Department of Defense (DoD)
Comments on the Interagency Science Consultation (Step 3)
Draft IRIS Toxicological Review of Perfluorononanoic Acid (PFNA) and Related Salts
Dated July 2023

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Section	Page(s)	Comment	Suggested Action	*Category
E.S.	xvii	EPA indicates that all methods for the assessment are provided in the Protocol, which was released in 2019. EPA performed study quality evaluations for each study; however, in 2019, the "Staff Handbook for Developing IRIS Assessments" had not been released. The Handbook, finalized in 2022, provides detailed study quality assessment guidance, among other substantive guidance following over a decade of IRIS reform. It is unclear whether EPA made changes to its quality evaluation methodology while the PFNA document was in development. The PFNA IRIS cites the Draft, but not the final Handbook.[1]	Suggest that EPA add information to the Appendix pertaining to any modifications to study quality assessment throughout the development of the PFNA IRIS process, if applicable.	S/E
E.S.	xvii	EPA provides simple tables depicting its quality evaluation ratings for animal and human studies, noting the ratings for each domain in the form of symbols, ranging from ++ (good) to - - (critically deficient). There are no accompanying tables in the draft or the appendices that describe how EPA determined the scores for each domain (i.e., explaining why each rating was given). In contrast, detailed tables describing the ratings of each domain were provided in the supplemental information of the IRIS Toxicological Review of Formaldehyde, released in April 2023.	Suggest EPA include detailed tables of the study evaluation findings for each study.	S/E

[1] U.S. EPA. ORD Staff Handbook for Developing IRIS Assessments (2022). U.S. EPA Office of Research and Development, Washington, DC, EPA/600/R-22/268, 2022.

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ES.1 **3.4**	xviii	EPA uses the term “causes” (l. 25) to describe the relationship between oral exposure to PFNA and health outcomes in humans. However, the epidemiological evidence of PFNA exposure and health effects is in many cases limited to a few studies, and often a mix of studies with varying overall methodological quality. Furthermore, few of the study authors present any conclusions regarding causation. Thus, it may for EPA to present conclusions as the weight of evidence for “associations,” rather than causes. Cohort studies are the best study design to assess causal inference, while cross-sectional studies cannot be used to establish causation. A mixture of cohort and cross-sectional studies were assessed in the PFNA literature. If EPA intends to assess causation based on the weight of the evidence, EPA must more clearly evaluate study design in drawing conclusions about PFNA and human health effects. There are several examples in the comments that follow that demonstrate that EPA may have overstated the strength and weight of some weaker study designs.	EPA should consider removing the word “causes;” EPA should also more carefully consider study design within the lens of causal inference.	S/E
ES.1	xviii	EPA concludes “[t]here is robust epidemiological evidence that PFNA exposure is associated with deficits in birth weight.” However, of the 21 studies EPA included, only six reported statistically significant associations between PFNA and birth weight decreases. Additionally, the studies analyzed the outcome of birth weight differently, so the effect estimates are not directly comparable, and this is not acknowledged in this section or in Section 3.2.2.	EPA must clarify how it is incorporating statistical significance in its assessment of associations. Additionally, EPA should consider and acknowledge that birth weight deficits were differently assessed among studies.	S/E
ES.1	xviii	A more recent article by Marshal et al. 2021 does not indicate PVDF fluoropolymer breakdown results in PFNA.	Marshall et al. 2021. On the Solubility and Stability of Polyvinylidene Fluoride. Polymers. 13:1354. https://doi.org/10.3390/polym13091354	S
ES.2	xxii	Does the estimation of CL_H negate the need for a 10-fold UF to account for intraspecies variability? At a maximum, the human susceptibility would require a UF of 3, not 10.	Suggest clarifying text be inserted here.	S

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ES.3	N/A	It is appreciated that EPA provides a high-level summary of the number of epidemiological studies and animal studies and their quality judgments at the outset of the study evaluation section, including their confidence in the oral reference dose.	N/A	O
ES.5	xxiii	EPA's conclusions on carcinogenicity, i.e., that there is "inadequate information to assess carcinogenic potential" for both oral and inhalation routes is supported. The lack of available evidence to quantitatively carcinogenic potential also is appropriate.	N/A	S
ES.5	xxiii	EPA's assessment that there is inadequate evidence to derive an inhalation RfC is appropriate given that only one acute exposure study was identified, and it was determined to be of low confidence.	N/A	S
1.1.1 Physical and Chemical Properties	Table 1-1	Soil adsorption coefficient (L/kg) is often referred to as K_{OC}.	Please add "K_{OC}" to Table 1-1.	E
1.1.1 Physical and Chemical Properties	Table 1-1	The US EPA CompTox Dashboard identifies PFNA (CASRN: 72007-68-2) as having water solubility of 1.64E-03 mol/L. This is very different from any of the values reported in Table 1-1.	Please resolve differences. Please check other values in Table 1-1 with those provided in the CompTox Dashboard.	E
1.1.2 Sources, Production, and Use	1-3	It is unclear to what extent (weight fraction percent) is PFNA a residual byproduct contained in PVDF products for industrial and consumer uses.	Unless the actual product PFNA content is reported, it should be considered a trace byproduct of processing.	S/M

Section	Page(s)	Comment	Suggested Action	*Category
1.1.2 Sources, Production, and Use	1-4	Given the vapor pressure and Henry's Law constant in Table 1-1, is PFNA sufficiently volatile to have been released to air? If it was released to air, was it bound to particulate, released as an aerosol, or a vapor?	Please clarify type and fraction of release to air.	S/M
1.1.2 Sources, Production, and Use	1-4	A more recent publication (Marshall et al. 2021) does not indicate PFNA is a significant breakdown product of PVDF. Does the reference of Kim and Kannan (2007) provided by USEPA support PFNA as a breakdown product? If so, why is it specifically associated with PVDF in ES-1 and not in this paragraph?	Please resolve.	S/M
1.1.2 Sources, Production, and Use	1-4	Does FTOH breakdown or degrade to PFNA? How much PFNA is produced by FTOH degradation?	What amount of PFNA is produced by degradation of FTOH? In Poothong et al (2020), the degradation of 8:2FTOH and 8:2diPAP is said to contribute to PFOA (a biotransformation factor of 0.003) and PFNA (a biotransformation factor of 0.0003). This suggests a very small amount of PFNA is produced during 8:2(FTOH/diPAP) degradation. The text should report on the relative amount of PFNA production via the biotransformation of precursor PFAS.	E
1.1.3 Environmental Fate and Transport	1-4	The statement that "*PFNA released to air will exist in the vapor phase given its vapor pressure*" might conflict with other statements in this section (Lines 35-37 this page) or with the rule of thumb – chemicals vapor pressures below 10 Pa (PFNA = 1.2 Pa) tend not to be volatile chemicals. What vapor pressure is required to predict that a chemical will preferentially exist as a vapor? Would PFNA released as an aerosol volatilize to vapor?	Please reconcile and cite why this statement is true or eliminate.	S

