DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR PERFLUOROALKYLS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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Comments provided by Peer Reviewer #1

Chapter 1. Relevance to Public Health

COMMENT: This is a useful chapter and generally a good summary of a voluminous toxicity data base. The MRLs have been derived appropriately.

RESPONSE: *No revisions were suggested.*

COMMENT: Some items should be made more explicit for the reader. The text should state that "PFAs are not metabolized in either experimental animals or humans, and the precise biologic mechanisms responsible for observed effects are incompletely understood". There are profound difference in the half-life of PFAs between humans and experimental animals that make it extremely difficult to compare the effects of exposure levels seen for human populations with those used in animals experiments. Moreover, exposure levels are generally measured and expressed differently: exposure data in animals is generally expressed as intake per unit of animal weight per day (e.g. mg/Kg/day), which is rarely comparable with the exposure dosage data available in humans, expressed as the blood or serum concentration of the relevant PFA (e.g. ppb in serum). There is a general lack of information as to how to accurately convert an intake of mg/Kg/day to concentrations of ppb in serum. Accordingly, the information base is not available to understand the significant dosages given to experimental animals in terms of observed serum concentrations in humans.

RESPONSE: *A discussion of the difficulties in comparing the toxicity observed in experimental animals and humans was added to the beginning of Section 1.2. This discussion includes species differences in elimination half-times, lack of mechanistic data for most endpoints, species differences in mechanisms involving PPARα activation, and differences in exposure measurement between epidemiology studies and experimental studies.*

COMMENT: The "increases in serum lipids, particularly total cholesterol and low density lipoprotein (LDL) cholesterol (PFOA, PFOS, PFNA, PFDeA)" observed in some human studies, and the underlying associations between PFAs with cholesterol and low density lipoproteins may have a physiologic explanation rather than result from toxicity. An apparent association with these lipids could be the result of the partitioning of PFAs with beta lipoproteins in blood described by Kerstner-Wood et al. 2003, and cited in Chapter 3 Toxicokinetics.

RESPONSE: *ATSDR did not identify studies that examined the possibility that the associations between serum lipid levels and the partitioning of perfluoroalkyls with beta lipoproteins. It is noted that blood PFOA is primarily bound (>90%) to albumin, with a small percentage bound to other plasma proteins. The results of the Kerstner-Wood et al. (2003*) in vitro *study found that PFOA was predominantly bound to albumin, whereas PFOS was bound to albumin and beta lipoproteins. If the association between serum lipids and perfluoroalkyls was due to partitioning with beta lipoproteins, one could expect differences between PFOA and PFOS; however, the available epidemiology data suggest similar associations for both compounds.*

COMMENT: When presenting the MRLs there should be an opening statement that all MRLs have been derived from experiments in animals and not from studies of humans. The MRLs derived in the

document are justifiable from these animal experiments. Extrapolation to humans is difficult because of the extreme differences in half-life across species and the lack of a generally comparable measurements of exposure between human populations and the animals used in experiments. In all instances where no MRLs have been derived, I know of no data sufficient to support an MRL.

RESPONSE: *A note was added to Section 1.3 that the MRLs were based on laboratory animal data. A detailed discussion of why epidemiology data were not considered adequate for MRL derivation and species differences are included in Appendix A.*

Chapter 2. Health Effects

COMMENT: This chapter presents a sound and detailed overview of the available literature.

RESPONSE: *No revisions were suggested.*

COMMENT: I strongly agree with the statement that plausibility of effects possibly seen in epidemiologic studies "depends primarily on experimental toxicology studies that establish a plausible biologic mechanism for the observed effects". It is also very important that experimental studies measure the relevant dosage (particularly internal dose) in a manner that supports extrapolation to plausible or observed doses in human exposure situations.

RESPONSE: *No revisions were suggested.*

COMMENT: The document describes "links" between exposures and effects in epidemiologic studies. These links are properly termed associations. There are many reasons for apparent associations, and the presence of an association is, of itself, insufficient to prove causation.

RESPONSE: *ATSDR agrees with the Reviewer that association is insufficient to prove causation; this is stated in numerous places in the profile.*

COMMENT: Early in this chapter the number of studies dealing with specific toxicologic endpoints is enumerated. Especially in the case of the human epidemiologic endpoints the figures cited may reflect the number of publications rather than the actual number of studies. This is an important distinction because many publications have come from some studies, and seemingly different publications may have used data from the same human population. Referring to these as separate studies may exaggerate the appearance of consistency among the studies, which is generally poor, for almost all endpoints.

RESPONSE: *A note was added to Figures 2-1, 2-2, and 2-3 to indicate that the number of human studies is referring to the number of publications.*

COMMENT: Two epidemiologic publications, (which may have included some of the same population), describe an association between ulcerative colitis and PFOA exposure. This association is described in the section on immune effects, but since ulcerative colitis is a gastrointestinal disease, this association should also be cross-referenced in the section on gastrointestinal effects.

RESPONSE: *A statement was added to Section 2.6 that studies examining ulcerative colitis are discussed in Section 2.14, Immunological.*

COMMENT: Animal experiments are described where PFAs were applied locally to the skin to determine irritancy. For these experiments the applied dosage would be more appropriately described in terms of amount applied per unit area of skin, rather than as mg per Kg of body weight.

RESPONSE: *It is ATSDR's practice for dermal experiments to use the unit of measurement reported by the investigators.*

COMMENT: A policy decision is necessary with respect to a number of opinions that have been cited from the "C8 Science Panel" with respect to whether certain health effects in humans are "probable" from PFOA. Opinions of this type appear on pages 128 line 16, page 140 line 31, page 166 line 21, page 178 line 4, page 205 line 16, page 232 line 31, page 265 line 1, page 267 line 11, page 268 line 29, page 405 line 30, and page 421. These opinions were developed for the purpose of introduction into legal proceedings in a specific civil suit. In at least some cases these "expert opinions" are not independent of the investigators who performed the studies that are the basis for those opinions. They do not appear to have been produced in the same manner and lack the authority of evaluations by US Governmental or International Scientific Agencies such as EPA, NTP, or IARC which are appropriately cited in the document. If C8 Science Panel opinions are to be included, ATSDR should at the least ensure that other expert opinions introduced into these or similar legal proceedings are also included. My preference would be to have the toxicologic profile remain a scientific document, consistent with the stated aims, and to exclude the "C8 Science Panel" opinions and all other expert opinions constructed for the specific purpose of civil litigation.

