U.S. Department of Defense (DoD)

Comments on the Interagency Science Consultation Draft

IRIS Assessment of Perfluorohexanoic Acid (PFHxA) August 2021

(Date Received September 21, 2021)

Department of Defense Comments on Toxicological Review of Perfluorohexanoic Acid (PFHxA) and Related Compounds Ammonium and Sodium Perfluorohexanoate (PFHxA-NH4 and PFHxA-Na) (August 2021)

Comments submitted by: Chemical Material Risk Management Program	Organization: Department of Defense	Date Submitted: 21 September, 2021

*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.

Comment No.	Section	Page(s)	Comment	Suggested Action	*Category
1	Overall comment	NA	The PFHxA draft is one of the first full IRIS assessments following the release of the Draft ORD Staff Handbook for Conducting IRIS Assessments (the "Handbook"). Overall, the draft provides clear descriptions of the methodology and EPA's analysis of the evidence. The PFHxA IRIS draft reflects many of the revised guidelines for IRIS assessments, including increased transparency and increased used of graphical representation of EPA's conclusions. In particular, the use of an "Evidence Integration" narrative and tabular summary of all evidence streams (evidence profile tables) for each health endpoint allows the reader to better identify and follow EPA's decision process, which is an important improvement in IRIS toxicological reviews.	N/A	E/S
2	Table ES-1 and associated text throughout the document	Pages xiv and xv	The adverse nature of the hepatic effects reported by Loveless et al. (2009), the hematopoetic effects reported by Klaunig et al (2015) and Chengelis et al 2009b) and the developmental effects identified by Loveless et al (2009) are not clear and appear to conflict with other references relied upon by the authors. None of the authors cited above identify the "selected" effects as either being of interest or being dose-related. The dose-response information presented in the Supplement is not convincing. Dose-responses that appear to be flat, without ever varying outside of the normal range of effect variance, should not be used to support selection of an effect. What we see is that Klaunig et al. (2015) does, in fact, report hematopoietic effects in the high female dose group (200 mg/kg-day), but the authors attribute these effects to the acid dose saying the effects are likely due to "slight blood loss from gastric erosion	The text needs to be revised to support the selected effects in the liver, hematopoietic system, and developmental effects. The Hall et al (2012) criteria should be used to identify adverse liver effects and the cited author's own words, where applicable, should be used in the justification of a "selected" effect. Hall, A.P., Elcombe, C.R., Foster, J.R., Harada, T., Kaufmann, W., Knippel, A., Kuttler, K., Malarkey, D.E., Maronpot, R.R., Nishikawa, A., Nolte, T., 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes, conclusions from the 3rd	S/M

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			and ulceration, and possibly from renal hemorrhage associated with papillary necrosis, although distinct hemorrhage was not observed microscopically in the renal pelvises." The hepatocellular hypertrophy observed in Chengelis et al. (2009b) and in Loveless et al (2009) were interpreted by Luz et al. (2019) to be indicative of a non-adverse (adaptive) response. Applying the criteria by Hall et al (2012), no necrosis was seen in Loveless et al. (2009) at doses up to 500 mg/kg-day, and only one rat was found to be necrotic in the Chengelis et al. study at 200 mg/kg-day. Similarly, NTP (2018) reported a dose-dependent increase in relative liver weights in males (250, 500, and 1,000 mg/kg-day) and in females (500 and 1,000 mg/kg-day). Hepatocellular hypertrophy was observed in males at 500 and 1,0000 mg/kg-day and in females at 1,000 mg/kg-day, but no necrosis was seen at any dose. Luz et al (2019) reported that a dose-dependent increase in liver acetyl-CoA activity (a marker of PPARalpha activation) was observed in male rats (250, 500, and 1,000 mg/kg-day. Female rats were not tested. Based upon the framework of Hall et al. (2012), the lack of necrosis and inflammation suggests that these liver effects are not adverse and are unlikely to be relevant in humans.	International ESTP Expert Workshop. Toxicol. Pathol. 40 (7), 971-994.	
3	1.1.2	1-3	It is noted that there is a small concentration of PFHxA in AFFF; however, no other specific uses are discussed for PFHxA. Is there any information about use patterns over time? Most of the data on uses and exposure are from 8-10 years ago; it is not clear whether PFHxA is still used in the same frequency in AFFF or other products in more recent years.	If possible, this section should be updated to provide information on specific uses of PFHxA (not just PFAS, generally).	S
4	1.2.3	1-10	The text states that: "Not all studies that meet the PECO criteria go through data extraction: For example, studies evaluated as being uninformative are not considered further and therefore do not undergo data extraction. The same could be true for low confidence studies if enough medium and high confidence studies (e.g., on an outcome) are available." How does EPA determine if there are "enough" studies? Generally, systematic review methods require the consideration and synthesis of the body of the evidence. While it is understandable that some evidence does not need to be extracted/evaluated for quality, the cutoff for sufficiency of database	Please provide additional justification for any studies that were not extracted/reviewed further, and define how EPA determines whether there are a sufficient number of higher-quality studies to warrant excluding low-quality studies.	S

