uncertainty is also associated with the mechanistic studies and supplemental information. Specifically, inconsistent results were observed for effects on T3 in rats exposed to PFDA via i.p. injection and results from the protein binding

	studies (Gutshall et al., 1989) suggest that PFDA decreased protein binding of T4, which could result in increased fT4 and decreased tT4, which is not consistent with the results from the NTP (2018) study. The mechanistic database is also limited in that there are no studies that investigated the effects of PFDA on deiodinase activity. The activities of thyroid-sensitive hepatic enzymes (e.g., L-glycerol-3-phosphate dehydrogenase) were increased in rats exposed to PFDA via the i.p. route suggesting that thyroid activity may not be decreased due to PFDA treatment. In general, the interpretation and relevance of the mechanistic studies and supplemental information to thyroid effects observed in the NTP (2018) study is unclear given that these studies used doses that were much higher (i.e., 20–80 mg/kg-day, as compared to ≤2.5 mg/kg-day) and associated with overt systemic toxicity. Additionally, the mechanistic studies and supplemental information are of shorter duration and rats were exposed to PFDA via i.p. injection rather than gavage as was done in the NTP (2018) study. In addition to the uncertainty in the available evidence in adults, due to the sparse evidence base available, concern remains for potential susceptible populations to PFDA-induced endocrine effects in susceptible populations including young individuals exposed during gestation, early childhood, and puberty. Importantly, T3 and T4 levels play critical roles in bone growth and brain development (O'Shaughnessy et al., 2019) at these various life stages. However, at the present time few epidemiological studies and no animal toxicological studies have addressed the potential for PFDA-induced effects in these populations. A primary delineating feature between adult animals was coherence with increased relative thyroid weight and decreased T4 serum levels at and developing offspring is that adults have a considerable reserve thyroid hormone capacity whereas developing offspring do not. Thus, there is an elevated concern regarding the potential for decreases in thyroid hormones during developmental life stages due to the critical endocrine dependency of in utero and neonatal development. (Page 3 -279) Associations between PFDA exposure and **impaired renal function** were reported in two low confidence epidemiological studies. However, there is considerable uncertainty in the interpretation of these findings due to the potential for reverse causation and some unexplained inconsistency in the direction of association across studies. The evidence for potential urinary system effects in experimental animals is limited to three high/medium confidence studies in rats (Frawley et al., 2018; NTP, 2018) and one high confidence study in mice with exposure for 28 days (Frawley et al., 2018). Although alterations in BUN and creatine levels were observed at ≥1.25 mg/kg-day in rats, there is no coherent pattern of effects (BUN levels increased and creatinine levels decreased) or supportive information (i.e., histopathology) to determine the toxicological relevance of the changes that occurred (NTP, 2018). Histopathological examinations of rat kidney and urinary bladder were mostly unremarkable across two studies (Frawley et al., 2018; NTP, 2018). Finally, the

	interpretation of the absolute and relative kidney weight changes in rats at doses ≥0.312 mg/kg-day is complicated by the lack of coherent histopathological findings (Frawley et al., 2018; NTP, 2018), inconsistencies in the direction of changes across experiments, and confounding effects from significant body weight reductions at the highest doses tested (≥1.25 mg/kg-day) (NTP, 2018). In summary, the sparse and uncertain evidence from animal studies is considered indeterminate. The absence of any long-term studies (subchronic/chronic) via the oral route or other relevant routes of exposure increases uncertainty in the evaluation of potential urinary system toxicity in animals following PFDA exposure. Altogether, based on the available human and animal studies, there is inadequate evidence to assess whether PFDA exposure can cause urinary system effects in humans. • **Tier 1: *Necessary Revisions*** –None • **Tier 2: *Suggested Revisions*** – None • **Tier 3: *Future Considerations*** – None
Leung	This Toxicological Review for PFDAs is overall clear and effective. This external reviewer especially appreciates the individual heatmaps denoting the EPA's level of confidence, as well as the summary tables listing the body of evidence separately for human and animal studies, in a clear and organized fashion for each health effect. However, I would recommend standardizing the layout of the heatmaps throughout the report; at present, the listing of the individual studies is inconsistently listed either across columns or rows, while the components for strength of evidence is on the other axis (it would be much easier to interpret the various heatmaps by uniformly showing them in the same format; **Tier 2 Recommendation**). The corresponding subsections on mechanistic studies/supplemental evidence before the evidence integration text for each health effect are excellent to support these complex data. a. Liver effects: The Review summarizes the results of 8 human studies and 10 animal studies that were included to examine this aggregate endpoint. Strengths and limitations of the studies are clearly presented and well-organized. This particular endpoint is challenging to study in humans, given the presence of likely multiple measured and unmeasured confounders that are difficult to control for in epidemiologic studies. The report has appropriately attempted to consider this through reasonable confidence ratings of the human studies. The animal studies overall are able to more strongly support the conclusions and appear mostly consistent across the hepatic outcomes, with the exception of histopathologic effects that were mostly low confidence studies. Furthermore, the review has also undertaken relatively extensive supplemental analyses (Appendix) for a proposed MOA/AOP approach, as well as analyses of relevant high-throughput screening assays from the EPA's CompTox Chemicals Dashboard, to support the stated conclusions. Overall, the report's conclusions (that the available evidence suggesting PFDA exposure has the potential to be associated with adverse hepatic effects in humans under sufficient exposure conditions), appear to be scientifically justified.

	b. Immune effects: The Review summarizes the results of several human studies and 2 animal studies that were included to examine this aggregate endpoint. Strengths and limitations of the studies are clearly presented and well-organized. The Report's conclusion that the available evidence suggesting that PFDA exposure is likely to be associated with adverse immunologic outcomes is appropriate. Notably, the human studies examining antibody response to vaccination, though they are of relatively small sample size and vulnerable to confounding, are medium confidence studies but show clinically significant and consistent decreases, even after adjustments for other possible PFAS toxicant exposures. These are further supported by the two available rat studies (high/medium confidence) demonstrating similar adverse effects. The body of available evidence for adverse sensitization and allergic response effects is appropriately summarized to be less consistent. Overall, the report's conclusions stating that the available evidence suggesting that PFDA exposure has the potential to be associated with adverse immune effects (specifically immunosuppression) in humans under sufficient exposure conditions, appear to be scientifically justified. c. Developmental effects: The Review summarizes the results of 46 human studies and 1 animal study that were included to examine this aggregate endpoint, which overlaps with the female reproductive outcomes assessed. Strengths and limitations of the studies are clearly presented and well-organized, including the additional methodological considerations relevant to the complex physiology associated with early fetal development during pregnancy. Given the multiple possible windows of exposure for this outcome, I recommend stating when possible whether the individual study evaluated and conclusions drawn were concerning maternal and/or neonatal PFDA exposure, especially in the Table 3-24 summary of the weight of evidence (**Tier 1 Recommendation**). Overall, the report's conclusions stating that the available evidence suggesting that PFDA exposure has the potential to be associated with adverse developmental effects in humans under sufficient exposure conditions, appear to be scientifically justified. Notable data gaps on this topic are appropriately acknowledged, including the scarce data regarding postnatal development. d. Male reproductive effects: The Review summarizes the results of 9 human studies and 1 animal study that were included to examine this aggregate endpoint. Strengths and limitations of the studies are clearly presented and well-organized. The Review's conclusions stating that PFDA exposure is likely to cause male reproductive effects (upon sufficient exposure conditions) in humans is based only on 3 studies (two medium confidence, one low confidence); the conclusion is primarily drawn instead from the single short-term (28 days) high confidence rat study, which showed negative effects on all male reproductive parameters studied except sperm evaluation, which could not be evaluated due to insufficient exposure duration (NTP 2018). The report acknowledges the relative scarcity of available human data to inform this endpoint, but the strength of the animal study across

	multiple parts of the male reproductive axis appears to appropriately provide the scientific support for the stated conclusions. e. Female reproductive effects: The Review summarizes the results of 22 human studies and 1 animal study that were included to examine this aggregate endpoint. Strengths and limitations of the studies are clearly presented and well-organized. It is appropriate that the literature search took into account a wide range of possible PFDA exposure developmental timepoints, given the long window for exposure effects in the female reproductive cycle. As the outcomes characterizing this aggregate outcome are quite heterogeneous, the Report proposes an there is indeterminate evidence of association when assessing the human (mostly low confidence) studies and a moderate evidence of association when assessing the single high confidence rat study (which was short-term [28 days], and examined only select adverse female reproductive effects). Based on the combination of these human and rat data, the Report overall concludes that the available evidence shows that PFDA exposure is likely to be associated with adverse female reproductive effects under sufficient exposure conditions. Although there is similar reproductive physiology between rats and humans, the Report's overall conclusion to suggest there is a likely association between PFDA exposure and female reproductive toxicity may be too strong, given the sparse data of the single rat study (a **Tier 2 Recommendation** is suggested to considering softening this conclusion). Indeed, the proposed types of studies stated at the end of the evidence integration text seem to be needed to more strongly support this conclusion (**Tier 3 Recommendation**). f. Cardiometabolic effects: The Review has synthesized the results of 22 human epidemiologic studies and 1 animal study that were included to examine this aggregate endpoint. The report is overall clear and effectively organized, and summarizes the included studies' strengths and limitations. The Review delineated reasons for noting why many studies were regarded only as medium confidence, such as the inclusion of some studies that may have included non-fasting (less accurate) serum lipid levels and potential lipid-lowering medication use. The clinical relevance of Tables 3-30, 3-33, and 3-34 could be made easier to interpret if the various measurement units for the various relevant outcomes (such the lipid subfractions, fasting blood glucose, and waist circumference, respectively) were provided for the studies that reported the ß effect estimate (**Tier 2 Recommendation**). It would be additionally helpful in Section 3.2.6 to provide more details of the lipid subfractions reported in the studies (e.g., the strength and directionality of association between PFDA exposure and not just total cholesterol levels, but also LDL, HDL, and triglycerides, since HDL elevation is protective while elevation of the other subfractions are adverse outcomes); similarly, the evidence integration section can be better clarified in this regard [**Tier 2 Recommendation**]). Overall, the report's conclusions, stating that the available evidence suggesting that PFDA exposure has the potential to be associated with adverse cardiometabolic

	effects in humans under sufficient exposure conditions, appear to be scientifically justified. g. Neurodevelopmental effects: The synthesized results of the 13 human and 0 animal studies that were included for this endpoint are clear and effectively organized. The Review has appropriate taken into account the available studies' strengths and limitations for this aggregate endpoint. Notably, only about half of the human studies were regarded as having good or high confidence in the exposure classification, as there was substantial variability in the timing of exposure (from early pregnancy to as late as age 11 years in the offspring), thus there was considerable heterogeneity across studies. Furthermore, the possible neurodevelopmental outcomes ascertained in these studies were in themselves quite heterogeneous. The report's conclusions, which state that the available evidence is insufficient to deem whether or not there is an association between PFDA exposure and adverse neurodevelopmental effects in humans, appear to be scientifically justified. h. Endocrine, urinary, and other noncancer effects: The Review summarizes the results of available human studies (23 studies on thyroid effects, 14 on urinary effects) and 2 animal studies (1 on thyroid effects, 1 on adrenal effects, 4 on urinary effects, 8 on general toxicity, 2 on other organ systems) that were included to examine this combined endpoint of multiple organ systems. Strengths and limitations of the studies are well-organized. However, the report may consider the following: 1. In the methodological considerations of the endocrine studies, serum thyroid hormone concentrations are not usually regarded to have diurnal variation, and this may have thus been an unnecessary limitation (it is a **Tier 1 Recommendation** to not regard thyroid-related studies that failed to consider diurnal variation as deficient); ** Table 3-77 already lists this; revise to state that downgrading them not necessary 2. Any included studies of thyroid hormone status in pregnancy should not be combined with the same assessments as non-pregnant adults (**Tier 1 Recommendation**), given the different reference ranges for TSH and of changes in the binding of serum thyroid hormone levels in gestation; 3. Similar to the developmental effects outcome, given the multiple possible windows of PFDA exposure on thyroid effects, it would be clearer to state when possible whether the individual study evaluated and conclusions drawn were concerning maternal and/or neonatal PFDA exposure (**Tier 1 Recommendation**). Though it is fairly unlikely, reevaluation of the confidence of the included human studies (in regards to the above considerations) may potentially alter the Report's strength of evidence for adverse endocrine/thyroid effects.

	Overall, the Report's conclusion that there is inadequate available evidence to support whether or not there are associations between PFDA exposure and urinary and other noncancer effects is scientifically justified.
Zoeller	a. Liver effects: The Agency's analysis of PFDA impacts of hepatic toxicity is described in section 3.2.1. This analysis is clearly stated, comprehensive and appears scientifically defensible and clear. **No recommendation.** a.i. This analysis was clear and defensible**. No Recommendation.** b. Immune effects: The Agency's analysis was covered in section 3.2.2. The Agency described the human and animal studies that provide information in this domain in detail including a consideration of confounding due to the presence of other PFAS. There was considerable discussion and clarification of this issue. The take-home lesson was that the Agency needs to explain more fully what they have done and what their thinking is. **Tier 2 Recommendation**. It may be useful for the Agency explain more fully in the main document their thinking about the issue of confounding by other PFAS (especially PFOS & PFOA). Although it is the goal of this assessment to develop an RfD for PFDA as if it is the only chemical causing an effect, mixture risk analysis procedures are being developed to explain the combined effect of more than one chemical exposure. The Agency may choose to strengthen their argument about PFDA using some of this logic. **Tier 2 Recommendation**. The agency explained their thinking about the use of PFDA effects on diphtheria and tetanus antibodies as an index of immune suppression, but this appeared to require strengthening based on some of the discussion. Thus, the Agency may opt to develop this thinking further in the main document. c. Developmental effects: The effect of PFDA on development was covered in section 3.2.3. The Agency identified 8 studies on postnatal growth, 12 studies on gestational duration, 6 on fetal loss, 3 or anogenital distance, 2 on birth defects and 31 on fetal growth restrictions. The Agency concludes that, "*Based on over 45 different epidemiological studies included here the evidence of an association between PFDA exposure and developmental effects in humans is considered slight but was supported by moderate evidence in animals."* I was not entirely convinced by the Agency's arguments. For example, "…9 out of 14 *medium* and *high* confidence studies" reported birth weight deficits. About this endpoint, the Agency stated that, "*Despite fairly consistent evidence of an association between PFDA and different BWT-related measures, and more mixed for other endpoints, there is considerable uncertainty given that some sample timing differences may explain some of the reported fetal growth restriction deficits examined here."* The argument here appears to be that a number of high-quality studies are disregarded – or "downgraded" – because there are no data to rule out the possibility that the primary observation (i.e., fetal growth restriction) is biased away from the null. Would the association between PFDA exposure and developmental effects in humans be more important (stronger) if it was known that sample timing differences were not confounding? **Tier 1 recommendation.** The argument that uncertainty around the relationship

	between sample timing differences and reported fetal growth restriction deficits needs to be strengthened. Is it more likely than not to be a confounding variable and what is the evidence for this opinion? If this isn't justified, then downgrading an important observation seems unwarranted. d. Male reproductive effects: The Agency covers the evidence indicating that PFDA is likely to cause male reproductive effects in humans in section 3.2.4. This conclusion was based primarily on a single high confidence study (NTP) in animals with a number of coherent endpoints of male reproduction being affected by PFDA exposure. Several epidemiological studies we less informative, but the Agency clearly articulated why these results were less informative. **No recommendation.** e. Female reproductive effects: The effect of PFDA on female reproduction is covered in section 3.2.5. Epidemiological studies were deemed inconclusive because of the variability among studies and lack of coherent observations. A single high confidence animals study identified a number of coherent endpoints reflective of adverse effects on female reproduction. **No recommendation.** f. Cardiometabolic effects: Cardiometabolic effects includes here risk factors for cardiovascular disease including obesity, serum lipids, diabetes, and others. Thus, this is a complex combination of studies in humans and animals. The Agency's analysis was clear and logical. **No recommendation.** g. Neurodevelopmental effects**:** The Agency's analysis of neurodevelopmental effects was clear. **No recommendation.** h. Endocrine, urinary, and other noncancer effects: In this case, "Endocrine" means "Thyroid". The Agency clearly and thoroughly described the data in humans and animals and justified their interpretation that the data were indeterminate. There are several issues here. The interpretation of the diurnal rhythm of thyroid hormones might be overly restrictive in the interpretation of epidemiological studies. First, the time-of-day differences in T3 and TSH reported by van Kerkhof (2015) do not seem large enough to downgrade a study. Perhaps more important is the work by Andersen et al. (JCEM 2002, 87(3):1068). They show that individual variation in T4 and TSH is about 10% of the population variation. Thus, in a population study, individual differences in the set-point around which T4 is regulated might be more important that time-of-day. Still, this variability would bias the results toward the null. The Agency captured well the inconsistency in the human data. However, the issue of "coherence" might be overstated. Specifically, the Agency notes that, "Even in the studies that observed associations, there is not clear coherence across outcomes, where one would expect a decrease in T4 and T3 to correspond with an increase in TSH, or vice versa, though this could be explained by secondary ("central") hypothyroidism…". First, serum T3 is not associated with TSH, while T4 is negatively associated (negative feedback). So, it is not necessarily surprising that T4 and T3 don't follow the same pattern with respect

	to PFDA exposure. Moreover, animal data for other chemicals (e.g., PCBs or PBDEs, or other PFAS) show that these chemicals can cause a reduction in serum total and free T4 without a change in serum T3 or TSH. Although the mechanism for this pattern is not understood, it is well documented. Thus, the Agency should not expect that "coherence" based on patterns of thyroid hormones in cases of disease or antithyroid drug therapy is observed. The Agency's interpretation of the relationship between free and total T4 seems overstated. Total T4 is dependent upon protein binding, but in the absence of information about protein binding, these measures should not be ignored. Also, the concept that free T4 "is available to be utilized by the body" is true on the surface, but there is a dynamic equilibrium between the two fractions much as there is in the case of "free" and protein bound oxygen. While T3 is "the more active form" of the hormone, this is not to say that serum T3 normally drives hormone action in tissues. A clear example is the case of MCT8 deficiency in humans where blood levels of T3 can be elevated, but the brain is deficient in thyroid hormone because the cellular transporter that moves T4 across membranes is missing. These two forms – T4 and T3 – are regulated differently and should not be considered in this concept of "coherence". Thus, the NTP observation in animals showing a dose response of PFDA and free T4 decrement in male rats should not be downgraded because T3 is elevated, or because TSH exhibits a decreasing trend. Moreover, thyroid gland histopathology is driven by TSH, so the observation that thyroid histopathology was not altered by PFDA is "coherent" with the observation that TSH levels were not significantly altered. The increased weight of the thyroid gland would not be expected, but this observation should not alter the conclusion that PFDA caused a reduction in serum free T4 in males. The observations of Gutshall et al., (1989) are interesting because it is quite similar to the data for PCBs and PBDEs in that serum T4 is reduced, but thyroid hormone-regulated genes (or enzymes) in liver are upregulated. In the case of PCBs, there is evidence that some congeners can be hydroxylated and then activate thyroid hormone receptor. Although we don't know the underlying effects of PFDA, Ren et al. show that PFDA can bind to the thyroid hormone receptor and while it didn't affect TR activity in GH-3 cells, the in vivo liver may be a different environment. Finally, the observation by Gutshall et al. that a T4 administration to PFDA-treated rats did not produce a normal increase in serum T4 indicates that the clearance rate of T4 is accelerated in PFDA-treated animals (as the Agency noted –"biliary excretion"). This is also similar to other liver enzyme inducers. Ultimately, the Agency concluded that, "*Taken together, there is* ***inadequate evidence*** *across human, animal, and mechanistic data to determine whether PFDA exposure would cause endocrine effects in humans. This conclusion is based on inconsistent evidence from human studies and from a single high confidence rat study investigating PFDA doses ≤2.5 mg/kg-day that reported largely incoherent effects on thyroid hormone homeostasis and thyroid structure (i.e., increased T3, decreased TSH and T4; increased thyroid weight; no*

	histopathology) that cannot be interpreted based on the currently available evidence base." The interpretation of the human and animal data as "incoherent" is based on a "model" of the thyroid hormone system responding to thyroidectomy or goitrogenic chemicals/drugs (e.g., PTU). There is enough high-quality data about the response of the thyroid hormone system responding to environmental chemicals (see work of Mary Gilbert at EPA and Marta Axelstad of the Danish Technical University) to recognize that the data in the NTP study is "coherent" to the response to various chemical exposures. Moreover, the Agency does have a policy of interpreting a decrease in serum T4 in a rat study as "adverse" independent of other endpoints. **Tier 1 Recommendation.** The Agency should incorporate the concept of "coherence" in terms of the response to chemicals that do not act as a goitrogen per se. Chemicals like PCBs or PBDEs as shown by EPA's Mary Gilbert is important to incorporate. **Tier 3 Recommendation.** The Agency should develop a strategy to incorporate new information about the thyroid hormone system into an Agency-wide policy of the way to perform a risk assessment for chemicals affecting the thyroid hormone system.

3.3 For PFDA, no RfC was derived for inhalation exposures. An RfD is derived based on studies by Budtz-Jorgensen and Grandjean (2018) and Grandjean et al. (2012) showing decreased serum antibody concentrations for both tetanus and diphtheria in children (male and female) at age seven years and PFDA measured at age five years and developmental effects (i.e., reduced birth weight in humans) from the Wikstrom (2020) study. Given the close proximity of the developmental and immune PODs and resulting osRfDs and because these effects are observed during the developmental period, they are selected as co-critical effects supporting the RfD. Are the selection of the studies for the immune (Budtz-Jorgensen and Grandjean, 2018) and developmental (Wikstrom, 2020) effects for use in deriving the RfD values for PFDA scientifically justified? Are the modeling approaches appropriate?

a. If so, please provide an explanation.

b. If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the lifetime RfD and detail the rationale for use of such an alternative.

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

change) and the scientific support for their selection[19]. Please also see charge questions 2b and 2c.

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

e. For liver, male reproductive and female reproductive effects, quantitative information was limited to studies in animals exposed to PFDA 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 organ-specific (os) RfD values was not attempted for liver, male reproductive and female reproductive effects. However, these endpoints were considered for the derivation of subchronic osRfDs. Does the provided scientific rationale support this decision? Please explain.

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

Noncancer Toxicity Value Data Selection and Modeling	
Reviewer	**Comments**
Adgate	**Tier 2**: The selection of the Budtz-Jorgensen and Grandjean (2018) for immune effects and Wikstrom (2020) for developmental effects for use in deriving the RfD values for PFDA is scientifically justified, with caveats on how the results should be interpreted in the context of the IRIS modelling analysis (see my response to 2b and c). **Tier 2**: Immune Effects: This section needs a clearer presentation of the extensive analysis that EPA did on dose-response and the link to the values that are later a major focus of Appendices. In particular, the presentation would be improved by providing a more detail on clinical relevance and justification for the choices made and the uncertainties associated with the determinations. **Tier 1**: Note that in the Supplemental section Table F.3 (Page F-25) is inconsistent with the text on Starling et al 2017—text says high confidence and table say low confidence. It seems like given that this same paper is listed as high confidence in the next table the right answer must be high confidence. More broadly this information on correlation

[19] For the decreased antibody responses, Selgrade (Tox Sci 2007;100:328–332) suggests that these specific immunotoxic effects may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards.
For developmental effects (i.e., fetal growth restriction), the human evidence was determined to be *slight*, primarily due to potential confounding by hemodynamic changes among studies showing birth weight deficits. For the study (i.e., Wikström, 2020) used to derive the developmental RfD, there is no presumed impact of pregnancy hemodynamics given the early sampling (96% from trimester 1). However, unlike the Wikström (2020) study, some uncertainty remains across many of the available human developmental studies given the predominance of associations that were detected were for studies with later pregnancy sampling.

	between PFASs is important for the discussion on confounding, so this summary information would be better addressed in the main document. d. **Tier 2**: The BMD approach is justified as a scientific process and fairly clearly described. e. **Tier 2**: The scientific rationale provided is reasonable given the underlying data. f. **Tier 3**: Given the lack inhalation data from animal or human studies and the likelihood that ingestion is the dominant human exposure pathway it is reasonable that no RfC is estimated. PFAS inhalation exposure, however, is an area that has had little research, and some planned future studies will likely explore this topic and whether precursors that may be transformed in to PFDA under some circumstances are an important consideration. At this time an analysis of PFDA physical constants and potential resulting air concentrations in indoor environments would be informative for decision-makings given PFDA's wide presence in consumer products.
Carignan	This reviewer agrees that in the absence of data an RfC cannot be derived. Future research should investigate inhalation toxicity for PFDA, current use PFASs and legacy PFAS mixtures to inform derivation of RfCs. [**Tier 3**] This reviewer agrees that derivation of an RfD based on studies for the immune (Budtz-Jorgensen and Grandjean 2018) and developmental (Wikstrom 2020) effects is scientifically justified and that the modeling approaches are appropriate. Future research should consider toxicity of PFAS mixtures, which may be cumulative or synergistic, to inform derivation of RfDs. [**Tier 3**] Table 4-1: Symbols (+ - +/-) need to be defined. [**Tier 1**] a. Selection of co-critical effects is reasonable given the close proximity of the PODs and that they are observed during the developmental period. b. Not applicable. c. Reduced antibody production in response to vaccinations is a sensitive, functional test in humans that indicates global suppression of the adaptive immune response and may indicate increased susceptibility to infectious diseases in general. Infant body weight is also an important and sensitive outcome for development. Lower birth weight has been linked to increased risk of neurodevelopmental effects including impaired language development, reduced cognitive abilities and IQ, and increased risk of cardiovascular disease and diabetes. d. In the absence of a minimal biological response to set the BMR for the immune endpoint, and given the monotonic dose response with no apparent threshold, use of ½ SD is scientifically justified and clearly described in Appendix C. Selection of 5% as the minimal biological change leading to an adverse effect is scientifically justified for the developmental endpoint as it is clinically

	predictive of health outcomes. e. This reviewer agrees that in the absence of longer term studies, derivation of lifetime osRfD values for liver, male reproductive and female reproductive effects would be uncertain and limitation to derivation of subchronic osRfDs is a reasonable approach and the scientific rationale supports this decision. Future research should involve longer term studies appropriate for derivation of lifetime osRfDs. [**Tier 3**] f. This reviewer agrees that in the absence inhalation toxicity studies for PFDA, no reference concentration (RfC) can be derived. For PFASs with similar properties (e.g., long chain) read-across could be considered where PFAS-specific data is unlikely to be generated. [**Tier 2**] **Additional comments**: Suggest providing prominent clarification in the immune section and this section which studies shown in the immune review were used to create the BDML analysis presented by Budtz-Jorgensen and Grandjean (2018). If available it would be helpful if effect estimates and CIs for the combined cohort used for that study could be provided. [**Tier 2**] Suggest addition of a sensitive endpoint figure for the summary that helps highlight data rich, moderate and poor areas. [**Tier 2**] Suggest adding clarification to the RfD table where the values came from and how they were derived (make things easier for your readers). [**Tier 2**]
Faustman	a. This reviewer agrees with USEPA's decision to use the two studies on serum antibody response as the most sensitive endpoints along with a study of reduced birth weight in humans for development of an RfD. This choice is consistent with WHO guidelines that identify such immunological changes as relevant for assessment of evidence for immunotoxicity and reduced body weight of children is also well-recognized by WHO as well as USEPA as an important indicator of alterations in the developmental trajectory. Using these three studies allowed for the incorporation of two antibody responses one to diphtheria and tetanus and this is supported by similarities in the dose response relationship and allowed for assessments in both males and female offspring and at a developmental time period where PFDA levels were assessed. c. This reviewer agrees with the choice of both reductions in birth weight as well as decreased antibody responses as well recognized endpoints of toxicological importance. Reduced birth weight is recognized as a clear sign of developmental toxicity in both human and animal studies in long-standing guidance from the USEPA developmental toxicity guidelines as well as WHO reports. It is recognized as a clinically important parameter. In the birth weight studies in humans, USEPA provides extended discussion on the hemodynamic changes during pregnancy

	however stops short of actually accepting a pregnancy based TKTD model. Biological discussion on such hemodynamic changes is again referred to in the foot note below in this section of charge questions and continues to focus primarily on volume of distribution differences however there are many pregnancy-related changes in PK factors and for this reviewer very little attention to these other factors is provided. An example is lack of attention to serum albumin changes that could also significantly alter biologically active PFDA at various stages of pregnancy. Also changes in gut motility and gut transit time also changes yet these other changes are not equally evaluated. **Tier 2 recommendation** is to acknowledge the broader range of PK differences associated with pregnancy that could also affect the interpretation of the human studies beyond the focused "hemodynamic" currently discussed. The EPA cites both the WHO guidelines for immunotoxicity as well as Selgrade, 2007 as examples of supportive evidence for identifying reduced antibody response as significant endpoints of immunotoxicity esp. when occurring in humans at early life stages. This reviewer supports the USEPA choice of these endpoints for development of RfD values. d. USEPA provides details about their choice of the Benchmark response appropriate for the decrease in antibody response (continuous endpoint) in the main IRIS document as well as in detail in Appendix C. In particular, their discussion includes justification for quantal responses of a choice of a BMR of between 10% (standard default value) to 1% based on modeled population level responses. They also discuss the identification of biologically relevant response levels for continuous responses based on standard deviation and variability. Appendix C cites biological references for the tetanus and diphtheria antibodies as protective levels at 0.1 IU/blood concentration from vaccine producers (Grandjean et al, 2017) as well as a discussion of uncertainty of immunological protection at specified levels of circulating antibodies. USEPA states that "in the absence of a clear definition of an adverse effect for a continuous endpoints like antibody concentrations" that they calculated a BMR at both a SD of 1 as well as ½ SD. This discussion was very well done with explanations of what endpoints and studies were modeled and what models were used and why as well as how choices between factors were made. The reviewers were also made aware of how USEPA obtained the datasets from the relevant studies from Grandjean et al and provided a searchable reference of this data posted in HAWC. This reviewer agrees with the transparency and choices that USEPA has made in these modeling responses for the POD. e. This reviewer agrees with the choice by USEPA to only use the data for these endpoints for the derivation of the subchronic os RfD. It was clear that the quantitative information for these endpoints was limited and human data inconclusive for this application. f. This reviewer would agree with this approach at this time due to lack of data.
Fisher	Selection of these studies are ok. The authors need to opine on the use of these data for extrapolation of immune suppression for a population. Is the suppression delayed or slowed or permanently suppressed? Is this an

	adverse response that is recognized clinically with clinical studies to backup the adverse findings? What are the problems with using a remote population of people for use in the US? Is this the first time EPA has used this endpoint for IRIS? **Tier 2.** b. My comments are restricted to the use of data for PNDA within a known serum mixture of PFAS. PNDA represents only a few percent of the total PFAS concentrations measured in serum of pregnant women. Budtz-Jorgensen and Greanjean (2018) acknowledge the mixture problem if one tries to tease out individual molecules. One way to overcome this is to assign a relative potency for each molecule, if possible. Please comment on relative potency for PFAS? Is this possible? Does computational toxicology help? **Tier 3.** c. I am not qualified to answer this. d. Selected aspects of the BMD process are clearly described. As a mixture issue, there should be a section with a header that pertains to mixtures. Text written on pages 1-11 and 1-12 are inadequate. This is a glaring weakness. The strengths and weaknesses of the assumptions should be clearly articulated using non-epidemiological language. From a toxicology perspective it appears unlikely that a molecule (PNDA) which represents only a few percent of a total mixture concentration in serum can be teased out for regulatory purposes. However, from an epidemiology perspective and a BMDL perspective this was accomplished. This needs to be explained in a lay language. Provide the strengths and weaknesses of this methodology. **Tier 1.** e. If subchronic RfDs are important, and even organ specific values, then using a GLP compliant NTP 28-day study is warranted. Granted, additional toxicity studies would provide more confidence. Listing all in vitro studies that may or may not support these findings would be of value in the future, if not now. **Tier 2**. The cost of not continuing to modify the Kim et al. published PBPK model for PFDA is problematic. To derive subchronic osRfDs a PBPK model would be superior to any other methodology because a PBPK model includes organs and tissue groups in a physiologically relevant manner and can predict internal organ dosimetry (**Tier 1**). f. Because of a lack of toxicity data for the inhalation route of exposure this route was not evaluated. This makes sense. However, from an exposure perspective one can simulate uptake of particles containing PFDA using physics based software, such as multi-path particle dosimetry (MPPD) software and this can be interfaced with a PBPK model.
Georgopoulos	a. The selection of the studies used in deriving the PFDA RfD values for immune effects (Budtz-Jorgensen and Grandjean, 2018) and for developmental effects (Wikstrom, 2020) is appropriate and scientifically justified. The rationale for selecting these studies is based on a thorough assessment of available data and information on immune and developmental effects from a substantial range of studies (presented, respectively, in Sections 3.2.2 and 3.2.3 of the Toxicological Review). **Note:** Concerns regarding the modeling component are discussed in the