RESPONSE: *Based on comments from several peer reviewers, ATSDR removed the C8 Science Panel conclusions from the profile. Note that the results of the C8 Health Study and C8 Health Panel studies are still discussed in the profile. The following statements were deleted:*

Section 2.5 (PFOA—Epidemiology Studies—Heart Disease)

> *The C8 Science Panel (2011) concluded that there was no probable link between PFOA and coronary heart disease (including its manifestations as myocardial infarction, angina, and coronary bypass surgery) or stroke among members in this community.*

Section 2.5 (PFOA—Epidemiology Studies—Hypertension)

> *The C8 Science Panel (2011) concluded that there were adequate data to suggest a probable link between PFOA exposure and pregnancy-induced hypertension; it is noted that the panel considered the Savitz et al. (2012a, 2012b) studies in their analysis. The Panel also concluded that there is not a probable link between PFOA exposure and diagnosed high blood pressure (hypertension).*

Section 2.8 (PFOA—Epidemiology Studies)

> *Based on the studies available at the time, the C8 Science Panel (2011) concluded that there is not a probable link between exposure to PFOA and osteoarthritis.*

Section 2.9 (PFOA—Epidemiology Studies—Liver Disease)

> *The C8 Science Panel (2011) concluded that there was not a probable link between PFOA exposure and liver disease.*

Section 2.9 (PFOA—Epidemiology Studies—Hepatic Serum Enzymes and Bilirubin Levels)
The C8 Science Panel (2011) concluded that the results of available studies show a positive association between PFOA concentrations and markers of hepatocellular damage, as measured by ALT serum levels.

Section 2.9 (PFOA—Epidemiology Studies—Serum Lipids)
The C8 Science Panel (2011) concluded that there is a probable link between exposure to PFOA and hypercholesterolemia.

Section 2.10 (PFOA—Epidemiology Studies—Kidney Disease)
The C8 Science Panel (2011) concluded that there is not a probable link between kidney disease and PFOA.

Section 2.13 (PFOA—Epidemiology Studies)
The C8 Science Panel (2011) has concluded that there is a probable link between PFOA exposure and thyroid disease.

Section 2.14 (PFOA—Epidemiology Studies—Immunosuppression Outcomes)
The C8 Science Panel (2011) concluded that there is a not a probable link between PFOA exposure and common infections, including influenza, in children or adults.

Section 2.14 (PFOA—Epidemiology Studies—Hypersensitivity Outcomes)
The C8 Science Panel (2011) concluded that there was no probable link between PFOA exposure and asthma; it is noted that many of the studies finding associations between PFOA and asthma were published after the C8 Science Panel report.

Section 2.14 (PFOA—Epidemiology Studies—Autoimmune Outcomes)
The C8 Science Panel (2011) concluded that there was a probable link between PFOA exposure and ulcerative colitis and there was no probable link between PFOA and other autoimmune diseases.

Section 2.18 (PFOA—Epidemiology Studies)
The C8 Science Panel concluded that there is not a probable link between exposure to PFOA and Type II (adult-onset) diabetes.

Section 2.19 (PFOA—Epidemiology Studies—Autoimmune Outcomes)
The C8 Science Panel (2011) concluded that there is a probable link between exposure to PFOA and testicular cancer and kidney cancer, but not any of the other cancers that were considered.

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

COMMENT: This is a strong and well-written Chapter with a contemporary analysis of the topic.

RESPONSE: *No revisions were suggested.*

COMMENT: The section could be enhanced by a brief discussion of the shortage of data relevant to the possibility that different PFAs could produce health effects through common physicochemical or biological mechanism such that additive effects might occur from structurally similar PFAs.

RESPONSE: *A note was added to Section 3.4 that studies examining interactions between perfluoroalkyl compounds have not been identified. It is also noted that this data gap was called out in the data needs section of the profile (Section 6.2).*

COMMENT: I am not aware of other studies or publications that should be referenced.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

COMMENT: This is a useful Chapter. I have no suggestions for improvement.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

COMMENT: This is a strong chapter with an appropriate level of detail.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

COMMENT (Section 6.1): I am not able to identify important data that is not already incorporated into the Toxicologic profile

RESPONSE: *No revisions were suggested.*

COMMENT (Section 6.2): The data needs are generally presented in a neutral, non-judgmental fashion. However, there are some weaknesses.

RESPONSE: *See responses to specific comments presented below.*

COMMENT: The chapter introduction refers to the number of studies relevant to certain health effects (Pages 607, 608, 609, 612). This numbers appear distorted because the number of publications appears to have been equated with the number of studies. In the case of epidemiologic observations there were often multiple publications from the same study, and multiple publications addressing an effect studied in the same population. Finding the same result on the same population does not fulfill the same scientific purpose as finding that the same phenomena in different populations with the same exposure. The latter is strongly suggestive of consistency of findings and is confirmatory of the findings, the former is not.

RESPONSE: *A noted was added to Figures 6-1, 6-2, and 6-3 that the number of human studies is referring to the number of publications*

COMMENT: The text states (p. 614 lines 32 to 34) that "Another limitation of the epidemiology studies is co-exposure to other perfluoroalkyl compounds; studies that statistically controlled for co-exposure to other pollutants would decrease this uncertainty'. In addition to ensuring that results are not attributed to the wrong PFA there is an important need to consider additive (and possibly multiplicative) effects of exposures to PFAs which have structurally similarities, could share the same mechanisms of biologic action, and therefore might act together to produce a toxic effect.

RESPONSE: *A note was added to the Epidemiology and Human Dosimetry Studies subsection that there is a need for studies examining potential interactions between perfluoroalkyls. Additionally, the Potential Interactions Between Perfluoroalkyls subsection has been expanded to call out possible interactions between perfluoroalkyls that may influence toxicity and toxicokinetic properties and to note that many of the perfluoroalkyls likely have similar mechanisms of action.*

COMMENT: The data needs do not sufficiently emphasize the importance of measuring doses in animal studies, and especially internal dose levels, in a manner that can be compared with the serum levels observed in human populations. This would allow better estimates of the exposure or dosage of structurally similar PFAs levels at which toxic effects of PFAs might be anticipated in humans.