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1.1.4 Potential for Human Exposure and Populations with Potentially Greater Exposure	1-5	Information exists concerning the relative intake of PFAS by different routes of exposure. Poothong et al. (2020) reports specifically identify the relative intake of PFNA via the diet, ingestion of house dust, inhalation of indoor air, and dermal absorption. Estimated daily intake of dermal PFNA exposure is 1/100th that of diet (Poothong et al 2020). It would be helpful to add this context to the statement regarding PFNA dermal exposure. ATSDR's Toxicological Profile for Perfluoroalkyls reports an estimated dermal penetration coefficient for PFOA of 9.49E-07 cm/hour. This is quite low and PFNA is expected to be even lower. The conditions of PFOA dermal absorption are also discussed by ATSDR (page 563) and are likely relevant to PFNA dermal absorption. These should be discussed or at least mentioned and cited by EPA.	Please add appropriate contextual information regarding the relative importance of the various routes of exposure. Poothong et al. 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. Environment International. 134:105244. https://doi.org/10.1016/j.envint.2019.105244	S/M
1.1.4 Potential for Human Exposure and Populations with Potentially Greater Exposure	1-5	Water: This information has been updated. The preliminary data for PFAS in water associated with the 5th UMCR should be included here.	Please update with current information	E/M
1.1.4 Potential for Human Exposure and Populations with Potentially Greater Exposure	1-5	Additional resources exist that describe dietary intake of PFAS and specifically PFNA (Poothong et al, 2020).	Please revisit the available literature and add appropriate reference materials. Poothong et al. 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. Environment International. 134:105244. https://doi.org/10.1016/j.envint.2019.105244	S/M

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1.1.4 Potential for Human Exposure and Populations with Potentially Greater Exposure	1-6	Please specify the source of the acronym "MRL" every time it is used. Confusion is caused by multiple uses of MRL. The US EPA refers to MRL as a Minimum Reporting Level (Page 1-5; Line 21) and ATSDR refers to MRL as a Minimum Risk Level. Is the concentration reported in parenthesis (0.096 ug/L) the EPA MRL or the detected concentration above the EPA MRL?	Please add citation for MRL wherever it is used so that the reader will know what MRL value is being used. Please clarify the concentration reported.	E
1.1.4 Potential for Human Exposure and Populations with Potentially Greater Exposure	1-7	The nearly 2,000 survey participants should be further characterized as reflecting locations throughout the US (~2,000 persons sampled annually). Furthermore, the stated limit of detection (LOD) for NHANES is 0.1 ng/mL.	Suggest EPA add the appropriate context to the annual NHANES population sampled.	E
1.1.4 Potential for Human Exposure and Populations with Potentially Greater Exposure	1-8 to 1-9	While the text on these pages identifies the relative levels of PFNA in the various populations, there is no information on the actual level of PFNA measured. For example, on lines 14-16 (this page), Inuit women were found to have a PFNA serum concentration 6.3-times higher than that for pregnant women in the Canadian Health Measure Survey (CHMS). There is no reporting, however, of the actual PFNA concentration measured in Inuit women or the pregnant women assessed in the CHMS.	Please report the PFNA concentrations actually measured.	E
3.1 Pharmacokinetics	3-1	The statement "*…which differ in the perfluoroalkyl chain.*" would benefit from further detail.	Suggest replacing "*the*" in this statement with "*branching of the*".	E
3.1 Pharmacokinetics	3-1	Free fatty acids also act as protein receptor co-factors and agonists. It is possible that PFNA might also have these properties.	Please add this additional functionality after the "(Papamandjaris et al., 1998)" reference. Reference: Niphakis et al. 2015. A Global Map of Lipid-Binding Proteins	S/M

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			and Their Ligandability in Cells. Cell. 161:1668-1680.	
3.1 Pharmacokinetics	3-1	The statement, "*However, unlike its fatty acid analog, PFNA is impervious to metabolism in mammals due to the perfluoro substitution of the alkyl chain.*" is not supported in any of the preceding or following text.	Recommend changing this statement to read "*However, unlike fatty acids, PFNA is not metabolized in mammals*.". A reference regarding the lack of PFNA metabolism in mammals to support the statement should be added.	E
3.1 Pharmacokinetics	3-1	The statement *"… relatively large concentrations …*". Is vague. It would be more useful to simply report the abundance of albumin in the blood	Medically, the normal range for albumin in blood is 3.4 to 5.4 grams per deciliter (g/dL). This amounts to 35 to 50% of all blood (serum) protein (Kaneko 1997). REFERENCE: Kaneko JJ. 1997. Serum proteins and the dysproteinemias. In: Kaneko JJ. Clinical biochemistry of domestic animals. San Diego (CA): Academic Press; p117–138.	S
3.1 Pharmacokinetics	3-2	Does the Genuis et al (2010) reference suggest that enterohepatic circulation of PFNA could increase levels of PFNA in the liver? Or does the Genuis et al. paper just refer just to the enterohepatic circulation of PFAS and PFNA? The finding of enterohepatic circulation does not, by itself, indicate liver accumulation.	Please clarify.	S/M
3.1 Pharmacokinetics	3-3	Unclear what "… a modest assumption." is. The assumption is **conservative** in that it assumes the higher absorption rate in mice is more similar to the human condition than the lesser absorption observed in the rat.	Suggest replacing "modest" with "conservative" in this sentence.	E

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3.1 Pharmacokinetics	3-4	What is meant by "*For consistency, …*"? This is not consistent with the calculations performed in the prior text. Perhaps a better phrasing is "*Because of the variation in reported and calculated Vd estimates in mice, Vd is calculated as …*"	Please consider clarifying this passage.	E
3.1 Pharmacokinetics	3-4	Do you mean "*robust*", commonly defined as "*strong and vigorous health*" or do you mean to suggest that the C_{MAX} is a good central tendency estimate (CTE) of this distribution.	Please clarify.	E
3.1 Pharmacokinetics	3-5	The argument for accepting a 20% error in the determination of dose is not convincing. This error is not modest. How does the acceptance of such error enable a more thorough evaluation of uncertainty in clearance? See also: test on Page 3-13 (Lines 26-28), where the error is estimated to be 25%. Appendix E should be re-read and modified to improve readability. Small errors (typographical) hinder reader understanding of what was done and why. For example: (Page E-8, line 16) the text is unclear *"… model the data assuming 100% or bioavailability appears consistent in that regard (no apparent …"*	Suggest EPA provide stronger justification for 20% error. Revise text in Appendix E.	S/M
3.1 Pharmacokinetics	3-7, 3-8	Generally, one places foot notes immediately following the applicable text. Footnote "9", regarding the preparation of hair samples, should be placed after the text "*The authors found that the hair concentration* **[PLACE FOOTNOTE HERE]**, *which could be …"*	Please revise.	E
3.1.2 Distribution – General Considerations	3-8	Additional detail should be added to the description of the Benskin et al. study here. The sentence "*One animal study which …*" can be re-written to include additional detail.	Suggest revising the text to read "*One study in rats, which distinguished between n- (linear) and iso-(branched)-PFNA isomer tissue levels showed that …*"	E

