Department of Defense
Comments on the Interagency Science Discussion (Step 6)
Draft IRIS Toxicological Review of Perfluorobutanoic Acid (PFBA)
October 2022
(Date Received November 22, 2022)

*Comment categories (CAT): 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	Pages	Comment	Suggested Action, Revision and References (if necessary)	*CAT
Global Comment - Applies to the entire document.	All	At numerous locations within the document text reference is made to "Appendix A", which often includes a Section number. For example, in section 1.2.5 Dose-Response Analysis, page 1-13, lines 17-18, the reader is instructed to "see Appendix A, Section 10.2 for exceptions." The reviewed document, however, has no Appendix A. In fact, it appears as though the authors intended the reader to refer to specific sections within US EPA (2019), i.e., "US EPA's Systematic Review Protocol for PFBA, PFHxA, PFHxS, PFNA, and PFDA IRIS Assessments" and not at all to any Appendix A.	Throughout the document all reference/citations referring to "Appendix A" and "Section" should be corrected to the appropriate source of the information to aid the reader in finding the information referenced.	E/M
Executive Summary	xii	If "available evidence indicates that developmental, thyroid and liver effects in humans are likely caused by PFBA in utero or during adulthood", then saying "there was inadequate evidence to determine whether reproductive effects might represent a potential human health hazard", is contradictory.	Recommend providing more details on the studies referred to in that statement.	S

Executive Summary	xii	To say that "the available evidence indicates that developmental, thyroid, and liver effects in humans are likely caused by PFBA exposure in utero or during adulthood." suggests that there is evidence for concluding that PFBA exposure will cause these effects. Given the first sentence in the following paragraph, it seems less certain that these effects are likely caused by PFBA exposure. The evidence available at this time is not sufficient to warrant stating that the effects are "likely caused by" PFBA exposure.	Recommend that the text be changed to more accurately reflect what is known; that the listed effects "may be associated with" PFBA exposure.	E
Executive Summary	xiii (lines1-2)	The statement: "From the identified human health hazards of potential concern for adults and developing offspring (liver, thyroid, developmental toxicity), ..." identifies human health hazards. Clarification is required here as to whether these are hazards identified in humans or hazards identified in animal bioassays that are used to select health hazards of potential concern in humans.	Please clarify the text to indicate what is identified and what is selected for use as potential health effects in humans.	E
Executive Summary	xii (lines 4-6)	Given that PFBA exposure can cause liver hypertrophy via PPARalpha activation and non-PPARalpha mechanisms in rodents and that PFAS appear to be a more active PPARalpha agonist in the rodent liver than in human liver, it is unclear whether it is reasonable to conclude that there is a one-to-one relationship between rodent and human PFBA PPARalpha and non-PPARalpha mechanisms inducing liver hypertrophy. There are PFAS studies performed in transgenic mice that can be used to address the role of PPARalpha in liver hypertrophy and more importantly, the relative potency of the PFAS compound to cause liver effects in mice and humans.	Suggest a review of how liver hypertrophy is selected as the POD and what uncertainty factors (UF) might be applied to account for differences in mouse and human sensitivity for liver hypertrophy. Suggest further justification for the selection of thyroid effects (changes in T4 levels) as a POD.	S/M

		Furthermore, selection of decreased T4 as a POD requires closer consideration. There do not appear to be any data related to decreases in T4 (total or free) in humans exposed to PFBA nor is there discussion of a potential mechanism of action for this effect in humans. Additionally, it is unclear whether human thyroid and animal model thyroids are similarly sensitive to exposure to PFBA.		
Executive Summary	xii (Lines 13-19)	The text should be clear about which effects were observed in animal models and which were observed in humans. The topical sentence could misdirect the reader to think of the remaining information as pertaining to epidemiological (human) study results.	Suggest rewording the topical sentence to read "Animal bioassays inform the potential or effects in the thyroid, liver, reproductive system, or developing offspring." A concluding sentence might be: "Except for liver effects and specifically dyslipidemia, epidemiological studies were not informative regarding these effects."	E
Executive Summary	xii (Table ES-1)	RfD Row/Basis Column: Explanation of how both hepatic effects and thyroid effects are considered to have a medium confidence level when all the thyroid studies are listed as only providing medium-low confidence is needed. Subchronic RfD Row/ Basis Column: Unclear why there are developmental effects (medium-low confidence) used to derive a subchronic RfD when there are medium confidence studies reporting hepatic effects that could be used for this endpoint. Alternatively, justification for the use of developmental effects as the basis for a subchronic	Suggest revision of this Table to address these comments or provide additional text to describe the rationale for why the basis for the RfD is presented in the way it appears in the Table.	S