RESPONSE: *A note was added in the Health Effects data needs subsection that future animal studies should include measurements of serum perfluoroalkyl levels to facilitate comparisons between epidemiology and experimental studies.*

Chapter 7. Regulations and Guidelines

COMMENT: I am not aware of other regulations or guidelines that would be appropriate for the table.

RESPONSE: *No revisions were suggested.*

Chapter 8. References

COMMENT: I have provided minor corrections.

RESPONSE: *The suggested revisions were made.*

Annotated Comments

COMMENT (Table of Contents): Give correct page numbers for 275 ff.

RESPONSE: *The table of contents was updated.*

COMMENT (page 5, line 20): The associations between PFAs and with cholesterol and low density lipoproteins may also be physiological because of partitioning of PFAs in the blood with beta lipoproteins

RESPONSE: *See response the same comment in Chapter 2.*

COMMENT (page 23, line 24): These links might be more correctly called associations, while making the point that there are many causes for associations and that association does not mean causation.

RESPONSE: *See response the same comment in Chapter 2.*

COMMENT (Figure 2-1): In the case of human studies this appears to be the number of publications rather than the number of studies. A number of publications come from the same individual epidemiologic study and the same population may be used for many studies. More correct to write publications, or if you could do the analysis, cite the number of studied populations.

RESPONSE: *See response the same comment in Chapter 2.*

COMMENT (page 101, line 4): It would be more correct to state: "No data unequivocally establish that PFAs cause deaths in human populations".

RESPONSE: *ATSDR disagrees that the available studies support this statement. Some studies have found disease-specific increases in the risk of death.*

COMMENT (page 127, line 10): This is not earlier than the 2009 study and not much different to the 2012 or 2013 studies. Either omit the term early, of if for some reason the chronology is important put the descriptions of all 4 studies in chronological order

RESPONSE: *The text was revised and the term "earlier" was deleted.*

COMMENT (page 130, line 23): One study reported an association with ulcerative colitis and PFOA exposure. It is described in the section on immunologic effects. However as ulcerative colitis is primarily a gastrointestinal disease that association should be cross-referenced in this section.

RESPONSE: *A statement was added that studies examining ulcerative colitis are discussed in Section 2.14, Immunological.*

COMMENT (page 139, line 6): Osteoarthritis is a disease of wear and tear. It is not caused by immune system alterations. The immune system reference here should be deleted.

RESPONSE: *The statement regarding the immune system was deleted.*

COMMENT (page 167, line 33): The epithet" small" should be removed. The study was not particularly small, and the exposure levels were very high. The wording small appears pejorative in this context!

RESPONSE: *The suggested revision was made.*

COMMENT (page 182, line 32): It should be noted that these were primarily studies of associations with PFOA, with high PFOA exposures rather than PFOS to which the studied population was not exposed more than the general population.

RESPONSE: *In numerous places in the profile, this population is described as being exposed to elevated PFOA levels. Additionally, serum PFOS levels for these studies are presented in the tables and figures.*

COMMENT (page 212, lines 32-34): These are not the most appropriate expressions of application to the skin for irritation studies. The applied dose should be expressed as dose per unit of area, or concentration of applied material, and not as mg/Kg/day.

RESPONSE: *The study presented the dose as mg/kg/day; it is ATSDR practice to report dermal doses using the same units as reported in the paper.*

COMMENT (page 261, line 5): This may have been to studies of the same population: see your discussion on page 267 in this chapter.

RESPONSE: *These are the same data as presented on page 267; the discussion on page 261 is part of the overview for the immune section. To avoid confusion, a subtitle was added to this section.*

COMMENT (page 607, line 19): The material presented for humans appears to refer to the number of publications, rather than the number of studies N, particularly for humans a relatively large number of publications emanated from the same study and the same exposed population. This does not constitute strong replication of findings

RESPONSE: *See response the same comment in Chapter 6.*

Comments provided by Peer Reviewer #2:

General Comments

COMMENT: All of the comments below are premised on the fact that this document brings together a significant body of information for a class of chemicals, and is clearly constitutes a significant, indeed heroic undertaking for which the authors are to be congratulated. In addition, these comments acknowledge the introduction's description of how these documents are typically formulated for the users for which they are intended. In general, while the MRLs ultimately derived seemed reasonable/ appropriate, many of my comments relate to the presentation of the logic used to make decisions that finally arrive at the MRLs.

RESPONSE: *No revisions were suggested.*

Chapter 1. Relevance to Public Health

COMMENT: The effects summarized on the basis of the literature appear to be the appropriate effects, and I am not aware of any references that have been omitted. In addition, the effects observed in animals and described as relevant to human health are appropriate. The inclusion of descriptions of where effects in rodents are seen in both wild type and PPARα knockout mice is a critical inclusion and underscores relevance to human outcomes. Exposure conditions are briefly but appropriated described.

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

COMMENT: P. 30 and others, the #R values on the plot apparently relate to the figure key column of the corresponding tables. The numbers/letters are not readable on the plots however. In addition, why not add the user guide to the beginning of this chapter instead of way far back at the end. Its not that much additional text.

RESPONSE: *Due to the number of data points in the figure, a larger font cannot be used. It is noted that the figures are a little blurry in the draft document because a pdf is inserted into a Word file. The final document is a pdf file and figures are clearer. ATSDR believes that inserting the user guide in Chapter 2 will disrupt the flow of the document; many of the users of the profile are familiar with the LSE figures and do not need to refer to the user's guide.*

COMMENT: My main concern is the adoption of the distinction of less serious vs. serious effects, which seems quite arbitrary, and in numerous cases very large effects are labeled in the Tables as 'less serious'. The document indicates that outcome measures are considered as serious vs. less serious, and admits that the distinctions may not seem objective. How these distinctions are being used to "define which levels at which major health effects start to appear" as stated in the text, is not clear how that is being used or interpreted. In fact some of the MRLs appear to be based on what are designated as less serious effects, in which case these arbitrary distinctions don't seem logical or needed. If these are based, as stated on the case that effects vary with dose and duration, then non-linearity in effect, e.g., U-shaped

curves, are by default already ignored and there are several examples within Chapter 2 that raise non-linearity of effects.