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3.1.2 Distribution – General Considerations	3-9	Additional detail added to the text can be useful for clearly communicating to the reader vital information. The sentence beginning with "*The resulting geometric mean …*" does not specifically identify what is being addressed.	Suggest adding to the subject sentence the fact that the statistic is for the volume of distribution. The revised text might read "*The resulting geometric mean and 95th% CI estimated by the authors for the human Vd is 0.19 (0.11-0.30) L/kg, which is lower than EPA mean values estimated in rats and mice, but within the range of reported values …*"	E
3.1.2 Distribution – General Considerations	3-9	What is meant by the sentence "*Another examination of humans revealed a mass fraction in plasma of 0.79 (Jin et al., 2016).*"? It is unclear what the mass fraction is of, and how it relates to the information presented in this paragraph. The paragraph appears to speak to two (2) topics and might be more easily interpreted by the reader as two separate paragraphs; (1) on serum plasma ratios and (2) regarding PFNA binding to serum proteins.	Please consider revising the text to read more clearly is meant by the statement.	E/M
3.1.2 Distribution – General Considerations	3-9	Given the earlier Benskin et al (2009) reference to the order of PFNA prevalence in various organs (page 3-7, lines 2-3), one might make the argument that PFNA preferentially resides in the blood and that the Benskin et al. rat study orders tissues by their relative blood content (blood perfusion). What is a reasonable explanation of the Yeung et al. (2013) data in Liver? How does the actual concentration of PFNA in liver tissue compare to that determined in the Perez et al. (2013) study (1.0 ng/g)? Presumably PFNA tissue levels should be limited to PFNA binding to tissue-specific proteins. For this reason, more weight should be placed on the Perez et al. (2013) study of human cadavers than on the available rat studies, which may have simply measured PFNA content in tissues containing more blood.	Please consider discussing if PFNA binding to tissue-specific protein explains the Yeung et al. (2013) data.	S/M
3.1.2 Distribution – General Considerations	3-11	Why assume the Vd in an adult woman is the same as in female rat when you have an estimated human Vd of 0.19 L/kg, which was earlier identified as lower than the rat Vd estimated by EPA?	Please justify assumptions made or re-work the calculation of an average maternal tissue concentration.	S/M

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3.1.3 Metabolism	3-12	Is PFNA the primary metabolite of 8:2FTOH metabolism? If it is, or is a secondary or tertiary metabolite, what is the amount of PFNA produced by metabolism of 8:2FTOH? In Poothong et al (2020), the metabolism of 8:2FTOH and 8:2diPAP primarily produces PFOA (a biotransformation factor of 0.003) and then PFNA (a biotransformation factor of 0.0003). These biotransformation factors suggest a very small amount of PFNA is produced by 8:2FTOH metabolism. Is the amount significant with regard to the PFNA human body burden?	Please clarify.	S/M
3.1.4 Excretion	3-12	The sentence: "*This suggests that there are sex-specific differences 27 in resorption of PFNA from bile in the gut.*" cannot be generalized to all animals since there are known resorption differences between rats and humans. The sentence requires added context.	Consider revising the sentence to read "*This suggests that there are sex-specific differences in **the rat for** resorption of PFNA from bile in the gut.*"	E/M
3.1.4 Excretion	Table 3-6	Critical to this discussion is the specific strain of rat and mouse used in each study. This information might be added to the Table for additional clarity or discussed in the text and eliminated as a potential variable in PFNA half-life determinations.	Please consider adding the relevant information to the table.	S/M
3.1.4 Excretion	3-16	Excretion in menstrual fluid (i.e., blood) is a significant route in women because a volume of blood containing PFAS is expelled from the body (loss of PFAS), not because urinary excretion is extremely low. It is true that if urinary excretion was higher the loss of PFAS in menstrual fluid (blood) would be less significant, but the relevance is unclear.	Consider revising the text to add this context.	E
3.1.4 Excretion	3-16	Fecal excretion of PFAS in humans is governed by enterohepatic circulation (i.e., the reabsorption of PFAS from the gut). Is the enterohepatic circulation of PFNA similar in rats and humans?	Please clarify.	E
3.1.5 ADME Summary	3-24	EPA concludes that "human Vd was assumed to be similar to the value in rats." There is a high degree of uncertainty given the sparse data available in humans. Accurately estimating Vd is critical to convert between half-life and clearance.	Suggest EPA consider caveat statements to acknowledge the limited human toxicokinetic information.	E

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		Further, EPA should clarify that the men in Table 3-7 are of all ages, since the women are broken out by age group (as is what is available in the data).		
3.2.2 Developmental Effects	3-27	EPA evaluated 24 epidemiological studies on the association between PFNA exposure and birth weight. Seventeen of these studies reported overall results for both sexes combined, while 12 analyses were sex specific. Ten of the 17 studies reported birth weight deficits in the population relative to increasing maternal PFNA exposure, six of which reported statistically significant associations. EPA evaluated the overall confidence in each study consistent with IRIS guidance; however, there were some inconsistencies in its application of its study evaluation framework. Kwon et al. (2016) reported a statistically significant decrease in mean birthweight. The Kwon et al. (2016) study was classified as medium confidence by EPA, notably based on its medium ratings in confounding analysis and exposure measurement methods. However, Kwon et al. (2016) is a cross-sectional study meaning the exposure and outcomes are measured at the same time. Because temporality cannot be established, cross-sectional studies cannot be used to assess causal relationships. Additionally, key potential confounding factors, such as GFR and blood volume, were not taken into consideration. The authors reported adjusting for alcohol consumption, gender, gestational age, maternal age, maternal pre-pregnancy body mass index, and parity. In contrast, another cross-sectional study by Shi et al. (2017), took into consideration similar confounders, including parity (a key confounder), but received a low confidence rating for confounding. Similarly, Kwon et al. (2016) used cord blood as a measurement of exposure assessment and received a high confidence rating for the exposure domain. However, Chen et al. (2012), a cross sectional study, also used cord blood and received a medium confidence rating for exposure assessment.	Recommend EPA consider re-evaluating its “medium confidence” determination for subdomains of the Kwon et al. (2016) study considering issues with confounding of PFNA exposure, among other limitations, to better match the assigned confidence to other cross-sectional studies in this assessment. An overall rating of low confidence should be assigned to this study. More globally, EPA should re-assess study quality ratings, given the inconsistent way it judged study quality among studies with similar quality features.	S
3.2.2 Developmental Effects	3-27	EPA evaluated 24 studies on the association between PFNA exposure and birth weight. Seventeen of these studies reported overall results for both sexes combined, while 12 analyses were sex specific. Ten of the 17 studies reported	Recommend EPA consider re-evaluating its “medium confidence” determination for subdomains of the	S

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		birth weight deficits in the population relative to increasing maternal PFNA exposure, six of which reported statistically significant associations. EPA evaluated its overall confidence in each study consistent with IRIS guidance; however, there were some inconsistencies in the application of its study evaluation framework. Gyllenhammer et al. (2018) reported a statistically significant decrease in mean birthweight. The Gyllenhammer et al. (2018) study was classified as medium confidence by EPA, based on its medium ratings in confounding analysis and exposure measurement methods. However, Gyllenhammer et al. (2018) is a cross-sectional study meaning the exposure and outcomes are measured at the same time. Because temporality cannot be established, cross-sectional studies cannot be used to assess causal relationships. Additionally, key potential confounding factors, such as GFR and blood volume, were not taken into consideration. The authors reported adjusting for sampling year, maternal age, pre pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, years of education, and total time of breastfeeding. In contrast, another cross-sectional study by Shi et al. (2017), took into consideration similar confounders, including parity (a key confounder), but received a low confidence rating for confounding. Similarly, Gyllenhammer et al. (2018) used 3-month postpartum blood samples as a measurement of exposure assessment and received a medium confidence rating for the exposure domain. However, Maekawa et al. (2017), a cross sectional study, which was ultimately excluded from analysis, received a low confidence rating despite collecting samples for PFAS exposure in the third trimester of gestation and sampling maternal blood and urine as well as cord blood and amniotic fluid. This demonstrates that EPA inconsistently in evaluated study quality in the IRIS assessment.	Gyllenhammer et al. (2018) study considering issues with confounding of PFNA exposure, among other limitations to better match the assigned confidence to other cross-sectional studies in this assessment despite inclusion of a sensitivity analysis. An overall rating of low confidence should be assigned to this study. More globally, EPA should re-assess study quality ratings, given the inconsistent way it judged study quality among studies with similar quality features.	
3.2.2 Developmental Effects	3-37	EPA should clarify how parity is taken into consideration as a confounder for assessment of the confounding domain. Although EPA states that parity is a key confounder, it is unclear what weight or role it specifically was evaluated, particularly among the many other potential confounders considered for the confounding domain.	EPA should include greater clarity regarding how key confounders affected the confounding domain confidence score	E
3.2.2 Developmental Effects	3-37	In the literature there is an ongoing debate on how to account for potential confounding by parity in reproductive epidemiological studies. Bach et al.	Suggest that EPA reevaluate the key confounder, parity, as part of the	S