	answers that follow. b. N/A (see response "a", above) c. The effects selected for deriving the lifetime RfD (antigen response for immune effects and low birth weight for developmental effects), in conjunction with the corresponding studies, are appropriate and scientifically justified. As stated in the Review (page 3-54, lines 29-32) *"[t]he production of antigen-specific antibodies in response to an immune challenge (e.g., vaccination in humans or injection with an antigen [...] in rodents) is a well-accepted measure of immune function included in risk assessment guidelines and animal testing requirements for immunotoxicity,*" and (page 3-55, lines 3-5) *"[r]educed antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious diseases generally (i.e., not limited to those specifically studied)."* For the selected developmental effect (low birth weight), the Toxicological Review assessed the human evidence to be slight, primarily due to potential confounding by hemodynamic changes among studies showing birth weight deficits. However, the study selected as the basis for the developmental effects RfD (Wikström et al. (2020), evaluated serum PFAS in the first trimester of pregnancy in 96% of study participants (and analysis of the impact of excluding those subjects who were not sampled in the first trimester showed no appreciable difference from the results including all subjects) thus avoiding the effect of altered pregnancy hemodynamics. d. EPA used benchmark dose modeling (BMD) to identify points-of-departure (PODs) for PFDA immune and developmental effects. Though the Toxicological Review follows established BMD procedures for this purpose (U.S. EPA, 2012), there are issues requiring further consideration: The most important concern in deriving the above-mentioned PODs is the presence of confounding effects associated with co-exposures to other PFAS, especially PFNA (and potentially to other immunomodulating agents). The Toxicological Review explicitly recognizes this concern; a relevant discussion (from page 3-57, line 34 to page 3-58, line 11) is quoted here: *"It is plausible that the observed associations with PFDA exposure could be explained by confounding across the PFAS. Exposure levels to other PFAS in the Faroe Islands populations were considerably higher (PFOS 17 ng/mL, PFOA 4 ng/mL, PFNA 1 ng/mL, PFDA 0.3 ng/mL at age 5 years in Grandjean et al. (2012), and there was a high correlation between PFDA and PFNA (r = 0.78) and moderate correlations with PFOS and PFOA (r = 0.39 and 0.35, respectively). The authors assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018a) that reanalyzed data from both Grandjean et al. (2012) and (Grandjean et al., 2017b) for benchmark analysis. In this re-analysis, estimates were adjusted for PFOS and PFOA. There were variable attenuation of the observed effect estimates across the*

	different analyses (though some of the adjusted estimates were not estimable, likely due to collinearity), and PFNA was not adjusted for in these models. However, associations with PFDA were stronger than for PFNA, and adjustment by PFOS and PFOA did not eliminate the association, so confounding by co-occurring PFAS is unlikely to fully explain the associations. Overall, while it is not possible to rule out confounding across PFAS, the available evidence suggests that it is unlikely to explain the observed effects. Other sources of potential confounding, including possible co-exposures such as PCBs, were controlled appropriately." The above quoted conclusion, that "associations with PFDA were stronger than for PFNA, and adjustment by PFOS and PFOA did not eliminate the association, so confounding by co-occurring PFAS is unlikely to fully explain the associations" may be qualitatively valid but needs further elaboration in the context of deriving quantitative dose-response metrics to support development of an RfD. The correlation between PFDA and PFNA was the highest of the reported correlations in the studies discussed above (and in fact such high correlations appear to be common in exposures studies); however, while the impact of PFOA and PFOS on the associations of PFDA with decreased vaccine response in the datasets used for RfD derivation was evaluated, it was reported that it was not possible to do the same for the corresponding impact of PFNA. Furthermore, though information on correlations of PFDA with other PFAS is not provided in Wikström et al. (2020), the same concern regarding confounding effects of co-exposures to other PFAS, and in particular PFNA, also applies to the derivation of the RfD for developmental effects; two relevant statements from the Toxicological Review document and its Appendix F on Additional Confounding Considerations (from page 3-99, lines 12-15 and page F-27, lines 24-32, respectively) are quoted here: *"For fetal growth restriction and other developmental endpoints, there may be more concern over potential PFAS co-exposure confounding due to PFNA given higher correlations with PFDA and associations that are fairly comparable in consistency and magnitude, as detailed in Appendix F."* *"11 of 22 studies showed evidence of some 24 associations with PFDA and mean birth weight in the overall population. Among these 11 studies [...] 7 showed deficits comparable in magnitude for PFNA and PFDA. Two studies showed larger deficits for PFDA compared to PFNA, and three studies showed larger deficits for PFNA compared to PFDA. Given these comparable results seen in most of these studies for both PFNA and PFDA and the moderately high correlations consistently reported between PFDA and PFNA, there is considerable uncertainty due to potential confounding by co-occurring PFAS in the existing literature."* EPA should address more clearly the uncertainties associated with the potential impact of confounding (especially from co-exposures to PFNA) on the derivation of the RfD for immune and developmental effects.

	e. The fact that insufficient information was available to evaluate the effects of chronic exposure on the liver, male reproductive organs, and female reproductive organs, as quantitative information was limited to studies in animals exposed to PFDA for only 28 days, makes the decision to not pursue the development of lifetime organ-specific (os) RfD values for these effects the appropriate one. The use of these endpoints for deriving subchronic osRfDs is discussed in my answer to Charge Question 4. f. The decision to not derive reference concentration (RfC) for PFDA is appropriate and represents the only reasonable option given the lack of relevant inhalation exposure studies. **Suggested Revisions** • ***Tier 1 Necessary Revision:*** *Expand the discussion and consideration of confounding factors (especially co-exposures to PFNA) in the context of their potential quantitative impact on the derivation of the RfD for immune and developmental effects. At a minimum clarify the rationale for evaluating the effects of PFNA co-exposures as non-significant based on the analytical details of the regression models performed by the authors of Budtz-Jorgensen and Grandjean 2018 (HERO 7276745)*
Haney	a. As I do not agree with the selection of these studies and endpoints for RfD derivation, please see my comments under subsection "b" below. b. <u>Immune Effect Endpoint</u> EPA indicates that the hazard judgment that "the ***evidence indicates*** that PFDA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans" (p. 3-92, lines 35-37) is driven primarily by consistent evidence of reduced antibody response from human epidemiological studies (mostly from two birth cohort studies)…" (pp. 3-92 and 3-93, lines 37-1). However, *it is not clear that EPA has sufficiently considered adversity for the primary basis of this hazard judgment, fully considered the weaknesses/limitations of this evidence, or fully considered the lack of supporting evidence from studies on potentially increased incidences of disease*. For example, even without considering potential confounding, 3 out of 4 ORs for PFDA and antibody concentrations falling below the generally cited protective level of 0.1 IU/mL for tetanus and diphtheria in children ages 5 years (n=510) or 7 years (n=386) contain 1, indicating that the WOE from this key study cohort is for no statistically significant associations with less-than-protective serum antibody concentrations in children (see eTable 4 of Grandjean et al. 2012).[20] Consistent with this, the more recent Grandjean et al. (2017a) study states [*emphasis added*] that, "With many antibody concentrations being close to the assumed clinically protective level of 0.1 IU/mL, *logistic regression showed only weak tendencies for antibody levels below the limit to be associated with serum PFAS*

[20] The confidence interval (CI) for the one statistically significant OR of 1.36 (age 5, diphtheria) is (1.04, 1.77), with the lower end of the CI practically equal to 1 (eTable 4 of Grandjean et al. 2012).

	concentrations."[21] Also consistent with a WOE for no statistically significant associations (much less being able to say "effects" as there are problems with causal attribution to PFDA), 6 of 8 confidence intervals (95% CIs) for both tetanus and diphtheria serum antibodies included 0% change per 2-fold increase in maternal and age 5 serum PFDA (see Table 3 of the study). Additionally, in Grandjean et al. (2012), PFDA was correlated with other PFAS (e.g., PFOS, PFOA, PFNA) that had some associations with antibody concentrations falling below the protective level of 0.1 IU/mL (see Table 2 and eTable 4 of Grandjean et al. 2012).[22] Moreover, the level of serum antibodies corresponding to a clinically protective level appears to be assay specific. For the ToBI assay apparently used in the Faroe Islands studies, ≥0.01 IU/mL is considered to be the clinically protective level, not the value of ≥0.1 IU/mL indicated by study authors. Considering the comments above, this means that the reported decreases in serum antibodies are even less likely to be biologically/clinically significant. This is not surprising given the rarity of tetanus/diphtheria cases, particularly in those fully vaccinated, and the WOE for PFDA not being associated with statistically significant increases in the incidences of diseases based on the epidemiological literature (all discussed below). To say the least, all this brings into serious question the validity of the EPA's 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 the 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.[23] 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-*

[21] Also, while Grandjean et al. (2017b) state that, "At age 5, 152 (44%) children had antibody concentrations lower than the protective level of 0.1 IU/mL for diphtheria and 126 (36%) for tetanus", this appears inconsistent with Table 1 of that study, which shows that the 25th percentiles for diphtheria and tetanus serum antibody concentrations were 0.1 IU/mL.

[22] A 2-fold increase in PFOS and PFOA concentrations at age 5 years was associated with odds ratios between 2.38 (95% CI, 0.89 to 6.35) and 4.20 (95% CI, 1.54 to 11.44) for falling below a clinically protective level of 0.1 IU/mL for tetanus and diphtheria antibodies at age 7 years.

[23] 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."

	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.[24] 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), even further calling into question the biological/clinical significance and adversity of the reported results. In regard to confounding and the ability to causally attribute associated effects to PFDA: • 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*." • Similarly, Grandjean et al. (2017b) state [*emphasis added*], "The close correlations *prevented meaningful adjustment* for concomitant PFAS exposures." *Thus, it appears that effects may neither rise to the level of adversity nor be attributable specifically to PFDA.* Co-exposures to 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 IQR differences in blood sera concentrations of less than 1.6-fold each (e.g., 75th percentile blood concentration of PFOA/25th percentile blood

[24] 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."

	concentration of PFOA), and PFDA and PFNA had a correlation coefficient of 0.78 in the blood sera of 5-year olds and IQR differences in blood sera concentrations of less than 1.9-fold each (see Table 2 of the study). Despite Grandjean et al. (2017b) stating that the close correlations prevented meaningful adjustment for concomitant PFAS exposures, Budtz-Jørgensen and Grandjean (2018a) attempts to do just that for benchmark dose, and the results appear to help demonstrate the effects of confounding co-exposures and/or the inability to properly adjust for them. Table 2 of Grandjean et al. (2017b) reports the change (in percent) of the pre-booster serum-antibody concentrations at age 5 years associated with a doubling of the serum concentration of PFDA. Results for cohorts 5, 3, and joint results are in the negative direction for tetanus at age 5 but none are statistically significant, which already points to the unreliability of an effect having been demonstrated by these results and the unreliability of any RfD based on these results or association. Table 2 of Budtz-Jørgensen and Grandjean (2018a) reports benchmark results for the five prenatal PFAS concentrations in regard to antibody concentrations at age 5 years (pre-booster) both unadjusted and adjusted for PFOS/PFOA co-exposures. For tetanus antibodies, while unadjusted benchmark doses for three models for PFDA (linear, piecewise, conservative) appear to show excellent agreement (BMDs of 0.11-0.25 ng/mL) with insignificant reliance on choice of model, when adjusted for PFOS/PFOA the three models' best estimates of the PFDA serum concentrations associated with a 5% change go to infinity (although BMDLs can still be estimated). For diphtheria, Table 2 of Grandjean et al. (2017b) reports statistically significant changes for cohort 3 and joint results for the pre-booster serum-antibody concentrations at age 5 years associated with a doubling of the serum concentration of PFDA. However, similar to results for tetanus, Table 2 of Budtz-Jørgensen and Grandjean (2018a) reports that when benchmark results are adjusted for PFOS/PFOA, two of the three models' best estimates of the PFDA serum concentrations associated with a 5% change go to infinity (although BMDLs can still be estimated). While Table 1 of Budtz-Jørgensen and Grandjean (2018a; benchmark results for the age-5 serum concentrations of five PFASs in regard to tetanus and diphtheria antibody concentrations at age 7 years) provides no benchmark doses for PFDA that go to infinity, Table 2 results point to the unreliability of this endpoint and should be considered along with the other issues raised above. For example, the odds ratios for PFDA and inadequate antibody concentrations for diphtheria and tetanus at 7 years was not statistically significant (see eTable 4 of Grandjean et al. 2012). Key BMD results in the draft assessment itself demonstrate the importance of co-exposures to other PFAS. Tables C-1 and C-3 of the draft assessment (below) provide BMD results for the critical effects used for RfD derivation (e.g., see Table ES-1).

Table C-1. Results specific to the slope from the linear analyses of PFDA measured in serum at age 5 years and log_2(tetanus antibody concentrations) measured at age 7 years in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b).

Exposure	Model shape	PFOS & PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/m Lin serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at Age 5	Linear	No	-1.55	0.602	p = 0.01	-2.55
PFDA at Age 5	Linear	Yes	-0.98	0.681	p = 0.15	-2.10

PFOS and PFOA had correlation coefficients of 0.39 and 0.35 with serum PFDA at age 5, respectively (Table 2 of Grandjean et al. 2012). Despite these relatively low correlation coefficients (Mukaka 2012), Table C-1 shows that just controlling for co-exposures from these two PFAS (PFOS, PFOA) resulted in significant impacts on slope (β) and slope fit. The slope estimate for PFDA was reduced 37% and *PFDA is no longer a significant predictor of tetanus antibody concentrations* (p=0.15). The most likely explanation (Occam's razor) is classic confounding as PFOS and PFOA are documented immunotoxicants (e.g., per Budtz-Jørgensen and Grandjean 2018a), and the existence of some chance that correction for these co-exposures could create some confounding is not a scientifically robust justification for dismissing the important implications of the results of adjustments for PFOS/PFOA that the study authors themselves (Budtz-Jørgensen and Grandjean 2018a) thought it important to adjust for, and with good reason. *These results demonstrate the statistical unreliability of serum PFDA predicting tetanus antibody concentrations when just two other PFAS are controlled for*. Table C-3 concerns the critical effect for the RfD based on diphtheria antibody concentrations.

Table C-3. Results specific to the slope from the linear analyses of PFDA in serum measured at age 5 years and log_2(diphtheria antibodies) measured at age 7 years from Table 1 in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b).

Exposure	Model shape	PFOS & PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/mL in serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at Age 5	Linear	No	-0.894	0.561	p = 0.11	-1.82
PFDA at Age 5	Linear	Yes	-0.297	0.635	p = 0.64	-1.35

The implications of these results are worse. First, even when evaluated alone without accounting for co-exposures to relatively low correlated PFOS and PFOA, serum PFDA is not a significant predictor of diphtheria antibody concentrations (p=0.11). Table C-3 further shows that controlling for co-exposures from these two PFAS (PFOS, PFOA) resulted in significant impacts on slope (β) and slope fit. *The slope estimate for PFDA was reduced 67% and serum PFDA became a worse nonsignificant predictor of diphtheria antibody concentrations (p=0.64)*. The most likely explanation (Occam's razor) is classic confounding as PFOS and PFOA are documented immunotoxicants (e.g., per Budtz-Jørgensen and Grandjean 2018a), and the existence of some chance that correction for these co-exposures could create some confounding is not a scientifically robust justification for dismissing

	the important implications of the results of adjustments for PFOS/PFOA that the study authors themselves (Budtz-Jørgensen and Grandjean 2018a) thought it important to adjust for, and with good reason. *Thus, when co-exposures are taken into account for two modestly correlated PFAS (PFOS, PFOA), serum PFDA is not a significant (i.e., reliable) predictor of these critical effects serving as the basis of the RfD (i.e., decreases in serum tetanus and diphtheria antibody concentrations)*.[25] Moreover, Budtz-Jørgensen and Grandjean (2018a) also controlled for PFOS or PFOA when deriving BMDs/BMDLs for the other, and EPA did use those co-exposure-adjusted results for RfD derivation (i.e., PFOA adjusted for PFOS, PFOS adjusted for PFOA; see Tables B-1 and B-2 in Sections B.1.1 and B.1.2 of the PFOA and PFOS draft assessments USEPA 2021a,b) without expressing any similar concerns about creating confounding by adjusting for these co-exposures (low/moderate correlation coefficient of 0.50 (Mukaka 2012); see Table 2 of Grandjean et al. 2012) or citing Weisskopf et al. (2018) and Weisskopf and Webster (2017). Rather, these concerns have been selectively cited for PFDA in an attempt to provide some rationale for dismissal of the co-exposure-controlled results (e.g., slope (β) values are reduced; serum PFDA is a nonsignificant predictor of tetanus and diphtheria antibody concentrations) and thus for selection of BMDLs uncontrolled for PFOS and PFOA co-exposures (see Tables C-2 and C-4 of the draft), but this is not a scientifically robust rationale and is furthermore inconsistent with EPA's draft assessments for PFOA and PFOS (USEPA 2021a,b). Lastly, it is further noted that 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 PFDA and PFOS/PFOA are low (0.35 and 0.39, respectively). The results of Weisskopf et al. (2018) do not constitute reasonable doubt that for these PFDA results, the confounding from not adjusting for co-exposures to documented immunotoxicants (PFOS, PFOA) is significantly greater than the potential amplification of biases that remains undemonstrated under the same or similar circumstances. EPA should reevaluate the issues raised above in regard to implications for their draft PFDA assessment (**Tier 1 necessary revision**). Regarding potential immunosuppressive effects by PFDA, effects that rise to the level of adversity would be expected to result in *increased incidences of disease*, reflecting lower immunity and lower resistance to disease in the real world. However, consistent with the WOE for no statistically significant associations with antibody concentrations falling below the generally cited protective level of 0.1 IU/mL based on results from Grandjean et al. (2012), almost all ORs in Table 3-13 of the draft assessment include 1, indicating that *the WOE from studies on PFDA*

[25] Knowing this, disparate results are not particularly surprising, such as the 18.7% increase in tetanus antibodies predicted for children (age 13) with a 2-fold increase in serum PFDA based on the same study and type of analysis used for the RfD critical effects (see Table 3-12, p. 3-59).

	and infectious disease in humans is for no statistically significant associations. Consistent with this, host resistance was unaffected by PFDA based on the limited animal study data available (p. 3-73, lines 8-9), and host resistance assays are considered highly relevant to the evaluation of immunotoxicity in the context of human health assessment (p. 3-72, lines 15-18; IPCS 2012). EPA should reevaluate the adversity of these presumed antibody level effects, including the association with PFDA itself, and do so within the context of potential confounding, other limitations, and available human/animal data on disease incidence (**Tier 1 necessary revision**), as this has important implications for the hazard judgment, the strength of human evidence descriptor for immunosuppression (listed as "moderate" in Table 3-19), and whether an RfD should be based on these effects. Moreover, it is noted that the PODs for immunosuppressive effects from these epidemiological studies range from 2.57E-04 mg PFDA/L blood serum to 7.02E-04 mg PFDA/L blood serum ($BMDL_{1/2\ SD}$ values from Table 5-8, pp. 5-16 to 5-17), and when intrahuman variability is considered (through application of a UF_H of 10), the resulting values range from 2.57E-05 to 7.02E-05 mg PFDA/L blood or 0.0257 to 0.0702 µg PFDA/L blood serum.[26] Data from NHANES show that GMs representative of the U.S. population are well above these blood serum levels (see Appendix A to these comments).[27] Most notably, 2005-2018 population GMs range from 0.154-0.355 µg/L, which are 2.2- to 13.8-fold higher than the PODs adjusted for intrahuman variability (cited above). Despite these exceedances, tetanus and diphtheria appear to be quite rare in the U.S. population. The average annual number of tetanus cases in the U.S. from 2009-2018 was 29, with the CDC attributing most cases to individuals who either have not been vaccinated or who are not current on their boosters (e.g., only 3% of the cases from 2001-2008 were in people who had received a complete tetanus toxoid series with the last dose within 10 years; Tiwari et al. 2021). Tetanus also appears rare in U.S. children specifically, occurring primarily in older adults. Per Liang et al. (2018): "During 2001-2016, three neonatal tetanus cases and 459 non-neonatal tetanus cases were reported to the National Notifiable Diseases Surveillance System (NNDSS). The median age for non-neonatal cases was 44.0 years (range: 2-95 years)… The risk for both tetanus disease and mortality was higher among persons aged ≥65 years than among persons aged <65 years. Tetanus occurs almost exclusively among persons who are unvaccinated or inadequately vaccinated or in those whose vaccination histories are unknown or uncertain." The incidence of U.S. diphtheria cases is even more rare. The CDC reported only 14 cases from 1996 through 2018 (Acosta et al. 2021). *Thus, consistent with the WOE for the lack of statistically significant associations from the epidemiology study data discussed above and despite the NHANES blood serum data showing exceedances of the draft assessment human PODs adjusted for intrahuman*

[26] Based on $BMD_{1/2\ SD}$ values (Table C-9, p. C-16), these values are 0.0385 to 0.226 µg PFDA/L blood serum.
[27] See NHANES Biomonitoring Data Tables at https://www.cdc.gov/exposurereport/data_tables.html. Budtz-Jørgensen and Grandjean (2018a) also acknowledge that, "Our BMDL results, both before and after adjustment are generally below current exposure levels…"

	variability (e.g., toxicodynamic) for most of the U.S. population for a prolonged period of time (see the 50th percentile concentrations in Appendix A), U.S. surveillance disease incidence data are not supportive of adversity. That is, U.S. surveillance disease incidence data do not support that serum PFDA (or any other serum PFAS) is suppressing tetanus and diphtheria vaccine responses and leaving people vulnerable to infection from these diseases. The apparent lack of adversity/ consequence for the effects reported for tetanus and diphtheria certainly does not provide support for an expectation of adversity/consequence for other effects not measured/observed (e.g., for vaccines for other diseases and their incidences). Additionally, it appears that EPA has not fully considered *all the relevant evidence* or the weaknesses/limitations of the epidemiological evidence, which in turn are relevant for the hazard judgment and whether an RfD should be derived based on these effects. Table 3-19 indicates that human data provide "moderate" evidence and that "the inconsistent and low confidence evidence on infectious disease did not influence this judgment." However, this points to the fact that *EPA has not duly considered the implications of the null findings on human and laboratory animal infectious disease and other relevant considerations (e.g., some discussed above) for the scientific WOE, which is not a scientifically supportable approach* as it does not consider all relevant data, directly relevant human data in particular. EPA should consider such null findings (and other relevant considerations) in their WOE (**Tier 1 necessary revision**). Combined with the "slight" human data for sensitization and allergic response, the "slight" laboratory animal data for immunosuppression, and the "indeterminate" animal data for sensitization and allergic response (Table 3-19), *it does not appear that PFDA exposure is "likely to cause" adverse immune effects in humans is sufficiently supported*. EPA should reevaluate this determination (**Tier 1 necessary revision**) as *"may cause" might very well be the better supported hazard judgement*, and also reconsider their use of the serum antibody endpoints for quantitative risk assessment/derivation of toxicity factors (**Tier 1 necessary revision**). This would appear more consistent with the data discussed above and recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021). *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."

Based on the information in the draft assessment and reviewed elsewhere, this reviewer finds it difficult to disagree with the recent conclusions of the Australian government (FSANZ 2021) and ATSDR (2021) that the epidemiology literature (e.g., on PFAS blood levels and impaired vaccine response) *is inadequate for quantitative risk assessment and use as the basis for deriving toxicity factors* (e.g., RfDs). 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals.

The above comments provide relevant rationale for use of an alternative study(ies) or effect(s) for lifetime RfD derivation. While the draft assessment provides various but limited candidate PODs, due consideration of the comments above appears to require a more complete evaluation of alternative studies/effects (**Tier 1 necessary revision**). One method to begin such an evaluation may entail sorting the study data extracted by NOAEL/LOAEL, BMD or a similar criterion that would allow EPA to readily identify the next most sensitive effects based on less problematic studies that are adequate for RfD derivation. However, any newly derived toxicity factor should be subject to external expert peer review (**Tier 1 necessary revision**).

<u>Birth Weight Endpoint</u>

EPA's hazard conclusion for developmental effects primarily relies on in vivo animal data (i.e., the mouse study of Harris and Birnbaum 1989). *Based on over 45 different epidemiological studies included in the draft assessment, the evidence of an association between PFDA exposure and developmental effects in humans is considered only "slight"* (p. 3-156, lines 10-11). Despite the "slight" evidence in humans, Table ES-1 indicates that RfDs were nevertheless calculated based on decreased birth weight in male and female children (Wikström et al. 2020). While data in humans, as the species of ultimate interest, are usually preferred as the basis for derivation of toxicity factors, in this case it appears that the mere "slight"

totally of the evidence for developmental effects from the over 45 different epidemiological studies included in the draft assessment should not be considered sufficient for quantitative dose-response assessment (e.g., birth weight), but rather only for potentially supportive information for hazard identification. EPA should reconsider their use of these epidemiological data for quantitative risk assessment/derivation of toxicity factors (**Tier 1 necessary revision**). EPA Figure 3-28 (p. 3-108), reproduced below, helps illustrate the weakness and incoherence of the overall epidemiological database for demonstrating decreased birth weight, particularly statistically significantly decreased birth weight, associated with PFDA levels and how no one epidemiological study could possibly be representative of these inconsistent and disparate results or the results of a meta-analysis of relevant studies.

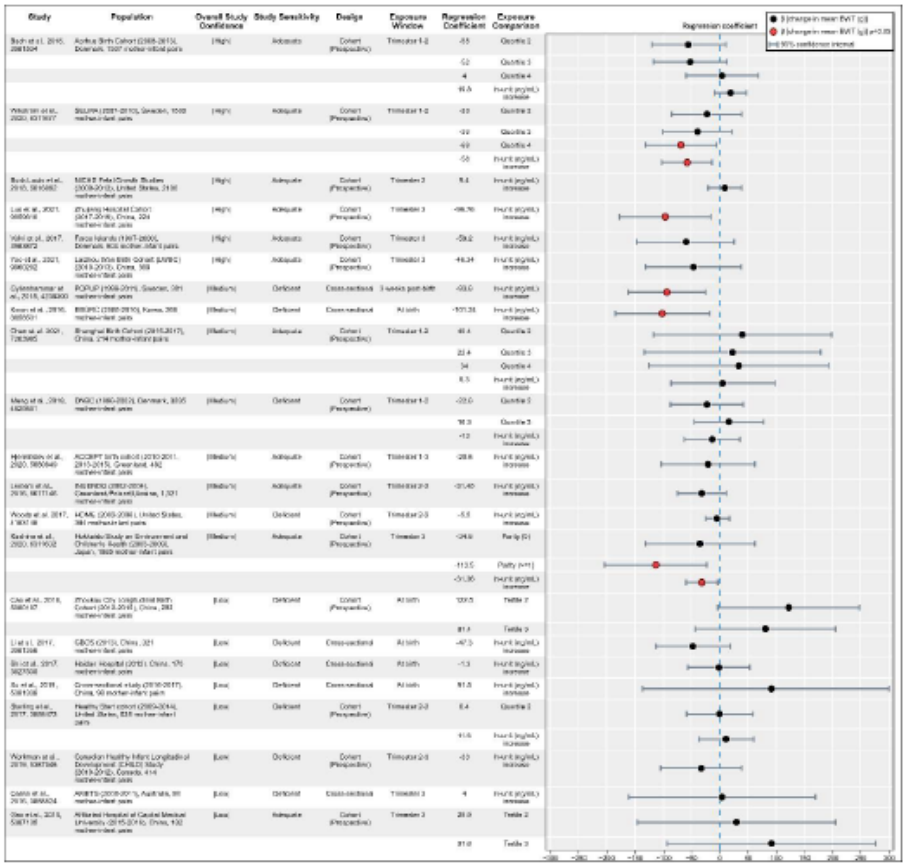

Figure 3-28. Overall study population mean birth weight results for 22 PFDA epidemiological studies[a-e]. (results can be viewed by clicking the HAWC link).

Abbreviation: BWT = Birth Weight

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b] Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during the first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c] If a study presented regression coefficients for continuous exposure with multiple exposure units, only one unit change is shown (e.g., (Bach et al., 2016), with the exception of (Li et al., 2017), which displays both IQR and ln-unit (ng/mL) values.

[d] The results displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared to a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[e] Xu et al. (2019a) results are truncated for the 210.7 gram increase; the complete 95% CI ranges from -314.3 to 735.8 grams.

Similarly, Figure 3-26 (p. 3-104) below illustrates how the results of Bach et al. (2016), the other high confidence prospective cohort for trimester 1-2 with adequate study sensitivity, had a nonmonotonic response, no statistically significant quartile results, and a regression coefficient of +0.03 per each ln-unit (ng/mL) (compared to -0.147 per each ln-unit increase for Wikström et al. 2020), all of which does not support the Wikström et al. (2020) study results or their use in toxicity factor derivation. The Bach et al. (2016) and Wikström et al. (2020) studies are the first two entries in Figure 3-26, respectively.

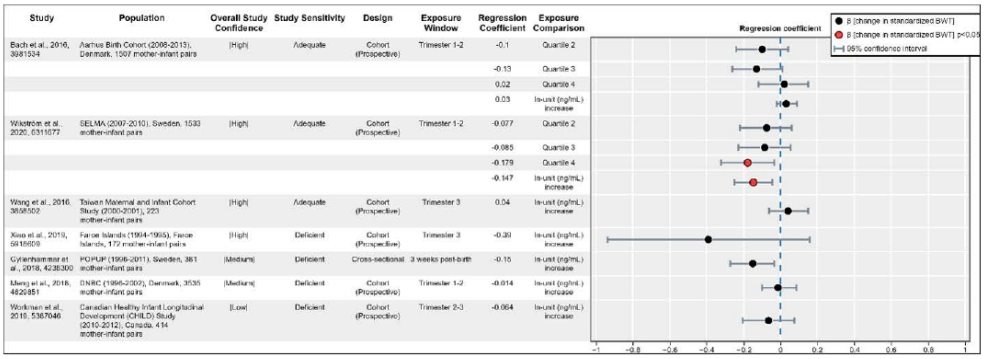

Figure 3-26.PFDA and birth weight z-scores (overall population)[a]. Refer to Birth Weight-Z for details on the individual study evaluation review.