RESPONSE: *ATSDR categorizes adverse health effects into two groups based on the severity of the effect. A serious LOAEL is defined as effects that evoke failure in a biological system and can lead to morbidity or mortality; a less serious LOAEL is used for adverse effects that do not result in significant dysfunction or death. ATSDR acknowledges that for many effects, the distinction between serious LOAEL and less serious LOAEL is somewhat subjective and has established guidelines for classifying the degree of adversity for certain endpoints; this guidance document (ATSDR 2003) is cited in Section 2.1. The statement in the introductory text of Section 2.1 that "LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration" is referring to evaluating dose-response relationships; however, it is not used to ignore U-shaped dose-response curves or for effects that are nonlinear.*

COMMENT: While all appropriate studies are cited, major study limitations are not consistently mentioned. It is difficult based on the tables to ascertain anything about quality of studies other than use of multiple doses for the most part. Currently the tables list studies for each perfluoroalkyl by alphabetical order for each endpoint. It would be helpful and easier for the user of the document to arrange these by quality of the study within each of those categories instead. In addition, it might be particularly useful to add a column that indicates some metric of study quality. Also, it would be helpful to add some row offset that differentiates the acute studies summarized from intermediate from chronic.

RESPONSE: *More detailed summaries of the studies are presented in the supplemental documents. The studies are listed by species and alphabetical order for ease of locating a particular study in a table that spans a number of pages. A shaded row is used to indicate a change in the exposure duration.*

COMMENT: Adequately defined human studies are identified in the text and in general limitations are described, although these are most often related to exposure conditions. There is little indication of study quality based on other epidemiological or methodological parameters. What is less described are other potential study conditions, e.g., adequate controls for confounding except in limited cases. Here, as in the Tables provided for animal studies, it might be useful to add an additional column to the human studies tables that indicate strengths/limitations of the studies; the 'results' column currently takes up a bit of room on these tables and these could be indicated more succinctly. In addition, it might be easier for users if the table inputs were arranged in order of decreasing study quality/reliability as judged by ATSDR.

RESPONSE: *As noted in response to the previous comment on the LSE tables, more detailed descriptions of the epidemiology studies are presented in a supporting document, which will be available from ATSDR's website; these descriptions include a list of confounding variables with statistical adjustments. The studies are listed alphabetically for ease of locating a specific study. Ranking studies by quality/reliability would require a systematic review of each cited study and may decrease the ease of locating a particular study in the table.*

COMMENT: P. 139, lines 2-6 states: "…provide some suggestive evidence of a relationship between serum perfluoroalkyls and osteoarthritis. However, there are no mechanistic data to support this association and it is noted that there are a number of factors that contribute to the osteoarthritis risk…" seems to suggest that in the absence of a known mechanism, an effect of these compounds has no validity, which if carried to its logical conclusion would eliminate many of the health outcomes.

RESPONSE: *The text was revised to indicate that assessing whether there is a link between perfluoroalkyl exposure and osteoarthritis is complicated by the lack of mechanistic data and that there are a number of factors that contribute to the overall risk.*

COMMENT: Based on the fact that the animal literature is primarily in rodents and studies in non-human primates have no real dose response to evaluate, the studies used are appropriate. I am not aware of studies that were omitted.

RESPONSE: *No revisions were suggested.*

COMMENT: Another suggestion for consideration is the inclusion of some interpretations of health outcomes. I understand a totally species-specific health outcome (e.g., PPARα-mediated effects being deemed irrelevant to human health. However, in other cases, it raises questions/concerns.

- For example, the fact that delayed mammary development is ok because dams were still able to nurse later seems potentially short-sighted. First, it suggests that delayed development is 'ok'. That delay was clearly a function of some biological consequence of the exposure. So how do we know that that delayed development is truly the end of the line of mammary gland health effects and doesn't relate to some other effect that wasn't measured?
- Similarly, somehow body weight changes of less than 10% are ok. If someone experiences these as side effects of a drug, would they not be reported?
- On. P. 24, it is stated (line 23) that significant health-related changes in response to one of these compounds were adaptive. What does that mean? Is that ok if exposures of humans are non-voluntary? Does it consider that it could indicate other biological changes that aren't being measured?
- Huge changes for example in serum estradiol (e.g., 184%, 63%, p. 33) are indicated as "less serious". As are almost 90% changes in thymus weight?
- Another example, on p. 133 lines 20-21: …"of unlikely biological significance". Again, it's not clear how biological significance is being defined. An additional example, p. 135 lines 14-15: "These hematological effects were considered minor and not evidence of an adverse effect…".
- These examples in the paragraph above raise another consideration. There are several examples in the description of health effects where health effects are dismissed since the changes, whether significant or not are essentially dismissed because the changes are still within the range of normal values. However, from a population point of view, this indicates a shift of the population distribution toward extreme values and thus with sufficient sample sizes could be indicative of a significant shift in biological values. Another example of this is on p. 234, lines 24-25 which states "…induced a significant increase in serum TSH (approximately twice control value, but still within reference range).

RESPONSE: *The Reviewer raised a number of issues regarding interpretations of health outcomes; below are responses to these issues:*

- *The delays in mammary gland development were considered as adverse effects and the lowest doses associated with these alterations were categorized as LOAELs. However, for MRL derivation, the biological significance of the changes observed at the lowest LOAELs was questioned since it did not appear to result in functional impairment.*
- *Regarding body weight effects, it is standard practice by ATSDR and EPA to consider body weight changes of less than 10% to be non-adverse.*

- *The statement on page 24 that the liver effects observed were considered adaptive was revised to indicate that the effects were considered to be specific to rodents and were not considered relevant to humans.*
- *As noted in a previous response, serious LOAELs are effects that are indicative of serious organ dysfunction. The changes in estradiol levels were not considered a serious health effect. The marked decrease in thymus weight was re-categorized as a serious effect as it was indicative of thymic lymphocyte atrophy.*
- *Regarding the biological significance of altered hematological effects discussed on page 133 of the profile, ATSDR does not consider all statistically significant alterations to be of toxicological significance. In this example, the changes were small and were not likely to impact the health of the animal.*
- *Many of the epidemiological studies examined very large populations and small changes in hematological or serum chemistry parameters were often statistically significant. ATSDR utilized the normal range as a guideline for assessing the toxicological significance of the effect. ATSDR also took into consideration the consistency of the alteration across studies.*

COMMENT: Adequate attention has been paid to dose-response relationships for both human and animal data.