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			in which low quality studies are set aside is not specified and is not clear. There may be concern for a substance like PFHxA that the weight of the evidence is skewed by limiting data extraction and discussion of most/all studies.		
5	2.2	2-3	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.	N/A	E
6	3.1	3-1	The first sentence indicates that PK studies in humans provide sufficient data to estimate half-life; however, subsequent sentences pertaining to human studies seem to indicate that the data are insufficient.	Review and revise the opening summary statement.	S
7	3.1.1	3-2	The description of PK study by Dzierlenga et al. (2019) is not clear.	Please clarify which PFAS were administered via which route. It is also not clear whether plasma samples were collected at all doses and routes of exposure. Lastly, for the sentence noting "Tmax slightly increased", it needs to be clarified whether the increase was statistically significant or a trend.	S
8	3.1.2 (Distribu-tion in animal and in vitro studies)	3-5	On line 36, it states that rats and monkeys were "also given PFHxA (10 mg/kg) via a single i.v. injection". It's not clear whether Chengelis et al. (2009a) performed separate sets of experiments for different routes of exposure, or whether the same animals were exposed via multiple routes of exposure.	Please clarify the route(s) of exposure for Chengelis et al. (2009a)	E
9	3.1.2 (Distribu-tion in humans)	3-3, 3-4	The subsection is titled as 'Distribution in humans' but there is a substantial discussion on animal studies pertaining to Fabrega et al. (2015). It would be clearer if comparisons of animal and human data were discussed separately.	Consider separating sections for human evidence and the integration of human and animal evidence.	E
10	3.1.2. Distribution / Distribution in Humans	3-4, Lines 6 to 14.	The authors seem to be confused about what physical properties of a tissue (e.g., blood) are significant in PFHxA partitioning or distribution. Contrary to the assertion, that "lipid content is a significant component", for many PFAS like PFHxA the significant component of interest in blood are PFHxA binding sites (i.e., principally albumin, but also including other circulating proteins). PFHxA does not partition into fats like PCBs or dioxins and therefore	Please revise the text to reflect the partitioning and distribution of PFHxA in relation to its binding to serum proteins and integrating into cellular membranes.	S/M

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			the lipid component of blood is not a significant determinant of either partitioning or distribution - at least not at environmental PFHxA concentrations (not saturated). As a consequence, the author's topical sentence for this paragraph "The average Vd for rats (0.33 L/kg) is only 40% lower than the average for monkeys (0.56 L/kg), a modest species difference that could occur due to differences in the relative lipid content in blood vs. the rest of body." has no basis in fact. In addition, the summary sentence regarding the difference in volumes of distribution between humans, monkeys, rat and mice is not knowable given the author's incorrect assumption related to lipids.		
11	3.1.2. Distribution / Role of Plasma Protein Binding	3-5, Lines 7 to 33.	The authors, by their wording of this section, appear to suggest that plasma protein binding ".... could affect its [PFHxA] pharmacokinetics." The text reports the result of Bischel et al (2011) demonstrating 99% of PFHxA is bound to serum proteins, but then suggests that that is inconsistent with PFHxA's fast elimination rate. No reference/citation is provided. The percent plasma protein binding of PFHxA (distribution) has nothing to do with its fast elimination (urine), but the avidity of the PFHxA to serum proteins and OATs does. Why is this not discussed? The authors surmise that "If glomerular filtration could remove only 1% (i.e., the free f[r]action) of PFHxA carried in the corresponding serum flow, the elimination half-life should be much longer.", but fail to provide the rationale or any citation for this statement. Intuitively, one would have to estimate the flow of blood through the kidney and the rates (avidity) of serum protein binding and OAT binding to know what the relative half-life might be as a result of elimination in urine. The authors did not justify the use of empirically determined distribution and elimination rates for PFHxA rather than albumin binding.	This section requires revision and the addition of text critically addressing the author's concerns and supposition regarding why the Bischel et al. paper should not be used.	S/M
12	3.1.2. Distribution / Distribution in Humans	3-4, Lines 15 to 26	It is not clear how the most likely explanation for the differences in PCs in Fabrega et al (2015) is an artifact. It seems likely that the combining of data from non-matched human samples (Perez et al 2013) is simply an indication of variation within the human population. How uncertain are the results of Fabrega et al. and Ericson et al (2007) and how was that uncertainty measured? How does a change in PFHxA exposure (serum concentration) over time change the estimated volume of distribution? The later statement by	The text needs clarification.	S/M