		RfD that would not allow its use as the basis for the chronic RfD is required. It is unusual for a subchronic RfD to be lower (more toxic) than the chronic RfD.		
Chronic Oral RfD for Noncancer Effects	Xiii (lines 1-15)	Throughout this section discussing Butenhoff et al., it is unclear whether the decrease in T4 and increase in liver hypertrophy are significant.	Please clarify the significance of these changes.	S
Chronic Oral RfD for Noncancer Effects	Xiii (line 9)	A brief description of how the serum clearance values were determined would add valuable context to this section.	Please clarify as to how the clearance values were determined	S
Chronic Oral RfD for Noncancer Effects	xiii (line 15)	The UF of 10, extrapolating from a subchronic-to-chronic duration, requires more adequate justification given the relatively short body half-life in rodent species and in humans. In other words, clarification is needed as to when serum PFBA reaches steady state (intake = elimination), and whether the PFBA serum level at steady state is the same for both subchronic and chronic exposures to the same dose.	Justification for this UF is required given the very short half-life of PFBA in rodents and humans. Please note that depending on when steady state is reached in animal and human serum, justification may be specific for the exposure time used in the animal bioassay.	S/M
Table ES-1	xii-xiii	Table ES-1 should provide the citations for the basis to facilitate review, especially since the ES section does not include them.	Consider including more explanation on how studies with med-low confidence provide evidence indicating likely, such as how many studies are they based on.	E

Confidence in the Oral Reference Dose	xiv (line 3)	The method of arriving at the overall confidence rating in the RfD of medium requires more explanation, to include how liver hypertrophy (medium confidence) and changes in T4 levels (medium-low confidence) are used to arrive at this conclusion. Clarification is also needed as to whether thyroid effects, at medium-low confidence, support the RfD based on liver hypertrophy.	Please clarify the medium confidence rating.	S
Confidence in the Oral Reference Dose	xiv (lines 7-9)	The medium confidence rating for the oral toxicity database requires more explanation. Table ES-1 does not demonstrate either consistent or coherent effects within both (presumably liver and thyroid) organ systems with the same level of confidence. The "important uncertainties" that remain are not adequately laid out.	Please clarify the medium rating and the nature of the "important uncertainties" mentioned in the text.	S
Subchronic Oral Reference Dose for Noncancer Effects	xv (lines 10-12)	Developmental effects of a lower confidence are used here as the POD for a subchronic RfD rather than higher confidence liver effects.	Please consider including additional justification regarding this decision.	S
Subchronic Oral Reference Dose for Noncancer Effects	xv (line 19)	If an effect on delayed time to vaginal opening (developmental effect) is used as the POD for the development of a subchronic RfD, the relative importance of this effect should be stated. The fact that delayed vaginal opening apparently has no effect on the reproductive success in rats suggests that its importance in humans might be overstated.	Please consider including a statement justifying the use of this developmental effect.	S/M
1.1.1 Physical	1-1 (lines 21-22)	While the statement: "Concerns about PFBA and other PFAS stem from the resistance of these compounds to 21 hydrolysis, photolysis, and biodegradation, which leads to their persistence in	Strongly recommend a statement regarding the half-life of PFBA in humans and its importance	S/M

Chemical Properties		the environment Sundstrom et al (2012a)" is true, it does not include critical information relating to the toxicology of PFBA; namely its relatively short half-life in humans.	in the determination of toxicity.	
1.1.1 Physical Chemical Properties	1-2 (Table 1-1)	Soil adsorption Row: The soil adsorption coefficient or Kd, describes the amount of a substance that binds to soil per amount of water. It is typically defined as including some fraction of organic carbon (a percent). This should be clearly defined in the footnotes. The following value for the BCF is predicted (include footnote b). Additionally, clarification is required regarding whether there is supporting evidence for a BCF of this magnitude given the relatively quick half-life of this PFAS.	Please clarify regarding the fraction of organic carbon and consider including some potential supporting evidence for the predicted BCF.	S
1.1.2 Sources, Production, and Use	1-3	It would be nice to have more detail on pathways for this breakdown of other PFASs that result in PFBA. It is unclear whether the majority of environmental PFBA is from these degradation pathways or from de novo manufacturing. It is also unclear which specific PFAS compounds are known or potential parents of PFBA or whether these processes can occur in vivo.	Please consider including the reference for the original research as well as more information regarding PFAS degradation pathways and processes which can lead to PFBA formation.	S/M
1.1.3. Environmental Fate and Transport	1-3 (lines 29-31)	It is unclear how the referenced information relates to PFBA. While it is true that PFAS released to the air can exist as a vapor phase in the atmosphere, they do not prefer to remain in the vapor phase. Most PFAS prefer to be bound to particulate in the atmosphere (the possible exceptions to this generalization are the fluorotelomer alcohols). And, while PFAS generally resist photolysis in the atmosphere, some like some specific fluorotelomer alcohols, are degraded to more persistent and more degradation resistant PFAS compounds (e.g.,	Consider revising the text to provide specific information regarding the likelihood of PFBA existing as a vapor phase in the atmosphere, or bound to particulate, and whether it is resistant to photolysis.	S