Moreover, the BMD_{5RD} values for decreased birth weight based on Wikström et al. (2020) range from 0.54 to 0.71 ng/mL (note that BMD_{5RD} values are not treated like NOAELs for purposes of RfD derivation; Table C-10, p. C-22).[28] By comparison, PFDA blood serum concentrations in quartile 4 of the Bach et al. (2016) study range from 0.43 to 2.87 ng/mL (see Table 1 of the study). Despite these quartile 4 blood serum concentrations being up to 5.3-fold higher than the BMD_{5RD} values for decreased birth weight based on Wikström et al. (2020), birth weight and birth weight z-scores for quartile 4 in Bach et al. (2016) were actually increased (nonsignificantly) (see Table 3 of the study). Again, this is from the other high confidence, prospective cohort for trimester 1-2 with adequate study sensitivity. These results from Bach et al. (2016), obtained from the other high confidence study in the same PFDA blood serum concentration range (and higher) compared to EPA's BMD_{5RD} values, are inconsistent with and unsupportive of EPA's use of the PODs for adverse birth weight effects based on Wikström et al. (2020) for RfD derivation. Sex-specific results from Bach et al. (2016) do not provide strong support for use of Wikström et al. (2020) results for quantitative dose-response assessment either (e.g., Figure 3-29, p. 3-109 of the draft assessment).

Given the drastically different results across epidemiological studies and the mere "slight" evidence of developmental effects across more than 45 such studies, EPA should reconsider use of a single epidemiological study (Wikström et al. 2020) for dose-response assessment of birth weight and RfD derivation and consider a meta-analysis and/or using the more definitive dose-response data from the mouse study for dose-response assessment of birth weight and RfD derivation (**Tier 1 necessary revision**). It is remarkable that per EPA, the single mouse study by Harris and

[28] For comparison, the $BMDL_{5RD}$ values range from 0.31 to 0.37 ng/mL (Table C-10, p. C-22).

Birnbaum (1989) gives rise to a greater level of evidence for developmental effects ("moderate") than the results of over 45 epidemiological studies. This fact alone, in this case, justifies use of the animal data for dose-response assessment and osRfD derivation. Based on EPA Figure 3-56 (p. 3-152), reproduced below, the dose-response data for fetal body weight (as an example) appear well suited for benchmark dose analysis.

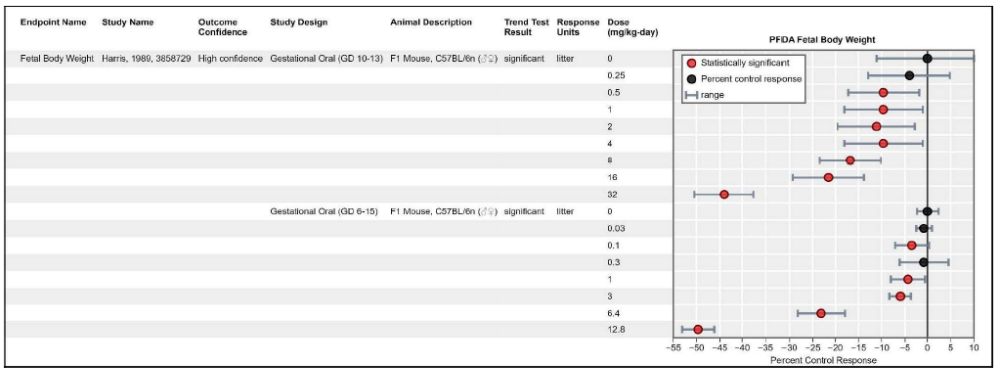

Figure 3-56. PFDA fetal body weight after gestational exposure (results can be viewed by clicking the HAWC link).

More specifically, Figure 3-56 shows statistically significant and progressive decreases/trends in fetal body weight with dose across a range that includes the >5% decrease historically used by regulatory agencies as the demarcation of adversity in dose-response assessment (i.e., as a >5% reduction in fetal body weight is usually considered adverse, a benchmark response of 5% is typically used in benchmark dose analysis), from -1% to -50% and including doses that resulted in decreases of -4% and -6% (Table 3-23, p. 3-153). Furthermore, the changes in fetal body weight were at doses not associated with maternal toxicity (p. 3-151, lines 5-6).

Harris and Birnbaum (1989) is a medium/high-confidence study (p. 3-150, lines 9-12), and EPA should consider mouse data from the Harris and Birnbaum (1989) study for use as the primary basis for osRfD development (**Tier 1 necessary revision**) based on developmental growth effects consistent with: (1) this mouse study providing the primary basis for EPA's developmental effects hazard conclusion; (2) the lack of factors that decrease certainty for fetal growth as evaluated in the mouse study (see Table 3-24); and (3) the extensive epidemiological database merely being able to provide support for this mouse study with what amounts to "slight" evidence for developmental effects across over 45 epidemiological studies. Afterall, per the draft assessment, it is the mouse data that primarily support that assuming sufficiently high exposure over a sufficiently long duration (i.e., "given sufficient exposure conditions"), PFDA exposure is likely to cause developmental toxicity in the general human population, which includes potentially susceptible subpopulations (e.g., developing fetuses of pregnant women).

My suggestion to use the mouse data (Harris and Birnbaum 1989) instead of epidemiological study data (Wikström et al. 2020) for dose-response assessment and osRfD derivation based on developmental growth effects (**Tier 1 necessary revision**) would make EPA's assessment somewhat more consistent with ATSDR

(2021),[29] who found *the epidemiology literature inadequate for use as the basis of deriving 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."

Getting back to use of Wikström et al. (2020) for dose-response assessment of birth weight and RfD derivation, Tables 2 and 3 from that study appear below (unaltered; see license at https://creativecommons.org/licenses/by/4.0/) and demonstrate: (1) significant co-exposures to other PFAS (e.g., PFOS, PFOA) associated with birth weight deficits, an ATSDR concern and significant uncertainty; and (2) few statistically significant ORs for decreased body weight in the PFDA exposure quartiles; that is, only 2 out of 9 ORs showed statistically significant birth weight decrements, which were for quartile 4 where both PFOS and PFOA were certainly co-exposures and also showed statistically significant decreases for birth weight. In regard to this latter point concerning potential confounding by co-exposure to other PFAS, which was cited by ATSDR as a significant uncertainty, Wikström et al. state [*emphasis added*]:

> "*Another limitation is the compound-by-compound approach*. Theoretically, *a health outcome is simultaneously influenced by multiple environmental factors*. Nevertheless, the exposure to several PFASs may be correlated with each other due to common sources. Our findings were consistent across different PFAS compounds, and we regard correction for multiple comparisons overly conservative to be suitable for the investigations on such interrelated compounds. If the single compound's level could represent the levels of several other compounds, *our findings based on single compound analyses may still shed some light on the joint effects of multiple PFAS compounds*. However, carefully designed statistical models, such as *mixture-based approaches* within the PFAS compound class, *should be explored in follow-up studies*."

Thus, the study authors are acknowledging confounding by other PFAS as an important limitation of their compound-by-compound approach as joint effects of

[29] To be entirely consistent with this conclusion from ATSDR (2021) and my own comments above, the EPA assessment would further need to omit the highly uncertain RfD derivations based on PFDA associations with decreases in antibody levels/vaccine response (**Tier 1 necessary revision**) reported in some epidemiological study results that appear overall to be inconsistent, non-adverse, and uncertain in nature (e.g., causal attribution problems, unsupported by epidemiological disease incidence studies).

	multiple PFAS compounds may be occurring such that mixture-based approaches should be explored for data analysis. *The compound-by-compound approach used in this study gives rise to significant uncertainty that precludes use of these data for dose-response assessment and RfD derivation*. It cannot be confidently said scientifically that the presumed effects are due to PFDA exposure (e.g., PFOS and PFOA were certainly co-exposures and also showed statistically significant decreases for birth weight). Consistent with my opinion on the significant uncertainty and confounding associated with these Wikström et al. results and the weakness/limitations of the epidemiological evidence overall, Section F.3 of the draft assessment states that, "In the six studies using mutually adjusted PFAS approaches to address coexposures, there was not consistent evidence for birth weight deficits associated with increased exposure to PFDA." (p. F-27, lines 13-14), and acknowledges that "there is considerable uncertainty due to potential confounding by co-occurring PFAS in the existing literature." (p. F-27, lines 30-32). Indeed, significant co-exposure to multiple PFAS is not the exception but the rule, and is just the condition to result in significant bias away from the null for adverse effects.[30] The high confidence study of Luo et al. (2021) is an example that serves as a cautionary tale on the obvious importance of PFAS co-exposures, which reported large statistically significant birth weight deficits (-97 g; -178, -16 per each ln-unit PFDA increase) in a single-pollutant PFDA model, but results were null and their direction reversed in the multipollutant model with a nonsignificant increase in birth weight associated with PFDA (Table F-2).[31] As the data from epidemiological studies provide only "slight" evidence of developmental effects, including data from Wikström et al. (2020) that appears too uncertain and unsuitable for quantitative dose-response assessment, I suggest use of the mouse data (Harris and Birnbaum 1989) for dose-response assessment and osRfD derivation based on developmental growth effects (**Tier 1 necessary revision**). 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals.

[30] To be a confounder, the co-occurring PFAS would need to be associated with both the PFAS of interest and the outcome, but not an intermediate in the causal pathway; such PFAS would be considered positive confounders if their effect estimate with the endpoint of interest is in the same direction as the primary PFAS of interest. If positive confounders are not accounted for, the anticipation is that any resultant bias would be away from the null. (p. F-24, lines 16-21). The stronger the association between co-exposures, the larger the effect size for the co-exposure of interest (i.e., the greater the confounding) (p. F-25, lines 14-15).

[31] The other high confidence study in Table F-2 (Starling et al. 2017) showed that adjustment for PFAS co-exposures in the multipollutant model resulted in a large statistically significant increase in birth weight for PFDA (+97.5 g; 31.5, 163.6), which for some reason is not discussed in Section F.3 (*PFDA and PFAS Coexposure Study Results*) but serves as yet another example of the obvious, commonsensical importance of accounting for PFAS co-exposures.

Table 2. Prenatal exposure to eight PFAS, measured as maternal serum concentrations (ng/mL) during early pregnancy.

Compound	Geometric mean [95% CI]	Median (IQR)	95th %	LOD	Above LOD (%)
PFOS	5.35 [5.21–5.50]	5.38 (3.97–7.60)	10.34	0.06	100
PFOA	1.60 [1.56–1.65]	1.61 (1.11–2.30)	3.18	0.02	100
PFHxS	1.31 [1.28–1.35]	1.23 (0.86–1.99)	2.94	0.03	100
PFNA	0.54 [0.53–0.56]	0.53 (0.39–0.73)	1.02	0.01	100
PFDA	0.26 [0.26–0.27]	0.26 (0.19–0.34)	0.50	0.02	100
PFUnDA	0.21 [0.21–0.22]	0.23 (0.15–0.33)	0.44	0.02	99.5
PFHpA	0.018 [0.017–0.019]	0.02 (<LOD–0.04)	0.077	0.01	73.9
PFDoDA	0.027 [0.026–0.027]	<LOD (<LOD–0.05)	0.08	0.03	46.7

LOD limit of detection

Table 3. Associations between prenatal PFAS exposure[a] and birth weight[b], together with odds ratios for birth weight small for gestational age in 1533 children.

	All children		Girls		Boys	
	BW (g) β (95% CI)	SGA OR (95% CI)	BW (g) β (95% CI)	SGA OR (95% CI)	BW (g) β (95% CI)	SGA OR (95% CI)
PFOS						
Per ln-unit	−46 (−88; −3)	1.19 (0.87; 1.64)	−85 (−145; −25)	1.40 (0.83; 2.35)	−13 (−73; 47)	1.08 (0.72; 1.63)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−27 (−89; 35)	0.69 (0.43; 1.08)	−32 (−115; 52)	0.89 (0.39; 2.03)	−28 (−118; 63)	1.26 (0.67; 2.37)
Q3	−22 (−84; 41)	0.79 (0.53; 1.18)	−51 (−137; 34)	0.82 (0.36; 2.03)	5 (−86; 96)	0.86 (0.45; 1.67)
Q4	−80 (−144; −16)	1.56 (1.09; 2.22)	−142 (−231; −54)	2.05 (1.00; 4.21)	−28 (−119; 63)	1.30 (0.70; 2.40)
PFOA						
Per ln-unit	−68 (−112; −24)	1.43 (1.03, 1.99)	−86 (−145; −26)	1.96 (1.18; 3.28)	−49 (−113; 15)	1.16 (0.75; 1.78)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	27 (−35; 89)	0.77 (0.45; 1.32)	30 (−55; 115)	1.00 (0.40; 2.51)	26 (−66; 116)	0.67 (0.34; 1.31)
Q3	−41 (−106; 23)	0.96 (0.57; 1.61)	−36 (−124; 52)	1.64 (0.71; 3.83)	−44 (−139; 50)	0.66 (0.33; 1.29)
Q4	−90 (−159; −91)	1.44 (0.86; 2.40)	−136 (−231;−40)	2.33 (1.00; 5.43)	−47 (−147; 54)	1.04 (0.54; 2.01)
PFHxS						
Per ln-unit	−0.1 (−38; 38)	0.96 (0.72; 1.27)	−14 (−68; 39)	1.14 (0.73; 1.80)	−13 (−67; 41)	0.84 (0.58; 1.22)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−4 (−66; 58)	1.37 (0.86; 2.20)	30 (−56; 116)	1.77 (0.78; 3.99)	−39 (−129; 50)	1.24 (0.69; 2.23)
Q3	−15 (−78; 48)	0.89 (0.54; 1.47)	28 (−59; 115)	1.05 (0.44; 2.49)	−51 (−141; 39)	0.82 (0.44; 1.54)
Q4	−6 (−69; 57)	1.04 (0.63; 1.69)	−16 (−104; 71)	1.76 (0.79; 3.90)	1 (−90; 92)	0.73 (0.38; 1.41)
PFNA						
Per ln-unit	−46 (−89; −4)	1.38 (1.02, 1.87)	−52 (−117; −2)	1.34 (0.85; 2.11)	−50 (−113; 14)	1.42 (0.94; 2.17)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	7 (−55; 69)	0.83 (0.49; 1.38)	−2 (−86; 82)	0.66 (0.29; 1.52)	15 (76; 106)	0.97 (0.50; 1.89)
Q3	−39 (−102; 24)	1.14 (0.70; 1.85)	−49 (−137; 38)	1.39 (0.66; 2.90)	−28 (−119; 64)	0.95 (0.50; 1.82)
Q4	−33 (−96; 31)	1.23 (0.77; 1.99)	−66 (−153; 20)	1.22 (0.59; 2.53)	1 (−94; 95)	1.24 (0.66; 2.33)
PFDA						
Per ln-unit	−58 (−103; −13)	1.46 (1.06, 2.01)	−69 (−133; −6)	1.62 (0.98, 2.67)	−47 (−112;17)	1.36 (0.90; 2.07)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−23 (−85; 39)	1.03 (0.62; 1.69)	−42 (−126; 42)	0.86 (0.37; 2.00)	−8 (−99; 82)	1.18 (0.63; 2.23)
Q3	−39 (−101; 23)	1.07 (0.65; 1.76)	−74 (−160; 13)	1.20 (0.54; 2.67)	−8 (−98; 81)	0.99 (0.52; 1.89)
Q4	−69 (−132; −5)	1.50 (0.94; 2.38)	−116 (−204; −27)	1.95 (0.94; 4.06)	−27 (−118; 64)	1.21 (0.66; 2.23)
PFUnDA						
Per ln-unit	−13 (−49; 22)	1.21 (0.92; 1.58)	−24 (−75; 27)	1.08 (0.70; 1.67)	−6 (−55; 42)	1.29 (0.82; 1.83)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−67 (−153; 19)	1.24 (0.77; 2.01)	−67 (−153;19)	2.09 (0.94; 4.63)	41 (−48; 130)	0.90 (0.48; 1.68)
Q3	−42 (−128; 44)	0.85 (0.51; 1.43)	−42 (−128; 44)	1.01 (0.42; 2.44)	46 (−44; 136)	0.81 (0.43; 1.56)
Q4	−46 (−110; 17)	1.52 (0.95; 2.44)	−93 (−183; −3)	1.92 (0.86; 4.25)	−10 (−100; 80)	1.36 (0.76; 2.46)
PFHpA						
Per ln-unit	−1 (−24; 21)	1.06 (0.90; 1.25)	−4 (−34; 26)	1.06 (0.83, 1.36)	−0.03 (−33; 32)	1.07 (0.86; 1.37)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−0 (−62; 61)	0.92 (0.57; 1.48)	17 (−70; 103)	0.92 (0.44; 1.92)	−15 (−102; 73)	0.94 (0.50; 1.75)
Q3	3 (−59; 65)	0.78 (0.48; 1.27)	−21 (−106; 65)	0.75 (0.35; 1.65)	23 (−65; 112)	0.78 (0.42; 1.45)
Q4	31 (−31; 93)	1.25 (0.85; 1.84)	27 (−57; 112)	1.31 (0.66; 2.60)	33 (−58; 124)	1.15 (0.65; 2.04)

All analyses were adjusted for maternal weight, parity (three categories) and cotinine levels. Analyses including both boys and girls were in addition adjusted for sex and analyses of BW were adjusted for GA. SGA was defined as BW 10th percentile for sex and GA
[a]Associations with PFAS are presented per ln-unit and by quartiles of exposure, as related to
[b]Birth weight (g) and odds ratios (adjusted) for birth weight small for gestational age

The above comments provide relevant rationale for use of an alternative study(ies) or effect(s) for lifetime RfD derivation. While the draft assessment provides various but limited candidate PODs, due consideration of the comments above appears to require a more complete evaluation of alternative studies/effects (**Tier 1 necessary revision**). One method to begin such an evaluation may entail sorting the study data extracted by NOAEL/LOAEL, BMD or a similar criterion that would allow EPA to

	readily identify the next most sensitive effects based on less problematic studies that are adequate for RfD derivation. However, any newly derived toxicity factor should be subject to external expert peer review (**Tier 1 necessary revision**). c. As I do not agree with the selection of these studies/endpoints for RfD derivation, please see my comments under subsection "b" above, many of which concern the considerations of adversity and scientific support (as well as this question's footnote). That being said, speaking more generally in regard to deriving a lifetime RfD, lifetime RfDs must also be protective against adverse effects occurring over a shorter duration of exposure, such as in childhood or during development. Thus, if effects that occur over a shorter duration of exposure are truly adverse, relevant to humans, and are the first to occur as the dose rate increases (i.e., the critical effect), they can appropriately serve as the basis for a lifetime RfD. Lastly, since I suggest use of the mouse study of Harris and Birnbaum (1989) above as the basis of an osRfD based on decreases in fetal body weight (with various gestational exposures), I note that Figure 3-56 of the draft assessment shows statistically significant and progressive decreases/trends in fetal body weight with dose across a range that includes the >5% decrease historically used by regulatory agencies as the demarcation of adversity in dose-response assessment (i.e., as a >5% reduction in fetal body weight is usually considered adverse, a benchmark response of 5% is typically used in benchmark dose analysis), from -1% to -50% and including doses that resulted in decreases of -4% and -6% (Table 3-23, p. 3-153). Furthermore, the changes in fetal body weight were at doses not associated with maternal toxicity (p. 3-151, lines 5-6). Harris and Birnbaum (1989) is a medium/high-confidence study (p. 3-150, lines 9-12). d. Table 5-7 (pp. 5-13 to 5-18) contains the benchmark response (BMR) levels for BMD modeling. For immuno-toxicity, the draft assessment indicates [*emphasis added*] that *in the absence of a clear definition of an adverse effect* for a continuous endpoint like antibody concentrations, a default BMR of 1 SD change from the control mean may be selected, and a BMR lower than 1 SD can be used if it can be justified on a biological and/or statistical basis (p. 5-5). For decreased antibody tetanus vaccine response in children, the draft seems to primarily use three considerations to justify a BMR of ½ SD (pp. 5-5 to 5-6): (1) characterization as a developmental effect; (2) severity of the associated disease for which the vaccine is designed to protect as a biological consideration (but not severity/adversity of the effect itself); and (3) a BMR of ½ SD results in 7.9% of the tetanus antibody distribution values being below a 0.1 IU/mL cutoff, which is 5.1% extra risk, showing that the generic BMR of ½ SD can provide a reasonably good estimate of 5% extra risk (perhaps their statistical consideration). Accordingly, the draft assessment: (a) is not using the demonstrated severity/adversity of the effect itself (decreased antibodies) as support for the lower BMR (i.e., ½ SD); and (b) is using an admittingly unclear definition of an adverse effect (antibodies <0.1 IU/mL)[32] in an attempt to justify/support the lower BMR of ½ SD. (a) is problematic and (b) is internally

[32] Per EPA's draft assessment (p. 5-5, lines 260-28).

	inconsistent as the draft does not scientifically support the definition of <0.1 IU/mL as the adverse cutoff. These justifications/support for the BMR should be removed (**Tier 1 necessary revision**). Additionally, while the draft assessment suggests that specific immuno-toxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards (citing Selgrade 2007), this appears speculative on EPA's part as the draft does not provide robust scientific support for such an assumption. In fact, the draft does not even provide evidence that the antibody decreases reported result in any inability for children to protect themselves against the very diseases for which the antibody decreases are reported (diphtheria and tetanus). This is true for both the study populations as well as the U.S. population. For the U.S. general population, the CDC reported that about 10% of children for ages 6- to 11-years old had tetanus or diphtheria antibody titer measurements below 0.1 IU/mL[33], and EPA argues that even 0.1 IU/mL may not be protective (p. 5-6, lines 17-25). The implication is that a significant percentage of U.S. children are at risk of contracting tetanus or diphtheria. Yet as discussed in detail under question "3.b" in regard to the adversity (or lack thereof) of the reported antibody effects, U.S. surveillance disease incidence data do not support that serum PFDA (or any other serum PFAS) is suppressing tetanus and diphtheria vaccine responses and leaving people vulnerable to infection from these diseases. As a corollary to EPA's reasoning, the lack of demonstrable real-world effects on the incidences of these diseases when NHANES data show most blood serum levels well above the PODs adjusted for intrahuman variability (see comments under question "2.b") suggests that these specific effects observed in children certainly may not be broadly indicative of immunosuppression impacting these children's ability to protect against a range of other diseases (i.e., "immune hazards"). In fact, this is consistent with the WOE from scientific studies on PFDA and disease incidence, which is for no significant effects (again, see comments under question "2.b" above). Also see comments above under question "3.b" above citing rationale for the use of mouse data for reduced fetal weight (**Tier 1 necessary revision**). With all this being said, otherwise, the modeling approaches, model selection process, and BMRs used to derive PODs for toxicity value derivation are scientifically justified. 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).[34] Aside from the

[33] CDC (Centers for Disease Control and Prevention). National Health and Nutrition Examination Survey, Immunizations. Available at: https://www.cdc.gov/nchs/data/nhanes/databriefs/immuniza.pdf

[34] An adequate fit is judged on the basis of a $\chi 2$ goodness-of-fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined BMR (absolute value <2.0), and visual inspection of the model fit. Among all models providing adequate fit, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL estimates differed by less than threefold. When BMDL estimates differed by greater than threefold, the model with the lowest BMDL was selected to account for model uncertainty (see Section C.2.1, p. C-24).

BMR for immune effects (see comments above), reasonable scientific justifications for the BMRs utilized are provided in Table 5-7 (pp. 5-13 to 5-18). Since I suggest use of the mouse study of Harris and Birnbaum (1989) as the basis of an osRfD based on decreases in fetal body weight (with various gestational exposures), I note here that a 5% relative deviation BMR in markers of growth/development in gestational studies (e.g., fetal weight) has generally been considered a minimally biologically significant response level and has historical precedence for use as a BMR (EPA 2012b, 2004, 2003).

e. Yes, the section *Derivation of Candidate Lifetime Toxicity Values for the RfD* (p. 5-19) provides reasonable rationale for only considering liver effects data and male/female reproductive effects data for subchronic osRfD derivation. More specifically, p. 5-19 (lines 10-20) states [*emphasis added*]:

> "For liver and male and female reproductive effects, *quantitative information is limited to* studies in which animals were exposed for *≤28 days*. For each of these identified hazards, *very little information is available to assess the extent to which the specific changes caused by PFDA exposure for 28 days might be expected to worsen* with PFDA exposure for a lifetime. Separately, *human equivalent PODs for these endpoints were much less sensitive* (several orders of magnitude) than the PODs for developmental and immune effects from the epidemiology studies (see Table 5-9). As such, for liver, male reproductive, and female reproductive effects, derivation of candidate lifetime values was not attempted given the *high degree of uncertainty associated with using PODs from a 28-day rodent study to protect against effects observed in a chronic setting*. However, these endpoints were considered for the derivation of the subchronic RfD (see Section 5.2.2)."

Taken together, these considerations provide a reasonable rationale to support the decision not to attempt the derivation of *lifetime* osRfD values (only for derivation of *subchronic* osRfD values). While the total composite uncertainty factor for these effects is already considerable (1,000; Table ES-1), if lifetime osRfDs based on these effects (i.e., liver, male and female reproductive effects) are desired by EPA and/or considered useful for more complete risk assessments, EPA could consider increasing the total composite uncertainty factor to the upper end of their total uncertainty factor range (3,000) to estimate chronic osRfDs **(Tier 2 suggestion)**.

f. Section 5.2.4 (p. 5-41, lines 35-36) states, "No studies examining inhalation effects of short-term, subchronic, chronic or gestational exposure for PFDA in humans or animals have been identified, precluding the derivation of an RfC." Furthermore, it appears from Sections 3.1.6 and 3.1.7 of the draft assessment that a reliable PBPK/PK model has not been identified for PFDA that could be used for route-to-route extrapolation. If EPA has not identified a reliable PFDA PBPK/PK model that could be used for route-to-route extrapolation, then EPA's decision not to derive an RfC would be fully justified. However, this is not explicitly stated in Section 5.2.4, which should clearly state whether or not EPA has identified a reliable PFDA PBPK/PK model for route-to-route extrapolation (**Tier 1 necessary revision**).

Hoberman	The selection of the three studies covering the response to decreased serum antibody concentrations for both tetanus and diphtheria in children and reduced birth weight in humans to determine the RfD values for PFDA is believed to be scientifically justified. The modeling approach appears appropriate. a. The modeling appears to be appropriate based on the nature of the endpoints and fact that the changes in the endpoints occurred during development, a critical period. b. Not applicable. c. The effects selected were appropriate for use in deriving the lifetime RfD considering that the effects occurred during development of the immune system and the effects were presumed continue throughout the lifetime of the affected subjects. There are no long term or lifetime studies of these effects to confirm this modeling. d. The BMD modeling approaches, selection and justification of benchmark response levels, and selection of the BMD models used to identify each POD for toxicity value derivation scientifically were justified and clearly described. e. Not deriving a lifetime organ-specific RfD for the liver, male and female reproductive effects appears appropriate since there were no lifetime studies and no fully supportive human studies for these endpoints. The 28-day study in mice was supportive of subchronic os RFDs since four weeks of exposure is an appropriate subchronic exposure. f. With no inhalation studies of adequate quality to review not deriving an RfC appears to be appropriate.
Leung	Toxicological modeling is not my area of expertise, and I defer this question to the other external reviewers. From an overall perspective, I agree with the point made in the panel meeting, that clearly conveying the additional data obtained from the authors of the original papers would improve the clarity of the sections used to derive the RfD.
Zoeller	a. Derivation of the RfD values based on immune suppression and birth weight are reasonable and the Agency has justified the use of the presented in Budtz-Jorgensen and Grandjean and Wikstöm. As the Agency points out, the endpoint of decreased antidiphtheria and tetanus titers is not an adverse endpoint per se and there is no minimal biological response that the Agency can identify to set the BMR. Therefore, the choice of a ½ SD as discussed and justified in Appendix C.1.1. was clear and reasonable. In addition, while this is an intermediate endpoint, it is clear that diphtheria and tetanus represent potential severe including fatal infections that can occur during development. Thus, there is significant justification for the use of this intermediate endpoint to establish the RfD. The scientific support for this was

	well-developed in C.1.1., including the recognition that these responses may be broadly indicative of immune suppression. Likewise, the use of Wikström (birth weight) was clear and scientifically justified. A 5% change was chosen as the minimal biological change leading to an adverse effect because this endpoint occurs during a developmental stage and because this endpoint is clinically predictive of health outcomes. **No recommendation**. d. This issue was clearly and thoroughly presented by the Agency. **No Recommendation.** e. It is reasonable that the Agency used these endpoints to derive subchronic RfDs only. The endpoints captured for liver and male and female reproductive endpoints are scientifically justified. **No Recommendation.** f. The Agency was justified in not developing an RfC where no studies are available. **No Recommendation**.

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

a. If so, please provide an explanation.

b. If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the subchronic RfD and detail the rationale for use of such an alternative.