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			the authors, that "...given the biochemical properties of tissues that determine the relative affinity for PFHxA in tissue vs. blood are more similar between humans and a nonhuman primate than between humans and rats or mice." is not consistent with use of rats and mice as animal models useful in the assessment of human adverse health endpoints and is inconsistent with the expectation of conservation across species of fatty acid binding sites in albumin to which PFHxA binds.		
13	3.1.2 (Distribu-tion in animal and in vitro studies)	3-6	On lines 30-32, an *in vitro* study by Sanchez Garcia et al. (2018) is described. It is noted the lack of accumulation and retention of PFHxA in lung epithelial cells and adipocytes was similar to what was noted in the animal studies. The accumulation of PFHxA *in vitro* could be affected by experimental factors (e.g., pH, culture medium, incubation time/temperature); it is unclear whether these factors were considered.	It is recommended that additional information be provided on Sanchez Garcia et al. (2018) to clarify how the *in vitro* results are relevant to the results observed *in vivo*.	S
14	3.1.4	3-11	Lines 23 and 24 indicate that 94 blood samples were collected from 11 ski wax technicians.	Please provide the number of samples that were taken from each individual participant.	S
15	3.2	3-17	The text notes: "Some organs/systems for which data were available (i.e., dermal, musculoskeletal/connective tissue, sensory, ocular) had no evidence of an effect even at the highest administered dose, and others (i.e., respiratory, gastrointestinal system, cardiovascular, and metabolic effects) were limited findings of unclear toxicological relevance (e.g., outcome not necessarily adverse or considered nonspecific). Thus, these data are not synthesized in detail below, but are summarized in the animal literature inventory." The tables presented in the literature inventory online provide helpful summaries of studies not summarized in detail in the narrative. It would be helpful, however, if EPA provided the NOAELs/LOAELs in order of magnitude (or perhaps even plotted the results), so the range of NOAELs/LOAELs in these studies could be more easily scanned.	Consider re-arranging the tables in order of NOAEL/LOAEL magnitude.	E
16	3.2.1	3-19	In Table 3-2, Peroxisomal beta oxidation for Chengelis et al. (2009b) is noted as '++', but the color coding implies 'medium' confidence.	Update color coding	E
17	3.2.1	3-21	The IRIS draft indicated that, "Increased hepatocellular hypertrophy was observed in adult male and female rats in the high confidence	EPA should consider defining the point of departure for liver effects based on necrosis	S/M

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			short-term (NTP, 2018) and high confidence subchronic (Loveless et al., 2009) studies at doses ≥100–500 mg/kg-day. In the low confidence subchronic study, centrilobular hepatocellular hypertrophy was found at 200 mg/kg-day in male rats only (Chengelis et al., 2009b). In the chronic study (Klaunig et al., 2015), no change in hepatocellular hypertrophy was found, although the highest administered dose was 2–10 times lower (100 mg/kg-day in males or 200 mg/kg-day in females) than the highest dose in other studies where effects on hypertrophy were observed. Coherent with findings on liver weight, the observations of hepatocellular hypertrophy were dose-dependent and male rats were more sensitive than females." Hepatocellular hypertrophy (increased cell size) is typically only considered adverse only when accompanied by marked histopathological changes (most importantly, necrosis) (Hall et al., 2002[1]). In the absence of other effects, hepatocellular hypertrophy can sometimes be considered adaptive. Chengelis et al. (2009) noted only a single animal at the high dose with necrosis; the chronic study Klauning et al. (2015) observed necrosis in females at the high dose (500 mg/kg/day) but no hypertrophy, and NTP found hypertrophy at 500 mg/kg/day in males and 1,000 mg/kg in females, but no necrosis. Loveless et al. (2009) did not observe necrosis, but rather only minor hypertrophy at ≥100 mg/kg/day in males and 500 mg/kg/day in females. Liver enzymes were significantly increased in male rats only, but resolved within 3 months of cessation of dosing (Loveless et al., 2009).	rather than hypertrophy, or more fully justify the selection of hypertrophy as an adverse effect based on mode of action arguments.	
18	3.2.1	3-31	Were the bioactivity data obtained from ToxCast specific to liver cells? It appears as though lines derived from renal cells are the only type mentioned (COS-1).	Please provide clarity on ToxCast cell types.	S
19	3.2.1 Considera-tion for Potentially	3-33 to 3-34	Luz et al (2019) provides an analysis of the adaptive responses of rodents to PFHxA, and principally concerning the activation of PPARalpha. Luz et al. used the Hill et al (2012) criteria for liver hypertrophy as a guide for determining when this effect should be	In the consideration of adaptive vs. adverse responses, the response of interest should not only be adverse, but also of the same/similar magnitude with respect to	S/M