RESPONSE: *No revisions were suggested.*

COMMENT: Animal data used to draw support for known human effects is described and provides valid support.

RESPONSE: *No revisions were suggested*

COMMENT: The selection of endpoints carried forward appears to be appropriate.

RESPONSE: *No revisions were suggested*

COMMENT: Its not clear what is being referred to in Appendix B as this contains the Literature Search Strategy.

RESPONSE: *The charge question is referring to the systematic review that is included in Appendix B of some profiles; the question is not relevant for the perfluoroalkyls profile, which does not include a systematic review of health effects studies.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

COMMENT: Chapter 3 presents an in depth presentation of toxicokinetics and the differences in the parameters of these processes between species.

RESPONSE: *No revisions were suggested*

COMMENT: I cannot comment on the validity or inclusiveness of the modeling.

RESPONSE: *No revisions were suggested*

COMMENT: Biomarkers of exposure/effect are adequately addressed.

RESPONSE: *No revisions were suggested*

COMMENT: The one known interaction of these chemicals is described. However, the fact that perfluoroalkyls are related compounds raises the question of the potential for additive or synergistic effects between/among them. While I am not specifically aware of any such studies, it might be important to add them if there are, or to mention it if there are not.

RESPONSE: *Studies examining the potential interaction between perfluoroalkyl compounds were not identified. ATSDR agrees with the Reviewer that this is an important issue, especially for interpreting the epidemiology data; a need for interaction studies was identified in Section 6.2.*

Chapter 4. Chemical and Physical Information

COMMENT: I am not aware of any missing information.

RESPONSE: *No revisions were suggested*

Chapter 5. Potential for Human Exposure

COMMENT: This Chapter does a very thorough presentation of sources and levels of exposure in various media as well as in various populations. I am not aware of any relevant information that is missing.

RESPONSE: *No revisions were suggested*

Chapter 6. Adequacy of the Database

COMMENT: I don't have any issues with the description of the adequacy of the databases. Its good to see two particular points listed in the research needs. One is the need to examine potential interactions of effects of this group of compounds. Another is the need for more dose response data and this should be explicitly addressed to the potential for non-linear dose effect curves as have been suggested by some of the studies to date. These add to the need for more conservative approaches to MRLs.

RESPONSE: *No revisions were suggested*

Chapter 7. Regulations and Guidelines

COMMENT: The presentation is appropriate and useful as an overview. I don't necessarily have issues with the particular MRLs that are derived here, except for the issue raised above as to 'serious' vs 'less serious' distinctions. In assessment of the data for chronic oral exposure, I agree with the decision not to derive an MRL based on available data.

RESPONSE: *No revisions were suggested*

Chapter 8. References

COMMENT: I don't know of missing references.

RESPONSE: *No revisions were suggested*

Comments provided by Peer Reviewer #3

General Comments

COMMENT: Overall, this is an impressively, even uniquely, comprehensive report on the research addressing potential health effects of perfluoroalkyls. The tables in particular seem to have captured all relevant reports and provided helpful summaries, recognizing that there is a need to distill the evidence to make it useful. The reference list alone provides a valuable resource for researchers and those who wish to dig more deeply into the original literature. The material is organized in a logical manner, making it clear where to find information on specific issues (specific fluoroalkyls, specific health endpoints) which will ensure its value as a reference, obviously the primary use for this document.

RESPONSE: *ATSDR thanks the Reviewer for their kind comments.*

COMMENT: The overall tone and judgment is consistently even-handed and careful, acknowledging where there is insufficient or contradictory evidence, but also noting where the weight of evidence is leaning in a particular direction, towards or away from suggesting an adverse effect. The comments generally reflect an appreciation of the methodologic concerns and relative strengths of the studies, but in a comprehensive document of this nature, such considerations are infused broadly through the document without being able to dig more deeply into any one of them.

RESPONSE: *No revisions were suggested.*

COMMENT: While I cannot claim familiarity with the entire scope of studies that were considered, where I do have a good working knowledge, the material that is presented is accurate and it is reasonable to presume that the acquisition and tabulation of information on the studies is accurate throughout.

RESPONSE: *No revisions were suggested.*

COMMENT: It is stated that the intended audience for the report is "community-level public health officials, physicians, and concerned citizens." However, the comprehensive, largely technical discussion is likely to be challenging to all but the most sophisticated among those audiences. It may be easier to imagine that expert groups advising physicians or public health leaders will use the document to inform those to whom they report, but it's hard to imagine practicing physicians or public health personnel broadly making use of such a resource. The sheer volume of material is daunting, and it is not reasonable to expect those types of readers to be able to go from the detailed reports to their own bottom line inferences. This is probably the strongest argument for helping such readers, perhaps by having some form of a "lay summary" for each health endpoint of concern that conveys what we know. In other words, ATSDR could consider providing the distilled information that practitioners would need but that would need to be a new and separate section. There could be a separate chapter that would provide the sort of summary such audiences would find most useful.