		PFOA). The high Kd and the low Henry's Law Constant (Table 1-1) suggests that very little if any PFBA would be found as a vapor/gas in air and that the vast majority of it would be found bound to particulate or other solids.		
1.1.3. Environmental Fate and Transport	1-4 (line 1)	The statement that PFBA would be expected to be mobile in soil based on its soil adsorption coefficient is not supported by the physicochemical parameters provided in Table 1-1. A water solubility of 2.09E-03 mol/L does not suggest significant water solubility and the Kd (soil adsorption coefficient) of 47.9 (L/kg) suggests that PFBA prefers to be bound to soil constituents and not dissolved in water.	Recommend revising this statement to be consistent with the physicochemical parameters provided in Table 1-1.	S/M
1.1.3. Environmental Fate and Transport	1-4 (lines 14-18)	The bioaccumulation of PFBA in plant biomass is likely a result of its uptake into plant roots and leaves, which can only occur when PFBA is free to dissociate from soil constituents.	Blaine et al (2013) likely discussed why PFBA tended to bioaccumulate to a higher degree in plant tissues than other PFAS. Consider including these authors' rationale for why PFBA bioaccumulates in plant tissues to a higher degree than other PFAS in this section.	S
1.1.3. Environmental Fate and Transport	1-4 (lines 28-29)	The importance of this statement is unclear: "PFBA levels in water at these sites seem to exceed those identified in drinking water."	If the levels in groundwater not used for drinking water are higher than that which was used for drinking water, please consider clarifying the relevance of this statement.	S

1.1.3. Environmental Fate and Transport	1-4 (line 30)	The fact that PFBA is found in fish at 16% of sites sampled in the Great Lakes is not informative of the levels found.	Recommend including the range of values found in fish tissues. Typically, such studies also identify whether the fish tissue sampled were fillets, whole fish or some target fish tissue, information that should also be included here.	S
1.1.3. Environmental Fate and Transport	1-4 (lines 34-35)	The source of the PFBA concentrations detected in seven municipal wells in Oakdale, Minnesota is unclear.	Please clarify as to the source of the PFBA.	S
1.1.3. Environmental Fate and Transport	1-4 (line 38)	Generally, it is worthwhile to identify the detection limit for concentrations that are identified as "non-detectable."	Please include the detection limit (e.g., method detection limit, reporting limit, limit of detection?) or lower bound value for the range cited by Post et al (2013).	S
1.1.3. Environmental Fate and Transport	1-5 (Table 1-2)	The number of NPL sites sampled and analyzed for PFBA levels in water, soil, and air would be beneficial to include in this table.	Please add a footnote to indicate the depth of the information from which the number of NPL sites with PFBA detections are derived. Alternatively, one might report the percentage of NPL sites demonstrating an impact (i.e., a detectable concentration of PFBA). The number of NPL sites sampled and analyzed are still identified in the	E

			footnotes. Inherent in the identification of the number of PFBA detections is the applicable non-detection limits that are typical of analytical analysis performed at NPL sites (i.e., the reporting limit for each media sampled).	
1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure	1-5 (lines 8-9)	While the "... oral route of exposure has been considered the most important one among the general population." there is very little information of any PFBA exposure in the general population. The ATSDR no longer analyzes the 2,000 persons from general population for PFBA (NHANES) since PFBA is rarely detected in the serum of this population.	Please include information about PFBA in NHANES to further inform the reader of the scope of PFBA detections in the general population.	S/M
1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure	1-5 (lines 10-11)	The statement that "Due to the high water solubility and mobility of PFAS in groundwater ..." is in direct conflict with the tabled values provided in Table 1-1. The water solubility (S = 212.03 g/mol x 2.09 E-03 mol/L x 1000 mg/g = 443 mg/L) does not indicate "high water solubility," but moderate water solubility. According to the National Pesticide Information Center (NPIC), a compound is identified as having low water solubility if its water solubility is less than 10 mg/L, moderate water solubility if between 10 to 1000 mg/l and high water solubility if over 1000 mg/L.	Please define what is meant by "high" water solubility and reference.	S/M