[1] Hall AP, Elcombe CR, Foster JR, et al. Liver Hypertrophy: A Review of Adaptive (Adverse and Non-adverse) Changes—Conclusions from the 3rd International ESTP Expert Workshop. Toxicologic Pathology. 2012;40(7):971-994. doi:10.1177/0192623312448935

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	Adaptive Versus Adverse Responses		considered adverse (e.g., the appearance of necrosis and/or inflammation). Additional discussion of the relative sensitivity of rats and humans to PPARalpha activation is relevant and should also occur in this section. While the text includes the work by Foreman et al. (2009), who demonstrated the ability of PFBA to cause hepatocellular hypertrophy in wild type and humanized mice, but necrosis only in wild type mice - indicating a lower sensitivity for adverse effect in humanized mice, other relevant references were not discussed. There are significant differences in the genes activated by PPARalpha in mice and humans only a few of which are commonly regulated in both mice and humans (Rakhshandehroo et al 2009). These authors concluded that "PPARalpha regulates a mostly divergent set of genes in mouse and human hepatocytes". A related study by Bility et al. (2004) demonstrated that mouse PPARalpha is generally activated at a much lower concentration of agonist than is human PPARalpha. This result is supported by the earlier result of Lawrence et al. (2001).	equivalent doses. The authors have not established either. Bility et al. 2004. Activation of Mouse and Human Peroxisome Proliferator-Activated Receptors (PPARs) by Phthalate Monoesters. Toxicological Sciences. 82:170-182. Lawrence et al. 2001. Differential Gene Regulation in Human Versus Rodent Hepatocytes by Peroxisome Proliferator-activated Receptor (PPAR) alpha. The Journal of Biological Chemistry. 276(34):31521-31527. Rakhshandehroo et al. 2009. Comparative Analysis of Gene Regulation by the Transcription Factor PPARa between Mouse and Human. PLoS ONE. 4(8):6796.	
20	3.2.2	3-42	EPA indicated that decreased offspring body weights were observed in several animal studies. In some cases, these body weight changes resolved after weaning. The one-generation reproductive/developmental study by Loveless et al. (2009) reported statistically significant reductions in body weight at 500 mg/kg/day postnatally (but not after weaning); however, maternal toxicity was also apparent at this dose (body weight loss); indicating this is not a selective developmental effect. Iwai and Hoberman (2014) reported pup body weight losses in mice at all doses, but these effects only persisted at doses also causing substantial maternal toxicity (≥350 mg/kg/day). The authors indeed stated, "Results of this study support what has been generally observed for other PFAAs in that developmental toxicity has generally only been seen in the presence of maternal toxicity."	Please consider the limitations of using reduced pup weight as a basis for an RfD. Suggest providing additional discussion of the uncertainty surrounding this endpoint and justification for its use.	S/M
21	3.2.2	3-46	The conclusion that "PFHxA likely causes developmental effects in humans" overstates the weight of the evidence. In animal studies, developmental effects were typically noted at high doses that are not likely to be applicable to exposure in humans. Epidemiological studies of developmental toxicity in humans were not identified.	Same as in comment above, please reconsider using reduced pup weight as a basis for an RfD.	S/M