RESPONSE: *Chapter 1, Relevance to Public Health, is intended as a summary of the available information on the toxicity and potential for exposure to perfluoroalkyls. The chapter is intentionally short (typically 10–20 pages including tables and figures) and written as an executive summary.*

Chapter 2. Health Effects

COMMENT: A feature that is not entirely clear is the desired level of detail and specificity for the "bottom line" conclusion for each section. As a reader, I would await the final word which often did not come, either at the beginning or end. It would be useful to set appropriate expectations at the outset for what to expect in the way of final, integrative assessments. This would be an issue for the perfluoroalkyls in the aggregate and for each specific perfluoroalkyls, and of course have to be specific for each of the health endpoints under discussion. The question of whether conclusions are drawn for the human and animal literature separately, or for the two lines of research in combination, needs to be clarified. While comments are made in places on the volume of research, in many cases quite limited, and whether the evidence that is available is consistent or leans towards or away from an association, this is not done consistently. The contrast of leaving the issue open versus drawing a conclusion is especially clear when the judgments of the C8 Science Panel are provided, almost as though their conclusions were adopted and accepted by ATSDR in that areas that they did not address (i.e., chemicals other than PFOA, many of the health endpoints of concern) are not provided with such definitive assessments. I presume that it would not be in the scope of ATDSR's effort to methodically assess the evidence for the dozens of potential causal associations under consideration (as IARC does, for example) but even a less formal take of whether the volume of research is sufficient to draw any judgments and whether the evidence is leaning toward or away from an association could be done using standard terminology.

RESPONSE: *Each section in Chapter 2 begins with an overview of the data and a bottom-line conclusion. A subsection title "Overview" was added for ease of use. Additionally, short summaries of the larger sections have been added to this chapter. In response to several peer reviewers, the C8 Science Panel conclusions regarding associations between PFOA and specific health effects have been removed from the profile.*

COMMENT: This raises a few more specific points to consider to ensure maximum value, consistency, and clarity in the health effects section of the report:

1) In a preamble to the health effects chapter, it would be useful to set the stage to let the reader know how it is being approached and what to expect in each section in the way the evidence is summarized and what sorts of conclusions are or are not going to be provided.

2) The organization of the text to cover the whole class of perfluoroalkyls and then the evidence on individual chemicals in the class should be explained with some comment on the rationale. Presumably that is because we don't know if the chemical class allows for generalization (i.e., they act similarly) or whether each chemical needs to be considered independently. This is especially important as the number of perfluoroalkyls of concern continues to expand and unless we can find a way to address them broadly as a class or predict toxicity based on chemical structure, we have a huge problem since the number of them goes beyond what is feasible to evaluate on a chemical-by-chemical basis.

3) The presentation and synthesis of human and animal evidence offers an opportunity for a unique contribution that is only partially realized. It would be quite helpful to comment on the compatibility of those lines of research, even to note that there are places where the human evidence is pointing in a particular direction but there's little animal evidence to help interpret it, or where the animal evidence suggests a pathway that has been insufficiently examined in human studies. And of course, noting where there is compatibility or incompatibility between the findings from those different lines of research.

RESPONSE: *A discussion of how Chapter 2 is organized was added to the chapter introduction. A statement was also added that each compound is treated separately due to qualitative and mechanistic*

differences between compounds, despite the similar outcomes for some compounds. Differences in health outcomes between humans and animals are noted in the health effects text.

COMMENT: Some of the general methodologic issues could be addressed in general terms prior to presenting the evidence. It would be tedious and unnecessary to note such issues in each section that follows, so a broader comment on what is coming would be the best way to go about this. In that way, as the reader goes to the section of interest they would be better prepared to make sense of the literature that is there.

1) The sequence of the human studies presented seems to be occupational, highly exposed community populations, then general community populations. Again, a preamble that lays this out clearly and provides the rationale for doing so would be helpful. For example, it would be noted that occupational populations often have the highest exposure but are selective in nature, often underrepresenting women, excluding children, subject to the healthy worker effect, etc.
2) The approach to classifying exposure should be discussed as well to help interpret the literature, noting the strengths and limitations of the methods used. Most rely on biomarkers, which have their own considerations, but others use job title or location in contaminated area as the exposure indicator.
3) There is a heavy (excessive) reliance on statistical significance testing to screen results. Acknowledging that some approach is needed to handle the hundreds of studies being considered, it might be useful to provide an explanation of how and why this was done, and what else is worth considering as the reader looks at the tables. For example, where exposure was categorized and there is not a monotonic gradient of increasing risk with increasing exposure, the likelihood of a causal effect is reduced, so even when a statistically significant elevation in risk is found for the 2nd or 3rd quartile, not the 4th, it provides little evidence of an adverse effect. Similarly, some studies are so large that tiny, inconsequential associations are statistically significant, whereas other studies are quite small with very limited power. Again, this can't be discussed on a study-by-study basis but could be presented in advance of the evidence in a general way.

RESPONSE: *In a number of sections discussing PFOA health outcomes, the epidemiology data are ordered by occupational, highly exposed, and general population; this is not true for all sections. For other perfluoroalkyls, there are no occupational (except PFOS and PFNA) or highly exposed populations. With few exceptions, epidemiology data used serum levels as the biomarker of exposure; this is discussed in the chapter introduction (Section 2.1). ATSDR believes that it is beyond the scope of the profile to include a general discussion of how to interpret the statistical analyses used in epidemiology studies.*

Additional Comments

COMMENT (page 5, list of health outcomes): it seems the cancers should be included (kidney, testicular)

RESPONSE: *Testicular and kidney cancers are noted in the paragraph listing the IARC and EPA cancer classifications.*

COMMENT (page 119): where there's an ordered set of categories, observation associations in the middle ranges doesn't seem to be that notable – could argue it's evidence against a dose-response gradient

RESPONSE: *Table 2-8 reports the lowest exposure category with a significant finding; odds ratios for higher exposure categories ae reported in the Supporting Document for Epidemiology Studies. If there were no significant findings, the odds ratio for the highest exposure category is reported.*

COMMENT (Chapter 5): Comment on occupational exposure levels, not just general population levels

RESPONSE: *The text of Section 5.7 was revised to include occupational monitoring data.*

Comments on the Minimal Risk Levels

COMMENT: The need to look to the animal literature (rather than human studies) to determine MRLs is well-justified since the level of detail on exposure in human studies, volume of studies, and clarity of results is insufficient to quantify thresholds of effect. It is also appropriate to focus on "intermediate duration" given what we know about potential health effects.