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

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

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

Noncancer Toxicity Value Data Selection and Modeling	
Reviewer	**Comments**
Adgate	**Tier 3**: Given that the same studies are used for both lifetime and subchronic RfDs, the main issue is the significance of a short-term adverse effect that may or may not be reversible relative to a lifetime adverse effect RfD derivation. Given the persistence of PFDA leading to ~ lifetime exposure (even if declining) a subchronic RfD seems less important and harder to understand than a lifespan RfD.

	d. See above comment. e. **Tier 3**: Given the lack inhalation data from animal or human studies and the likelihood that ingestion is the dominant human exposure pathway it is reasonable that no subchronic RfC is estimated. PFAS inhalation exposure, however, is an area that has had little research, and some planned future studies will likely explore this topic and the potential for precursors that are often present in the environment to transform to PFDA. At this time an analysis of PFDA physical constants and potential resulting air concentrations in indoor environments would be informative for decision-makings given PFDA's presence in many consumer products.
Carignan	a. Use of the same studies to derive a subchronic RfD is reasonable and scientifically justified given the persistence of PFDA. d. The rationale and approach provided is reasonable. e. In the absence of data it is reasonable not to derive a subchronic RfC for PFDA. For PFASs with similar properties (e.g., long chain) read-across could be considered where PFAS-specific data is unlikely to be generated. [**Tier 2**]
Faustman	a. This reviewer supports the inability to derive a meaningful subchronic RfC. This reviewer does support the development of subchronic RfDs and in particular is supportive of the choice of human studies for both the immunotoxicity and developmental endpoints and likewise supportive of the choice to use the animal study to derive the RfDs for hepatotoxicity and male and female reproductive toxicity. These choices were well described and resulted from the evidence summary tables at the end of each of the toxicity endpoint sections as well as the relevant endpoint discussions in Section 5. b. No alternative suggestions. c. Section 5.2.1 details the choice of the studies used by USEPA to proceed for toxicity value calculations. Table 5-2 details the immune antibiotic response data sets that were used for the deriving subchronic endpoints for immunotoxicity. For example, the combined birth cohort data was used for the POD derivation for the human immunotoxicity endpoint as the log-log transformed data could not be used for calculating the BMDs (see notes above about availability of the original data in order to do these calculations). Examples for developmental toxicity outcomes are provided in Table 5-3 and two studies Valvi et al 2017 and Wikstrom et al 2020 both high confidence epi studies are available and were modeled. Animal studies were cited as being supportive for the choice of these endpoints to model and Table 5-4 notes one animal study (Harris and Birnbaum, 1989) as modeled for decreased fetal body weight. Notes provided in these tables summarize the rational for choosing these studies for toxicity value calculations. d. For hepatic effects, Table 5-1 details the endpoints for which PODs are derived for the high confidence studies. Four endpoints were chosen and included increased serum ALT and ALP values (NTP, 2018) as well as increased liver weight (NTP, 2018 and Fawley et al 2018) from two high confidence rodent studies. Rationale for

	choosing these endpoints included the presence of dose response as well as consistency across studies and biological significance of endpoints affected. Male reproductive endpoints are presented in Table 5-5 and two endpoints with dose response data for decreased absolute epididymis sperm counts and Leydig cell atrophy were chosen from the NTP, 2018. Note that only the Leydig cell atrophy endpoint study was identified as a high confidence study but the epididymis sperm count endpoint from the same study was identified as low confidence due to potential insensitivity however both endpoints were identified as biologically important and both endpoints were modeled. Table 5-6 lists four endpoints from the NTP, 2018 study that are available for female reproductive endpoints and all but one endpoint are modeled due to the studies being high confidence, of biological relevance and of interest due to the presence of dose-response data. Increased testosterone as an endpoint was not modeled as the toxicological significance of this endpoint in this study was unclear. This reviewer supports all of these choices. e. This reviewer supports this lack of action due to lack of relevant studies to model.
Fisher	a. This has been done before, using the same study for subchronic and lifetime exposures. It is not a great position to be in, but this is the state of the toxicology for PNDA. The chronic RfD is more uncertain than the subchronic. c. Table 5-16 lists organ specific endpoints for subchronic RfD consideration. The table and text provide good descriptions about the evidence base supporting the toxicity, along with confidence in the POD for a human and a summary confidence statement based on these factors. If there are in vitro studies supporting the toxicity endpoints (Confidence in evidence base supporting this hazard), it is worthwhile to include. **Tier 2**. d. There is a good discussion about these endpoints relative to an organ specific RfD. The liver is the most studied endpoint in animals. The use of human data (immune/developmental) is preferred by me over animal data. e. This is the proper course of action. See comment above about exposure and the use of MPPD simulation software.
Georgopoulos	a. The rationale for selecting the same studies and outcomes for deriving the lifetime and the subchronic RfDs for PFDA is appropriate and scientifically justified. As stated in the Toxicological Review (page 5-29, lines 27-32): *"Datasets considered for the subchronic RfD were based on endpoints advanced for RfD derivation in Table 5-8. Given that the developmental and immune effects were observed in humans exposed to PFDA during susceptible lifestages (postnatal growth/development and immune system effects in children at ages 5–7), these endpoints were also considered for the derivation of candidate subchronic values, applying identical uncertainty factors to those used for the lifetime candidate values."* b. N/A. c. The appropriateness of the selected effects in representing adverse changes is

	discussed adequately in the corresponding sections of the Toxicological Review. d. The studies and effects selected for deriving other subchronic osRfDs (i.e., for liver, male reproductive, and female reproductive effects) are also appropriate; their relevance in representing adverse changes is discussed adequately in the corresponding sections of the Toxicological Review. It should be recognized that the available evidence base is the determining factor limiting the ability to derive osRfDs. For example, regarding liver effects, the Toxicological Review (page 3-49, lines 29-32) states: *"The evidence base is limited in that there is an absence of studies via relevant exposure routes with durations longer than 28 days (i.e., no subchronic and chronic exposure studies) examining potential hepatic effects of PFDA exposure."* Given such constraints, the selected studies and effects represent the best available options. e. The decision to not derive a subchronic RfC is the only reasonable option since no relevant inhalation exposure studies to PFDA have been identified.
Haney	This question states that, "The same studies and outcomes were chosen for use in deriving the lifetime and subchronic RfDs." However, question "3.e" states, "the derivation of lifetime organ-specific (os) RfD values was not attempted for liver, male reproductive and female reproductive effects." Therefore, while correct for the final RfD value, this question (4) would be incorrect in regard to candidate RfDs as liver and male/female reproductive effects were not chosen for use in deriving osRfDs for consideration in selecting the lifetime RfD. The cited sentence of this question "4" was not abundantly clear and open for interpretation, hence this comment. a. As with the lifetime RfD, I do not agree with the selection of these same studies and endpoints for subchronic RfD derivation (i.e., decreased serum antibody concentrations in Grandjean et al. 2012, decreased birth weight in Wikström et al. 2020). Please see my comments under subsection "b" below, which I feel compelled to reiterate here. b. <u>Immune Effect Endpoint</u> EPA indicates that the hazard judgment that "the ***evidence indicates*** that PFDA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans" (p. 3-92, lines 35-37) is driven primarily by consistent evidence of reduced antibody response from human epidemiological studies (mostly from two birth cohort studies)…" (pp. 3-92 and 3-93, lines 37-1). However, it is not clear that EPA has sufficiently considered adversity for the primary basis of this hazard judgment, or fully considered the weaknesses/ limitations of this evidence. For example, even without considering potential confounding, 3 out of 4 ORs for PFDA and antibody concentrations falling below the protective level of 0.1 IU/mL for tetanus and diphtheria in children ages 5 years (n=510) or 7 years (n=386) contain 1, indicating that the WOE from this key

	study cohort is for no statistically significant associations with less-than-protective serum antibody concentrations in children (see eTable 4 of Grandjean et al. 2012).[35] Consistent with this, the more recent Grandjean et al. (2017a) study states [*emphasis added*] that, "With many antibody concentrations being close to the assumed clinically protective level of 0.1 IU/mL, *logistic regression showed only weak tendencies for antibody levels below the limit to be associated with serum PFAS concentrations*."[36] Also consistent with a WOE for no statistically significant associations (much less being able to say "effects" as there are problems with causal attribution to PFDA), 6 of 8 confidence intervals (95% CIs) for both tetanus and diphtheria serum antibodies included 0% change per 2-fold increase in maternal and age 5 serum PFDA (see Table 3 of the study). Additionally, in Grandjean et al. (2012), PFDA was correlated with other PFAS (e.g., PFOS, PFOA, PFNA) that had some associations with antibody concentrations falling below the protective level of 0.1 IU/mL (see Table 2 and eTable 4 of Grandjean et al. 2012).[37] Moreover, the level of serum antibodies corresponding to a clinically protective level appears to be assay specific. For the ToBI assay apparently used in the Faroe Islands studies, ≥0.01 IU/mL is considered to be the clinically protective level, not the value of ≥0.1 IU/mL indicated by study authors. Considering the comments above, this means that the reported decreases in serum antibodies are even less likely to be biologically/clinically significant. This is not surprising given the rarity of tetanus/diphtheria cases, particularly in those fully vaccinated, and the WOE for PFDA not being associated with statistically significant increases in the incidences of diseases based on the epidemiological literature (all discussed below). To say the least, all this brings into serious question the validity of the EPA's 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 the 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

[35] The confidence interval (CI) for the one statistically significant OR of 1.36 (age 5, diphtheria) is (1.04, 1.77), with the lower end of the CI practically equal to 1 (eTable 4 of Grandjean et al. 2012).

[36] Also, while Grandjean et al. (2017b) state that, "At age 5, 152 (44%) children had antibody concentrations lower than the protective level of 0.1 IU/mL for diphtheria and 126 (36%) for tetanus", this appears inconsistent with Table 1 of that study, which shows that the 25th percentiles for diphtheria and tetanus serum antibody concentrations were 0.1 IU/mL.

[37] A 2-fold increase in PFOS and PFOA concentrations at age 5 years was associated with odds ratios between 2.38 (95% CI, 0.89 to 6.35) and 4.20 (95% CI, 1.54 to 11.44) for falling below a clinically protective level of 0.1 IU/mL for tetanus and diphtheria antibodies at age 7 years.

	• 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.[38] 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.[39] 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), even further calling into question the biological/clinical significance and adversity of the reported results. In regard to confounding and the ability to causally attribute associated effects to PFDA: • 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*." • Similarly, Grandjean et al. (2017b) state [*emphasis added*], "The close correlations prevented meaningful adjustment for concomitant PFAS

[38] 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."

[39] 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."

	exposures." *Thus, it appears that effects may neither rise to the level of adversity nor be attributable specifically to PFDA.* Co-exposures to 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 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 PFDA and PFNA had a correlation coefficient of 0.78 in the blood sera of 5-year olds and IQR differences in blood sera concentrations of less than 1.9-fold each (see Table 2 of the study). Despite Grandjean et al. (2017b) stating that the close correlations prevented meaningful adjustment for concomitant PFAS exposures, Budtz-Jørgensen and Grandjean (2018a) attempts to do just that for benchmark dose, and the results appear to help demonstrate the effects of confounding co-exposures and/or the inability to properly adjust for them. Table 2 of Grandjean et al. (2017b) reports the change (in percent) of the pre-booster serum-antibody concentrations at age 5 years associated with a doubling of the serum concentration of PFDA. Results for cohorts 5, 3, and joint results are in the negative direction for tetanus at age 5 but none are statistically significant, which already points to the unreliability of an effect having been demonstrated by these results and the unreliability of any RfD based on these results or association. Table 2 of Budtz-Jørgensen and Grandjean (2018a) reports benchmark results for the five prenatal PFAS concentrations in regard to antibody concentrations at age 5 years (pre-booster) both unadjusted and adjusted for PFOS/PFOA co-exposures. For tetanus antibodies, while unadjusted benchmark doses for three models for PFDA (linear, piecewise, conservative) appear to show excellent agreement (BMDs of 0.11-0.25 ng/mL) with insignificant reliance on choice of model, when adjusted for PFOS/PFOA the three models' best estimates of the PFDA serum concentrations associated with a 5% change go to infinity (although BMDLs can still be estimated). For diphtheria, Table 2 of Grandjean et al. (2017b) reports statistically significant changes for cohort 3 and joint results for the pre-booster serum-antibody concentrations at age 5 years associated with a doubling of the serum concentration of PFDA. However, similar to results for tetanus, Table 2 of Budtz-Jørgensen and Grandjean (2018a) reports that when benchmark results are adjusted for PFOS/PFOA, two of the three models' best estimates of the PFDA serum concentrations associated with a 5% change go to infinity (although BMDLs can still be estimated). While Table 1 of Budtz-Jørgensen and Grandjean (2018a; benchmark results for the age-5 serum concentrations of five PFASs in regard to tetanus and diphtheria antibody concentrations at age 7 years) provides no benchmark doses for PFDA that go to infinity, Table 2 results point to the unreliability of this endpoint and should be considered along with the other issues raised above. For example, the odds ratios for PFDA and inadequate antibody concentrations for diphtheria and tetanus at 7

years was not statistically significant (see eTable 4 of Grandjean et al. 2012).

Key BMD results in the draft assessment itself demonstrate the importance of co-exposures to other PFAS. Tables C-1 and C-3 of the draft assessment (below) provide BMD results for the critical effects used for RfD derivation (e.g., see Table ES-1).

Table C-1. Results specific to the slope from the linear analyses of PFDA measured in serum at age 5 years and $\log_2$(tetanus antibody concentrations) measured at age 7 years in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b).

Exposure	Model shape	PFOS & PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/m Lin serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at Age 5	Linear	No	-1.55	0.602	p = 0.01	-2.55
PFDA at Age 5	Linear	Yes	-0.98	0.681	p = 0.15	-2.10

PFOS and PFOA had correlation coefficients of 0.39 and 0.35 with serum PFDA at age 5, respectively (Table 2 of Grandjean et al. 2012). Despite these relatively low correlation coefficients (Mukaka 2012), Table C-1 shows that just controlling for co-exposures from these two PFAS (PFOS, PFOA) resulted in significant impacts on slope (β) and slope fit. The slope estimate for PFDA was reduced 37% and *PFDA is no longer a significant predictor of tetanus antibody concentrations* (p=0.15). The most likely explanation (Occam's razor) is classic confounding as PFOS and PFOA are documented immunotoxicants (e.g., per Budtz-Jørgensen and Grandjean 2018a), and the existence of some chance that correction for these co-exposures could create some confounding is not a scientifically robust justification for dismissing the important implications of the results of adjustments for PFOS/PFOA that the study authors themselves (Budtz-Jørgensen and Grandjean 2018a) thought it important to adjust for, and with good reason. *These results demonstrate the statistical unreliability of serum PFDA predicting tetanus antibody concentrations when just two other PFAS are controlled for*. Table C-3 concerns the critical effect for the RfD based on diphtheria antibody concentrations.

Table C-3. Results specific to the slope from the linear analyses of PFDA in serum measured at age 5 years and $\log_2$(diphtheria antibodies) measured at age 7 years from Table 1 in a single-PFAS model and in a multi-PFAS model from (Budtz-Jørgensen and Grandjean, 2018b).

Exposure	Model shape	PFOS & PFOA adjusted	Slope (β) per ng/mL in serum	SE(β) ng/mL in serum	Slope (β) fit	Lower bound slope (β_{LB}) per ng/mL in serum
PFDA at Age 5	Linear	No	-0.894	0.561	p = 0.11	-1.82
PFDA at Age 5	Linear	Yes	-0.297	0.635	p = 0.64	-1.35

The implications of these results are worse. First, even when evaluated alone without accounting for co-exposures to relatively low correlated PFOS and PFOA, serum PFDA is not a significant predictor of diphtheria antibody concentrations (p=0.11). Table C-3 further shows that controlling for co-exposures from these

	two PFAS (PFOS, PFOA) resulted in significant impacts on slope (β) and slope fit. *The slope estimate for PFDA was reduced 67% and serum PFDA became a worse nonsignificant predictor of diphtheria antibody concentrations (p=0.64)*. The most likely explanation (Occam's razor) is classic confounding as PFOS and PFOA are documented immunotoxicants (e.g., per Budtz-Jørgensen and Grandjean 2018a), and the existence of some chance that correction for these co-exposures could create some confounding is not a scientifically robust justification for dismissing the important implications of the results of adjustments for PFOS/PFOA that the study authors themselves (Budtz-Jørgensen and Grandjean 2018a) thought it important to adjust for, and with good reason. *Thus, when co-exposures are taken into account for two modestly correlated PFAS (PFOS, PFOA), serum PFDA is not a significant (i.e., reliable) predictor of these critical effects serving as the basis of the RfD (i.e., decreases in serum tetanus and diphtheria antibody concentrations)*.[40] Moreover, Budtz-Jørgensen and Grandjean (2018a) also controlled for PFOS or PFOA when deriving BMDs/BMDLs for the other, and EPA did use those co-exposure-adjusted results for RfD derivation (i.e., PFOA adjusted for PFOS, PFOS adjusted for PFOA; see Tables B-1 and B-2 in Sections B.1.1 and B.1.2 of the PFOA and PFOS draft assessments USEPA 2021a,b) without expressing any similar concerns about creating confounding by adjusting for these co-exposures (low/moderate correlation coefficient of 0.50 (Mukaka 2012); see Table 2 of Grandjean et al. 2012) or citing Weisskopf et al. (2018) and Weisskopf and Webster (2017). Rather, these concerns have been selectively cited for PFDA in an attempt to provide some rationale for dismissal of the co-exposure-controlled results (e.g., slope (β) values are reduced; serum PFDA is a nonsignificant predictor of tetanus and diphtheria antibody concentrations) and thus for selection of BMDLs uncontrolled for PFOS and PFOA co-exposures (see Tables C-2 and C-4 of the draft), but this is not a scientifically robust rationale and is furthermore inconsistent with EPA's draft assessments for PFOA and PFOS (USEPA 2021a,b). Lastly, it is further noted that 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 PFDA and PFOS/PFOA are low (0.35 and 0.39, respectively). The results of Weisskopf et al. (2018) do not constitute reasonable doubt that for these PFDA results, the confounding from not adjusting for co-exposures to documented immunotoxicants (PFOS, PFOA) is significantly greater than the potential amplification of biases that remains undemonstrated under the same or similar circumstances.EPA should reevaluate the issues raised above in regard to implications for their draft PFDA assessment (**Tier 1 necessary revision**).

[40] Knowing this, disparate results are not particularly surprising, such as the 18.7% increase in tetanus antibodies predicted for children (age 13) with a 2-fold increase in serum PFDA based on the same study and type of analysis used for the RfD critical effects (see Table 3-12, p. 3-59).

	Regarding potential immunosuppressive effects by PFDA, effects that rise to the level of adversity would be expected to result in increased incidences of disease, reflecting lower immunity and lower resistance to disease in the real world. However, consistent with the WOE for no statistically significant associations with antibody concentrations falling below the protective level of 0.1 IU/mL based on results from Grandjean et al. (2012), almost all ORs in Table 3-13 of the draft assessment include 1, indicating that *the WOE from studies on PFDA and infectious disease in humans is for no statistically significant associations*. Consistent with this, host resistance was unaffected by PFDA based on the limited animal study data available (p. 3-73, lines 8-9), and host resistance assays are considered highly relevant to the evaluation of immunotoxicity in the context of human health assessment (p. 3-72, lines 15-18; IPCS 2012). EPA should reevaluate the adversity of these presumed antibody level effects, including the association with PFDA itself, and do so within the context of potential confounding, other limitations, and available human/animal data on disease incidence (**Tier 1 necessary revision**), as this has important implications for the hazard judgment, the strength of human evidence descriptor for immunosuppression (listed as "moderate" in Table 3-19), and whether an RfD should be based on these effects. Moreover, it is noted that the PODs for immunosuppressive effects from these epidemiological studies range from 2.57E-04 mg PFDA/L blood serum to 7.02E-04 mg PFDA/L blood serum ($BMDL_{1/2\ SD}$ values from Table 5-8, pp. 5-16 to 5-17) that when intrahuman variability is considered (through application of a UF_H of 10) results in values of 2.57E-05 to 7.02E-05 mg PFDA/L blood or 0.0257 to 0.0702 µg PFDA/L blood serum.[41] Data from NHANES show that GMs representative of the U.S. population are well above these blood serum levels (See Appendix A NHANES tables).[42] Most notably, 2005-2018 population GMs range from 0.154-0.355 µg/L, which are 2.2- to 13.8-fold higher than the PODs adjusted for intrahuman variability (cited above). Despite these exceedances, tetanus and diphtheria appear to be quite rare in the U.S. population. The average annual number of tetanus cases in the U.S. from 2009-2018 was 29, with the CDC attributing most cases to individuals who either have not been vaccinated or who are not current on their boosters (e.g., only 3% of the cases from 2001-2008 were in people who had received a complete tetanus toxoid series with the last dose within 10 years; Tiwari et al. 2021). Tetanus also appears rare in U.S. children specifically, occurring primarily in older adults. Per Liang et al. (2018): "During 2001-2016, three neonatal tetanus cases and 459 non-neonatal tetanus cases were reported to the National Notifiable Diseases Surveillance System (NNDSS). The median age for non-neonatal cases was 44.0 years (range: 2-95 years)… The risk for both tetanus disease and mortality was higher among persons aged ≥65 years than among persons aged <65 years. Tetanus occurs almost exclusively among persons who are unvaccinated or inadequately

[41] Based on $BMD_{1/2\ SD}$ values (Table C-9, p. C-16), these values are 0.0385 to 0.226 µg PFDA/L blood serum.
[42] See NHANES Biomonitoring Data Tables at https://www.cdc.gov/exposurereport/data_tables.html. Budtz-Jørgensen and Grandjean (2018a) also acknowledge that, "Our BMDL results, both before and after adjustment are generally below current exposure levels…"

	vaccinated or in those whose vaccination histories are unknown or uncertain." The incidence of U.S. diphtheria cases is even more rare. The CDC reported only 14 cases from 1996 through 2018 (Acosta et al. 2021). Thus, consistent with the lack of statistically significant effects from the epidemiology study data discussed above and despite the NHANES blood serum data showing exceedances of the draft assessment human PODs adjusted for intrahuman variability (e.g., toxicodynamic) for most of the U.S. population for a prolonged period of time (see the 50th percentile concentrations in Appendix A), U.S. surveillance disease incidence data are not supportive of adversity. That is, U.S. surveillance disease incidence data do not support that serum PFDA (or any other serum PFAS) is suppressing tetanus and diphtheria vaccine responses and leaving people vulnerable to infection from these diseases. The apparent lack of adversity/ consequence for the effects reported for tetanus and diphtheria certainly does not provide support for an expectation of adversity/consequence for other effects not measured/observed (e.g., for vaccines for other diseases and their incidences). Additionally, it appears that EPA has not fully considered all the relevant evidence or the weaknesses/limitations of the epidemiological evidence, which in turn are relevant for the hazard judgment and whether an RfD should be derived based on these effects. Table 3-19 indicates that human data provide "moderate" evidence and that "the inconsistent and low confidence evidence on infectious disease did not influence this judgment." However, this points to the fact that EPA has not duly considered the implications of the null findings on human and laboratory animal infectious disease and other relevant considerations (e.g., some discussed above) for the scientific WOE, which is not a scientifically supportable approach as it does not consider all relevant data, directly relevant human data in particular. EPA should consider such null findings (and other relevant considerations) in their WOE (**Tier 1 necessary revision**). Combined with the "slight" human data for sensitization and allergic response, the "slight" laboratory animal data for immunosuppression, and the "indeterminate" animal data for sensitization and allergic response (Table 3-19), it does not appear that PFDA exposure is "likely to cause" adverse immune effects in humans is sufficiently supported. EPA should reevaluate this determination (**Tier 1 necessary revision**) as "may cause" might very well be the better supported hazard judgement, and also reconsider their use of the serum antibody endpoints for quantitative risk assessment/derivation of toxicity factors (**Tier 1 necessary revision**). This would appear more consistent with the data discussed above and recent conclusions by the Australian government (FSANZ 2021) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 2021). 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." Based on the information in the draft assessment and reviewed elsewhere, this reviewer finds it difficult to disagree with the recent conclusions of the Australian government (FSANZ 2021) and ATSDR (2021) that the epidemiology literature (e.g., on PFAS blood levels and impaired vaccine response) is inadequate for quantitative risk assessment and use as the basis for deriving toxicity factors (e.g., RfDs). 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals. The above comments provide relevant rationale for use of an alternative study(ies) or effect(s) for subchronic RfD derivation. While the draft assessment provides various but limited candidate PODs, due consideration of the comments above appears to require a more complete evaluation of alternative studies/effects (**Tier 1 necessary revision**). One method to begin such an evaluation may entail sorting the study data extracted by duration, then NOAEL/LOAEL, BMD or a similar criterion that would allow EPA to readily identify the next most sensitive effects based on less problematic studies that are adequate for subchronic RfD derivation. However, any newly derived toxicity factor should be subject to external expert peer review (**Tier 1 necessary revision**). **<u>Birth Weight Endpoint</u>** EPA's hazard conclusion for developmental effects primarily relies on in vivo animal

	data (i.e., the gestational exposure mouse study of Harris and Birnbaum 1989). Based on over 45 different epidemiological studies included in the draft assessment, the evidence of an association between PFDA exposure and developmental effects in humans is considered only "slight" (p. 3-156, lines 10-11). Despite the "slight" evidence in humans, Table ES-1 indicates that RfDs were nevertheless calculated based on decreased birth weight in male and female children (Wikström et al. 2020). While data in humans, as the species of ultimate interest, are usually preferred as the basis for derivation of toxicity factors, in this case it appears that the mere "slight" totally of the evidence for developmental effects from the over 45 different epidemiological studies included in the draft assessment should not be considered sufficient for quantitative dose-response assessment, but rather only for supportive information for hazard identification. EPA Figure 3-28 (p. 3-108), reproduced below, helps illustrate the weakness and incoherence of the overall epidemiological database for demonstrating decreased birth weight, particularly statistically significantly decreased birth weight, associated with PFDA levels and how no one epidemiological study could possibly be representative of these inconsistent and disparate results or the results of a meta-analysis of relevant studies.

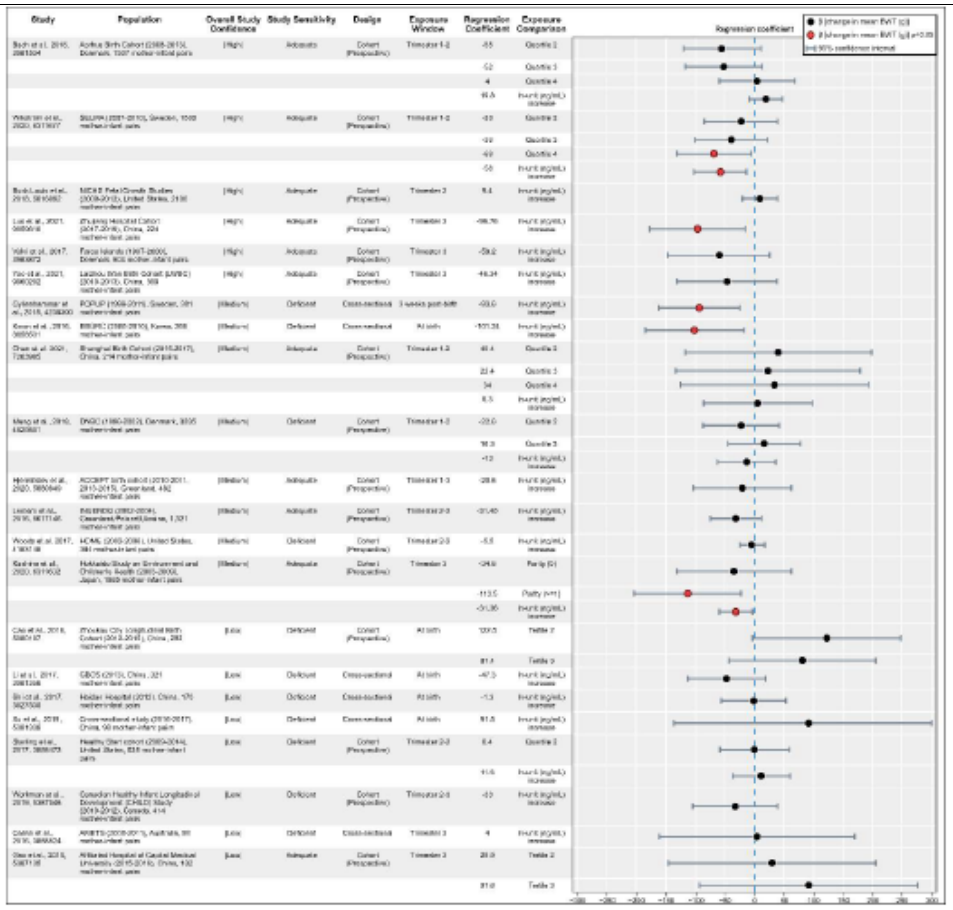

Figure 3-28. Overall study population mean birth weight results for 22 PFDA epidemiological studies[a-e]. (results can be viewed by clicking the HAWC link).

Abbreviation: BWT = Birth Weight

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b] Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during the first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c] If a study presented regression coefficients for continuous exposure with multiple exposure units, only one unit change is shown (e.g., (Bach et al., 2016), with the exception of (Li et al., 2017), which displays both IQR and ln-unit (ng/mL) values.

[d] The results displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared to a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[e] Xu et al. (2019a) results are truncated for the 210.7 gram increase; the complete 95% CI ranges from -314.3 to 735.8 grams.

Similarly, Figure 3-26 (p. 3-104) below illustrates how the results of Bach et al. (2016), the other high confidence, prospective cohort for trimester 1-2 with adequate study sensitivity, had a nonmonotonic response, no statistically significant quartile results for the overall population, and a regression coefficient of +0.03 per each ln-unit (ng/mL) (compared to -0.147 per each ln-unit increase for Wikström et al. 2020), all of which does not support use of the Wikström et al. (2020) study results. The Bach et al. (2016) and Wikström et al. (2020) studies are the first two entries in Figure 3-26, respectively.

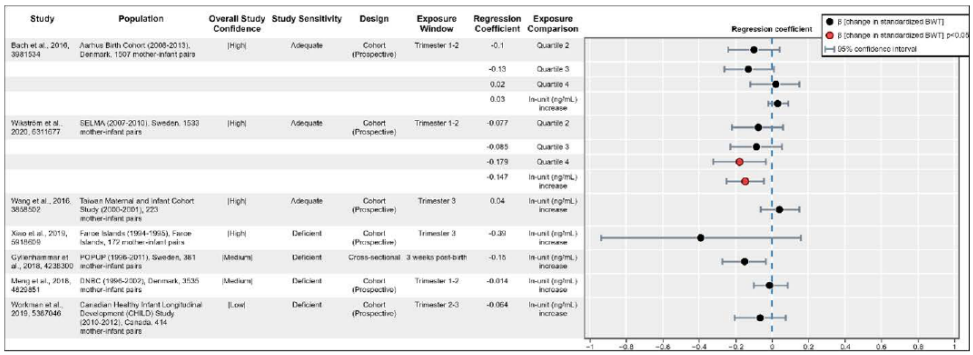

Figure 3-26.PFDA and birth weight z-scores (overall population)[a]. Refer to Birth Weight-Z for details on the individual study evaluation review.

Moreover, the BMD_{5RD} values for decreased birth weight based on Wikström et al. (2020) range from 0.54 to 0.71 ng/mL (note that BMD_{5RD} values are not treated like NOAELs for purposes of RfD derivation), while the $BMDL_{5RD}$ values range from 0.31 to 0.37 ng/mL (Table C-10, p. C-22). By comparison, PFDA blood serum concentrations in quartile 4 of the Bach et al. (2016) study range from 0.43 to 2.87 ng/mL (see Table 1 of the study). Despite these quartile 4 blood serum concentrations being up to 5.3-fold higher than the BMD_{5RD} values for decreased birth weight based on Wikström et al. (2020), birth weight and birth weight z-scores for quartile 4 in Bach et al. (2016) were actually increased (nonsignificantly) (see Table 3 of the study). Again, this is from the other high confidence, prospective cohort for trimester 1-2 with adequate study sensitivity. These results from Bach et al. (2016), obtained from the other high confidence study in the same PFDA blood serum concentration range (and higher) compared to EPA's BMD_{5RD} values, are entirely inconsistent with and unsupportive of EPA's use of the PODs for adverse birth weight effects based on Wikström et al. (2020) for RfD derivation. Sex-specific results from Bach et al. (2016) do not provide strong support for use of Wikström et al. (2020) results for quantitative dose-response assessment either (e.g., Figure 3-29, p. 3-109 of the draft assessment).

Given the drastically different results across epidemiological studies and the mere "slight" evidence of developmental effects across more than 45 such studies, EPA should reconsider use of a single epidemiological study (Wikström et al. 2020) for dose-response assessment of birth weight and RfD derivation and consider a meta-analysis and/or using the more definitive dose-response data from the mouse study for dose-response assessment of birth weight and RfD derivation (**Tier 1 necessary revision**). It is remarkable that per EPA, the single mouse study by Harris and Birnbaum (1989) gives rise to a greater level of evidence for developmental effects ("moderate") than the results of over 45 epidemiological studies. This fact alone, in this case, justifies use of the animal data for dose-response assessment and osRfD derivation. Based on EPA Figure 3-56 (p. 3-152), reproduced below, the dose-response data for fetal body weight (as an example) appear well suited for BMD analysis.

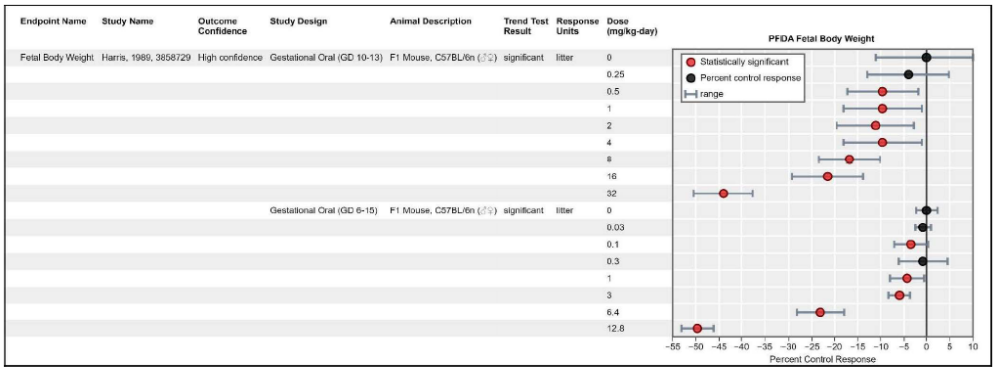

Figure 3-56. PFDA fetal body weight after gestational exposure (results can be viewed by clicking the HAWC link).

More specifically, Figure 3-56 shows statistically significant and progressive decreases/trends in fetal body weight with dose across a range that includes the >5% decrease historically used by regulatory agencies as the demarcation of adversity in dose-response assessment (i.e., as a >5% reduction in fetal body weight is usually considered adverse, a benchmark response of 5% is typically used in BMD analysis), from -1% to -50% and including doses that resulted in decreases of -4% and -6% (Table 3-23, p. 3-153). Furthermore, the changes in fetal body weight were at doses not associated with maternal toxicity (p. 3-151, lines 5-6).