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22	3.2.3 (Renal Effects)	3-49	EPA indicates that two of three epidemiological studies of renal effects were considered uninformative "due to critical deficiencies in multiple study evaluation domains (Seo et al., 2018; Zhang et al., 2019). EPA provided no summary of the findings of these studies; the HAWC link was broken so study details could not be reviewed. While it is very important to thoroughly review the quality of studies, the "exclusion" of low quality studies for hazard identification may not be appropriate, particularly for a chemical such as PFHxA, which has relatively little information. Very low quality studies clearly should not be used for any quantitative analysis, but it seems EPA could retain and summarize them, providing the caveat that there is low confidence in this study. Further, because EPA's quality evaluation system does not weigh any particularly study quality domain more than others, epidemiological studies with deficiencies in a single domain (e.g., "selective reporting") but that are otherwise strong, may be useful; in contrast, studies with many critically deficient judgments, or critical deficiencies in very important domains such as exposure measurement may indeed be relatively uninformative.	Consider summarizing, at least briefly, the epidemiological evidence for renal effects, including low quality studies.	S
23	3.2.4	3-61, 3-67	EPA stated that, "Collectively, the animal toxicological information provided coherent evidence indicative of macrocytic anemia (characterized by low hemoglobin and large red blood cells) that is consistent across multiple laboratories and experimental designs." These findings were largely observed ≥200 mg/kg/day PFHxA. EPA further concluded that, "Overall, the currently available evidence indicates that PFHxA likely causes hematopoietic effects in humans under relevant exposure circumstances." Conclusions are largely based on one 28-day study and two 90-days studies. EPA stated that hematopoietic findings from the 2-year chronic study (Klaunig et al., 2015) "were generally null" but suggested that measures could have been complicated due to natural disease and test variability. No other detail is provided. Further, given that the single chronic study was largely null except for some findings in females, whereas the subchronic studies showed decreases in RBCs, HGB, and HCT, did EPA consider the	Given Klaunig et al. is the only chronic study, please discuss the study findings and potential limitations in more detail (particularly considering that the study was given a high confidence rating). Suggest re-evaluating the adversity of the hematopoietic findings and providing additional language regarding the uncertainties as they pertain to the selection of this endpoint as a critical effect.	S/M

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			possibility that the hematopoietic effects are a dose- and duration-related phenomenon, and that animals' adapt, and hematopoietic effects reverse upon chronic exposure through adaption?		
24	3.2.5 (Endocrine Effects)	3-70	Li et al. (2017) reported low exposures to PFHxA (0.01 [LOD-1.1]) with 47% of samples below the limit of detection. This issue is common for some of the short-chain PFAS with short half-lives (and in some cases, less widespread use). Given that low or no detection often precludes analysis, this is a substantial uncertainty regarding the relevance of PFHxA effects measured in animals at relatively high doses to the human population at these low exposure levels.	Suggest providing additional discussion regarding the uncertainty in the human relevance of high-dose animal studies considering the epidemiological studies reporting low or non-detects for PFHxA in serum.	S
25	3.2.5	3-74	Thyroid hormone effects were observed only in males, and with only certain hormones showing a dose-response relationship (FT4). Thyroid epithelial cell hypertrophy was observed in rats exposed to 500 mg/kg/day PFHxA for 90 days, but there was no clear dose-response relationships. Similarly, there were no clear treatment-related findings for organ weights. EPA concluded that "***evidence suggests***, but is not sufficient to infer, that PFHxA could cause endocrine effects in humans under relevant exposure circumstances." There is some uncertainty regarding whether the level of evidence required for an "evidence suggests" conclusion was reached. According to the IRIS Handbook, this conclusion is usually reserved for endpoints with at least "moderate" evidence of an effect in one species (for PFHxA and thyroid effects, EPA called the animal evidence "slight" and the human evidence "indeterminate."	Suggest re-evaluating the thyroid evidence against the IRIS Handbook guidance to determine whether the current hazard conclusion is appropriate.	S/M
26	3.2.6 (Male Reproductive Effects); 3.2.7 (Female Reproductive Effects)	3-82, 3-89	EPA concluded that, "Overall, the currently available ***evidence is inadequate*** to assess whether PFHxA might cause male reproductive effects in humans under relevant exposure circumstances." Similarly, EPA concluded that "currently available ***evidence is inadequate*** to assess whether PFHxA might cause female reproductive effects in humans under relevant exposure circumstances." These conclusions are supported by the evidence; EPA's explanations of study and endpoint limitations were concise and clear.	N/A	S