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		(2015), cited by EPA in the IRIS draft, recommended that researchers should limit analyses to nulliparous participants to limit potential bias. Similarly, other authors such as Velez et al. (2016) indicate that any conditioning on parity is redundant as parity is often associated with both the exposure to PFAS and developmental or reproductive outcome being evaluated. However, several studies evaluated by EPA do adjust for parity, including it as a covariate or confounder in their models, often noting that no difference was seen whether parity was or was not included in the overall model. The lack of standardization surrounding parity in reproductive epidemiology makes it challenging to assess it as a confounder. Rather than consider parity as a key confounder, we recommend EPA reassess the sensitivity analyses for each of these studies, which would be a more valuable insight into if parity should or should not be adjusted for in various models. Stratification of parity or other approaches may be more appropriate. Since this covariate seems to impact results differently across cohorts and study types, it warrants individualized assessment.	sensitivity analysis domain to better understand if it was assessed and appropriately included or excluded in the models.	
3.2.2 Developmental Effects	3-37	Additionally, there is concern for confounding by maternal physiology, for example blood volume and kidney function. Given that PFAS are excreted via blood loss and urine, there is potential for unmeasured or inappropriate handling of these confounders in studies that assessed maternal PFAS exposure and outcomes of offspring. Although EPA acknowledged biomarkers were assessed in the confounding domain, biomarkers of exposure to birth metrics like weight or length are prone to additional confounding by maternal physiology since individual variation can result from differential uptake and excretion mechanisms that vary throughout participants. This is supported by an editorial written by Savitz (2007) that specifically called out PFAS and birthweight studies, such as Apelberg et al. (2007) and Feiet al. (2007), which reported varying individual PFAS serum levels that may have resulted from this individualized difference in uptake and excretion.	Recommend EPA consider how maternal physiology is addressed in the confounding and sensitivity domains to better assess if this confounding variable is handled appropriately.	S

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3.2.2 Developmental Effects	3-37	It is unclear how the participant selection domain was evaluated. EPA should explain its assessment in further detail beyond the appendix considering the participant selection domain discussed in Section 3.2.2. For example, Cao et al. (2018) received a low confidence score for the participant selection domain, and it is unclear why as the domain requirements and scoring details are not included, as they are in other sections.	Please clarify and explicitly state what is in the criteria for participant selection domain scoring.	E
3.2.2 Developmental Effects	3-37	Two cohort studies, Buck Louis et al. (2018) and Cao et al. (2018), came to different conclusions regarding the relationship between PFNA exposure and birthweight. Buck Louis et al. (2018) reported a null association while Cao et al. (2018) reported a positive association between PFNA exposure and birthweight. This difference, considering the similarity of study design, could have been due to confounding adjustment including under- or over-adjustment for confounders linked to the outcome, such as parity. In developmental epidemiological literature, parity is often a mediator in the exposure-outcome relationship, and if not adjusted for in all models it can be inappropriate and cause overadjustment without sensitivity analysis. Given the lack of sensitivity analysis in Cao et al. (2018), it is likely that the model used in Cao et al. (2018) which adjusted for parity is an overadjustment biasing results away from the null. A high confidence study, Shaoff et al. (2018) included parity and biomarkers in their adjusted model and found a non-significant inverse association between PFNA exposure and birthweight. However, their sensitivity models did not include an assessment of parity which, like Cao et al. (2018), could have biased the results toward or away from the null depending on the distribution of women who were multiparous vs nulliparous.	Recommend EPA reconsider the use of parity as a key confounder and should assess whether this confounder was handled appropriately in sensitivity analyses by the studies being evaluated. Secondly, recommend it be discussed in this section how over- or under-adjustment for confounders can bias results and how this should be considered when weighing the overall evidence to reach conclusions on hazard potential.	S/E
3.2.2 Developmental Effects	3-39	As noted above, many of the studies reporting an “association” between PFNA exposure and lowered birthweight were not statistically significant. EPA commented on this as follows: “[s]ome of the reported deficits were not statistically significant, especially in sex-specific analyses with smaller sample sizes. This may be due to limited sensitivity as only two of these studies were	This sentence contains information pertinent to related materials but needs to be separated and clarified. It is recommended that EPA delineate which studies are being referred to here in reference to “smaller sample	S/E

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		considered to have good overall study sensitivity (Robledo et al., 2015); (Wang et al., 2016)" (p. 3-39, I. 7-9). There are two issues with this statement. First, study sensitivity would not have been limited in the cohort studies that were null, which had large sample sizes. For example, Buck Louis et al. (2018), which sampled over 2000 women and included a sensitivity analysis which was classified by EPA as "medium confidence" and an overall confidence rating higher than Robledo et al (2015) reported null findings in the overall population. Additionally, a sex-specific study by Valvi et al. (2017) sampled over 600 participants and included a sensitivity analysis which was classified by EPA as "medium confidence" with an overall confidence rating higher than Robledo et al. (2015). This study found no statistically significant association for both boys and girls. Second, EPA should provide discussion on the relationship between sample size and sensitivity analysis since this may be unclear to some readers.	size," EPA should also more clearly discuss sensitivity, confounding, and exposure domain ratings in the beginning of this section such that it is clear how these impacted EPA's overall weight of evidence assessment for this endpoint.	
3.2.2 Developmental Effects	3-40	Regarding Sagiv et al. (2018), which EPA ultimately selected to derive the toxicity value, the authors reported a critical study limitation: "We detected associations of PFNA with birth outcomes in the current study; however, given the low plasma concentrations of PFNA in Project Viva compared with other, more commonly studied PFAS, such as PFOS and PFOA, these results should be interpreted with caution."	Recommend that EPA provide additional discussion of the uncertainties in the Sagiv et al. (2018) analysis given the low plasma concentrations.	S
3.2.2 Developmental Effects	3-45	EPA conducted a meta-analysis that assessed the relationship between PFNA exposure and birthweight using all 23 epidemiological studies it identified. The meta-analysis yielded statistically significant decreases in mean birth weight of 29 grams per natural log-unit increase of PFNA. When stratified by pregnancy sampling period the results were only statistically significant for mid- and -late pregnancy period, but not for early trimesters and postpartum samples, which were not statistically significant. Since many studies evaluated in the meta-analysis did not adjust for important confounders like maternal blood volume, which changes significantly during late pregnancy, this could have had a significant impact, biasing results.	Recommend EPA strengthen its discussion of the impact of study-specific confounding and biases on the overall meta-analysis results.	S