Harris and Birnbaum (1989) is a medium/high-confidence study (p. 3-150, lines 9-12), and EPA should consider mouse data from the Harris and Birnbaum (1989) gestational exposure study for use as the primary basis for subchronic osRfD development (**Tier 1 necessary revision**) based on developmental growth effects consistent with: (1) this mouse study providing the primary basis for EPA's developmental effects hazard conclusion; (2) the lack of factors that decrease certainty for fetal growth as evaluated in the mouse study (see Table 3-24); and (3) the substantial epidemiological database merely being able to provide support for this mouse study with what amounts to "slight" evidence for developmental effects across over 45 epidemiological studies. Afterall, per the draft assessment, it is the mouse data that primarily support that assuming sufficiently high exposure over a sufficiently long duration (i.e., "given sufficient exposure conditions"), PFDA exposure is likely to cause developmental toxicity in the general human population, which includes potentially susceptible subpopulations (e.g., developing fetuses of pregnant women).

My suggestion to use the mouse data (Harris and Birnbaum 1989) instead of epidemiological study data (Wikström et al. 2020) for dose-response assessment and osRfD derivation based on developmental growth effects (**Tier 1 necessary revision**) would make EPA's assessment somewhat more consistent with ATSDR (2021),[43] who found *the epidemiology literature inadequate for use as the basis of deriving MRLs*

[43] To be entirely consistent with this conclusion from ATSDR (2021) and my own comments above, the EPA assessment would further need to omit the highly uncertain RfD derivations based on PFDA associations with decreases in antibody levels/vaccine response (**Tier 1 necessary revision**) reported in some epidemiological study results that appear overall to be inconsistent, non-adverse, and uncertain in nature (e.g., causal attribution problems, unsupported by epidemiological disease incidence studies).

	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." Getting back to use of Wikström et al. (2020) for dose-response assessment of birth weight and RfD derivation, Tables 2 and 3 from that study appear below (Table 2 unaltered, Table 3 *statistically insignificant results* for PFDA associations with decreased birth weight and small for gestational age *are highlighted in red*; see license at https://creativecommons.org/licenses/by/4.0/) and demonstrate: (1) significant co-exposures to other PFAS (e.g., PFOS, PFOA) associated with birth weight deficits, an ATSDR concern and significant uncertainty; and (2) few statistically significant ORs for decreased body weight in the PFDA exposure quartiles; that is, only 2 out of 9 ORs showed statistically significant birth weight decrements, which were for quartile 4 where both PFOS and PFOA were certainly co-exposures and also showed statistically significant decreases for birth weight. In regard to this latter point concerning potential confounding by co-exposure to other PFAS, which was cited by ATSDR as a significant uncertainty, Wikström et al. state [*emphasis added*]: "*Another limitation is the compound-by-compound approach*. Theoretically, *a health outcome is simultaneously influenced by multiple environmental factors*. Nevertheless, the exposure to several PFASs may be correlated with each other due to common sources. Our findings were consistent across different PFAS compounds, and we regard correction for multiple comparisons overly conservative to be suitable for the investigations on such interrelated compounds. If the single compound's level could represent the levels of several other compounds, *our findings based on single compound analyses may still shed some light on the joint effects of multiple PFAS compounds*. However, carefully designed statistical models, such as *mixture-based approaches* within the PFAS compound class, *should be explored in follow-up studies*." *Thus, the study authors are acknowledging confounding by other PFAS as an important limitation of their compound-by-compound approach* as joint effects of multiple PFAS compounds may be occurring such that mixture-based approaches should be explored for data analysis. *The compound-by-compound approach used in this study gives rise to significant uncertainty that precludes use of these data for dose-response assessment and RfD derivation*. It cannot be confidently said scientifically that the presumed effects are due to PFDA exposure (e.g., PFOS and PFOA were certainly co-exposures

and also showed statistically significant decreases for birth weight). Consistent with my opinion on the significant uncertainty and confounding associated with these Wikström et al. results and the weakness/limitations of the epidemiological evidence overall, Section F.3 of the draft assessment states that, "In the six studies using mutually adjusted PFAS approaches to address coexposures, there was not consistent evidence for birth weight deficits associated with increased exposure to PFDA." (p. F-27, lines 13-14), and acknowledges that "there is considerable uncertainty due to potential confounding by co-occurring PFAS in the existing literature." (p. F-27, lines 30-32). Indeed, significant co-exposure to multiple PFAS is not the exception but the rule, and is just the condition to result in significant bias away from the null for adverse effects.[44] The high confidence study of Luo et al. (2021) is an example that serves as a cautionary tale on the obvious importance of PFAS co-exposures, which reported large statistically significant birth weight deficits (-97 g; -178, -16 per each ln-unit PFDA increase) in a single-pollutant PFDA model, but results were null and their direction reversed in the multipollutant model with a nonsignificant increase in birth weight associated with PFDA (Table F-2).[45]

As the data from epidemiological studies provide only "slight" evidence of developmental effects, including data from Wikström et al. (2020) that appears too uncertain and unsuitable for quantitative dose-response assessment, I suggest use of the mouse data (Harris and Birnbaum 1989) for dose-response assessment and subchronic osRfD derivation based on developmental growth effects due to gestational exposure (**Tier 1 necessary revision**). See previous comments about the apparent amenability of these mouse data to BMD analysis. 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 definitively showing cause-and-effect for unquestionably adverse effects, unconfounded by significant co-exposures to similar chemicals.

Table 2. Prenatal exposure to eight PFAS, measured as maternal serum concentrations (ng/mL) during early pregnancy.

Compound	Geometric mean [95% CI]	Median (IQR)	95th %	LOD	Above LOD (%)
PFOS	5.35 [5.21–5.50]	5.38 (3.97–7.60)	10.34	0.06	100
PFOA	1.60 [1.56–1.65]	1.61 (1.11–2.30)	3.18	0.02	100
PFHxS	1.31 [1.28–1.35]	1.23 (0.86–1.99)	2.94	0.03	100
PFNA	0.54 [0.53–0.56]	0.53 (0.39–0.73)	1.02	0.01	100
PFDA	0.26 [0.26–0.27]	0.26 (0.19–0.34)	0.50	0.02	100
PFUnDA	0.21 [0.21–0.22]	0.23 (0.15–0.33)	0.44	0.02	99.5
PFHpA	0.018 [0.017–0.019]	0.02 (<LOD–0.04)	0.077	0.01	73.9
PFDoDA	0.027 [0.026–0.027]	<LOD (<LOD–0.05)	0.08	0.03	46.7

LOD limit of detection

[44] To be a confounder, the co-occurring PFAS would need to be associated with both the PFAS of interest and the outcome, but not an intermediate in the causal pathway; such PFAS would be considered positive confounders if their effect estimate with the endpoint of interest is in the same direction as the primary PFAS of interest. If positive confounders are not accounted for, the anticipation is that any resultant bias would be away from the null. (p. F-24, lines 16-21). The stronger the association between co-exposures, the larger the effect size for the co-exposure of interest (i.e., the greater the confounding) (p. F-25, lines 14-15).

[45] The other high confidence study in Table F-2 (Starling et al. 2017) showed that adjustment for PFAS co-exposures in the multipollutant model resulted in a large statistically significant increase in birth weight for PFDA (+97.5 g; 31.5, 163.6), which for some reason is not discussed in Section F.3 (*PFDA and PFAS Coexposure Study Results*) but serves as yet another example of the obvious, commonsensical importance of accounting for PFAS co-exposures.

Table 3. Associations between prenatal PFAS exposure[a] and birth weight[b], together with odds ratios for birth weight small for gestational age in 1533 children.

	All children		Girls		Boys	
	BW (g) β (95% CI)	SGA OR (95% CI)	BW (g) β (95% CI)	SGA OR (95% CI)	BW (g) β (95% CI)	SGA OR (95% CI)
PFOS						
Per ln-unit	−46 (−88; −3)	1.19 (0.87; 1.64)	−85 (−145; −25)	1.40 (0.83; 2.35)	−13 (−73; 47)	1.08 (0.72; 1.63)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−27 (−89; 35)	0.69 (0.43; 1.08)	−32 (−115; 52)	0.89 (0.39; 2.03)	−28 (−118; 63)	1.26 (0.67; 2.37)
Q3	−22 (−84; 41)	0.79 (0.53; 1.18)	−51 (−137; 34)	0.82 (0.36; 2.03)	5 (−86; 96)	0.86 (0.45; 1.67)
Q4	−80 (−144; −16)	1.56 (1.09; 2.22)	−142 (−231; −54)	2.05 (1.00; 4.21)	−28 (−119; 63)	1.30 (0.70; 2.40)
PFOA						
Per ln-unit	−68 (−112; −24)	1.43 (1.03, 1.99)	−86 (−145; −26)	1.96 (1.18; 3.28)	−49 (−113; 15)	1.16 (0.75; 1.78)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	27 (−35; 89)	0.77 (0.45; 1.32)	30 (−55; 115)	1.00 (0.40; 2.51)	26 (−66; 116)	0.67 (0.34; 1.31)
Q3	−41 (−106; 23)	0.96 (0.57; 1.61)	−36 (−124; 52)	1.64 (0.71; 3.83)	−44 (−139; 50)	0.66 (0.33; 1.29)
Q4	−90 (−159; −91)	1.44 (0.86; 2.40)	−136 (−231;−40)	2.33 (1.00; 5.43)	−47 (−147; 54)	1.04 (0.54; 2.01)
PFHxS						
Per ln-unit	−0.1 (−38; 38)	0.96 (0.72; 1.27)	−14 (−68; 39)	1.14 (0.73; 1.80)	−13 (−67; 41)	0.84 (0.58; 1.22)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−4 (−66; 58)	1.37 (0.86; 2.20)	30 (−56; 116)	1.77 (0.78; 3.99)	−39 (−129; 50)	1.24 (0.69; 2.23)
Q3	−15 (−78; 48)	0.89 (0.54; 1.47)	28 (−59; 115)	1.05 (0.44; 2.49)	−51 (−141; 39)	0.82 (0.44; 1.54)
Q4	−6 (−69; 57)	1.04 (0.63; 1.69)	−16 (−104; 71)	1.76 (0.79; 3.90)	1 (−90; 92)	0.73 (0.38; 1.41)
PFNA						
Per ln-unit	−46 (−89; −4)	1.38 (1.02, 1.87)	−52 (−117; −2)	1.34 (0.85; 2.11)	−50 (−113; 14)	1.42 (0.94; 2.17)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	7 (−55; 69)	0.83 (0.49; 1.38)	−2 (−86; 82)	0.66 (0.29; 1.52)	15 (76; 106)	0.97 (0.50; 1.89)
Q3	−39 (−102; 24)	1.14 (0.70; 1.85)	−49 (−137; 38)	1.39 (0.66; 2.90)	−28 (−119; 64)	0.95 (0.50; 1.82)
Q4	−33 (−96; 31)	1.23 (0.77; 1.99)	−66 (−153; 20)	1.22 (0.59; 2.53)	1 (−94; 95)	1.24 (0.66; 2.33)
PFDA						
Per ln-unit	−58 (−103; −13)	1.46 (1.06, 2.01)	−69 (−133; −6)	1.62 (0.98, 2.67)	−47 (−112;17)	1.36 (0.90; 2.07)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−23 (−85; 39)	1.03 (0.62; 1.69)	−42 (−126; 42)	0.86 (0.37; 2.00)	−8 (−99; 82)	1.18 (0.63; 2.23)
Q3	−39 (−101; 23)	1.07 (0.65; 1.76)	−74 (−160; 13)	1.20 (0.54; 2.67)	−8 (−98; 81)	0.99 (0.52; 1.89)
Q4	−69 (−132; −5)	1.50 (0.94; 2.38)	−116 (−204; −27)	1.95 (0.94; 4.06)	−27 (−118; 64)	1.21 (0.66; 2.23)
PFUnDA						
Per ln-unit	−13 (−49; 22)	1.21 (0.92; 1.58)	−24 (−75; 27)	1.08 (0.70; 1.67)	−6 (−55; 42)	1.29 (0.82; 1.83)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−67 (−153; 19)	1.24 (0.77; 2.01)	−67 (−153;19)	2.09 (0.94; 4.63)	41 (−48; 130)	0.90 (0.48; 1.68)
Q3	−42 (−128; 44)	0.85 (0.51; 1.43)	−42 (−128; 44)	1.01 (0.42; 2.44)	46 (−44; 136)	0.81 (0.43; 1.56)
Q4	−46 (−110; 17)	1.52 (0.95; 2.44)	−93 (−183; −3)	1.92 (0.86; 4.25)	−10 (−100; 80)	1.36 (0.76; 2.46)
PFHpA						
Per ln-unit	−1 (−24; 21)	1.06 (0.90; 1.25)	−4 (−34; 26)	1.06 (0.83, 1.36)	−0.03 (−33; 32)	1.07 (0.86; 1.37)
Q1	Reference	Reference	Reference	Reference	Reference	Reference
Q2	−0 (−62; 61)	0.92 (0.57; 1.48)	17 (−70; 103)	0.92 (0.44; 1.92)	−15 (−102; 73)	0.94 (0.50; 1.75)
Q3	3 (−59; 65)	0.78 (0.48; 1.27)	−21 (−106; 65)	0.75 (0.35; 1.65)	23 (−65; 112)	0.78 (0.42; 1.45)
Q4	31 (−31; 93)	1.25 (0.85; 1.84)	27 (−57; 112)	1.31 (0.66; 2.60)	33 (−58; 124)	1.15 (0.65; 2.04)

All analyses were adjusted for maternal weight, parity (three categories) and cotinine levels. Analyses including both boys and girls were in addition adjusted for sex and analyses of BW were adjusted for GA. SGA was defined as BW 10th percentile for sex and GA
[a]Associations with PFAS are presented per ln-unit and by quartiles of exposure, as related to
[b]Birth weight (g) and odds ratios (adjusted) for birth weight small for gestational age

The above comments provide relevant rationale for use of an alternative study(ies) or effect(s) for subchronic RfD derivation. While the draft assessment provides various but limited candidate PODs, due consideration of the comments above appears to require a more complete evaluation of alternative studies/effects (**Tier 1 necessary revision**). One method to begin such an evaluation may entail sorting the study data extracted by duration, then NOAEL/LOAEL, BMD or a similar criterion that would allow EPA to readily identify the next most sensitive effects based on less problematic studies that are adequate for subchronic RfD derivation. However, any newly derived toxicity factor should be subject to external expert peer review (**Tier 1 necessary revision**).

c. As I do not agree with the selection of these studies/endpoints for lifetime or subchronic RfD derivation, please see my comments under subsection “b” above, many of which concern the considerations of adversity and scientific support.

	That being said, speaking more generally in regard to deriving a subchronic RfD, subchronic RfDs must be protective against adverse effects occurring over subchronic and shorter durations of exposure, such as during childhood or during development (e.g., critical windows of development during gestation). Thus, if effects that occur over a subchronic or shorter duration of exposure are truly adverse, relevant to humans, and are the first to occur as the dose rate increases (i.e., the critical effect), they can appropriately serve as the basis for a subchronic RfD. Lastly, since I suggest use of the mouse study of Harris and Birnbaum (1989) as the basis of a subchronic osRfD based on decreases in fetal body weight (with various gestational exposures), I note that Figure 3-56 of the draft assessment shows statistically significant and progressive decreases/trends in fetal body weight with dose across a range that includes the >5% decrease historically used by regulatory agencies as the demarcation of adversity in dose-response assessment (i.e., as a >5% reduction in fetal body weight is usually considered adverse, a BMR of 5% is typically used in BMD analysis), from -1% to -50% and including doses that resulted in decreases of -4% and -6% (Table 3-23, p. 3-153). Furthermore, the changes in fetal body weight were at doses not associated with maternal toxicity (p. 3-151, lines 5-6). Harris and Birnbaum (1989) is a medium/high-confidence study (p. 3-150, lines 9-12). d. The section *Derivation of Candidate Lifetime Toxicity Values for the RfD* (p. 5-19) provides reasonable rationale for only considering liver effects data and male/female reproductive effects data for subchronic osRfD derivation. While the total composite uncertainty factor for these effects is already considerable (1,000; Table ES-1), EPA could consider increasing it to the upper end of their total uncertainty factor range (3,000) to estimate chronic osRfDs. e. Section 5.2.4 (p. 5-41, lines 35-36) states, "No studies examining inhalation effects of short-term, subchronic, chronic or gestational exposure for PFDA in humans or animals have been identified, precluding the derivation of an RfC." Furthermore, it appears from Sections 3.1.6 and 3.1.7 of the draft assessment that a reliable PBPK/PK model has not been identified for PFDA that could be used for route-to-route extrapolation. If EPA has not identified a reliable PFDA PBPK/PK model that could be used for route-to-route extrapolation, then EPA's decision not to derive a subchronic or chronic RfC would be fully justified. However, this is not explicitly stated in Section 5.2.4, which should clearly state whether or not EPA has identified a reliable PFDA PBPK/PK model for route-to-route extrapolation (**Tier 1 necessary revision**).
Hoberman	The selected studies to derive the subchronic RfD for PFDA were scientifically justified. a. The studies used to derive the subchronic RfD were typically of 4 weeks in duration and appropriate for use to derive a subchronic RfD. b. Not applicable.

	c. The effects selected were 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. This reviewer agrees that "the subchronic osRfDs for liver, male reproductive and female reproductive effects derived from short-term animal data were several orders of magnitude higher than the subchronic osRfDs for immune and developmental effects in humans; therefore, they were not considered to be sufficiently protective for consideration in the selection of the overall subchronic RfD. Also, in the case of male and female reproductive effects, confidence in the respective osRfDs was lower compared to the immune osRfD (medium-low versus medium) due to deficiencies in the evidence base for these health effects" (Page 5-41) e. This reviewer agrees that with "no studies examining inhalation effects of short-term, subchronic, chronic or gestational exposure for PFDA in humans or animals have been identified, precluding the derivation of an RfC." (Page 5-41)
Leung	Toxicological modeling is not my area of expertise, and I defer this question to the other external reviewers.
Zoeller	a. The Agency clearly explained the use of the same studies for derivation of lifetime and subchronic RfDs. The issues regarding adversity etc are developed under Question 3 above. **No Recommendation.** d. This is covered in Question 3 above. e. This is a rational decision. **No Recommendation.**

3.5 Appendix G identifies the potential for pharmacokinetic (PK) differences across species and sexes as a key science issue and lays out a hierarchy for using relevant PK data in extrapolating doses between laboratory animals and humans. 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. However, the evaluation of existing PBPK models and a one-compartment PK model found that these options were not sufficiently reliable for use. Given the information available on potential interspecies differences in PFDA PK, EPA applied a data-derived extrapolation factor (DDEF) to POD values from toxicity studies in laboratory animals to estimate corresponding human equivalent doses (HEDs) in the derivation of the respective RfDs. Similarly, the estimated human clearance (CL) was used to convert internal dose POD (PODint) values from epidemiological analyses to corresponding HEDs.

After publicly releasing the draft IRIS PFDA assessment, the EPA evaluated recently published data for several other long-chain PFAS, described here (U.S. EPA, 2023, HERO ID

11181055), that are potentially relevant to evaluating PFDA dosimetry in women of childbearing age (see question 5c below).

a. Is applying the estimated DDEF values for PFDA 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 application of the human CL to estimate HEDs from PODint values scientifically justified? If not, please provide an explanation and detail on a more appropriate approach.

c. Have the uncertainties in the DDEFs and human CL been adequately evaluated and described? In answering this question, please provide an explicit recommendation on whether or not EPA should expand its adjustment for menstrual fluid loss as outlined in (U.S. EPA, 2023, HERO ID 11181055) prior to finalizing the assessment. As these newer data are from other PFAS, note that such an expansion would be based on the assumption that the pharmacokinetic effect of pregnancy and lactation on PFDA is similar to that of the other PFAS (i.e., a read-across based interpretation).

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors	
Reviewer	**Comments**
Adgate	**Tier 1**: The presentation of the complex relevant data landscape on PFDA and shortcomings of the single available PK model can be improved substantially. Given the shortcomings of the underlying data from both animals and humans the DDEF approach is justified in this case, with the caveat that there needs to be an Evidence Integration section in the Appendix AND main PFDA document that lay out the logic of the choices made more clearly. Most importantly, this section needs to explicitly lay out the uncertainties as judged by the scientists who have done this extensive evaluation of the existing data. EPA's remarks, especially the summary provided by Dr Dzierlenga, should be used as a basis for this improved Evidence Integration section. This EI section should also contain a table that explicitly summarizes the key uncertainties and judgments on the relative importance of each uncertainty for model performance and for informing future efforts to improve the existing model or create new models that can provide insight into PFDA pharmacokinetics. It is notable that the DDEF approach was chosen due to the uncertainties of the model parameters and quality of the underlying data. The presentation could be improved if the DDEF approach were applied to another, more data rich, PFAS (e.g., PFOA or PFOS) to see how the DDEF performs under those circumstances. Lastly, this section should clearly acknowledge that half-life for PFAS in general and likely PFDA in particular is often represented by a mean that often understates the wide observed variability in half-lives observed for many similarly persistent PFAS in humans. At a minimum half-life should be reported as a mean or median, standard deviation and/or geometric standard deviation, and, if possible, range of observed values.

Carignan	a. As explained during the meeting the approach seemed reasonable, however it was complicated and not readily apparent from reading the document. Modeling should be sure to consider enterohepatic recirculation and kidney resorption. However, this type of modeling is not my main area of expertise. [**Tier 2**] b. It was not clear from the description that assumptions are based on animal data, which introduces substantial uncertainty. [**Tier 1**] c. This seems like a reasonable assumption. It is important to consider that not all women menstruate, bear children or breast feed. Those who do not would of course have lower clearance than those who do. [**Tier 1**] Of course these considerations are essential in order to protect the fetus, infants and children. **Additional comments:** Discussion of the magnitude and directionality of uncertainty. [**Tier 2**] Recommend adding a table of uncertainties and prioritization for evidence integration. [**Tier 2**]
Faustman	a. This reviewer supported the actions by the USEPA to use DDEF values. Ideally, as the report states a specific PBPK model would be ideal however this review was convinced by the extensive discussion that any existing models are of limited application. b. This reviewer was supportive of the actions by the USEPA to use human CL values to estimate HEDs. In particular, the discussion on distribution and known roles of Oat transporters was of interest in the discussion of PFDA as there is increasing information regarding developmental differences in the developmental time of expression. This reviewer was also glad to see discussion on the degree of protein binding of PFDA and how it affects both distribution and elimination on page 3-3, however minimal discussion of pregnancy related changes in protein binding was not especially emphasized (see earlier comments about hemodynamics during pregnancy). This reviewer was looking for discussion of transfer of PFDA via breast milk but minimal to no comments were made on this aspect of PK. This issue was raised in the public comments but does need to be discussed in the document. A **Tier 2 recommendation** is made to acknowledge the finding of differences in a variety of pharmacodynamic parameters including protein binding during pregnancy with linked comment on potential for breast milk lactational pharmacokinetic considerations. Addition of references and several sentences would be suggested. This reviewer was less convinced about the importance of menstrual blood loss and increased elimination in women reportedly due to this process. It was listed as an exercise to look at potential impact however there are so many factors that affect population dynamics and potential loss by this pathway that further thought would be need to determine human impact. Due to various underlying conditions, there is

	tremendous variability across the female proportion of women and menstrual flow. For example, many women do not experience regular periods. Would the USEPA use a population distribution to determine this variation in more details. This reviewer did not determine that this was a consideration that was "ready for prime-time application "and was glad to see that this was not applied as an adjustment. c. This reviewer did not identify other areas or factors to be examined. This reviewer appreciated the efforts by USEPA to evaluate available PBPK models as well as characterize both a two and one compartment model with Bayesian modeling efforts. Due to the ultimate need to improve cross compound PFAS comparisons such a model would provide important tools to accomplish these comparisons. This reviewer however agreed with the ultimate decisions to apply a more simple approach in the document due to lack of fully supported pregnancy based PBPK model availability.
Fisher	a. The reasons stated why data-derived extrapolation factors (DDEFs) were used are because of the publication of a poor quality PBPK model for PFDA (Kim et al. 2019) and the inability to predicted plasma levels in rats gavage dosed with PFDA daily for 28 days (NTP 2018) using a compartment model calibrated using single dose PFDA PK data (IV and oral). I think the EPA should continue to resolve these issues and use a PBPK model (**Tier 1**) beyond what has been published by EPA authors (Bernstein et al. 2021). It would be useful to have a PBPK model that can describe several PFAS compounds, both single doses and repeated dosing scenarios. It would provide substantial utility in the future. It appears that the EPA modeling group started down this path (Bernstein et al. 2021) using the Kim model along with other PBPK models for PFOA and PFNA. I would call this harmonization of existing models. I was struck by this finding because this is what we found when working with perchlorate (single dose versus multi-day drinking water exposures), which inhibits thyroid uptake of iodide. Our conclusion was that the transporter which transports iodide into the thyroid gland (sodium iodide symporter (NIS) protein) was upregulated by the thyroid stimulating hormone, TSH. In this case with PFDA there is potential involvement of transporters in the kidney and the liver, which may be altered by PFDA itself. The liver was a target for toxicity, which is discussed in detail in this report and may be associated with the altered PK profiles of PFDA (biliary transporters, for example). The accumulated PFDA concentrations (in plasma) were well below model predictions (Fig. G-8) even at the lowest doses, suggesting alterations in binding profiles in plasma or tissues and/or transporter proteins that are key for PFDA pharmacokinetics. Without seeking resolve for the PFDA pharmacokinetics for repeated daily exposure in animals I am skeptical of using a DDEF methodology that relies on the ratio of calculated whole body total clearance rates (CLhuman/CLrat). More work is needed to better understand the pharmacokinetics of PFDA for repeated animal exposures in the context of a PBPK model. **Tier 1.** I would look at the protein transporters involved and determine if there is experimental evidence for the up or downregulation (gene level or protein), including PFOA and PFNA. I would evaluate where the transporters are located and their functions. If protein binding is saturated in plasma, the liver or other organs this could lead to increased

	clearance. Is there experimental evidence with PFNA or PFOA? Is a 3X increase in CL approaching the GFR? Is there evidence for changes in clearance with PFNA or PFOA in rats exposed daily for weeks? This might be called read across, but I call this harmonization. b. HED= PODint (serum concentration of PFDA) x CLhuman. The downside is that no dose-response information was obtained. This is a serum concentration associated with a single response. The only way to strengthen this would be to have all the individual data on serum concentrations and immune response. Then a POD could be estimated, perhaps (**Tier 2**). Another assumption is that the PODint garnered from human blood sampling is robust. The human CL values encompassing the values reported by Fujii et al. (2015) and Zhang et al. (2013b) are valuable. I would prefer healthy individuals with spot urine samples (86 individuals, Zhang et al. (2013b)) over a smaller number of older hospitalized individuals (bile/serum, n=5) with a 24 hr urine /blood samples from 10 young volunteers (Fujii et al (2015). Both studies should be used to capture the potential variability in CL. I believe this leads to a probabilistic type assessment of HEDs using human serum concentrations of PFDA. **Tier 2.** c. Yes, the authors discuss the shortcomings of the studies used and how CL was calculated. The big problem is that protein transporters (liver and kidney) and serum and tissue (?) protein binding are responsible for the slow whole body CL rate. A PBPK model is greatly favored over the current approach because the key biological factors can be explicitly described and used in predictions. Animals are much different than humans. The use of allometric relationships is of little or no value for across species scaling. **Tier 2.** HERO 10410674 does have data on PFDA in the supplemental table (S2). In pregnancy many changes occur that can affect the measured plasma levels of a drug or chemical. In this case, albumin concentrations decrease (PFDA is highly bound to albumin), plasma volume increases by up to 50%, GFR increases and renal blood flow. These changes alone may account for the observed decreases in PNDA (in this paper) and other PFAS. There are many good reviews of pregnancy, physiology, and pharmacokinetics (Ward and Varner 2019, Clin Perinatol 46 383-398, Pariente et al., 2016). Pariente et al. report pharmacokinetic parameter changes for drugs in pregnancy. In lactation, lactational transfer and physiology both play a role in the measured levels of PFAS and PNDA. I would not call it menstrual fluid loss during pregnancy, but apparent pregnancy related changes in distribution and clearance. Table S2 shows a slight trend of decreasing serum PFDA. Again what are the observations, if any, for PFNA and PFOA?