1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure	1-5 (lines 15-19)	The modeled exposure to PFBA in adults from the general population is interesting but lacks context. For instance, the relationship between the exposure and potential releases would be useful. Data such as the median inputs for all exposure parameters would also add context in addition to the assurance that the use of these data are consistent with risk assessment-related exposure analysis. Finally, the estimate from direct and indirect sources was used to estimate an intake of 19 pg/kg-day; a comparison to what is measured in the adult general population (ages 12 and over) for which we have data (NHANES) would also add to the context.	Please add the necessary context to this modeled effort so that the reader can understand its significance.	S/M
1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure	1-5 (line 21)	The lack of context under which the direct intake of PFBA in water was assessed in the population provides little useful information about why the largest portion of the individuals total exposure from three unidentified exposure scenarios is from water.	Please revise to add additional context regarding the source of exposure and the exposure scenarios considered.	S
1.2 Summary of Assessment Methods	1-6	It would seem that generic (i.e. not PFAS-specific) frameworks that may resolve apparent conflicts in the PFAS literature, and aid in its understanding and interpretation, could be missed. Examples are theoretical frameworks for the quantitative analysis of acid/base dissociation and the quantitative kinetics of protein binding, and their impact on pharmacokinetics (PK) through tissue distribution (partitioning) and metabolism.	Ensure that such theoretical frameworks for the quantitative analysis are included.	S

1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure	1-6 (line 2)	Clarification is required as to whether PFBA was not measured or not detected; but should it be the latter, the limit of detection would be appropriate to include. ATSDR ceased assessing the general population for PFBA since it was rarely if ever detected in human serum. Earlier NHANES records should be reviewed to see if PFBA was ever analyzed and/or detected in human serum.	Please review earlier NHANES records to see if PFBA was ever analyzed for and detected in human serum.	S
1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure	1-6 (lines 10 and 14-15)	There is no reference supporting the statement that PFAS break down to PFBA in either line 10 or in lines 14-15. It should be clarified whether ski wax contains PFBA to begin with or is its occurrence the result of some degradation of a parent PFAS that occurs in the atmosphere or via metabolism in the individual (noting that these options are both in contradiction to earlier statements).	Please add a reference that supports the fact that PFAS can break down into PFBA. Furthermore, it would be informative to the reader to know what PFAS break down into PFBA.	S
1.2.1, Table 13	1-8	Under the PECO Exposures element: This is the only mention in the document of PFAS mixtures as a source of experimental data.	Perhaps a comment could be added somewhere on the advisability of ultimately using a "mixtures" approach in assessing these compounds (see EPA EPA-822-P-22-002: Framework for Estimating Noncancer Health Risks Associated with Mixtures of Per- and Polyfluoroalkyl Substances (PFAS)), particularly in light of interconversions	S

			between individual compounds.	
1.2.4. Evidence Synthesis and Integration	1-11	The apparent inability of "the process" used for evidence synthesis and Integration to correctly identify the fact that PFBA has "moderate" water solubility suggests that the process is not fully defined, explained, or prone to error. Regardless, "the process" may not provide useful information from which to derive appropriate toxicity values.	Suggest checking "the process" used for evidence synthesis and integration; defining inputs and referencing information prior to arriving at conclusions relating to the information used in toxicity assessment.	S
1.2.4. Evidence Synthesis and Integration	1-12 (lines 27-28)	It is unclear whether the relative sensitivity of rodent and human PPAR activity to PFAS in the expression of liver hypertrophy was considered.	Recommend clarifying whether this was considered.	S
1.2.4. Evidence Synthesis and Integration	1-12 (lines 29-30)	The lack of human serum data reported in NHANES historical records suggests that the only significant exposures occur when an individual is regularly consuming PFBA contaminated drinking water. The short half-life of PFBA in humans may not result in measurable PFBA levels in human serum with intermittent or single exposures to PFBA. Serum samples collected just after an intermittent exposure might detect PFBA, but those samples collected months after a single exposure might not. Of course, the magnitude of exposure may have a great deal to do with what might be detected, but evidence regarding PFOA suggests that very high exposures to PFOA saturate organic anion transport (OAT) proteins in the kidney responsible for PFOA reabsorption, which along with enterohepatic circulation is thought to be responsible for the longer half-life of PFOA in the	Please consider further description and explanation of the assumption of human relevance of animal findings.	S