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		Stratified results indicated that the statistically significant findings are being driven partially by samples taken in mid-to-late pregnancy, which is confounded heavily by maternal physiological factors which several studies assessed in the meta-analysis did not account for.		
3.2.2 Developmental Effects	3-39 to 3-41	While discussing the studies that reported a null association between PFNA exposure and birthweight, EPA stated, "The four null studies reported no discernible patterns based on exposure sample timing or the biological matrix in which PFNA was measured; nor did low study sensitivity (including consideration of the mean/median PFNA exposure levels and variability within populations) appear to be a reasonable explanation for why associations were not detected" (I. 9-13). EPA has stated that these four high confidence null studies, with no apparent limitations, support no association. Five high confidence studies report a statistically significant association. Given the inconsistency of findings across these high-quality studies, there does not seem to be enough robust evidence of an effect. Given this finding, EPA should reassess whether there is sufficient evidence to demonstrate PFNA causes developmental growth impairments in humans. It appears the weight of the evidence would be more supportive of a lower tier classification of "moderate" evidence. The IRIS Handbook describes moderate evidence as, "A set of evidence that does not reach the degree of certainty required for robust, but which includes at least one high or medium confidence study reporting an association and additional information increasing certainty in the evidence" (EPA 2022, p. 6-13).	Reevaluate the available evidence and give studies with more confidence higher weight in the causal conclusions, relative to those with less confidence. The results of the high-quality studies are inconsistent and therefore do not provide robust evidence of an effect.	S
3.2.2 Developmental Effects	3-42 & 3-43 (Figs. 3-5 through 3-8)	Figure 3-5 and Figure 3-6 report the PFNA exposure and mean birth weight results. Studies descend from high to low overall confidence rating and study time, but that is not clear from first glance of the figure. Figure 3-6 and Figure 3-8 are blurry upon closer inspection and makes it challenging to understand the information	Consider using color or boarders to distinguish high quality from low quality studies to make it clear to the reader which studies are more "influential" in assessing the weight of evidence. Additionally, include a new figure for 3-6 and 3-8 that is not pixelated to allow for clearer communication of results.	E

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3.2.2 Developmental Effects	3-42 (Fig. 3-5)	Wang et al. (2016) is not included in Figure 3-5 for PFNA exposure and mean birth weight.	Wang et al. (2016) birth weight (β = –0.08, 95% CI: –0.16, 0.00) should be included in this figure unless there is a clearly stated reason as to why it is not.	E
3.2.2 Developmental Effects	3-47	In their concluding remarks on birth length, EPA stated that "[o]verall, despite some unexplained inconsistency across studies, there is supportive evidence of inverse associations between PFNA exposure and birth length in the overall population" (p. 3-47, l. 37-38). This statement is inconsistent given that only two studies, one rated high confidence and one rated medium confidence, evaluated by EPA were statistically significant, out of a total of 13 studies (Figure 3-8). Further, some of the reported associations for birth length were findings pertaining to specific limbs, including arms and legs, as reported in Buck Louis et al. (2018). Buck Louis et al. (2018) is the only high-confidence study that reported statistically significant results of any kind. It is unclear how limb length correlates to full body length and its relevance in the overall weight of evidence for birth length, traditionally the full size of the body.	Please re-evaluate this conclusion and consider removing or creating a separate conclusion for limb specific conclusions regarding birth length and PFNA exposure.	S
3.2.2 Developmental Effects	3-47	Although there may be differing results in analyses of PFNA exposure and developmental outcomes among different racial groups, this is the only racial subgroup analysis EPA presents or discusses in the entire document. It is unclear why EPA chose to discuss this issue as it pertains to developmental outcomes, but not any other health effects.	EPA should either remove race-specific findings from the developmental (birth length) section of the IRIS document or be more consistent and incorporate discussions of this issue throughout the document.	S,E
3.2.2 Developmental Effects	3-52	The EPA comments on birth weight deficits in the section on "Fetal growth restriction summary." It should be noted here and in Section 3.2.2 that birth weight deficits and low birth weight are categorized differently across the papers that are being evaluated. These differences range from the use of the clinical definition of LBW (< 2,500 g) to comparison to a control group. One cannot determine whether a small average deficit in birth weight (e.g., a few grams to a few hundred grams) observed across individuals in a study would	Consider adding a paragraph clarifying the different methods papers used to assess birth weight deficits and why they are comparable (not not). Alternatively, consider separating the outcomes or discussing them individually.	S

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		be considered adverse or correspond to long-term effects. There needs to be more discussion in this section about the appropriateness of comparing results generating using different outcome measurements to one another and a discussion of what is considered clinically relevant and what may not be clinically relevant.		
3.2.2 Developmental Effects	3-60	EPA should include a concluding remark based on the information available about the relationship between PFNA exposure and gestational age, as this was done in other sections of the IRIS report.	Based on the results and discussion noted by EPA, consider a concluding sentence along the lines of…" Given the inconsistent findings and small magnitude of effects that were reported, the potential for adverse associations between PFNA exposure and gestational age could not be determined."	E
3.2.2 Developmental Effects	3-61	EPA should include a concluding remark based on the information available about the relationship between PFNA exposure and preterm birth as this was done in other sections of the IRIS report.	EPA should edit the concluding sentence to state, "Given the inconsistent findings and small magnitude of effects that were reported, the potential for adverse associations between PFNA exposure and preterm birth could not be determined."	E
3.2.2 Developmental Effects	3-64	EPA should be consistent and include a concluding or closing sentence on the subsection for anogenital distance as it does for all the subcategories of developmental outcomes and PFNA exposure.	Consider including a concluding sentence such as "the considerable uncertainty related to the inconsistency in results between the two studies means that the potential for effects of PFNA exposure on this outcome cannot be inferred."	E
Table 3-15	3-88		Please discuss the differences in birthweight versus post-natal weight outcomes in mammalian models in	S

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		EPA concludes that there is *moderate evidence of* developmental effects in animals exposed to PFNA. Available studies reported reduced postnatal survival and decreased weight gain pre- and post-weaning in rodents exposed to PFNA *in utero*. However, there are inconsistencies in the developmental effects across strains (including strains of PPARα knockout mice), which complicates interpretation of the overall hazard conclusions. Specifically, EPA indicated that "there were some differences in sensitivity across species, sex, and offspring life stage gradients for some endpoints" but for postnatal body weight, EPA reports there is "unexplained consistency" across strains and sex. Despite uncertainties, developmental effects were selected as the critical endpoint in the derivation of toxicity values (RfDs) for PFNA. Upon review of the experimental animal data, the effects on birthweight and/or post-natal weights are not entirely coherent with the epidemiological data, which was considered *robust* for developmental effects. Most studies observed no statistically significant decreases in birthweight in rodent. While Rogers et al. (2014) reported statistically significant reductions in birth weight of rats exposed to 5 mg/kg-day PFNA, dams also experienced toxicity as indicated by reduced weight gain. EPA acknowledges, “the potential influence in this study of the reduced weight gain in dams during pregnancy on birth weights is unknown”. All mouse studies showed no effect of PFNA on fetal body weight and birthweight. Further, this “*generally medium*” confidence study reported that the weight differences were no longer statistically different than controls by PND 21. It is unclear whether the statistically significant deficits in postnatal body weight in rats and mice, either pre- or post-weaning, correlate directly with the purported birth weight changes in epidemiological studies (although note that the epidemiological studies reported deficits that were not statistically significant, i.e., not supportive of a causal association). Only one study of “*generally medium*” confidence showed significant reductions birth weight in male and female rats, although weight differences were no longer statistically different than controls by PND 21.	more detail, as well as their interspecies relevance for deriving PODs.	
Table 3-15	3-88	While EPA conclusion that there is *moderate* evidence of developmental effects in animal studies generally is supported, the overall evidence	Suggest EPA reconsider selection of POD and evidence summary	S