	Clarification: Since there is data on PFDA as mentioned above, you do not need read-across, use the data directly. Read-across is helpful to provide evidence of going down the right path in terms of PK. Tier 2. Because the CL or half-life is so long in humans for PFDA, menstrual fluid represents a significant pathway for clearance or loss from the body because PFDA is highly bound to protein in blood. You can calculate what fraction of total CL menstrual fluid represents. Childbirth itself represents a significant loss of blood from the body. If mechanistic models were used to interpret the PK for animal studies for extrapolation to humans, more confidence would be achieved in the HEDs based on animal studies.
Georgopoulos	a. Applying estimated data-derived extrapolation factor (DDEF) values for conversion of PODs from animal toxicity studies to human equivalent doses (HEDs) is in general appropriate in cases when reliable pharmacokinetic models (either physiologically-based or traditional) are not available for animal to human extrapolation. The Toxicological Review reports (in Section 3.1 and in particular in subsection 3.1.6) that EPA evaluated existing PBPK models and a one-compartment PK model and concluded that these options were not sufficiently reliable for use. This conclusion is appropriate, despite the fact that certain issues require clarification and additional information that is not currently included in the Review (I summarize related concerns in my response to Charge Question 6). Since this is the case, EPA proceeded to apply a data-derived extrapolation factor (DDEF) to point of departure (POD) values from toxicity studies in laboratory animals for estimating corresponding human equivalent doses (HEDs) in the derivation of the respective RfDs. The DDEF essentially (assuming similar absorption fractions) represents the ratio of human clearance (CLH) over animal (rat) clearance (CLA). Although, as mentioned above, the use of the ratio of human to animal clearance factors for interspecies toxicokinetic extrapolation (in the absence of reliable PK/PBPK models) appropriate, in this case there are concerns that should be discussed and addressed in the revision of the Toxicological Review. The human clearance for PFDA was calculated by combining information from human data reported in Fujii et al. (2015) and from animal (rat) data reported in Kim et al. (2019); it involves critical assumptions that are discussed next, in my answer to Charge Question 4b. The calculation of the animal clearance for PFDA raises more serious concerns as it was performed employing a steady-state assumption but using data from short-term animal studies (involving only 28 days of exposure, i.e., duration much shorter than the half-life of PFDA in either male or female rats). Serum PFDA levels will not reach steady-state at the end of the 28-day dosing period studies, since a reasonable approximation of steady-state is not attained until after 4 to 5 half-lives of dosing. Using this approach when serum PFDA levels in rats have not reached steady-state will result in overprediction of the HED. The Toxicological Review recognizes the issue but proceeds with the calculation by assigning an uncertainty factor to account for the fact that that the required conditions for steady-state are not fulfilled; specifically the Review (page 3-24, lines 7-12) states:

	"… application of a DDEF assumes that a steady-state concentration is reached, equal to dose/CL. When the end-of-study PFDA levels observed by NTP (2018) are compared to the corresponding dose/CL using the mean CL estimated for male rats the resulting dose/CL values are 2.7 to 1.4 times higher than the data. Hence the uncertainty in use of the male rat CL in the current analysis is considered to be less than a factor of 3." An alternative to assuming steady-state conditions could involve using average measured PFDA serum levels over the course of the study, or maximum serum PFDA level at the end of the dosing period to determine the POD for benchmark dose modeling (BMD). b. The approach of using the human clearance (CLH) for extrapolation of the serum PFDA level point of departure (PODint) from human epidemiological studies via HED = PODint x CLH is appropriate, assuming of course, that a reliable value of CLH can be estimated. According to the Toxicological Review, for this purpose a *"health-protective estimate for human clearance*" was used, equal to CLH = 2.6 x 10-5 L/kg-d, i.e., *"the value estimated for pregnant and breast-feeding women and their female children."* (page 3-24, lines 1-4). It should be taken into account however that these estimates of human clearance are based on critical assumptions, such as that the ratio of fecal/urinary clearance is the same in humans and rats; specifically, as per the Toxicological Review (page 3-16, lines 8-15) it was assumed: *"that the ratio of fecal/urinary clearance is similar in humans as in rats. Kim et al. (2019) observed a mean fecal excretion 1.63 times higher than urinary excretion in male rats, but only 0.742 times urinary excretion in female rats. Both of these ratios are considerably lower than the ratio of 3.3 estimated by Fujii et al. (2015). Given the uncertainty [...] for the estimated fecal clearance of Fujii et al. (2015), these sex-specific ratios will be applied to the estimated human urinary clearance from Fujii et al. (2015) (0.015 mL/kg/day) to obtain total estimated urinary plus fecal clearance rates of 0.039 mL/kg/day in men and 0.026 mL/kg/day in women."* The Toxicological Review also states (page 3-18, lines 3-7) that: *"The estimated urinary clearance from Fujii et al. (2015) is considered particularly reliable because 24-hour urine samples were used to determine the rate. Adding the menstrual clearance of 0.030 mL/kg-day based on the results of Verner and Longnecker (2015) yields total CL = 0.056 mL/kg-day for women between 12.4 (menarche) and 50 years of age, except during pregnancy and until menstruation resumes postpartum."* The question whether the ratio of fecal/urinary clearance of PFDA is the same in humans and rats deserves further inquiry and certainly contributes to the uncertainty associated with the estimates of CLH. c. Uncertainties and concerns associated with calculating the human clearance (CLH) and the data-derived extrapolation factors (DDEFs) for animal-to human extrapolation were discussed above, in my answers to Charge Questions 5a and 5b. Regarding the specific question on whether or not EPA should expand its

	adjustment for menstrual fluid loss as outlined in (U.S. EPA, 2023, HERO ID 11181055) prior to finalizing the assessment, my recommendation is positive. It should be pointed out that EPA recognizes (in IRISPFDA_ChargeQuestion5c_SupplementalInformation.PDF, page 3) that the overall impact of adopting this alternative approach is minor: *"If the alternative dosimetric approach is recommended by the peer reviewers, this would result in no quantitative change to the draft RfD or subchronic RfD; the highlighted minor change, an approximate 2-fold increase in the draft Developmental osRfD, would be implemented…"* **Suggested Revisions and Future Considerations** • ***Tier 1 Necessary Revision:*** *Edit and expand the discussion on the estimation of human clearance to state explicitly and clarify that that this estimation incorporates uncertainties associated with assumptions that are based on animal data regarding the ratio of fecal/urinary clearance of PFDA.* • ***Tier 2 Suggested Revision****: Evaluate and, if feasible, apply options for alternative animal to human extrapolation approaches that do not require steady-state assumptions inconsistent with short-term study data.*
Haney	a. Generally speaking, yes. As discussed in Section 3.1.7 of the draft assessment (starting on p. 3-22), evaluation of a published PBPK model (Kim et al., 2019) and a one-compartment PK model showed significant errors in the PBPK model and that the simpler PK modeling approach did not reliably predict PFDA serum concentrations. An alternative to PBPK/PK modeling for dosimetric extrapolation is data-derived extrapolation factors (DDEFs). The estimated population average values of CL_{tot} for male and female rats, female mice, and male and female humans are considered to be sufficiently sound for use in such extrapolations, while use of default $BW^{3/4}$ scaling would lead to significant errors in HED calculations. Therefore, application of DDEFs calculated from the clearance values listed in Table 3-3 (p. 3-18) of the draft was considered by EPA to be the next preferred option for extrapolation from laboratory animal doses to HEDs. The corresponding DDEFs appear in Table 3-4 (p. 3-23). This hierarchy of preferences appears consistent with, and therefore is generally justified by, environmental regulatory agency practice (e.g., EPA, TCEQ). Additionally, it is noted that in the absence of good scientific information on which laboratory animal species/sex/life-stage best predicts similar effects in humans, regulatory agencies often use the most sensitive species/sex/life-stage for each adverse effect. Consistent with this type of assumption, EPA used CL values generally matched for animals and humans based on sex and life stage.[46] In the absence of additional information, this may be the most reasonable approach.

[46] "Liver effects observed in adult female rats are assumed to be relevant to older women, hence the same CL_H (0.026 mL/kg-day) will be used to extrapolate those. Liver and reproductive effects observed in adult male rats will be extrapolated using the CL_H for men, 0.039 mL/kg-day. Finally, reproductive effects observed in adult female rats will be extrapolated using the CL_H for women of reproductive age, 0.056 mL/kg-day." (p. 3-23, lines 16-20)

	b. As I am not a PBPK/PK modeler, on this question I think it best to ultimately defer to those with expertise in PBPK/PK and its implementation in the context of dose-response assessment. I simply note that the draft assessment indicates that application of a DDEF assumes that a steady-state concentration is reached (p. 3-24, lines 7-8), that a chemical "achieves a given steady-state concentration after approximately 4 to 5 half-lives" (Hallare and Gerriets 2022), and that PFDA half-lives are relatively long depending on species (e.g., 44 days to 23 years per Table 3-3; p. 3-18). Accordingly, it seems that if steady-state was not reached in the underlying study made basis for an RfD, this extrapolation method might be a source of significant uncertainty. c. The draft assessment section *Uncertainty in HED calculations for PFDA* discusses the evaluation of various uncertainties (pp. 3-24 to 3-25), which by and large seems to adequately highlight those aspects of uncertainty. At the same time, I note that the uncertainty appears appreciable. For example, this section states [*emphasis added*], "While the PK model parameter estimates seek to make the best use of the available chemical- and species-specific data, *there are also many uncertainties* noted above, *in particular for humans*." (p. 3-25, lines 3-5), and ultimately acknowledges *that it is plausible that human clearance is actually as high as that predicted by* $BW^{3/4}$ *scaling* (p. 3-25, line 10), which would result in HEDs *20- to 40-fold higher* (p. 3-19, lines 33-35). Thus, there appears to be significant uncertainty. EPA should expand its adjustment for menstrual fluid loss (as outlined in U.S. EPA, 2023, HERO ID 11181055) prior to finalizing the assessment if EPA judges the alternative/modified adjustment to be associated with less uncertainty than that associated with not conducting such an adjustment (**Tier 1 necessary revision**). The assumption that the PK effect of pregnancy and lactation on PFDA is similar to that of the other PFAS likely to have similar PK properties seems to be a reasonable one, and this new alternative/modified adjustment would appear to result in a greater degree of scientific refinement.
Hoberman	a. Applying the estimated DDEF values for PFDA was scientifically justified for selection of the PODs when extrapolated from animal toxicity studies to HEDs. b. The application of the human CL to estimate HEDs from PODint values is scientifically justified. c. The uncertainties for the DDEFs and human CL have been adequately evaluated. However with the newer data from other PFAS, especially the adjustment for menstrual fluid should considered on the assumption that the pharmacokinetic effect of PFDA on pregnancy and lactation can be extrapolated using read-across based interpretation. **Clarification: Tier 3** - All future evaluations should include the adjustment for menstrual fluid for each PFAS.

Leung	Toxicological pharmacokinetics is not my area of expertise, and I defer this question to the other external reviewers.
Zoeller	a. This is outside my area of expertise. c. This is outside my area of expertise.

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

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

i. Please comment specifically on whether the methods used to derive toxicity values for PFDA appropriately account for uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans?

b. For immune effects, a UFS of 1 and 3 were considered to account for extrapolation from less than lifetime human data; ultimately a UFS of 1 was selected. 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). Also important is the fact that, given PFDA's long half-life and the expectation that the children and their mothers have been exposed to elevated levels of PFDA for many years, the observed effects on immune response are considered to be the result of a cumulative, prolonged exposure. Uncertainties with regards to additional susceptible life stages (e.g., old age) are addressed as part of the UFD. Does the provided scientific rationale support this decision? Please explain.

c. For liver effects, 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 if that were the sole pathway leading to these effects, evidence for the involvement of non-PPARα pathways is available in the PFDA database. Thus, uncertainty remains regarding the potential differences in sensitivity across species due to the involvement of both PPARα-dependent and PPARα- independent mechanisms. As such, the Toxicological Review concludes the available data are not adequate to determine if humans are likely to be equally or less sensitive than laboratory animals with respect to the observed 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 is clearly documented.

d. For liver, male reproductive, and female reproductive effects, a default 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 (prolonged diestrus, sperm measures and increased liver weight) to worsen with increasing duration and the large uncertainty associated with the lack of chemical-specific data to evaluate the effects of subchronic exposure on liver, male reproductive and female reproductive outcomes, the Toxicological Review concludes that application of a UFs of 10 is supported for the

purposes of deriving the subchronic RfD from the 28-day toxicity data. Does the provided scientific rationale support this decision? Please explain.

e. Are the provided rationales for the remaining uncertainty factors (UFL, UFD, UFH) scientifically justified and clearly described (to inform the UFH, the assessment evaluates and considers the available evidence on potential susceptibility to PFDA within different populations or lifestages, including any potential impacts from early life exposure to PFDA on children's health or health later in life, although few studies on susceptibility were available)? If not, please explain.

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors	
Reviewer	**Comments**
Adgate	a. The derivation of tox values is clearly described and scientifically justified. b. This is a reasonable scientific rationale that supports this decision. My response is also consistent with my answer above on the question about subchronic RfDs and their utility. I also concur with Dr Zoeller's comments on this topic. c. The available animal data and the write up support this conclusion with enough clarity to provide adequate scientific support for this decision. d. The scientific rational here is clear and supported by this analysis. e. The scientific rationale for these UFs is clear and scientifically justified.
Carignan	a. No comment, this is not my main area of expertise. b. The scientific rationale supports application of a UFS of 1. c. Available studies support this conclusion and the analysis is clearly documented. d. The scientific rationale supports application of a UFS of 10. e. The provided rationales are scientifically justified and clearly described. **Additional comments**: Suggest defining the abbreviations in Table 5-9 (this is buried and hard to find), explain why the math doesn't add up (adding uncertainty factors as shown does not produce the sum shown), and make it clear that the one to the right is the sum. [**Tier 2**]
Faustman	a. This reviewer thought the derivation of toxicity values was scientifically justified and were clearly presented by USEPA. Please see the sections above that address these choices. Tables 5-14 and 5-15 are very clear and provide documentation of these choices. b. This reviewer was supportive of the choice of uncertainty factors for immune effects. The use of human data to set the immune response and to define the response during early development supports the choice of 1 for UFS. Also, this

	reviewer did feel that the immunotoxicity endpoint represented a biologically important and sensitive endpoint for derivation of toxicity values. c. This reviewer was not supportive of the use of the value of 3 to account for lack of a chronic study in animals. A **Tier 1 recommendation** is for EPA to review this choice. Please note that there was not even a 90 day study for extrapolation. This reviewer feels that the absence of such studies severely compromised our ability to evaluate more chronic impacts and would suggest a factor of 10 applied to extrapolate the available data for chronic considerations. This reviewer was supportive of the use of a value of UPA of greater than 1 to account for the complexities of PPAR alpha and non PPAR alpha receptor involvement. This was also supported by observations across other PFAS compounds. The factor of three is thus supported by complexity of the toxicodynamics across species and the report provides justification. d. This reviewer supports USEPA's choice of a default factor of 10 to account for the lack of a chronic study. The text supports this choice. e. This reviewer supports the rationales for the remaining uncertainty factors. In regards to the potential lifestage susceptibility, the report clearly discusses how an endpoint of altered antibody response in early childhood was considered a sensitive and relevant endpoint to id developmental impacts.
Fisher	a. The lack of fit to pharmacokinetic data for repeated exposures suggests that the acute dosing CL values do not predict repeated exposures. This is an unfortunate situation that needs resolved to deal with PFDA pharmacokinetics in animals. I think virtual simulations of populations would be helpful in the future (**Tier 3**), Monte Carlo, for example. b. I am not able to answer this question. c. I am not an expert on this issue, but my readings indicate that PPARα is most likely not an irrelevant endpoint for human health considerations. A UF of 3 is a good compromise. d. With a long half-life PFDA may accumulate in the body over time, that is, intake exceeds excretion from the body. Thus, a lifetime exposure may result in an internal exposure that is greater than in early life, if the rate of intake exceeds the excretion rate. I recommend that a calculation be completed in this regard using an animal/human PBPK model for PFDA to gain insights into subchronic and chronic exposures. If no accumulation appears likely (quasi-steady state) (then justify your UF by giving examples of chemicals that have 28-day, 90-day and 2-year toxicity information. Is a UF of 10 justified? **Tier 2**. e. I can see the rationale, more or less, for PFDA. I am stuck with the mixture issues and the uncertainty about PFDA potency when found in a mixture. I think the epidemiologic tools used to tease out its contribution need to be more transparent, using a language that all can understand. **Tier 2**.
Georgopoulos	a. It should be recognized that the Toxicological Review tries to evaluate and synthesize information from an extensive body of literature that is often

	inconsistent and associated with significant uncertainties; this fact is also true for the available information on the pharmacokinetics of PFDA for humans as well as for laboratory animals. Unfortunately, certain statements across the Toxicological Review and its Appendices reflect the inconsistencies in the literature. A characteristic example is the following: In the first page of the section on Pharmacokinetics (page 3-1, lines 18-23) one reads *"While female rats administered PFDA tended to have a higher dose-normalized area under the plasma concentration time curve (AUC) than males, (Dzierlenga et al., 2019) suggested that there was no sex difference in PFDA half-life. However, calculating the average clearance across studies, doses, and routes, the EPA obtains a value of 6.1 mL/kg-d in male rats and 4.3 mL/kg-day in female rats, i.e., 30% lower in female rats. The elimination half-life of PFDA is generally much longer in humans (4.5–12 years) than in rats (20–59 days) or mice (63–222 days)."* There are issues regarding the consistency of pharmacokinetic information within the above quote. In fact, the last sentence of the above statement is repeated on page 3-56 (lines 10-11) but no relevant references are provided in either case. The source is probably the article (included in the references of the Toxicological Review) by Zhang et al. (2013b), who estimated the PFDA half-lives being 4.5 years for women <50 years old and 12 years for males and women >50 years old. In IRISPFDA_ChargeQuestion5c_SupplementalInformation.PDF (page 2) one can read: *"…the half-life of PFDA in humans is estimated to be 10–20 years; see External Review Draft"* This statement contradicts the last sentence of the earlier quote but agrees (approximately) with the values of half-lives for humans reported in Table 3-3 of the Toxicological Review (page 3-18) that are 21 years for men, 23 years for women < 12.4 or > 50 years, and 10.6 years for women 12.4–50 years old. The half-lives reported for laboratory animals in Table 3-3 are 72 days for male rates, 54 males for female rats, 44 days for male mice and 63 days for female mice. One should compare these values for rats and mice with those reported on page 3-14 (lines 1-14) of the Toxicological Review: *"While Vanden Heuvel et al. (1991) also evaluated the elimination of PFDA in rats, they did not report clearance values nor AUC values that could be used to calculate clearance. The half-lives estimated from the decline in total body burden (based on 14C activity) were 23 and 43 days in males and females, respectively, while the half-lives based on blood concentrations were 22 and 29 days, respectively (Vanden Heuvel et al., 1991). These female half-lives are comparable to the beta-phase half-lives reported for female rats by Dzierlenga et al. (2019) (18–44 days), though somewhat lower than reported for female rats by Kim et al. (2019) (50–75 days). The half-life estimates of Vanden Heuvel et al. (1991) for male rats are between the alpha-phase (1.7–2.1 days) and beta-phase values (80–110 days) reported by Kim et al. (2019), but much less*

	than those reported by Dzierlenga et al. (2019) (215–300 days beta- or single-phase half-life). This range of half-life values reflects the fact that half-life estimates are sensitive to noise in the experimental data and study design, with Vanden Heuvel et al. (1991) having only measured elimination for 28 days, while Dzierlenga et al. (2019) measured plasma concentrations to 105 days and Kim et al. (2019) to 150 days." The above quotes confirm that uncertainties in interspecies and intraspecies differences are very significant; so, assumptions extrapolating pattern appearing in an animal species (e.g., the relationship of urinary to fecal clearance) to another should be questioned and evaluated. Pharmacokinetics of PFAS in general are primarily driven by processes such as serum protein binding and renal reabsorption, and these processes can differ dramatically between animal models and humans. Dzierlenga et al. (2019) and Fujii et al. (2015) fitted rat and human time-course data, respectively, with two-compartment pharmacokinetic models, as PFDA displays the biphasic elimination pattern typical of many PFAS, with a rapid decline in an initial (alpha) phase and a slower decline in a second (beta) phase, calculating the two corresponding half-lives. However, this "piecewise linear" approximation should not lead to a conclusion that there is a "universal" set of two values for PFDA biological half-lives for a given species/gender. PFAS kinetics in general 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). **Note a1:** The Toxicological Report (caption of Figure 3-2 on page 3-21) states that: *"EPA's replication of the PBPK model exactly reproduces the PBPK model results of Kim et al. (2019) for oral dosimetry hence is considered an accurate reproduction of the model. The discrepancy between the PBPK model prediction for a 1 mg/kg dose and the data demonstrates that the published model structure and parameters are very inconsistent with the empirical data, hence that there is a significant flaw in the model."* More specific information is provided on page 3-20, lines 25-33: *"The over-prediction (approximately three to four times higher than these key pharmacokinetic data for male rats) of the IV data by the Kim et al. (2019) model indicates that distribution into the body is significantly under-predicted by the model, which was offset in the simulations of oral dosimetry data by using an unrealistically low oral bioavailability. Initial efforts to re-fit the model to the data did not produce acceptable fits to both the IV and oral dose PK data and involved changing model assumptions in a way that would require separate experimental validation before use. It was therefore determined that the published model structure and underlying assumptions did not allow a sufficiently sound calibration of the model to the PK data, given the currently available data."* It would be useful to clarify how EPA replicated the model of Kim et al. (2019)

	and specifically if the EPA PBPK templates (coded in R) provided in Bernstein et al. (2021) were used for that purpose, as in fact these templates correct certain errors present in the formulation of the Kim et al. (2019) model and provide a valuable platform for model experimentation and refinement. Appendix G provides extensive descriptions of the analyses performed for the one- and two-compartment pharmacokinetic models; it would be informative to describe what procedures were followed in adjusting the EPA PBPK template in the effort to develop predictions matching the data reported in Kim et al. (2019). The statement in the above quote that "[i]nitial efforts to re-fit the model to the data did not produce acceptable fits to both the IV and oral dose PK data" should not be interpreted that this is the main reason for not adopting the Kim et al. (2019) PBPK model. In fact, using the PFDA model template provided in Bernstein et al. (2021) one can achieve reasonable fits to both the IV and oral dose PK data by adjusting simultaneously the values of a subset of model parameters (specifically the fraction unabsorbed, lung-plasma partition coefficient, transport maximum, and transport affinity constant), but this does not imply that the "re-parameterized" model fits the data for the right reasons. It should also be clarified whether EPA considered the possibility that enterohepatic reabsorption of PFDA may have affected the bioavailability measurements/estimates reported in Kim et al. (2019). Finally, it should be recognized that even if a PBPK model with a new calibrated set of parameters were considered acceptable for PFDA pharmacokinetics in rats, there is currently no scientifically defensible way to extrapolate that model to humans since the allometric scaling of parameters used by Kim et al. (2019) is not applicable to the parameters associated with renal reabsorption processes. **Note a2:** Regarding human pharmacokinetics, Fabrega et al. (2015) presented PBPK modeling results for PFDA; it should be clarified if EPA considered this source during the development of the Toxicological Review. **Note a3:** Finally, it should be pointed out that EPA's httk (high-throughput toxicokinetics) R package (September 22, 2022 version) includes a human PBPK formulation for PFDA; however it appears that the PFDA half-life in that model is approximately only 18 days. Information on whether EPA plans to align future versions of httk with the findings and approaches adopted in the Toxicological Review would be useful. b. The selection of UFS=1 to account for extrapolation from less than lifetime human data in assessing immune effects should be considered appropriate. The rationale provided by EPA, i.e., exposures during the developmental period, which is a susceptible lifestage for immune effects, can be considered more relevant than exposures in adulthood and that the observed effects on immune response are considered to be the result of a cumulative, prolonged exposure. It is also appropriate to address uncertainties associated with additional susceptible lifestages (e.g., old age) as part of the UFD. c. The selection of UFA = 3 for extrapolating liver effects from laboratory animals to humans in the derivation of the subchronic RfD is appropriate and accounts for residual uncertainty in interspecies toxicodynamic differences. The rationale

	provided, i.e., the evidence for the involvement of both PPARα-dependent and PPARα- independent pathways in liver effects, and the associated uncertainties regarding potential differences in sensitivities across species, support the conclusion that available data are not adequate to determine if humans are likely to be equally or less sensitive than laboratory animals with respect to the observed liver effects. The available animal and mechanistic studies support this conclusion and the analysis presented in the Toxicological Review is clearly documented. d. The value UFS = 10 applied when extrapolating from 28-day animal data to subchronic exposures for liver, male reproductive, and female reproductive effects, is appropriate. The reasons provided, i.e., (a) the potential for effects such as prolonged diestrus, sperm measures and increased liver weight to worsen with increasing duration and (b) the large uncertainties associated with the lack of data for evaluating effects of subchronic exposures on liver, male reproductive and female reproductive outcomes, support this decision. e. The rationales provided for the remaining uncertainty factors (UF_L, UF_D, UF_H) are scientifically justified and clearly described. **Suggested Revisions and Future Considerations** • ***Tier 1 Necessary Revision****: Briefly summarize and explain the approach followed for replicating and adjusting the PBPK model of Kim et al. (2019), i.e., using the R templates provided in Bernstein et al. (2021); incorporate this information in Appendix G following the analysis of the -1 and 2-compartment PK models. (Please see Answer to Charge Question 6a for more details).* • ***Tier 1 Necessary Revision****: Explain (in Appendix G) why other published models (e.g., Fabrega et al., 2015), were evaluated as not adequate for supporting pharmacokinetic calculations.* • ***Tier 1 Necessary Revision:*** *Check and ensure that values of pharmacokinetic properties listed in the document match those reported in their cited sources: an example is such as, e.g. the value of 215–300 days for beta- or single-phase half-life of PFDA in male rats attributed to Dzierlenga et al. (2019).*
Haney	a. As described in section *Derivation of Candidate Lifetime Toxicity Values for the RfD* of the draft assessment and EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), five possible areas of uncertainty and variability were considered in deriving the candidate values for PFDA. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as a designated UF to be applied to the candidate lifetime POD_{HED} values are listed In Table 5-9 (pp. 5-19 to 5-20) of the draft. Table 5-9 contains reasoned justifications for the values used for the various UFs in deriving lifetime candidate RfDs. The rationales are also clearly discussed in the text of that section.

	Similarly, an explanation of the five possible areas of uncertainty and variability and the values assigned to each as a designated UF to be applied to the candidate subchronic POD_{HED} values are listed in Table 5-13 (pp. 5-30 to 5-31) of Section 5.2.3 (*Subchronic Toxicity Values for Oral Exposure (Subchronic Oral Reference Dose [RfD]) Derivation*). Table 5-13 contains reasoned justifications for the values used for the various UFs in deriving subchronic candidate RfDs. The rationales are also clearly discussed in the text of Section 5.2.3. a.i. Beyond simply adopting a relatively more conservative approach, as was done in the draft assessment (e.g., DDEFS versus $BW^{3/4}$ scaling), I am not sure there is an acceptable way for EPA to further "account" for the total uncertainty surrounding interspecies differences in PK (e.g., through quantitative methods). However, an external PBPK/PK modeler, another peer review panel member, or a public commenter may be aware of an alternative method/approach. b. Yes, EPA provides the rationale needed to support a UF_S of 1 on pp. 5-20 (lines 7-18) to 5-21 (lines 1-14) of the draft assessment. The reasoning is clear, multifactorial, and strong. For example, the observed immune effects in children are considered the result of prolonged exposure to PFDA and the enhanced susceptibility/sensitivity of the developmental immune system, attenuating concerns of potentially increased sensitivity with longer-term exposures.[47] c. At present, it appears that available animal and mechanistic studies support use of a UF_A of 3 as "uncertainty remains regarding the potential differences in sensitivity across species due to the involvement of both PPARα-dependent and PPARα-independent mechanisms." EPA provides the rationale supporting a UF_A of 3 for liver effects on pp. 5-31 (lines 18-25) to 5-32 (lines 1-19) of the draft assessment. In addition to PPARα-dependent MOAs, other MOAs may be active in PFDA-induced liver effects[48], and the uncertainty surrounding a potential multifaceted MOA for PFDA-induced liver effects supports the value of 3 selected for the UF_A for the purposes of deriving candidate subchronic RfDs for hepatic effects. d. EPA provides a rationale supporting a UF_S of 10 for these effects on pp. 5-32 (lines 20-38) to 5-33 (lines 1-12) of the draft assessment. EPA states (p. 5-33, lines 7-12), "Considering the potential for some health effects (prolonged diestrus, sperm measures and increased liver weight) to worsen with increasing duration and the large uncertainty associated with the lack of any chemical-specific data on whether the effects observed in the short-term study worsen after subchronic

[47] However, as I do not agree with the selection of these studies and endpoints for RfD derivation, please see my previous comments.

[48] "Although PPARα appears to be an important mechanism of PFDA-induced liver toxicity in animals and reduced sensitivity in PPAR activation in humans compared to rodents has been suggested, available evidence for PFDA in PPARα null mice, human in vitro assays and in vivo animal models more relevant to humans with respect to PPARα sensitivity (i.e., guinea pigs and Syrian hamsters) suggest that liver effects occur, at least in part, independent of PPARα (see Summary of mechanistic studies for PFDA in Section 3.2.1). A plausible PPARα-dependent and independent MOA for liver effects is also supported by studies in null and humanized animal models of structurally related long-chain PFAS [C7–C9] (see *Evidence for Other PFAS* in Section 3.2.1), which are mostly lacking for PFDA (a few studies in null mice but no humanized models)." (p. 5-32, lines 6-15).

	exposure, a UFs of 10 is selected for the purposes of deriving candidate subchronic toxicity values from the 28-day toxicity data." However, while this quote refers to "chemical-specific data", it is unclear if EPA attempted to consider whether data from structurally related PFAS could help inform the UF_S value for one or more of these effects (e.g., as they did to some extent for the UF_A value for liver effects). EPA should consider whether data from structurally related PFAS could help inform the UF_S value for one or more of these effects, or if they have already done so, explicitly state this in Section 5.2.3 of the draft assessment (**Tier 1 necessary revision**). e. Yes, as alluded to under subsection "a", Table 5-9 contains reasoned justifications for the values used for the various UFs, including the UF_L, UF_D, and UF_H, in deriving lifetime candidate RfDs. The rationales are also clearly discussed in the text of that draft assessment section (*Derivation of Candidate Lifetime Toxicity Values for the RfD*). Similarly, Table 5-13 (pp. 5-30 to 5-31) of Section 5.2.3 (*Subchronic Toxicity Values for Oral Exposure (Subchronic Oral Reference Dose [RfD]) Derivation*) contains reasoned justifications for the values used for the various UFs, including the UF_L, UF_D, and UF_H, in deriving subchronic candidate RfDs. The rationales are clearly discussed in the text of Section 5.2.3 of the draft.
Hoberman	a. It is scientifically justified to consider uncertainty in the derivation of the toxicity values. The review clearly described these uncertainties. The method used to derive toxicity values for PFDA seem to appropriately account for uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans. a.i. The methods used to derive toxicity values for PFDA appropriately account for uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans. **Tier 1 necessary revision: The lack of consideration of women who are not menstruating due to amenorrhea, exercise or being on contraception should be noted in the analysis.** b. The scientific rationale supports the decision to use a UFS of 1 for immune effects. c. The available animal and mechanistic studies support the conclusion and is clearly documented. As noted on page 5-38,"Uncertainties remain due to the absence of longer-term toxicity studies (28 d) and limited information from available epidemiological studies and in vivo models to characterize the role of PPARα and other signaling pathways in the mechanisms of hepatotoxicity of PFDA in both humans and animals." d. The UF of 10 is supported for the purpose of deriving the subchronic RfD as these values are based on a single 28 day study in one species with no additional species or any longer term study in mice. A 90 day study is normally considered to provide better data for a subchronic RfD. e. The provided rationales for the remaining uncertainty factors appear to scientifically justified and are clearly described. With all the different populations

	and life stages that can occur with exposure to PFDA (neonates, children, adolescents, adults) it would take a significant more studies to cover all these life stages. **Tier 1 necessary revision**: Data from structurally related PFAS could help inform the UFS value for one or more of these effects.
Leung	Toxicological pharmacokinetics is not my area of expertise, and I defer this question to the other external reviewers.
Zoeller	a. This is outside my area of expertise. b. The justification for a UF of 1 for a sensitive life stage was not clear. **Tier 2 Recommendation.** The Agency should better support the justification for this UF. c. The justification for a UFA = 3 was clear. **No Recommendation**. d. The justification for this decision was clear. **No Recommendation**. e. Yes, these were clear. **No Recommendation.**

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

Carcinogenicity Hazard Identification and Toxicity Value Derivation	
Reviewer	**Comments**
Adgate	I concur that that there is inadequate information to assess the carcinogenic potential of PFDA at this time.
Carignan	This reviewer agrees that there is inadequate information to assess carcinogenic potential for PFDA at this time. To avoid readers confusing 'inadequate information' with 'not carcinogenic', recommend that determinations for PFASs with similar properties (e.g., PFOA and PFOS) be noted. [**Tier 1**]
Faustman	In general, this reviewer agrees with the conclusion that it is not possible at this time to assess carcinogenic potential however this reviewer also felt "limited" in options to explain how the observations of chromosomal abnormalities and clastogenic activity are to be handled that are reviewed in this section. For example, in Table 3-46 studies that showed positive genotoxicity in vitro and in in vivo for endpoints such as DNA strand breaks, chromosomal aberrations, oxidative damage and cell cycle changes were discounted as it was difficult to link with long

	term impacts and a chronic endpoint of cancer but these are of concern for their genotoxicity. Please see statement lines 9-11 page 3-289. Yes this is true for linkage with cancer however such impacts can have significant health implications and should be put into the correct hazard identification endpoint section. Were these reviewed elsewhere in a genotoxicity section? I may have missed this. Alternatively, this could be added to the male reproductive toxicology endpoints as supplemental data. The importance of this information is indirectly emphasized again in comments from the public that were circulated to us in June 2023. On page 2 and 3 of Table 2 there are comments about missing references. Although the comments from NRCD 3-4 are identified as relevant to charge question 1b and the literature search approaches and findings, the specific example of a missing reference Pan et al (see clarification below) that reports DNA fragmentation in semen in the section on male reproductive toxicity were sperm impacts are identified is of relevance there but also when combined with the genotoxicity and chromosomal aberrations above (now currently discussed in cancer) would support additional, separate review of genotoxicity. Please note that such "stove piping" can occur with the more mechanistic data if careful cross inclusion of data is not undertaken**. A Tier 1 recommendation is given here to first determine where in the hazard identification process such DNA impacts will be reviewed and second to undertake the evaluation of such data for the additional endpoint.** This reviewer would suggest to add to the supplemental information considered for reproductive impacts. **Clarification:** Pan Y, Cui Q, Wang J, Sheng N, Jing J, Yao B, Dai J. Profiles of Emerging and Legacy Per-/Polyfluoroalkyl Substances in Matched Serum and Semen Samples: New Implications for Human Semen Quality. Environ Health Perspect. 2019 Dec;127(12):127005. doi: 10.1289/EHP4431. Epub 2019 Dec 16. PMID: 31841032; PMCID: PMC6957285.
Fisher	I am not qualified to answer this.
Georgopoulos	The Toxicological Review appropriately concludes that there is inadequate information to assess carcinogenic potential for PFDA and that this descriptor applies to both oral and inhalation routes of human exposure. This decision is scientifically justified and clearly described in the Toxicological Review.
Haney	Yes, the available human, animal and mechanistic studies along with the analysis presented in the Toxicological Review support the conclusion that "the evidence is *inadequate to assess carcinogenic potential* of PFDA in humans" (Section 5.3, p. 5-42). Briefly, as indicated in Section 3.3 of the document: (1) the available epidemiologic evidence on PFDA and the risks of cancer is limited and generally uninformative (p. 3-288, lines 33-34); (2) there are no long-term animal bioassay studies available for PFDA (p. 3-289, line 2); and (3) in summary, PFDA does not appear to elicit a strong genotoxic response as demonstrated by the lack of activity in most assays described above, including mutagenicity tests in prokaryotic organisms and mammalian cells; SCE and cell transformation assays in vitro; and

	UDS, oxidative DNA damage and micronucleus assays in rats (p. 3-291, lines 4-7). This limited evidence amounts to inadequate information to confidently assess the carcinogenic potential of PFDA 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 oral and inhalation exposure to PFDA and is clearly scientifically justified.
Hoberman	The available human, animal and mechanistic studies are appropriately analyzed and clearly described, but these studies are inadequate to assess carcinogenic potential for PFDA.
Leung	Overall, the Review's conclusions that there is inadequate information for a potential clinical carcinogenic effect of PFDA exposure is scientifically justified, mostly due to the confounding potential in the human studies and the overall lack of longterm studies for this outcome, as has been clearly summarized. However, I agree with Dr. Faustman's excellent points raised regarding a possible genotoxic effect of PFDA observed in some studies, and agree with a **Tier 1 recommendation** to evaluate where in the hazard identification process this endpoint will be evaluated.
Zoeller	Yes, this decision was well justified. **No Recommendation.**

3.8 Given the conclusion there was inadequate information to assess carcinogenic potential for PFDA, 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**
Adgate	This scientific decision is clearly described and scientifically justified.
Carignan	This reviewer agrees estimates could not be derived. To avoid readers confusing 'inadequate information' with 'not carcinogenic', recommend that values for PFASs with similar properties (e.g., PFOA and PFOS) be noted. [**Tier 1**]
Faustman	This reviewer agrees with this approach at this time. Nevertheless, this reviewer is always concerned that the public confuses the category of "inadequate information to assess carcinogenic potential" as a statement of "no carcinogenicity" and encourages the EPA to be careful in how this information is translated to the public. It is also the opinion of this reviewer that as more reviews of longer chain PFAS compounds are published that discuss carcinogenic findings such as the IRAC listing of

	PFOA as a Group 2B carcinogen and the NCI PFAS Exposure and Risk of Cancer documents are published there will be more interest in determining how and when SAR approaches and read across extrapolations across the many PRAS compounds will be implemented. Thus this review proposes a **Tier 3 recommendation** that future documents clearly document the chemical domain for when such cross compound groups are conducted. It maybe helpful to enhance the Chemistry and structure information for these individual PFAS reports in anticipation of such efforts. It could pull from and highlight information already being generated within the Computational Toxicology program. USEPA states the intention to implement. This reviewer applauded the addition to Appendix D.2 and D.3 however some of these details almost get lost when just located in the Appendix. Also, Appendix E provides such Comp Tox data however it was interesting that seemed to be only put into context for informing the liver effects as it would seem that these assays could also start to inform neurodevelopmental and also endocrine such as thyroid endpoints. A **Tier 2** recommendation is for the authors to add an explanatory paragraph that states how the Computational Toxicology information, now primarily in the appendix, is or will be incorporated across endpoints and effects. Such a paragraph is needed to ensure the intended user of the current IRIS review is clear and can articulate how and when the additional information from the appendix was used and also perhaps when it was not used across endpoints.
Fisher	Yes, the lack of animal data or human data justifies this response currently.
Georgopoulos	Given the conclusion that there was *inadequate information to assess carcinogenic potential* for PFDA, the only reasonable option is to not derive quantitative estimates for cancer effects for oral or inhalation exposures. This decision is scientifically justified and clearly described in the Toxicological Review.
Haney	Yes, the decision to not derive quantitative estimates for cancer effects for oral or inhalation exposures is scientifically justified. Section 5.3 (p. 5-42) states: "Considering the limitations in the evidence base across human, animal, and mechanistic studies of PFDA (see Section 3.3) and in accordance with the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), EPA concluded that the evidence is *inadequate to assess carcinogenic potential* of PFDA in humans. The lack of adequate carcinogenicity data for PFDA precludes the derivation of quantitative estimates of either oral (oral slope factor, OSF) or inhalation (inhalation unit risk; IUR) exposure." *Consistent with "lack of adequate carcinogenicity data for PFDA", the derivation of quantitative risk estimates for either oral (oral slope factor, OSF) or inhalation (inhalation unit risk; IUR) exposure is precluded*. 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 exposures is both reasonable and scientifically justified*.