		human body. The result of OAT saturation is that there is significant urinary release of PFOA. A similar release may also occur at high exposures of PFBA, which would undoubtedly reduce the amount of PFBA at steady state in the body. Furthermore, the relative sensitivity of PFBA to cause liver hypertrophy in rodent test species and humans should be discussed here.		
1.2.4. Evidence Synthesis and Integration	1-12 (lines 31-33)	The suggestion that evidence demonstrates, or that evidence indicates (likely), human health effects as a result of exposure to PFBA should be further justified, since no human effects are identified in exposed persons and the only evidence is based upon animal responses to PFBA in bioassays, where the animal used does not respond in a manner similar to humans, thereby suggesting either a different MOA or a different sensitivity to a common MOA.	Please clarify and discuss.	S
1.2.5. Dose-Response Analysis	1-13 (lines 14-18)	This reference provided is incorrect. The correct reference citation is US EPA (2019), which refers to the US EPA's Systematic Review Protocol for PFBA, PFHxA, PFHxS, PFNA, and PFDA IRIS Assessments. Throughout the document all reference citations to "Appendix A" and "Section" should be corrected to aid the reader in finding the information provided.	Please correctly cite referenced information.	S
1.2.5. Dose-Response Analysis	1-14 (lines 14-17)	Justification is required for limiting the modeling effort to the use of the lower doses when the model fit is poor. It should be clarified whether this only occurs if the higher doses are shown to be influenced by competing toxicity. Additionally, discussion of how the different sensitivity of PPARalpha activation in rodent species and	Please clarify when it is appropriate to eliminate dose-response data in applied modeling efforts.	S

		humans is as addressed by modeling would be appropriate to include here.		
3.1 Pharmacokinetics	3-1 (lines 4-5)	"…the salts immediately dissociate after dissolution and analytic measurements are of the acid." PFBA is an acid with a pKa of 0.08, The references provided offer a detailed understanding of how the dissociation affects PFBA distribution.	See Ruark's or Schmitt's analyses of effect of dissociation on tissue partition coefficients (PCs). These studies provide a detailed understanding of the processes leading to the distribution of both the neutral and ionized forms of the chemical in the lipid and aqueous phases of specific tissues: Ruark CD, et al. Predicting Passive and Active Tissue:Plasma Partition Coefficients: Interindividual and Interspecies Variability. J Pharm Sci, 103, 2189-2198, 2014 Schmitt W. 2008. General approach for the calculation of tissue to plasma partition coefficients. Toxicol In Vitro 22:457-467.	S/M
3.1 Pharmacokinetics	3-1 (lines 26-27)	Toward bottom of page, it states that the distribution is predominantly extracellular (Chang et al., 2008). Other PFAS do partition into the phospholipid membranes and may bind with proteins other than serum proteins; It should be clarified whether this is the case with PFBA.	Provide more details on the distribution.	E

3.1.2. Distribution	3-2 (line 29)	Distribution: Burkemper et al. appears to be the wrong citation. It could possibly be Bartels et al., 2017.	Please check and correct if necessary.	S
3.1.5. Summary	3-13 (lines 20-37)	(Protein Binding): Clearance differences between rodent and human not fully explained by (SS) free fraction differences: "... clearance is not strictly limited to the free fraction (estimated from an in-vitro binding constant). Binding and dissociation are dynamic processes, and it may be that as blood passes through the glomerulus and filtration occurs, some portion of the albumin-bound PFBA is sufficiently labile to dissociate and also be cleared. If only 5% of the bound PFBA is available for clearance, that would be consistent with the empirical data and estimated clearance rates."	The key concept here is the comparison of finite on/off rates compared with (the distribution of) tissue transit times, rather than just considering the equilibrium free fraction as available for uptake. Recommend reviewing a detailed discussion and quantitative analysis of this process (in the context of the brain, but applicable to other tissues), can be found in Robinson & Rapoport, Am J Physiol. 1986 Dec; 251(6 Pt 2):R1212-20.	S
References	R-2	Burkemper et al., 2017 may need to be replaced with Bartels et al., 2017.	Please verify if the correct reference is used.	E