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		integration summary conclusion that the "evidence suggests" PFNA causes developmental effects is less well supported by the underlying studies, given the inconsistencies noted above. Moreover, there remain questions regarding the coherence across species and biological significance of this particular endpoint, given that mice experienced postnatal growth deficits, rather than birthweight deficits, and these effects appear PPAR-alpha-dependent. EPA should have considered the other candidate endpoints for derivation of the developmental RfD. We recommend revisiting the evidence integration conclusion and possibly demoting to *evidence indicates, but is not sufficient to infer,* based on the interspecies differences and biologically plausible significance.	integration conclusion for developmental effects.	
3.2.3 Immune Effects	3-93	EPA concluded that the "evidence suggests" that PFNA is associated with immune effects, largely based on studies reporting lower antibody response to routing vaccination, including 4 medium confidence studies in children and 2 low confidence studies in adults. EPA indicated there was consistency across vaccine type, timing of vaccination, and age at antibody response measurement. In EPA's discussion of immunosuppression, EPA also states, "Although many of the results were not statistically significant, a general trend across vaccination type was apparent" (I. 2-3). Grandjean et al. (2012) and Grandjean et al. (2017) reported overwhelmingly **non statistically** significant associations between PFNA exposure in utero or postnatally and vaccine response in children followed between ages 5 and 13, across numerous analyses investigating varying timepoints of exposure and outcome measurement. Because these are large studies generally considered high confidence, the non-significant findings suggest there is no causal association. Given that there are no studies with high overall confidence, and few studies were statistically significant, there appears to be little evidence of an association between PFNA and immunosuppression in the epidemiological literature.	Please rephrase the sentence to inform the reader explicitly of what is trying to be communicated and consider the quality of evidence that is presented before making this statement.	E
3.2.3 Immune Effects	3-103	EPA also discussed the association between PFNA exposure and asthma. EPA specifically highlights a study by Dong et al. (2013), which reported a relatively strong association (OR>2 in the highest quartile of exposure) and an	Consider including the 95% CI into this statement so that readers can put the strong association reported by	S

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		exposure-response gradient between PFNA exposure and asthma incidence. The OR, including 95% CI is reported as Q4: 2.56 (1.41, 4.65). Notably, this is considered a wide confidence interval. Confidence intervals can be impacted by the sample's variance and size. Dong et al. (2013) had a relatively small sample size of 231 participants, limiting the power of the study and reducing confidence that the sample was representative of the population being evaluated. Additionally, the wideness could be due to outliers in the data or a poorly specified model. Other risk estimates reported in this study for other PFAS being assessed were also extremely wide suggesting that this OR, reported for asthma in relation to PFNA exposure, was not the only measure of association affected.	Dong et al. (2013) into context or think about including a short sentence to describe the implication of wide confidence intervals or limitations of the study.	
3.2.3 Immune Effects	3-103	Regarding the relationship between asthma and PFNA exposure, EPA concludes, "While uncertain due to inconsistency in the results across studies, the null results are not interpreted to reduce confidence in the positive findings from this study given the better sensitivity and specificity in Dong et al. (2013) (p. 3-103, l. 33-36)." This conclusion does not align the statement made at the beginning of this section and additionally gives more weight to Dong et al. (2013), a study with a positive association and medium confidence, over all the other studies with a null association and medium confidence. All the studies of asthma appear to be weighted the same in EPA's evidence synthesis process. However, multiple null studies, relative to a single statistically significant study, should reduce confidence that there is a causal association between PFNA and this health effect.	Please rephrase this sentence as it is inconsistent with the weight that has been assigned to studies evaluating asthma outcomes. If there is another reason Dong et al. (2013) is given more higher weight than the other 11 studies evaluated for this outcome-exposure relationship, the measure or scale must be explicitly stated beyond its "better sensitivity and specificity."	S
3.2.3 Immune Effects	3-110 & 3-124	EPA concluded that, "The epidemiological evidence of an association between PFNA exposure and immune effects is based on generally consistent evidence of immunosuppression, driven primarily by a reduced antibody response following vaccination" (p. 3-124). In contrast, EPA concluded that there was no clear association between PFNA and increased incidence of infectious disease – i.e., the clinical outcome of immunosuppression. EPA stated, "There was also no consistency within each specific outcome. Where two studies per outcome were available, one study reported a positive association while the other reported null or inverse	Recommend that the EPA reevaluate the evidence they have and draw a clearer conclusion based on the epidemiological evidence.	S

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		associations. Given the considerable inconsistency of the findings, the available evidence on infectious diseases does not inform the immunosuppression observed in the antibody response studies" (p. 3-99, l. 35-37, p. 3-99, l. 1-2).		
3.2.4 Hepatic Effects	3-123	EPA ended the epidemiological study section on hepatic effects with the following sentence, "No evaluation of confounding across PFAS was performed due to the high level of uncertainty in evidence from other sources." It is not clear what EPA is referring to and what uncertainty limited their ability to assess confounding.	Recommend EPA include more detail regarding why confounding could not be assessed.	E
3.2.3 Immune Effects	3-125	EPA concludes that "the currently available evidence suggests that PFNA may cause immunosuppression in humans given sufficient exposure conditions." However, it is unclear how the evidence is suggestive of such when animal evidence is indeterminate and epidemiological evidence is "slight" and largely null. According to the IRIS Handbook, an overall conclusion of "evidence suggests" corresponds to "An evidence base that suggests that [chemical] exposure might cause [health effect] in humans, but there are very few studies that contributed to the evaluation, the evidence is very weak or conflicting, or the methodological conduct of the studies is poor." Given that the epidemiological evidence is largely null and includes several large cohort studies (i.e., should be able to detect an effect, if there is one), this conclusion appears stronger than the evidence supports.	Recommend EPA reevaluate its conclusion of "evidence suggests, as the overall hazard conclusion for PFNA does not appear to match the findings of the animal and epidemiological studies.	S
3.2.4 Hepatic Effects	3-130	EPA evaluated the exposure-outcome relationship between PFNA exposure and hepatic outcomes. Of the ten available studies, four were cross-sectional studies, and EPA noted "Cross-sectional studies were considered appropriate for this outcome due to the long half-life of PFNA and the potential for short term response in liver enzymes." While PFNA does have a long half-life, serum concentrations can vary over time, and we do not currently know what dose metric is associated with the health effects of PFNA, if any (e.g., whether effects result after a peak in exposure, or whether they only occur after chronic exposures that reach	Please add a sentence that details this limitation of cross-sectional studies. Additionally, as mentioned in other comments, consider this limitation when assigning weight to different studies.	S/E