RESPONSE: *No revisions were suggested.*

COMMENT: The menu of toxicologic findings that are used seem reasonable but some rationale would be helpful. For example, these might be a range of the best-established effects or represent the endpoints most pertinent to human health effects for each of these agents.

RESPONSE: *The "Selection of the Critical Effects" section of the MRL worksheet (Appendix A) contains specific endpoints that were considered as the critical effect for MRL derivation. The section was revised for PFOA and PFOS to clearly state why these endpoints were considered the primary targets of toxicity (adverse outcomes observed at lower doses than other tissues). For PFNA and PFHxS, all observed effects were considered as potential critical effects.*

COMMENT: To be most informative for public health officials, these numbers would need to be extrapolated to both blood concentrations in humans and ultimately to concentrations in the water, assuming a standard consumption level. That is, the question frequently arises in response to detection of perfluoroalkyls in the drinking water supply, and even approximate guidance on how those levels correspond to human exposure "of concern" would be a great service to those who must respond to the public. While there would have to be abundant caveats given the uncertainty, even a rough sense of the level of concern with health effects would be beneficial.

RESPONSE: *The toxicological profile does not include conversions of MRLs into drinking water concentrations or blood concentrations.*

Comments provided by Peer Reviewer #4

General Comments

COMMENT: The Table of Contents needs to be updated as nearly all page references are inaccurate.

RESPONSE: *The Table of Contents was updated.*

COMMENT: Sometimes the term "perfluoroalkyl substances" is used in the document and sometimes the term "perfluoroalkyls" is used in the document. It may improve clarity to use the same term throughout or to use a widely accepted abbreviation (i.e., PFASs).

RESPONSE: *The profile was revised to eliminate the term "perfluoroalkyl substances".*

COMMENT: The figures in this document are **absolutely amazing**. They should be easy to understand for both scientific and most lay audiences, summarize a huge amount of information, and are visually pleasing. Commendations to the team that drafted this document and adopted these figures. They really truly are outstanding in quality and clarity.

RESPONSE: *ATSDR thanks the Reviewer for the compliment.*

COMMENT: The overall organization by chemical within health endpoints makes it very easy to navigate to find specific information related to chemical and health effect. Again, commendations to the team for this outstanding organizational strategy.

RESPONSE: *No revisions were suggested.*

COMMENT: Throughout the document, when referring to findings, links, and conclusions made by the C8 Science Panel for PFOA *in regard to their specific study population*, the results often are presented in a way that makes it seem as if the C8 Science Panel is reaching conclusions about PFOA *in any PFOA-exposed population*. The authors may want to consider including language that indicates that C8 Science Panel studies refer to a specific study population, perhaps "The C8 Science Panel found no associations between PFOA and asthma in their study cohort" or something similar.

RESPONSE: *In response to comments from several peer reviewers, ATSDR has removed the C8 Science Panel conclusions from the profile.*

COMMENT: Throughout the document the assertion is made that PFASs are not metabolized. This is thought to be true for the PFASs in the profile, but is not true for PFASs as a whole as some PFASs may degrade or be metabolized to other PFASs (as stated on Page 1 Line 28 of the profile). A caveat such as "the PFASs evaluated in this profile are not known to be metabolized."

RESPONSE: *The statement is referring to the 14 compounds discussed in the profile. A note was added in several places in the profile that the term "perfluoroalkyls" used in the profile is referring to the 14 compounds examined and information may not be applicable to other perfluoroalkyl compounds. In Chapters 1 and 2, this statement was bolded.*

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text?

COMMENT: As with many chemicals of environmental and/or occupational concern, it can be challenging to identify effects "known" to occur in humans. However, as with many chemicals of environmental and/or occupational concern, studies of highly exposed human populations often are available in the published literature. The C8 Science Panel studies and studies of the same population to which the C8 Science Panel had access have provided the bulk of information for PFOA and to an extent, PFOS, in the U.S. This is reflected in the profile as the bulk of human effects are attributable to the findings of the C8 Science Panel studies. It may be helpful to note that the majority of data on human effects that establish probable links in the U.S. population arise from the C8 Science Panel studies and studies of the same population. Therefore, while the effects listed in humans have been associated with exposed humans, they come mostly from the study of a single (albeit large cohort) population.

RESPONSE: *For most of the endpoints, epidemiology studies examining possible associations with PFOA include both the C8 population and the general population. For the other perfluoroalkyls, health outcome data come almost exclusively from general population studies. In data summary tables (and in the text), C8 population studies are identified for the reader.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: On Page 10, Lines 1-6, it is noted that high PFAS serum concentrations in experimental animal models compared to exposed humans, differences in toxicokinetics between human and experimental animal models, and the issues related to the putative mode of action in experimental animal models compared to humans make it difficult, at this time, to determine the "true" relevance of some effects reported in animal studies to human health. While these points do contribute to uncertainty regarding the exposure dose associated with human health effects, it is critical to note that for several human findings, notably, liver toxicity, immunotoxicity, and developmental effects (i.e., effects on birth weight), concordance exists between human studies and studies of experimental animal models for these health effects. Concordance suggests similar modes or mechanisms of action, at least for a suite of health effects that are not highly dependent on serum concentration or on serum half-life. Additionally, the question "are the effects only observed in animals likely to be of concern to humans?" is not really captured in the profile as ALL of the effects listed as primary effects in experimental animals models (liver toxicity, developmental toxicity, and immunotoxicity) have been reported to occur in exposed humans. Maybe the question is about the relevancy of PPARα-associated effects in experimental animal models to humans. In this case, it is questionable whether effects that are known to be modulated via a PPARα mechanism in experimental animal models are highly relevant to humans, unless exposure doses are quite high.

RESPONSE: *ATSDR agrees with the Reviewer that there is considerable uncertainty about the relevance to humans of PPARα-associated effects observed in experimental animals. This is further complicated since effects within a target tissue can be due to PPARα-dependent and -independent mechanisms. ATSDR believes that additional data are needed, and the need for mechanistic studies is discussed in the data needs section of the profile (Section 6.2).*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT: Given that human serum concentrations are largely the biomarker for as of yet incompletely described exposure pathways and doses, the description of the exposure conditions reflect the current state of knowledge.