	5.2.1 Oral Reference Dose (RfD) derivation	5-1 to 5-8	PODs were determined by applying the linear/frequentist BMD.	Traditionally, the no observed adverse effect level (NOAEL) is chosen as the POD, but the statistical lower bound of an estimated benchmark dose (i.e., BMDL) has become the default choice for POD to replace the NOAEL. We suggest EPA consider as an option using the now widely accepted Bayesian BMD modeling (BBMD) system (Shao and Shapiro, 2018), which provides a more reliable way to derive the distribution of the RfD or Human Dose. The BBMD model directly generates the posterior sample of the BMD distribution, which can be more smoothly integrated with the distributional uncertainty factors using Monte Carlo simulation to generate the distribution of RfD (which is not necessarily log-normally distributed). Theoretically, the distributions of these uncertainty factors can be more flexible and are not necessarily to be log-normal. References: NRC, 2014. Review of EPA's Integrated Risk Information System (IRIS) Process. The National Academies Press, Washington, DC. WHO-IPCS, 2014. Guidance Document on Evaluating and Expressing Uncertainty in Hazard Characterization. WHO, Geneva, Switzerland. Simon, TW et al., (2016) Bayesian methods for uncertainty application for derivation of reference values. Regulatory Tox and Pharmacol 80: 9-24.	S

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				Shao, K; Shapiro, A. 2018. A Web Based System for Bayesian Benchmark Dose Estimation. Environmental Health Perspectives. 126 (1): 017002. https://doi.org/10.1289/EHP1289	
27	5 (Derivation of Toxicity Values)	5-7	EPA selected a 1% benchmark response for offspring mortality, noting "Although 5% ER is generally supported for developmental and reproductive outcomes (U.S. EPA, 2012a), a lower BMR of 1% ER was considered appropriate for modeling offspring mortality in light of the severity of the frank effect." Offspring mortality occurred in the presence of maternal toxicity, indicating it is not a selective developmental effect. Justification for the use of the 1% BMR would need to be made on statistical grounds related to the power of the study.	Please re-evaluate and/or provide justification for selection of the 1% BMR for offspring mortality.	S/M
28	5.2.1	5-13, 5-14	The approach to deriving a dosimetric-adjustment factor appears sound. For the preferred DAF, however, what is the range of estimated clearance levels (above and below)? The derivation of the DAF and the toxicity value are highly sensitive to the clearance value used.	Given the limited information on human PFHxA pharmacokinetics (PK), the assumptions used and range of uncertainty, we recommend a thorough discussion of the uncertainties associated with the human clearance value and DAF.	S
29	5.2.1	5-14	EPA used data from other PFAS to "check" their assumption regarding differences in clearance between humans and animals. The chain length and functional group of PFAS can affect their physicochemical properties and toxicity. The use of PFHxS clearance data to inform PFHxA may not be appropriate, given that PFHxS is a long-chain sulfonate with a very long half-life. PFNA and PFDA are also long-chain PFAS with long half-lives (several years). There appears to be substantial uncertainty in inferring these data are informative for PFHxA.	Provide additional justification for the use of long-chain PFAS PK data to inform the PK of PFHxA in humans.	S
30	5.2.1	5-19	Regarding the selection of red blood cell decreases as the POD, EPA stated, "The magnitude of change for RBCs (~8% decreased) or HGB (~5% decrease) was similar when comparisons were made between chronic and subchronic studies…*The biological significance of the magnitude of change for both RBC and HGB in rats is uncertain*, but the effect on red blood cell parameters had a	Consider re-evaluating the selection of hematopoietic effects for derivation of the RfD, considering statement in the Toxicological Review regarding unknown biological significance.	S/M

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			slightly lower POD than HGB and was concurrent with increased reticulocyte levels, a compensatory response to anemia" [emphasis added]. Despite this uncertainty, EPA indicated that they had high confidence in the evidence base for hematopoietic effects and the candidate RfD derived. NOAELs and LOAELs, and the points of departure/toxicity values based on them, are intended to apply to biologically significant effects. As noted by the National Academies of Science (NAS) in its 2014 review of the IRIS process, "EPA develops toxicity values for health effects for which there is "credible evidence of hazard" after chemical exposure *and of an adverse outcome*" [emphasis added].[2]		

[2] National Research Council. 2014. *Review of EPA's Integrated Risk Information System (IRIS) Process*. Washington, DC: The National Academies Press. https://doi.org/10.17226/18764.