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		steady state). Studies with a single PFNA measurement cannot provide information on internal doses over time in the population due to time-varying exposures. Overall, one cannot establish that PFNA exposure preceded the disease outcome and therefore this study remains of limited use for causal inference.		
Table 3-24; Figure 3-36; Figure 3-37	3-136; 3-147; 3-151	Regarding hepatic outcomes, the basis of the *robust* evidence conclusion is based on short-term and varied confidence studies in rats and mice. Taking into consideration of criteria from Hall et al. (2012)[2] on adaptive versus adverse liver effects, it is notable that while many of these studies are of medium and high confidence for organ weight, the majority of studies did not measure or were rated as uninformative or of low confidence for histopathology and clinical chemistry. Histopathology and clinical chemistry findings are critical to evaluating whether hepatic responses are adaptive (non-adverse) or adverse. In the 28-day NTP study, there high rates of mortality in numerous high-dose groups, indicating the maximum tolerated dose was exceeded. Hepatocellular necrosis was minimal to mild after exposure to ≥2.5 mg/kg-day in adult male rats and ≥12.5 mg/kg-day in female rats; however, severe body weight loss in males dosed with 2.5 mg/kg-day and females at 6.25 mg/kg-day impeded the ability to conclusively assess necrosis. Histopathological findings in other studies were considered of such low confidence that they were not discussed. Further, without any long-term animal studies to provide additional supporting evidence, *robust* evidence does not seem to be supported. Overall, evidence appears to be too limited to support a finding of "robust" evidence of liver effects in experimental animals.	Suggest revisiting evidence synthesis conclusion and confidence of hepatic outcomes, particularly for histopathology and clinical chemistry.	S
3.2.5 Male Reproductive Effects	3-175	The EPA framework for summary evidence integration judgements for an overall conclusion of "evidence indicates" includes scenarios in which moderate animal evidence combined with moderate to indeterminate human evidence (for male reproductive outcomes this was considered *sligh*t) could be used to determine an *indicating* summary judgement. Regarding male reproductive outcomes, EPA notes that subchronic studies in animals "are preferred to fully evaluate chemical effects on male reproductive tissue histopathology and associated sperm parameters." However, the only	Suggest EPA revisit male reproductive effects evidence integration summary judgement	S

[2] Hall, A. P., Elcombe, C. R., Foster, J. R., Harada, T., Kaufmann, W., Knippel, A., Küttler, K., Malarkey, D. E., Maronpot, R. R., Nishikawa, A., Nolte, T., Schulte, A., Strauss, V., & York, M. J. (2012). Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop. *Toxicologic pathology*, *40*(7), 971–994. https://doi.org/10.1177/0192623312448935

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		available subchronic study (Singh and Singh,2019) was determined to be of *low* confidence. Further, the PFNA dose of 2.5 mg/kg-day was the highest dose available to assess male reproductive outcomes due to mortality at higher doses. The *moderate* evidence judgement for animal studies and male reproductive effects is not supported based on the limitations in the evidence. These issues, combined with the *slight* human evidence and "*some*" mechanistic evidence supporting biological plausibility (primarily related to disrupted spermatogenesis), indicates that "evidence is inadequate" may be a more appropriate evidence integration judgment. Further, as depicted in Table 4.1 of the PFNA Draft, other PFAS IRIS assessments have concluded inadequate for male and female reproductive effects (Table 4-1, p. 4-5).		
3.2.6 Endocrine Effects	3-203 & 3-204; Figs. 3-53 & 3-54	In an assessment of T4 and T3 levels in adults in relation to PFNA exposure, only Crawford et al. (2017) reports positive statistically significant regression coefficients between PFNA exposure and altered thyroid hormones. It should be noted that the small sample size is a limitation in this study design. Additionally, the lack of inclusion of confidence intervals, despite inclusion of p-values, does not fully communicate the study findings.	Consider using a different figure for 3-53 and 3-54. The lack of confidence intervals makes it challenging to appreciate the uncertainty association with the measures reported, especially by Crawford et al. (2017), which seems to be the outlier in this dataset.	S/E
3.2.6 Endocrine Effects	3-208 (Figs. 3-53 through 3-60)	Figures 3-53 through 3-60 do not list the study evaluation rating with their reported results.	Please include an overall study confidence column in figures 3-53 through 3-60	E
3.2.7 Nervous System Effects	3-242	EPA assessed the relationship between social behavior (neurodevelopment) and PFNA exposure. Despite reporting no statistically significant associations in the epidemiological literature, EPA concludes "Overall, there is some largely imprecise evidence of an association between PFNA exposure and social behaviors, but not with autism diagnosis." EPA appears to be considering evidence that is imprecise as evidence of an association between PFNA and neurodevelopment; in fact, the evidence does not support this conclusion.	Please rephrase this concluding remark to represent the evidence evaluated in a more clear and concise way. For example, of the five studies assessing the relationship between PFNA exposure and social behavior, none reported statistically significant findings. These studies were largely imprecise and do not allow for clear	E

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			evidence of an association with social behavior or autism.	
3.2.8 Cardiometab-olic Effects	3-270 (Fig. 3-78)	Lin et al. (2009) is classified by Figure 3-78 as a medium confidence study however, in the write-up Lin et al. (2009) is classified as low confidence. Please correct the figure, or in-text discussion.	Clarify if Lin et al. (2009) is a low confidence or medium confidence study.	E
4.1 Summary of Conclusions of Noncancer Health Effects	4-1	EPA concludes that "The currently available evidence demonstrates that PFNA causes developmental growth impairments in humans given sufficient exposure conditions, and also that the evidence indicates that hazards likely exist with respect to the potential for hepatic and male reproductive effects in humans given sufficient exposure conditions." Given that the evidence provided is insufficient to infer causation (because it is largely comprised of cross-sectional studies), we recommend rephrasing the concluding remarks to remove "cause."	Please remove causal language from the conclusions.	S/E
3.2.3 Immune Effects	3-110 & 3-124	EPA concludes in the summary of human immune studies and in the combination of human and animal studies that, "The epidemiological evidence of an association between PFNA exposure and immune effects is based on generally consistent evidence of immunosuppression, driven primarily by a reduced antibody response following vaccination." However, as noted above, the human data is limited and includes many null analyses, and the animal data are limited by a lack of gold standard immunosuppression assays (i.e., there is no antigen challenge study). Further, the conclusions of the section on infectious diseases (i.e., the clinical outcome of immunosuppression), state, "There was also no consistency within each specific outcome. Where two studies per outcome were available, one study reported a positive association while the other reported null or inverse associations. Given the considerable inconsistency of the findings, the available evidence on infectious diseases does not inform the immunosuppression observed in the antibody response studies" (p. 3-99, l. 35-37, p. 3-99, l. 1-2). EPA must consider how the limited and largely null findings pertaining to actual clinical burdens of disease should be weight relative to the studies on antibody response to vaccination.	The conclusions in both sections need to be reframed to match the conclusions drawn in the epidemiology section on immunosuppression to match the evidence that EPA has referenced.	E

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		Overall, EPA overstates the evidence supporting immunosuppressive effects in humans.		
3.2.3 Immune Effects	3-110 & 3-124	Sagiv (2018) assessed plasma samples from first trimester women for PFOS, PFOA, PFNA, and PFHxS and fitted multivariable models to estimate associated with PFAS with birth weight for gestational age z score and length of gestation, adjusting for sociodemographic confounders and hemodynamic markers. a. *The study found that PFOS and PFNA were weakly inversely associated with birth weight for gestational age z scores. PFOS and PFNA were also associated with higher odds of preterm birth.* <u>However, the discussion specifically says the following:</u> b. "Given the low plasma concentrations of PFNA in Project Viva compare with other, more commonly studies PFAS, such as PFOS and PFOA, these results should be interpreted with caution. Only a few other studies have examined associated of PFNA with birth outcomes presumably because of the relatively low PFNA concretions with mixed findings." It seems inappropriate to use this study as the basis for an RfD given the degree of caution warranted by the authors specifically about PFNA data.	Please provide further justification for developing the RfD	S/M
3.2.3 Immune Effects	3-110 & 3-124	In general, we are concerned about the potential for confounding of outcomes associating effects of PFNA when other PFAS are clearly present. As mentioned in the main document, PFNA and PFDA are often correlated. a. In Sagiv (2018), PFNA was not strongly correlated with the other three PFAS assessed (which did not include PFDA), but the warning in its own discussion suggests (Sagiv) 2018 is inappropriate for the basis of the RfD. b. Although the objective of Appendix C was said to be: 1) to assess whether there is any direct evidence for confounding in the studies comparing results from multi-pollutant models and results from single pollutant models, and 2) to compare relationships between co-occurring PFAS and which may be associated with a primary endpoint of interest.	Please provide further justification on confounding factors	S/M