	In conclusion, it is obvious that the EPA has put a great deal of time and work into the draft PFDA 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 is the beneficiary of EPA staff having considered the most diverse set of scientifically credible perspectives possible. Thank you for the opportunity to have peer reviewed this important draft assessment.
Hoberman	The decision not to derive quantitative estimates for cancer effects for oral or inhalation exposures is scientifically justified and described in the text.
Leung	The inability to derive quantitative estimates for carcinogenic effects associated with PFDA exposure is scientifically justified and clearly described.
Zoeller	This decision was well justified. **No Recommendation.**

4.0 ADDITIONAL COMMENTS

Reviewer	Comments
Adgate	**Tier 3:** The overall PFDA document is the best that could be produced under the circumstances. This review process and its findings, which relied on limited animal and human data, demonstrates the key shortcoming of the IRIS chemical-by-chemical assessment approach: the assessment of PFDA is complicated by the multiple PFAS humans are routinely exposed to. Given it is hard to disentangle the effects of multiple similarly persistent PFAS (including PFDA), the Agency should strongly consider treating these PFAS as a mixture. **Tier 1**: All **Evidence integration** sections for each health endpoint: subheadings for human and animal determinations will improve the document, the clarity of the integration process, and judgments presented in each section, especially in sections with a large number of epidemiology studies. The Agency and section authors should pay special attention to these sections, as this summary of the determinations made is the most important part of each section devoted to a specific endpoint/set of endpoints. **Tier 2:** The Table of Acronyms is missing several definitions (e.g., DDEF), among others. This is especially important for relatively new or obscure acronyms that are not part of the standard risk assessment lexicon. The authors should do a systematic search for any that are defined but not included in the summary table. **Tier 2:** Many Tables and Figures do not stand alone as individual interpretable units that can be understood without consulting the text. This general editorial rule is widely

	accepted by scientific journals and should be EPA's rule for determining if a table or figure should be included and is interpretable by readers. **Clarification:** This applies to nearly every table and figure. Apply the following rules (which are the same one's any good scientific Journal uses): • Does the title describe the contents? • Are all acronyms defined? (master table of PFAS acronyms exempted) • Are all headers attached/existing on every page? • Figures: is everything (axes, colors, etc) clearly labelled. • And, as the comment says: can a reader figure out everything in the table WITHOUT consulting the text? **Tier 2:** In several places key tables that could fit on one page break across pages (e.g., Tables 3.24, 5.1, 5.5, 5.9, etc.; this list is not exhaustive). For tables that have to break across pages make sure column/sub column headers appear on every page, e.g., Table 3.20, 5-14 and others. **Tier 1:** Numbering of the Appendices with key information is incorrect in many places, e.g., page 1-10 references "Appendix A.6" – which can't be found in the Table of Contents of the main document or PFDA Supplement.
Faustman	This reviewer was tremendously impressed with the careful, transparent and in-depth IRIS review that the USEPA conducted for PFDA. On all levels from the transparency in the systematic review processes, clarity of the evidence reviews and synthesis, enhanced cross- talk across lines of evidence and detailed and thoughtful considerations on how toxicokinetics and dynamics are included in the review were all noteworthy. I have reviewed a series of the PFAS compounds and as these have been developed sequentially, the incorporation of cross compound information has become clearer and I applaud the IRIS team for these advances. These current reviews should serve as excellent examples of how these various integrations can occur. Please see my detailed comments however I wanted to start with this statement as in so many of the discussions in our open review sessions, the USEPA experts were available during the open discussion, they were prepared and thoughtful and provided many clarifications and were able to document additional points for our clarification. These additional details support my conclusions in reading this draft that at all aspects of the project the team is truly exceptional in their review and product. Thank you! One other comment on the linkage with the references and the newly modified HAWC system appears to be facilitating the translation of information from the original peer reviewed literature. Also USEPA's discussion and follow-up with authors now documented in retrievable documents in the reference system has also greatly documented the process. This reviewer also found the details in Appendix F.2 and F. 3 on confounding directionality of co-exposures to be of interest.

	Editorial comment: Please note that throughout the draft report the labeling for the evidence tables at the end of each of the non-cancer endpoints sections were consistently labeled and that was helpful to the reviewers however it appears that the evidence tables or figures were not. Note that sometimes this later table was labeled as a Figure and in the following examples different names were used including "heat maps" as well as evaluation results. Please look across the following example tables/figures to make these more consistent: Table 3-11 and 3-19, Table 3-41 and Figure 3-6. **Editorial recommendation** Please note that Figure 3-86, page 3-274 on Kidney histopathology… has a header that says "PFDA male reproductive organ histopathology". Please confirm this header?
Georgopoulos	**General Comment** ***Tier 3 Future Consideration: Future efforts and revisions of the assessment for PFDA (and other PFAS) should consider cumulative risks in the context of real-world population exposures to PFAS mixtures*** Individuals and communities who will experience high PFDA exposures should reasonably be expected to have above average (and above median) exposures to other PFAS, including the major legacy PFAS. Furthermore, it is recognized that different PFAS share multiple common Adverse Outcome Pathways. It is therefore very important for EPA to continue developing a consistent, integrative, framework for cumulative risk assessments of 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. This requires a concise but essential characterization of the distributions and of the geographical and temporal trends of real-world exposures to PFAS mixtures that include PFDA. As evaluation and assessment of environmental monitoring is not within the scope of the IRIS program, this characterization can consist of a summary of information from available biomarker data (e.g., from NHANES, from on-going CDC/ATSDR biomonitoring studies and NIH-funded studies across the US, and from international efforts in Canada, the European Union, China, etc.). *Finally, it should be recognized that the authors of the draft PFDA Toxicological Review have accomplished a most demanding task: they have developed a thorough and balanced document and they deserve our thanks*. **Selection of Editorial Notes and Recommendations on Specific Issues** • page xi, ABBREVIATIONS AND ACRONYMS: the list appears "generic" and it looks like the majority of acronyms and abbreviations used in the present Toxicological Review are not included; as one example CL is listed here as "Confidence Limit" whereas CL is used as abbreviation for clearance across the Review document. The list should be expanded if it is intended to be relevant to the present Toxicological Review

	• page 1-1: please ensure that the information appearing in Table 1-1 is consistent with the information provided on the CompTox Chemicals Dashboard • page 1-1: The 2018 reference to ATSDR should be updated • page 1-8, line 17: PFBA should be PFDA • page 1-10, line 29: the link to HAWC should be corrected as it takes the reader to https://hawc.epa.gov/assessment/100500073/ which is ORD IRIS Assessment PFBA (2022), instead of the PFDA assessment • page 2-2, Figure 2-1: inconsistencies in listed numbers of references should be addressed • page 3-1, line 15: shorted chain of PFAAs should be short chain PFAS • page 3-11, line 5: PFHxS should be PFDA • page 3-12, line 4: "appreciable" should be either explained/documented or deleted • page 3-12, line 28: PFSA should be PFDA • page 3-13, line 4: "compared to other short chain of PFAA compounds" should be corrected • page 3-14, line 19: why is PFDA compared to "short chained" PFAAs? • page 3-15, lines 15-16: "these half-life estimates of half-life" should be corrected • page 3-15, line 37: PFFDA should be PFDA • page 3-17, line 36: PFHxS should be PFDA • page 3-25, line 10: the statement "it is plausible that human clearance is actually that high" does not appear to be supported by any available data • page 3-26, line 5: "and are associated" should be "are associated" • page 3-44, line 6: pregnant should be pregnane • page 3-67, line, line 15: "four studies study" should be "four studies" • page 3-98, line 8: "examining examined" should be "examined" • page 3-98: it would be better to be consistent in using numerals when referring to the numbers of studies • page 3-16, lines 31-33: the sentences are incomplete and require editing • page 3-154, line 25: "Absence of the fifth sternebrae and delayed phalanges ossific- and mortality at" should be edited/corrected

- page 3-220, Figure 3-72: it should be explained why the study of Kang et al. (2018) is listed as medium in this figure but is described as low confidence on page 3-221 (line 29) and page 3-225 (Table 3-33)
- page 3-286, line 32: "to 28" should be "to 28 days"
- page 4-3, footnotes 14 and 15: "are not advance for" should be "are not advanced for"
- page 5-21, line 11: it is not clear if the cited PFHxS study is unique or if there are similar results from studies for other PFAS
- pages R-14, R-33, R-35 and elsewhere: on various occasions references to articles published in the same year that have different authors with the same last name, e.g. J. Yang and BY Yang, are cited incorrectly as Yang et al. (2022a) and Yang et al. (2022b)
- page F-23: the first page of Appendix F is F-23; page numbering should be corrected for this and the pages that follow
- page F-26, line 22: PDFA should be PFDA
- page G-12, line 2: "general" should be "generally"

Additional Studies for Consideration

HUMAN PHARMACOKINETICS

- Bernstein, A. S., Kapraun, D. F., & Schlosser, P. M. (2021). A model template approach for rapid evaluation and application of physiologically based pharmacokinetic models for use in human health risk assessments: A case study on per-and polyfluoroalkyl substances. Toxicological Sciences, 182(2), 215-228
- 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.
- Pérez, F., Nadal, M., Navarro-Ortega, A., Fàbrega, F., Domingo, J. L., Barceló, D., & Farré, M. (2013). Accumulation of perfluoroalkyl substances in human tissues. Environment international, 59, 354-362.

ANIMAL PHARMACOKINETICS

- Cao, H., Zhou, Z., Hu, Z., Wei, C., Li, J., Wang, L., ... & Liang, Y. (2022). Effect of enterohepatic circulation on the accumulation of per-and polyfluoroalkyl substances: evidence from experimental and computational studies. Environmental Science & Technology, 56(5), 3214-3224.

	IN VITRO STUDIES • 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). • Amstutz, V. H., Cengo, A., Sijm, D. T. H. M., & Vrolijk, M. F. (2022). The impact of legacy and novel perfluoroalkyl substances on human cytochrome P450: An in vitro study on the inhibitory potential and underlying mechanisms. Toxicology, 468, 153116. • Chen, H., Wang, Q., Cai, Y., Yuan, R., Wang, F., & Zhou, B. (2020). Investigation of the interaction mechanism of perfluoroalkyl carboxylic acids with human serum albumin by spectroscopic methods. International Journal of Environmental Research and Public Health, 17(4), 1319. • 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. • Modaresi, S. M. S., Wei, W., Emily, M., DaSilva, N. A., & Slitt, A. L. (2022). Per- and polyfluoroalkyl substances (PFAS) augment adipogenesis and shift the proteome in murine 3T3-L1 adipocytes. Toxicology, 465, 153044. • Pierozan, P., Cattani, D., & Karlsson, O. (2022). Tumorigenic activity of alternative per-and polyfluoroalkyl substances (PFAS): Mechanistic in vitro studies. Science of The Total Environment, 808, 151945. • Xie, M. Y., Lin, Z. Y., Liu, L. Y., Wu, C. C., Liu, Y. W., Huang, G. L., & Zeng, E. Y. (2022). Use of glioma to assess the distribution patterns of perfluoroalkyl and polyfluoroalkyl substances in human brain. Environmental Research, 204, 112011. ***IN SILICO STUDIES*** • Yu, S., Ren, J., Lv, Z., Li, R., Zhong, Y., Yao, W., & Yuan, J. (2022). Prediction of the endocrine-disrupting ability of 49 per-and polyfluoroalkyl substances: In silico and epidemiological evidence. Chemosphere, 290, 133366. ***HUMAN EXPOSURE STUDIES THAT MEASURED BIOMARKERS*** • Bao, J., Shao, L. X., Liu, Y., Cui, S. W., Wang, X., Lu, G. L., ... & Jin, Y. H. Target Analysis and Suspect Screening of Per-and Poly-Fluoroalkyl Substances in Paired Samples of Maternal Serum, Umbilical Cord Serum, and Placenta Near

Fluorochemical Plants in Fuxin, China. SSRN http://dx.doi.org/10.2139/ssrn.4091456

- Blomberg A.J., Norén E., Haug L.S., Lindh C, Sabaredzovic A., Pineda D., Jakobsson K., Nielsen C. (2023). Estimated Transfer of Perfluoroalkyl Substances (PFAS) from Maternal Serum to Breast Milk in Women Highly Exposed from Contaminated Drinking Water: A Study in the Ronneby Mother-Child Cohort. Environ Health Perspect.131(1):17005.
- Chen, X., Feng, X., Sun, X., Li, Y., Yang, Y., Shan, G., & Zhu, L. (2022). Quantifying Indirect Contribution from Precursors to Human Body Burden of Legacy PFASs Based on Paired Blood and One-Week Duplicate Diet. Environmental Science & Technology.
- Claessens, J., Pirard, C., & Charlier, C. (2022). Determination of contamination levels for multiple endocrine disruptors in hair from a non-occupationally exposed population living in Liege (Belgium). Science of The Total Environment, 815, 152734.
- Fiedler, H., Sadia, M., Krauss, T., Baabish, A., & Yeung, L. W. (2022). Perfluoroalkane acids in human milk under the global monitoring plan of the Stockholm Convention on Persistent Organic Pollutants (2008–2019). Frontiers of Environmental Science & Engineering, 16(10), 1-14.
- Li, N., Ying, G. G., Hong, H., & Deng, W. J. (2021). Perfluoroalkyl substances in the urine and hair of preschool children, airborne particles in kindergartens, and drinking water in Hong Kong. Environmental Pollution, 270, 116219.
- Macheka, L. R., Abafe, O. A., Mugivhisa, L. L., & Olowoyo, J. O. (2022). Occurrence and infant exposure assessment of per and polyfluoroalkyl substances in breast milk from South Africa. Chemosphere, 288, 132601.
- Peng, L., Xu, W., Zeng, Q., Sun, F., Guo, Y., Zhong, S., ... & Chen, D. (2022). Exposure to perfluoroalkyl substances in waste recycling workers: Distributions in paired human serum and urine. Environment international, 158, 106963.
- Xu, Y., Fletcher, T., Pineda, D., Lindh, C. H., Nilsson, C., Glynn, A., ... & Li, Y. (2020). Serum half-lives for short-and long-chain perfluoroalkyl acids after ceasing exposure from drinking water contaminated by firefighting foam. Environmental Health Perspectives, 128(7), 077004.
- Xu, Y., Nielsen, C., Li, Y., Hammarstrand, S., Andersson, E. M., Li, H., ... & Jakobsson, K. (2021). Serum perfluoroalkyl substances in residents following long-term drinking water contamination from firefighting foam in Ronneby, Sweden. Environment international, 147, 106333.
- Zheng, G., Schreder, E., Dempsey, J. C., Uding, N., Chu, V., Andres, G., ... & Salamova, A. (2021). Per-and polyfluoroalkyl substances (PFAS) in breast milk: Concerning trends for current-use PFAS. Environmental Science & Technology, 55(11), 7510-7520.

	HUMAN EXPOSURE/RISK STUDIES THAT ONLY MONITOR ENVIRONMENTAL AND MICROENVIRONMENTAL MEDIA • Lin, H., Taniyasu, S., Yamashita, N., Khan, M. K., Masood, S. S., Saied, S., & Khwaja, H. A. (2022). Per-and polyfluoroalkyl substances in the atmospheric total suspended particles in Karachi, Pakistan: Profiles, potential sources, and daily intake estimates. Chemosphere, 288, 132432. • Sonego, E., Simonetti, G., Di Filippo, P., Riccardi, C., Buiarelli, F., Fresta, A., ... & Pomata, D. (2022). Characterization of organophosphate esters (OPEs) and polyfluoralkyl substances (PFASs) in settled dust in specific workplaces. Environmental Science and Pollution Research, 1-15. • Tang, J., Zhu, Y., Li, Y., Xiang, B., Tan, T., Lv, L., & Luo, Q. (2022). Occurrence characteristics and health risk assessment of per-and polyfluoroalkyl substances from water in residential areas around fluorine chemical industrial areas, China. Environmental Science and Pollution Research, 1-11. • Wang, F., Wang, W., Zhao, D., Liu, J., Lu, P., Rose, N. L., & Zhang, G. (2022). Source apportionment and wet deposition of atmospheric poly-and per-fluoroalkyl substances in a metropolitan city centre of southwest China. Atmospheric Environment, 273, 118983. • Wang, Y., Gao, X., Liu, J., Lyu, B., Li, J., Zhao, Y., & Wu, Y. (2022). Exposure to Emerging and Legacy Polyfluoroalkyl Substances in the Sixth Total Diet Study—China, 2016–2019. China CDC Weekly, 4(9), 168. ***HUMAN POPULATION HEALTH STUDIES*** • Cao, L., Guo, Y., Chen, Y., Hong, J., Wu, J., & Hangbiao, J. (2022). Per-/polyfluoroalkyl substance concentrations in human serum and their associations with liver cancer. Chemosphere, 296, 134083. • Duan, Y., Sun, H., Yao, Y., Li, Y., Meng, Y., Lu, Y., ... & Chen, L. (2021). Serum concentrations of per-/polyfluoroalkyl substances and risk of type 2 diabetes: A case-control study. Science of The Total Environment, 787, 147476. • Jin, H., Mao, L., Xie, J., Zhao, M., Bai, X., Wen, J., ... & Wu, P. (2020). Poly-and perfluoroalkyl substance concentrations in human breast milk and their associations with postnatal infant growth. Science of the Total Environment, 713, 136417. • Liu, J. J., Cui, X. X., Tan, Y. W., Dong, P. X., Ou, Y. Q., Li, Q. Q., ... & Zhao, X. M. (2022). Per-and perfluoroalkyl substances alternatives, mixtures and liver function in adults: A community-based population study in China. Environment International, 163, 107179. • Nian, M., Zhou, W., Feng, Y., Wang, Y., Chen, Q., & Zhang, J. (2022). Emerging and legacy PFAS and cytokine homeostasis in women of childbearing age. Scientific Reports, 12(1), 6517.

	• Velarde, M. C., Chan, A. F. O., Sajo, M. E. J. V., Zakharevich, I., Melamed, J., Uy, G. L. B., ... & Gerona, R. R. (2022). Elevated levels of perfluoroalkyl substances in breast cancer patients within the Greater Manila Area. Chemosphere, 286, 131545 • Zhang, Y. T., Zeeshan, M., Su, F., Qian, Z. M., Geiger, S. D., McMillin, S. E., ... & Dong, G. H. (2022). Associations between both legacy and alternative per-and polyfluoroalkyl substances and glucose-homeostasis: The Isomers of C8 health project in China. Environment international, 158, 106913. ***REVIEWS*** • Appel, M., Forsthuber, M., Ramos, R., Widhalm, R., Granitzer, S., Uhl, M., ... & Gundacker, C. (2022). The transplacental transfer efficiency of per-and polyfluoroalkyl substances (PFAS): a first meta-analysis. Journal of Toxicology and Environmental Health, Part B, 25(1), 23-42. Review rodent and human toxicokinetics • Zhu, Q., Li, H., Wen, Z., Wang, Y., Li, X., Huang, T., ... & Ge, R. S. (2020). Perfluoroalkyl substances cause Leydig cell dysfunction as endocrine disruptors. Chemosphere, 253, 126764. Reviews rodent experiments and human epidemiological studies ***GENERAL METHODS FOR PFAS RISK ASSESSMENTS (NOT SPECIFIC TO PFDA)*** • Chou, W. C., & Lin, Z. (2020). Probabilistic human health risk assessment of perfluorooctane sulfonate (PFOS) by integrating in vitro, in vivo toxicity, and human epidemiological studies using a Bayesian-based dose-response assessment coupled with physiologically based pharmacokinetic (PBPK) modeling approach. Environment International, 137, 105581. • Neagu et al. (2021). Adverse outcome pathway in immunotoxicity of perfluoroalkyls. Current Opinion in Toxicology, 25, 23-29. ***LARGE SCALE BIOMONITORING PROGRAMS, FRAMEWORKS, REVIEWS and PROTOCOLS FOR PFAS THAT ARE RELEVANT TO PFDA*** • Calafat, A. M., Kato, K., Hubbard, K., Jia, T., Botelho, J. C., & Wong, L. Y. (2019). Legacy and alternative per-and polyfluoroalkyl substances in the US general population: paired serum-urine data from the 2013–2014 National Health and Nutrition Examination Survey. Environment international, 131, 105048. • Cousins, I. T., DeWitt, J. C., Glüge, J., Goldenman, G., Herzke, D., Lohmann, R., ... & Wang, Z. (2020). Strategies for grouping per-and polyfluoroalkyl substances (PFAS) to protect human and environmental health. Environmental Science: Processes & Impacts, 22(7), 1444-1460. • DeLuca, N. M., Angrish, M., Wilkins, A., Thayer, K., & Hubal, E. A. C. (2021). Human exposure pathways to poly-and perfluoroalkyl substances (PFAS) from

	indoor media: A systematic review protocol. Environment international, 146, 106308. • ECHA (2016). European Chemicals Agency. Member State Committee Support Document for identification of nonadecafluorodecanoic Acid (PFDA) and its sodium and ammonium salts as a Substance of Very High Concern Because of its toxic for reproduction (Article 57 C) and persistent, bioaccumulative, and toxic (PBT) (Article 57 D) properties. Adopted on 2 December 2016. https://echa.europa.eu/documents/10162/77191b64-4f79-0e53-0b89-57098caf46c4 • EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., ... & Schwerdtle, T. (2020). Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal, 18(9), e06223. • EPA SAB (2022). United States Environmental Protection Agency Science Advisory Board. Review of EPA's Analyses to Support EPA's National Primary Drinking Water Rulemaking for PFAS. EPA-SAB-22-008. August 22, 2022. • ITRC (2022) Interstate Technology and Regulatory Council Per- and Polyfluoroalkyl Substances Technical and Regulatory Guidance. ITRC, 50 F Street, NW, Suite 350, Washington, DC 20001.https://pfas-1.itrcweb.org/ • López, M. E., Göen, T., Mol, H., Nübler, S., Haji-Abbas-Zarrabi, K., Koch, H. M., ... & Castaño, A. (2021). The European human biomonitoring platform-Design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals. International Journal of Hygiene and Environmental Health, 234, 113740.
Haney	**Reviewer's References (not cited in EPA's draft assessment):** • Acosta AM, Moro PL, Hariri S, Tiwari TSP. 2021. Chapter 7: Diphtheria. In: Centers for Disease Control and Prevention's Epidemiology and Prevention of Vaccine-Preventable Diseases. Hall E, Wodi AP, Hamborsky J, et al., eds. 14th ed. Washington, D.C. Public Health Foundation, 2021. Available at: https://www.cdc.gov/vaccines/pubs/pinkbook/dip.html • 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 • 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

- Hallare J, Gerriets V. Half Life. 2022. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK554498/
- Hendriksen CF, vd Gun JW, Nagel J, Kreeftenberg JG. 1988. The toxin binding inhibition test as a reliable in vitro alternative to the toxin neutralization test in mice for the estimation of tetanus antitoxin in human sera. J Biol Stand. 16(4): 287-297.
- Mukaka MM. 2012. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 24(3): 69-71.
- Padula AM, Ning X, Bakre S, et al. 2023. Birth Outcomes in Relation to Prenatal Exposure to Per- and Polyfluoroalkyl Substances and Stress in the Environmental Influences on Child Health Outcomes (ECHO) Program. Environ Health Perspect. Mar 131(3): 37006.
- Tiwari TSP, Moro PL, Acosta AM. 2021. Chapter 21: Tetanus. In: Centers for Disease Control and Prevention's Epidemiology and Prevention of Vaccine-Preventable Diseases. Hall E, Wodi AP, Hamborsky J, et al., eds. 14th ed. Washington, D.C. Public Health Foundation, 2021. Available at: https://www.cdc.gov/vaccines/pubs/pinkbook/tetanus.html
- United States Environmental Protection Agency (USEPA). 2021a. External Peer Review Draft. Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water. United States Environmental Protection Agency, Office of Water. EPA Document No: 822D21001.
- United States Environmental Protection Agency (USEPA). 2021b. External Peer Review Draft. Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water. United States Environmental Protection Agency, Office of Water. EPA Document No: 822D21001.
- 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

Appendix A: NHANES Blood serum Data for PFDA

Serum Perfluorodecanoic acid (PFDA) (1999-2000, 2003-2010)

CAS Number 335-76-2

Geometric mean and selected percentiles of serum concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

Demographic Categories	Survey (Years)	Geometric Mean (95% CI)	50th Percentile (95% CI)	75th Percentile (95% CI)	90th Percentile (95% CI)	95th Percentile (95% CI)	Sample Size
Total population	99-00	*	<LOD	.200 (<LOD-.300)	.400 (.300-.600)	.600 (.400-1.00)	1432
Total population	03-04	*	<LOD	.300 (<LOD-.500)	.600 (.400-1.10)	.900 (.500-1.80)	2094
Total population	05-06	.355 (.297-.423)	.300 (.300-.400)	.500 (.400-.700)	.900 (.600-1.60)	1.50 (.900-2.60)	2120
Total population	07-08	.286 (.264-.309)	.300 (.300-.300)	.400 (.400-.500)	.700 (.600-.700)	.900 (.800-1.00)	2100
Total population	09-10	.279 (.258-.303)	.300 (.300-.300)	.400 (.400-.500)	.700 (.600-.800)	.900 (.800-1.10)	2233
Age 12-19 years	99-00	*	<LOD	<LOD	.300 (.200-.400)	.400 (.300-.700)	497
Age 12-19 years	03-04	*	<LOD	<LOD	.500 (<LOD-1.00)	.800 (.300-1.20)	640
Age 12-19 years	05-06	.295 (.258-.338)	.300 (.200-.300)	.500 (.400-.500)	.600 (.500-.800)	.800 (.600-1.60)	640
Age 12-19 years	07-08	.231 (.214-.248)	.200 (.200-.300)	.300 (.300-.400)	.500 (.400-.500)	.600 (.500-.700)	357
Age 12-19 years	09-10	.220 (.198-.245)	.200 (.200-.200)	.300 (.300-.300)	.400 (.400-.600)	.600 (.400-.800)	364
Age 20+ years	99-00	*	<LOD	.300 (.200-.300)	.400 (.300-.700)	.600 (.400-1.40)	935
Age 20+ years	03-04	*	<LOD	.400 (<LOD-.500)	.700 (.400-1.00)	.900 (.500-1.80)	1454
Age 20+ years	05-06	.364 (.303-.438)	.300 (.300-.400)	.500 (.400-.700)	1.00 (.600-1.70)	1.50 (.900-2.60)	1480
Age 20+ years	07-08	.295 (.271-.321)	.300 (.300-.300)	.400 (.400-.500)	.700 (.600-.800)	.900 (.800-1.10)	1743
Age 20+ years	09-10	.289 (.265-.314)	.300 (.300-.300)	.400 (.400-.500)	.700 (.600-.800)	.900 (.800-1.20)	1869
Males	99-00	*	<LOD	.300 (.200-.300)	.400 (.300-.700)	.500 (.300-1.90)	684
Males	03-04	*	<LOD	.400 (<LOD-.500)	.800 (.400-1.40)	1.10 (.600-2.10)	1053
Males	05-06	.381 (.318-.456)	.400 (.300-.400)	.600 (.400-.800)	1.00 (.600-2.20)	1.70 (1.00-2.60)	1048
Males	07-08	.306 (.283-.331)	.300 (.300-.300)	.400 (.400-.500)	.700 (.600-.800)	.900 (.800-1.20)	1059
Males	09-10	.289 (.264-.318)	.300 (.300-.300)	.400 (.400-.500)	.600 (.500-.800)	.800 (.600-1.20)	1075
Females	99-00	*	<LOD	.200 (<LOD-.300)	.400 (.300-.600)	.600 (.300-1.40)	748
Females	03-04	*	<LOD	.300 (<LOD-.400)	.500 (.400-.800)	.800 (.500-1.20)	1041
Females	05-06	.331 (.277-.396)	.300 (.300-.400)	.500 (.400-.600)	.900 (.600-1.40)	1.30 (.800-2.30)	1072
Females	07-08	.267 (.245-.292)	.300 (.200-.300)	.400 (.400-.500)	.600 (.500-.800)	.800 (.700-1.10)	1041
Females	09-10	.270 (.248-.295)	.300 (.200-.300)	.400 (.400-.500)	.700 (.600-.800)	1.00 (.800-1.10)	1158
Mexican Americans	99-00	*	<LOD	<LOD	.300 (<LOD-.400)	.300 (<LOD-1.50)	521
Mexican Americans	03-04	*	<LOD	<LOD	.500 (.400-.500)	.600 (.500-.800)	485
Mexican Americans	05-06	.283 (.245-.327)	.300 (.200-.300)	.400 (.300-.500)	.600 (.500-1.00)	1.00 (.500-2.40)	499
Mexican Americans	07-08	.253 (.222-.289)	.200 (.200-.300)	.400 (.300-.500)	.600 (.400-.700)	.600 (.500-1.40)	391
Mexican Americans	09-10	.242 (.215-.272)	.200 (.200-.300)	.400 (.300-.400)	.500 (.500-.600)	.700 (.600-.800)	461
Non-Hispanic Blacks	99-00	*	.200 (<LOD-.300)	.400 (.200-.800)	.800 (.500-1.40)	1.10 (.600-2.30)	269
Non-Hispanic Blacks	03-04	*	<LOD	.400 (<LOD-.700)	.800 (.400-1.50)	1.00 (.500-3.10)	538
Non-Hispanic Blacks	05-06	.405 (.309-.531)	.400 (.300-.500)	.600 (.400-.900)	1.20 (.600-2.90)	2.30 (1.10-3.70)	544
Non-Hispanic Blacks	07-08	.331 (.298-.368)	.300 (.300-.400)	.500 (.400-.600)	.800 (.600-.900)	1.00 (.800-1.50)	419
Non-Hispanic Blacks	09-10	.336 (.298-.379)	.300 (.300-.300)	.500 (.400-.600)	.800 (.600-1.00)	1.10 (.800-2.60)	391
Non-Hispanic Whites	99-00	*	<LOD	.200 (<LOD-.300)	.400 (.300-.500)	.500 (.400-.800)	491
Non-Hispanic Whites	03-04	*	<LOD	.300 (<LOD-.500)	.600 (.400-1.00)	.900 (.500-1.80)	962
Non-Hispanic Whites	05-06	.350 (.294-.417)	.300 (.300-.400)	.500 (.400-.700)	.900 (.600-1.50)	1.40 (.800-2.30)	935
Non-Hispanic Whites	07-08	.276 (.254-.301)	.300 (.200-.300)	.400 (.400-.500)	.600 (.500-.700)	.900 (.700-.900)	931
Non-Hispanic Whites	09-10	.270 (.240-.304)	.300 (.200-.300)	.400 (.300-.500)	.600 (.500-.800)	.800 (.600-1.10)	1031

Limit of detection (LOD, see Data Analysis section) for Survey years 99-00, 03-04, 05-06, 07-08, and 09-10 are 0.2, 0.3, 0.2, 0.2, and 0.1 respectively.

<LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample.

* Not calculated: proportion of results below limit of detection was too high to provide a valid result.

Biomonitoring Summary: https://www.cdc.gov/biomonitoring/PFAS_BiomonitoringSummary.html

Factsheet: https://www.cdc.gov/biomonitoring/PFAS_FactSheet.html

Serum Perfluorodecanoic acid (PFDA) (2011 - 2018)

CAS Number 335-76-2

Geometric mean and selected percentiles of serum concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

Demographic Categories	Survey (Years)	Geometric Mean (95% CI)	50th Percentile (95% CI)	75th Percentile (95% CI)	90th Percentile (95% CI)	95th Percentile (95% CI)	Sample Size
Total population	11-12	.199 (.181-.220)	.190 (.170-.210)	.300 (.270-.340)	.480 (.420-.580)	.690 (.600-.770)	1904
Total population	13-14	.185 (.165-.208)	.200 (.200-.200)	.300 (.300-.300)	.500 (.400-.600)	.700 (.600-.900)	2168
Total population	15-16	.154 (.140-.169)	.100 (.100-.200)	.300 (.200-.300)	.400 (.400-.600)	.700 (.500-.900)	1993
Total population	17-18	.193 (.178-.209)	.200 (.200-.200)	.300 (.300-.300)	.400 (.300-.500)	.600 (.500-.900)	1929
Age 12-19 years	11-12	.146 (.126-.168)	.150 (.120-.170)	.200 (.180-.230)	.290 (.240-.340)	.360 (.290-.560)	344
Age 12-19 years	13-14	.136 (.122-.152)	.100 (.100-.200)	.200 (.200-.200)	.300 (.200-.400)	.400 (.300-.500)	402
Age 12-19 years	15-16	*	.100 (<LOD-.100)	.200 (.100-.200)	.200 (.200-.400)	.300 (.200-.500)	353
Age 12-19 years	17-18	.153 (.136-.173)	.200 (.100-.200)	.200 (.200-.300)	.300 (.200-.300)	.400 (.300-.600)	313
Age 20+ years	11-12	.209 (.189-.230)	.200 (.180-.230)	.320 (.280-.370)	.510 (.440-.590)	.730 (.630-.850)	1560
Age 20+ years	13-14	.193 (.171-.218)	.200 (.200-.200)	.300 (.300-.400)	.500 (.400-.600)	.800 (.600-.900)	1766
Age 20+ years	15-16	.160 (.144-.178)	.200 (.100-.200)	.300 (.200-.300)	.400 (.400-.600)	.700 (.500-1.00)	1640
Age 20+ years	17-18	.199 (.183-.216)	.200 (.200-.200)	.300 (.300-.300)	.400 (.300-.600)	.600 (.500-.900)	1616
Males	11-12	.206 (.184-.232)	.200 (.180-.230)	.310 (.260-.370)	.440 (.380-.550)	.620 (.480-.810)	966
Males	13-14	.198 (.175-.225)	.200 (.200-.200)	.300 (.300-.400)	.500 (.400-.700)	.800 (.600-1.00)	1032
Males	15-16	.153 (.137-.172)	.100 (.100-.200)	.300 (.200-.300)	.400 (.300-.600)	.700 (.400-1.00)	964
Males	17-18	.190 (.177-.204)	.200 (.200-.200)	.300 (.200-.300)	.400 (.300-.400)	.500 (.400-.600)	952
Females	11-12	.193 (.177-.211)	.190 (.170-.210)	.290 (.260-.340)	.530 (.410-.640)	.690 (.640-.830)	938
Females	13-14	.174 (.155-.195)	.200 (.100-.200)	.300 (.200-.300)	.500 (.400-.500)	.700 (.500-.900)	1136
Females	15-16	.154 (.141-.168)	.100 (.100-.200)	.200 (.200-.300)	.400 (.400-.600)	.800 (.500-1.10)	1029
Females	17-18	.196 (.176-.219)	.200 (.200-.200)	.300 (.200-.300)	.500 (.400-.600)	.700 (.500-1.10)	977
Mexican Americans	11-12	.176 (.150-.205)	.170 (.150-.200)	.260 (.210-.300)	.380 (.300-.530)	.530 (.360-.600)	211
Mexican Americans	13-14	.145 (.125-.169)	.100 (.100-.200)	.200 (.200-.300)	.300 (.200-.500)	.400 (.300-1.40)	332
Mexican Americans	15-16	.124 (.111-.138)	.100 (<LOD-.100)	.200 (.200-.200)	.300 (.200-.300)	.400 (.300-.800)	370
Mexican Americans	17-18	.162 (.138-.190)	.200 (.100-.200)	.200 (.200-.300)	.300 (.200-.500)	.400 (.300-.800)	297
Non-Hispanic Blacks	11-12	.214 (.193-.237)	.200 (.180-.220)	.330 (.280-.380)	.590 (.420-.850)	.880 (.670-1.06)	485
Non-Hispanic Blacks	13-14	.200 (.162-.246)	.200 (.200-.200)	.300 (.200-.500)	.600 (.400-.900)	.900 (.700-1.20)	455
Non-Hispanic Blacks	15-16	.155 (.140-.171)	.100 (.100-.200)	.300 (.200-.300)	.500 (.400-.500)	.800 (.500-1.10)	439
Non-Hispanic Blacks	17-18	.189 (.162-.220)	.200 (.100-.200)	.300 (.200-.300)	.500 (.400-.700)	.800 (.500-1.10)	430
Non-Hispanic Whites	11-12	.193 (.171-.219)	.190 (.170-.220)	.290 (.260-.340)	.440 (.360-.590)	.620 (.480-.740)	666
Non-Hispanic Whites	13-14	.184 (.158-.213)	.200 (.200-.200)	.300 (.200-.400)	.500 (.400-.500)	.700 (.500-.800)	862
Non-Hispanic Whites	15-16	.150 (.136-.166)	.100 (.100-.200)	.200 (.200-.300)	.400 (.300-.500)	.600 (.400-.700)	619
Non-Hispanic Whites	17-18	.196 (.178-.215)	.200 (.200-.200)	.300 (.200-.300)	.400 (.300-.600)	.600 (.400-.900)	667
All Hispanics	11-12	.182 (.156-.212)	.170 (.150-.200)	.270 (.210-.330)	.430 (.330-.530)	.540 (.440-.760)	406
All Hispanics	13-14	.150 (.135-.167)	.200 (.100-.200)	.200 (.200-.300)	.300 (.300-.400)	.400 (.300-1.00)	537
All Hispanics	15-16	*	.100 (<LOD-.100)	.200 (.200-.300)	.300 (.300-.400)	.500 (.400-.600)	629
All Hispanics	17-18	.176 (.154-.201)	.200 (.200-.200)	.200 (.200-.300)	.300 (.300-.500)	.400 (.300-.600)	473
Asians	11-12	.367 (.308-.438)	.350 (.280-.420)	.670 (.510-.870)	1.35 (.870-2.05)	2.05 (1.19-2.52)	291
Asians	13-14	.360 (.294-.439)	.400 (.300-.500)	.700 (.500-.900)	1.50 (1.00-2.10)	2.20 (1.60-3.30)	236
Asians	15-16	.308 (.254-.373)	.300 (.200-.400)	.700 (.400-.900)	1.30 (.800-1.90)	2.10 (1.00-4.60)	220
Asians	17-18	.278 (.227-.340)	.200 (.200-.300)	.500 (.300-.600)	1.10 (.600-1.20)	1.20 (.900-2.40)	257

Limit of detection (LOD, see Data Analysis section) for Survey years 11-12, 13-14, 15-16, and 17-18 are 0.100, 0.100, 0.100, and 0.100 respectively.

<LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample.

* Not calculated: proportion of results below limit of detection was too high to provide a valid result.

Biomonitoring Summary: https://www.cdc.gov/biomonitoring/PFAS_BiomonitoringSummary.html
Factsheet: https://www.cdc.gov/biomonitoring/PFAS_FactSheet.html

Serum Perfluorodecanoic acid (PFDA) (Special Sample of Serum PFAS in Children, 2013-2014)

CAS Number 335-76-2

Geometric mean and selected percentiles of serum concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

Demographic Categories	Survey (Years)	Geometric Mean (95% CI)	50th Percentile (95% CI)	75th Percentile (95% CI)	90th Percentile (95% CI)	95th Percentile (95% CI)	Sample Size
Total population	13-14	*	<LOD	.170 (.130-.230)	.310 (.240-.360)	.370 (.300-.440)	525
Age 3-5 years	13-14	*	<LOD	.170 (.120-.320)	.340 (.160-.470)	.410 (.330-.750)	149
Age 6-11 years	13-14	*	<LOD	.170 (.140-.200)	.290 (.240-.320)	.350 (.320-.380)	376
Males	13-14	*	<LOD	.160 (.120-.230)	.300 (.210-.360)	.360 (.250-.510)	284
Females	13-14	*	.100 (<LOD-.120)	.180 (.150-.230)	.320 (.240-.370)	.380 (.270-.470)	241
All Hispanics	13-14	*	<LOD	.180 (.130-.230)	.270 (.240-.340)	.400 (.270-.470)	186
Other	13-14	*	<LOD	.170 (.120-.240)	.320 (.230-.370)	.370 (.270-.470)	339

Limit of detection (LOD, see Data Analysis section) for Survey year 13-14 is 0.1.

<LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample.

* Not calculated: proportion of results below limit of detection was too high to provide a valid result.

Biomonitoring Summary: https://www.cdc.gov/biomonitoring/PFAS_BiomonitoringSummary.html
Factsheet: https://www.cdc.gov/biomonitoring/PFAS_FactSheet.html

	Reference: See NHANES Biomonitoring Data Tables at: https://www.cdc.gov/exposurereport/data_tables.html

APPENDIX A

LIST OF REVIEWERS

External Peer Review of the EPA Draft "IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA) and Related Salts"

Monday, July 10, 2023: 10:00 AM - 4:00 PM EDT
Tuesday, July 11, 2023: 10:00 AM - 4:00 PM EDT
Thursday, July 13, 2023: 11:30 AM - 2:15 PM EDT
Virtual Meeting via Zoom.gov

Reviewers

John L. Adgate, Ph.D., MSPH
Professor
Colorado School of Public Health
Department of Environmental and Occupational Health
University of Colorado Anschutz Campus
Aurora, CO

Courtney C. Carignan, Ph.D.
Assistant Professor
Department of Food Science and Human Nutrition
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

Elaine M. Faustman, Ph.D., DABT (Panel Chair)
Professor, Environmental and Occupational Health Sciences, and Director, Institute for Risk Analysis and Risk Communication
School of Public Health
University of Washington
Seattle, WA

Jeffrey W. Fisher, Ph.D.
Senior Research Fellow
Computational Biology Division
ScitoVation
Durham, NC

Panagiotis G. Georgopoulos, Ph.D.
Professor, Rutgers Biomedical and Health Sciences
Department of Environmental and Occupational Health and Justice
School of Public Health
Rutgers University
Piscataway, NJ

Joseph T. Haney, Jr., M.S.
Distinguished Toxicologist
Toxicology Division, Office of the Executive Director
Texas Commission on Environmental Quality
Austin, TX

Alan M. Hoberman, Ph.D., DABT
President
Argus International, Inc.
Horsham, PA

Angela M. Leung, MD, MSc
Associate Professor of Medicine
David Geffen School of Medicine
University of California Los Angeles
Los Angeles, CA

R. Thomas Zoeller, Ph.D.
Professor Emeritus
Department of Biology
University of Massachusetts Amherst
Amherst, MA

APPENDIX B

CHARGE TO REVIEWERS

Technical Charge to External Peer Reviewers

Contract No. EP-C-17-017
Task Order 68HERH20F0407
June 2023

External Peer Review of EPA's Draft IRIS Toxicological Review of Perfluorodecanoic Acid [PFDA, CASRN 335-76-2] and Related Salts

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of the draft *IRIS Toxicological Review of Perfluorodecanoic Acid (PFDA) and Related Salts* developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's Center for Public Health and Environmental Assessment within the Office of Research and Development. IRIS assessments contain information about chemicals that 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 perfluorodecanoic acid (PFDA). The draft Toxicological Review of PFDA 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 PFDA or salts of PFDA. The systematic review protocol for PFDA and appendices for dose-response modeling, mechanistic evaluations and pharmacokinetic information and other supporting materials are provided as Supplemental Information (see Appendices A to I) to the draft Toxicological Review.

CHARGE QUESTIONS

In response to the numbered charge questions below organized by topic area (italicized headers), the advice provided as part of this peer review would be most useful when prioritized to indicate its relative importance as follows:

- **Tier 1: *Necessary Revisions*** – Use this category for any revisions you believe are necessary to adequately support and substantiate the analyses or scientific basis for the assessment conclusions.
- **Tier 2: *Suggested Revisions*** – Use this category for any revisions you encourage EPA to implement to strengthen the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the PFDA 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 PFDA Toxicological Review, they could inform future reviews or research efforts.

Literature Search Methods and Documentation

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

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

    EPA 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 criteria or otherwise inform key assessment conclusions, but which were not addressed in the external review draft, for example due to publication after April 2022). EPA will characterize these studies in a document that will be provided to the peer review panel and the public and, following the review, included as an Appendix to the assessment prior to finalization. The characterization will focus on EPA's judgment of whether the studies would have a material impact on the conclusions (i.e., identified hazards or toxicity values) in the external review draft. Following receipt of this additional document after the review is underway, please:

    c. Review EPA's characterization and provide tiered recommendations regarding which studies, if any, would have a material impact on the draft's conclusions and should be incorporated into the assessment before finalizing, as well as your interpretation of the impact of those studies to be incorporated.

Noncancer Hazard Identification

2. For each health effect considered in the assessment and outlined below, please comment on whether the available data have been clearly and appropriately synthesized to describe the strengths and limitations, including whether the presentation and analysis of study results are clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies. Please comment on whether the study confidence conclusions for the PFDA studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes[1]. Please specify any study confidence conclusions that are not justified and explain any alternative study evaluation decisions. For each, please also comment on whether the weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified. Note that the data from studies considered informative to the assessment are synthesized in the relevant health effect-specific sections and available in the Health Assessment Workspace Collaborative (HAWC).

[1] The Toxicological Review provides an overview of individual study evaluations within each evidence synthesis section, and the results of those outcome-specific evaluations are made available in the Health Assessment Workplace Collaborative linked here HAWC. Note that a "HAWC FAQ for assessment readers" document, linked here (scroll to the bottom of the page, and the document is available for download under "attachments"), is intended to help the reviewer navigate this on-line resource.

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

    i. 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 PPARα receptors as a key science issue. To the extent supported by the PFDA literature (and to a lesser extent, literature for 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 evidence from *in vivo* and *in vitro* studies support a potential role for multiple pathways operant in the induction of hepatic effects from PFDA exposure, although how those pathways interact within a mode of action (MOA) cannot be specifically determined.

b. For immune effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause immunosuppression in humans given sufficient exposure conditions, primarily on the basis of consistent evidence of reduced antibody responses from two epidemiological studies in children and one study in adults. Although some evidence for coherent immunomodulatory responses consistent with immunosuppression was identified in short-term animal studies, the animal evidence overall is uncertain. The Toxicological Review concludes the immune effects are considered relevant to humans as the judgment is based on studies in humans.

    i. For nearly all epidemiology studies of PFDA, there is potential that exposure to other highly correlated PFAS could contribute to the observed effects. The evidence synthesis for potential PFDA-induced immune effects included evaluation of the potential for confounding across PFAS as well as other sources of confounding and, based on the available data, determined that residual confounding could explain part of the observed effect, but concern was minimal, and it was unlikely to fully explain the associations seen in the literature.

c. For developmental effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause developmental effects in humans given sufficient exposure conditions, based primarily on consistent findings of dose-dependent decreases in fetal weight in mice gestationally exposed to PFDA supported by some coherent evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy, although uncertainties in the available epidemiological evidence reduced the impact of these latter findings. The Toxicological Review concludes the developmental effects in mice are considered relevant to humans

given similar findings of fetal growth restriction in mice and humans.

i. As described in question 3.c and footnote to 3.c, the evidence synthesis for potential PFDA-induced developmental effects considered potential confounding factors and concluded that confounding across PFAS or from other potential sources of bias (e.g., pregnancy hemodynamics in studies where PFDA was measured during or after pregnancy) introduce significant uncertainty. These sources of uncertainty ultimately reduce the strength of the available human evidence to *slight* for an evidence base that might otherwise be interpreted as *moderate*.

d. For male reproductive effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause male reproductive effects in humans given sufficient exposure conditions, based on coherent evidence in adult male rats exposed to PFDA for 28 days. Although no direct information on the human relevance of the animal evidence is available, the findings in animals are presumed to be relevant based on the conserved role of androgen-dependent pathways in male productive functions across species.

e. For female reproductive effects, the Toxicological Review concludes that the available ***evidence indicates*** PFDA exposure is likely to cause female reproductive effects in humans given sufficient exposure conditions, based primarily on coherent evidence from a 28-day study in adult female rats. Although human studies are available examining associations between PFDA and female reproductive toxicity (e.g., fecundity), the results were mostly null, possibly due to their low sensitivity for observing effects. The Toxicological Review concludes the female reproductive effects are considered relevant to humans given that mechanisms of female reproduction are similar between rats and humans.

f. For cardiometabolic effects, the Toxicological Review concludes that the available ***evidence suggests*** but is not sufficient to infer that PFDA exposure may have the potential to cause cardiometabolic effects in humans given sufficient exposure conditions, based on associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis in a few epidemiological studies. However, the evidence is largely inconsistent across studies, which adds 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 PFDA exposure may have the potential to cause neurobehavioral effects in humans given sufficient exposure conditions, based on associations between PFDA and outcomes related to attention and behavior in epidemiological studies. However, the evidence is largely inconsistent across studies, which adds considerable uncertainty. No evidence was found in experimental animals to inform this outcome (*indeterminate)*.

h. For endocrine, urinary, and other noncancer effects (i.e., hematological, respiratory, digestive, dermal, musculoskeletal, and nervous systems), the Toxicological Review

concludes there is ***inadequate evidence*** to determine whether PFDA exposure has the potential to cause these effects in humans on the basis of the sparsity of available evidence.

Noncancer Toxicity Value Data Selection and Modeling

3. For PFDA, no RfC was derived for inhalation exposures. An RfD is derived based on studies by Budtz-Jorgensen and Grandjean (2018) and Grandjean et al. (2012) showing decreased serum antibody concentrations for both tetanus and diphtheria in children (male and female) at age seven years and PFDA measured at age five years and developmental effects (i.e., reduced birth weight in humans) from the Wikstrom (2020) study. Given the close proximity of the developmental and immune PODs and resulting osRfDs and because these effects are observed during the developmental period, they are selected as co-critical effects supporting the RfD. Are the selection of the studies for the immune (Budtz-Jorgensen and Grandjean, 2018) and developmental (Wikstrom, 2020) effects for use in deriving the RfD values for PFDA scientifically justified? Are the modeling approaches appropriate?

    a. If so, please provide an explanation.

    b. If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the lifetime RfD and detail the rationale for use of such an alternative.

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

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

    e. For <u>liver, male reproductive and female reproductive effects</u>, quantitative information was limited to studies in animals exposed to PFDA for 28 days and little to no information was

[2] For the decreased antibody responses, Selgrade (Tox Sci 2007;100:328–332) suggests that these specific immunotoxic effects may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards.
For developmental effects (i.e., fetal growth restriction), the human evidence was determined to be *slight*, primarily due to potential confounding by hemodynamic changes among studies showing birth weight deficits. For the study (i.e., Wikström, 2020) used to derive the developmental RfD, there is no presumed impact of pregnancy hemodynamics given the early sampling (96% from trimester 1). However, unlike the Wikström (2020) study, some uncertainty remains across many of the available human developmental studies given the predominance of associations that were detected were for studies with later pregnancy sampling.

available to evaluate the effects of chronic exposure on these health hazards. Therefore, the derivation of lifetime organ-specific (os) RfD values was not attempted for liver, male reproductive and female reproductive effects. However, these endpoints were considered for the derivation of subchronic osRfDs. Does the provided scientific rationale support this decision? Please explain.

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

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

   a. If so, please provide an explanation.

   b. If not, please provide an alternative study(ies) or effect(s) that should be used to support the derivation of the subchronic RfD and detail the rationale for use of such an alternative.

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

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

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

Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors

5. Appendix G identifies the potential for pharmacokinetic (PK) differences across species and sexes as a key science issue and lays out a hierarchy for using relevant PK data in extrapolating doses between laboratory animals and humans. 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. However, the evaluation of existing PBPK models and a one-compartment PK model found that these options were not sufficiently reliable for use. Given the information available on potential interspecies differences in PFDA PK, EPA applied a data-derived extrapolation factor (DDEF) to POD values from toxicity studies in laboratory animals to estimate corresponding human equivalent doses (HEDs) in the derivation of the respective RfDs. Similarly, the estimated human clearance (CL) was used to convert internal dose POD (PODint) values from epidemiological analyses to corresponding HEDs.

   After publicly releasing the draft IRIS PFDA assessment, the EPA evaluated recently published data for several other long-chain PFAS, described here (U.S. EPA, 2023, HERO ID 11181055), that are potentially relevant to evaluating PFDA dosimetry in women of childbearing age (see question 5c

below).

a. Is applying the estimated DDEF values for PFDA 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 application of the human CL to estimate HEDs from PODint values scientifically justified? If not, please provide an explanation and detail on a more appropriate approach

c. Have the uncertainties in the DDEFs and human CL been adequately evaluated and described? In answering this question, please provide an explicit recommendation on whether or not EPA should expand its adjustment for menstrual fluid loss as outlined in (U.S. EPA, 2023, HERO ID 11181055) prior to finalizing the assessment. As these newer data are from other PFAS, note that such an expansion would be based on the assumption that the pharmacokinetic effect of pregnancy and lactation on PFDA is similar to that of the other PFAS (i.e., a read-across based interpretation). See file named IRISPFDA_ChargeQuestion5c_SupplementalInformation.PDF

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

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

        i. Please comment specifically on whether the methods used to derive toxicity values for PFDA appropriately account for uncertainties in evaluating the pharmacokinetic differences between the experimental animal data and humans?

    b. For immune effects, a UF_S of 1 and 3 were considered to account for extrapolation from less than lifetime human data; ultimately a UF_S of 1 was selected. A UF_S of 10 was not considered as the developmental period is recognized as a susceptible lifestage for these types of effects and therefore exposure during this time window can be considered more relevant than exposure in adulthood (U.S. EPA, 1991). Also important is the fact that, given PFDA's long half-life and the expectation that the children and their mothers have been exposed to elevated levels of PFDA for many years, the observed effects on immune response are considered to be the result of a cumulative, prolonged exposure. Uncertainties with regards to additional susceptible life stages (e.g., old age) are addressed as part of the UF_D. Does the provided scientific rationale support this decision? Please explain.

    c. For liver effects, 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 if that were the sole pathway leading to these effects, evidence for the involvement of non-PPARα pathways is available in the PFDA database. Thus, uncertainty remains regarding the potential differences in sensitivity across species due to the involvement of both PPARα-dependent and PPARα- independent

mechanisms. As such, the Toxicological Review concludes the available data are not adequate to determine if humans are likely to be equally or less sensitive than laboratory animals with respect to the observed 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 is clearly documented.

d. For liver, male reproductive, and female reproductive effects, a default 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 (prolonged diestrus, sperm measures and increased liver weight) to worsen with increasing duration and the large uncertainty associated with the lack of chemical-specific data to evaluate the effects of subchronic exposure on liver, male reproductive and female reproductive outcomes, the Toxicological Review concludes that application of a UF_S of 10 is supported for the purposes of deriving the subchronic RfD from the 28-day toxicity data. Does the provided scientific rationale support this decision? Please explain.

e. Are the provided rationales for the remaining uncertainty factors (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 PFDA within different populations or lifestages, including any potential impacts from early life exposure to PFDA on children's health or health later in life, although few studies on susceptibility were available)? If not, please explain.

Carcinogenicity Hazard Identification and Toxicity Value Derivation

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

9. Given the conclusion there was *inadequate information to assess carcinogenic potential* for PFDA, 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 Perfluorodecanoic Acid and Related Salts" (PFDA)

Monday, July 10, 2023: 10:00 AM - 4:00 PM EDT
Tuesday, July 11, 2023: 10:00 AM - 4:00 PM EDT
Thursday, July 13, 2023: 11:30 AM - 2:15 PM EDT
Virtual Meeting via Zoom.gov

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

Final Agenda

DAY 1: Monday, July 10

10:00 AM	**Meeting Purpose, Peer Review Process & Reviewer Intros** *Jan Connery, ERG (facilitator)*
10:20 AM	**U.S. EPA Office of Research and Development (ORD) Background Presentation**
11:05 AM	**Public Comments** ..... *Jan Connery, ERG*
11:15 AM	**Reviewer Discussion Agenda and Process** ..... *Jan Connery, ERG*
11:25 AM	BREAK
11:45 AM	**Chair Opening Remarks to Panel** ..... *Peer Review Chair*
11:55 AM	**Reviewer Discussions:**
	Literature Search Methods and Documentation
11:55 AM	**Charge Question 1: Literature Search Methods and Documentation** *Peer Review Panel*
	Noncancer Hazard Identification
12:40 PM	**Charge Question 2(a): Liver Effects** ..... *Peer Review Panel*
1:10 PM	BREAK
1:25 PM	**Charge Question 2(b): Immune Effects** ..... *Peer Review Panel*
2:20 PM	BREAK
2:35 PM	**Charge Question 2(c): Developmental Effects** ..... *Peer Review Panel*
3:30 PM	**Charge Question 2(d): Male Reproductive Effects** ..... *Peer Review Panel*
4:00 PM	**ADJOURN Day 1**

DAY 2: Tuesday, July 11

10:00 AM	**Day 1 Recap**, **Day 2 Agenda and Process** ..... *Jan Connery, ERG*
10:05 AM	**Reviewer Discussions:**
	Noncancer Hazard Identification (cont.)

DAY 2: Tuesday, July 11 (cont.)

10:05 AM	**Charge Question 2(e): Female Reproductive Effects**	*Peer Review Panel*
10:35 AM	**Charge Question 2(f): Cardiometabolic Effects**	*Peer Review Panel*
	Reviewer Discussions (cont.):	
	Noncancer Hazard Identification (cont.)	
10:55 AM	**Charge Question 2(g): Neurodevelopmental Effects**	*Peer Review Panel*
11:15 AM	BREAK	
11:35 AM	**Charge Question 2(h): Endocrine, Urinary, and Other Effects**	*Peer Review Panel*
	Noncancer Toxicity Value Data Selection and Modeling	
11:50 AM	**Charge Question 3: Derivation of Lifetime RfD**	*Peer Review Panel*
12:55 PM	BREAK	
1:20 PM	**Charge Question 4: Derivation of Subchronic RfD**	*Peer Review Panel*
	Noncancer Toxicity Value Pharmacokinetic Extrapolation and Uncertainty Factors	
1:55 PM	**Charge Question 5: Pharmacokinetic Extrapolation**	*Peer Review Panel*
2:40 PM	BREAK	
3:05 PM	**Charge Question 6: Uncertainty Factors**	*Peer Review Panel*
3:55 PM	Day 3 Preview	*Jan Connery, ERG*
4:00 PM	**ADJOURN Day 2**	

DAY 3: Thursday, July 13

11:30 AM	**Day 2 Recap**, **Day 3 Agenda and Process**	*Jan Connery, ERG*
11:35 AM	**Reviewer Discussions:**	
	Carcinogenicity Hazard Identification and Toxicity Value Derivation	
11:35 AM	**Charge Question 7: Carcinogenicity Hazard Identification**	*Peer Review Panel*
11:55 AM	**Charge Question 8: Toxicity Value Derivation**	*Peer Review Panel*
	General Discussion and Closing	
12:10 PM	**Reviewer Integrative Comments and Discussion**	*Peer Review Panel*
12:40 PM	BREAK	
12:55 PM	**Individual Reviewer Recommendations**	*Peer Review Panel*
2:10 PM	**Closing Remarks**	*EPA, ERG*
2:15 PM	**ADJOURN DAY 3**	