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		i. Across four studies, not all PFAS consistently co-occur with PFNA; however, 1. "Not a lot of evidence that confounding by other PFAS is responsible for the birth weight deficits detecting with increasing PFNA exposure across studies." a. Given the lack of strength, we are not assured that confounding effects of other PFAS do not influence these results.		
3.2.5 Male Reproductive Effects	3-193	EPA concluded "Taken together, the currently available evidence indicates that PFNA likely causes male reproductive toxicity in humans given sufficient exposure conditions." However, this is inconsistent with the epidemiological evidence paragraph as there is unclear and imprecise information about this outcome and exposure than what was previously stated. EPA follows this conclusion by stating "The lack of association in most of the epidemiological studies does not decrease confidence in the animal results given the uncertainties in the epidemiological evidence base." Again, this sentence should be rephrased. Although the animal studies reported some evidence of a statistically significant association between PFNA exposure and reproductive toxicity in males, human epidemiological studies did not.	Please rephrase this sentence as it does not represent the underlying weight of the evidence.	S
4.1 Summary of Conclusions of Noncancer Health Effects	4-4	We agree with EPA's conclusions of *inadequate* evidence to evaluate PFNA exposures and the following effects: female reproductive, urinary, adrenal, and other noncancer health outcomes.	N/A	S
5.2.1	5-17 to 5-19		EPA should consider re-evaluating Das et al. (2015) and the evidence of	S

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Oral Reference Dose Derivation		Das et al. (2015) is the basis for several candidate PODs for toxicity value derivation. In this study, pregnant female CD-1 mice were exposed to PFNA via oral gavage at doses of 1, 3, 5 or 10 mg/kg/day from gestational day 1–17, and maternal and offspring outcomes measured. There were no changes to maternal weight or general health, and no significant increases in implants, live fetuses, % prenatal loss, pup birthweight, and other related endpoints. Postnatal survival was affected in the highest dose group (5 mg/kg/day). At and above 3 mg/kg/day, pup weight gain in pups was significantly reduced, relative to controls, from PND 1 to PND 24. Pups were separated at weaning and body weight was monitored until 41 weeks old. Weight deficits continued from PND 25 to PND 287 for males, but females recovered to control levels by 7 weeks old. The fact that females' weight deficits were reversed raises uncertainties regarding long-term adversity and sex differences. As noted by EPA in their developmental toxicity guidelines, "While there is always a question as to whether weight reduction is a permanent or transitory effect, little is known about the long-term consequences of short-term fetal or neonatal weight changes" (EPA, 1991, p.13[3]). Postnatal weight gains are dependent on litter size. Pup gender, birth weight, ability to efficiently suckle, and maternal milk product all affect postnatal weight gain. When litters are large, small or weak offspring may have more difficulty thriving, affecting growth more substantially than if they were part of a smaller litter (2012). Authors could have evaluated the neonatal growth curve in conjunction with litter size to control for the confounding effects of within-litter competition (Hood, 2012). Moreover, Das et al. (2015) did not report maternal or offspring food and water consumption. While maternal body weights did not differ in exposed versus controls, it is impossible to rule out confounding effects related to food or water consumption in either dams or weaned offspring.	adversity considering that the female weight gain deficits reversed by 41 days of age. EPA should also consider whether failure to report food and water consumption should be noted in the study evaluation.	
5.2.1 Oral Reference Dose Derivation	5-17 through 5-20	The derivation of a BMDL based on the epidemiological studies has numerous uncertainties. EPA used a hybrid approach with dichotomous and continuous data. Specifically, EPA used a method to model the PFNA serum level associated with a 5% increase in the percentage of babies falling below the clinical low birthweight cutoff of 2,500 g based on an epidemiological study looking at decrease in birthweight as a continuous measure. "The exact	Recommend EPA provide all calculations in the appendix, and explain where the source of the mean birthweight 3,169g used as "y" in the BMD equation.	S

[3] U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment. Fed Reg 56: 63798-63826.

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		percentage (8.27%) of live births in the U.S. in 2018 that fell below the cut-off of 2,500 g" was used "as the tail probability to represent the probability of extreme ("adverse") response at zero dose." (5-19) However, the underlying study (Sagiv et al., 2018) did not report individual birth weights, nor did it report the number of babies in the cohort that would be considered low birth weight babies (>2500 g or <2500 g). EPA used the Sagiv et al (2018) study to obtain the linear regression coefficient representing to deficit in grams of birthweight per ng/mL increase maternal serum PFNA (representing the purported relationship between PFNA exposure and birthweight). EPA then applied this slope to the mean birthweight in the US general population to derive the serum level of PFNA associated with 12.86% of children being born <2500 g. EPA does not sufficiently describe its approach in the main text or in Appendix D. The calculation in which they apply the regression coefficient to the population means is not shown.		
5.2.1 Oral Reference Dose Derivation	5-30	The uncertainty factors for the development of the candidate lifetime RfD values for PFNA appear justified based on the evidence.	N/A	S
Appendix D		EPA does not sufficiently discuss the uncertainties and potential limitations of its BMD analysis for birthweight using epidemiological data. EPA states only the following in Appendix D: "EPA does not have access to the individual-level data that would be necessary to model the data from these studies with standard BMDS-based approaches. Therefore, the regression coefficients reported in these studies were used to calculate BMD and BMDL values." However, this statement does not appear in the main text, and in no part of the document or Appendix D is there a discussion about the uncertainty and conservative nature of this analysis considering the selection of a 5% BMR for an endpoint that is not consistently observed across epidemiological studies.	Suggest that EPA add additional information to the main text of the IRIS assessments regarding the level of uncertainty in the calculation of the BMDL for birthweight based on Sagiv et al. (2018).	S
Appendix D	D-15	EPA justifies its selection of a 5% BMR as follows:	Recommend that EPA provide stronger justification for their departure from the typical 10% BMR.	S

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		"…an extra risk of 5% is selected given that this level of response is typically used when modeling developmental responses from toxicology studies, and that low birthweight confers increased risk for adverse health effects throughout life, thus supporting a BMR lower than the standard BMR of 10% extra risk." (p. D-15). However, EPA did not use a toxicology study. The 5% BMR in toxicological studies of birthweight is used because that is the BMR associated with sufficient statistical power with a sample size of 20 animals. In other words, it's a statistical rather than a biological justification based on small toxicology study sample sizes. To support a BMR lower than the 10% default, EPA needs biological data supporting that as a biologically relevant cutoff.		
Appendix D	D-14 through D-18	EPA uses the 95th percentile lower limit of the slope to calculate the BMDL. Notably, the BMDLs derived using animal data are all several orders of magnitude higher than that derived using the epidemiological data. If EPA intends to select the BMDL based on the human data despite the comments above regarding the consideration of selection of one based on animal data, at a very minimum EPA should derive and compare the BMDL using the upper limit (UL) of the slope from Sagiv et al. (2018) to compare to the BMDLs derived using toxicological data. If the BMDLs are more similar to the BMDLs based on the UL of the slope than the LL of the slope from the epidemiological study, the weight of the evidence approach would indicate that a BMDL value based on the UL of the slope in Sagiv et al. (2018) should be selected for toxicity value derivation.	EPA should consider deriving the BMDL using the upper limit of the slope from Sagiv et al. (2018) and compare it to the BMDLs derived from animal studies.	S