RESPONSE: *No revisions were suggested.*

QUESTION: If MRLs have been derived, are the values justifiable? If no MRLs have been derived, do you agree that the data do not support such a derivation?

COMMENT: The chosen points of departure, key studies upon which they are based, and the adequacy of the database for establishing intermediate oral MRLs for PFOA, PFOS, PFHxS, and PFNA justify the chosen values. It may be important to highlight that these are **intermediate** MRLs as opposed to chronic MRLs as they likely will be compared to chronic values derived by other state and federal governmental organizations. These intermediate MRLs for PFOA and PFOS are also a magnitude of order smaller than the reference doses for chronic toxicity derived by the U.S. Environmental Protection Agency (U.S. EPA) in their health effects documents for each of these compounds. This will undoubtedly create some confusion in the broader regulatory community, but the details on MRL derivation included in Appendix A are transparent and easy to understand. It may be useful to include some of the rationale used to choose/exclude studies for the MRLs as described in Appendix A in an earlier part of the document, especially since these studies differ from, for example, the studies chosen by the U.S. EPA for their reference doses.

RESPONSE: *The MRLs derived in the toxicological profile are always referred to as intermediate-duration MRLs. In the revised format of the toxicological profile, the discussion of the MRLs are presented in Appendix A; the values are listed with limited detail in Sections 1.3 and in Chapter 7 (with a pointer to Appendix A for more details). The detailed discussion of critical effect and principal study selection is at the end of the profile, because data from Chapters 2–6 are taken into consideration during MRL derivation.*

Chapter 2. Health Effects

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: The details related to epidemiological studies are sufficient to highlight major findings as well as limitations of major studies.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)? Please suggest appropriate changes.

COMMENT: It appears as if the conclusions of cited papers are appropriately and accurately reflected in the profile.

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Most epidemiological studies for PFASs do not identify NOAELs or LOAELs as they typically do not have accurate exposure doses and have to use a biomarker of exposure. Therefore, it is not possible to appropriately answer this question for human studies.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the appropriate statistical tests used in the studies? Would other statistical tests have been more appropriate? Were statistical test results of study data evaluated properly? **NOTE:** As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

COMMENT: It is unclear from this question if this refers to the cited studies used in the profile or to statistical approaches used within the profile. Statistical approaches in cited studies were hopefully vetted by the peer-review process used by the journal in which they were published. Within the profile, it appears as if approaches for modeling human equivalent doses and benchmark doses are consistent with currently acceptable approaches.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of other studies which may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: Not for human studies.

RESPONSE: *No revisions were suggested.*

QUESTION: For the health effects in humans exposed tables, are the study details and author conclusions presented accurately?

COMMENT: The tables appear to be accurate representations of study details and author conclusions.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: It appears as if the animal studies identified in the text were adequately designed; however, without reviewing every published study in any sort of experimental animal model, this question is challenging to answer. It does appear as if the majority of studies identified in the text and used to support, for example, MRLs, were adequately designed.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: As most of the experimental animal studies that evaluate health effects with potential translational relevance to humans were performed in mouse, rat, or non-human primate models, the animal species associated with the MRLs appear to be appropriate.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the text? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?

COMMENT: This was a particularly well-done aspect of the profile, especially in Appendix A. It may be useful to include some of the rationale used to choose/exclude studies for the MRLs as described in Appendix A in an earlier part of the document, especially since these studies differ from, for example, the studies chosen by the U.S. EPA for their reference doses.

RESPONSE: *See previous response to this comment.*

QUESTION: Were all appropriate NOAELs and LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies? If not, please explain.

COMMENT: It appears as if appropriate NOAEL, LOAELs, toxicological effects were identified.

RESPONSE: *No revisions were suggested.*

QUESTION: If appropriate, is there a discussion of the toxicities of the various forms of the substance? If not, please give examples of toxicological effects that might be important for forms of the substance.

COMMENT: This appears to be part of the discussion, i.e., differences between linear and branched isomers of PFASs.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the appropriate statistical tests used in the interpretation of the studies? If not, which statistical tests would have been more appropriate? Were statistical test results of study data evaluated properly? **NOTE:** As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

COMMENT: It is unclear from this question if this refers to the cited studies used in the profile or to statistical approaches used within the profile. Statistical approaches in cited studies were hopefully vetted by the peer-review process used by the journal in which they were published. Within the profile, it appears as if approaches for modeling human equivalent doses and benchmark doses are consistent with currently acceptable approaches.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of other studies that may be important in evaluating the toxicity of the substance? If you are citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: Developmental cardiotoxicity of PFOA in a chicken model is addressed in a series of papers by Jiang et al. (2012, 2013, 2015). The 2012 paper describes a developmental cardiotoxic outcome and the subsequent papers evaluate potential mechanisms. These papers would be relevant to sections on developmental and cardiovascular findings in experimental animal models for PFOA.

- Jiang Q et al. 2012. Perfluorooctanoic acid induces developmental cardiotoxicity in chicken embryos and hatchlings. Toxicology. 293:97-106.
- Jiang Q et al. 2013 Perfluorooctanoic acid induced-developmental cardiotoxicity: are peroxisome proliferator activated receptor α (PPARα) and bone morphogenic protein 2 (BMP2) pathways involved? Journal of Toxicology and Environmental Health Part A. 76:635-650.
- Jiang Q et al. 2016. Perfluorooctanoic acid-induced toxicity in primary cultures of chicken embryo cardiomyoctes. Environmental Toxicology. 31:1580-1590.

Benninghoff et al, 2011, 2012 and Tilton et al. 2008 each describe tumor studies of PFOA in a rainbow trout model and propose potential mechanisms for hepatocarcinogenesis. The rainbow trout is often used as a model of liver cancer (Williams, 2012), so these papers would be relevant to the section on cancer studies in experimental animal models exposed to PFOA.

- Benninghoff AD et al. 2011. Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro. Toxicological Sciences. 120:42-58.
- Benninghoff AD et al. 2012. Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. Toxicological Sciences. 125:69-78.
- Williams DE. 2012. The rainbow trout liver cancer model: Response to environmental chemicals and studies on promotion and chemoprevention. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 155:121-127.

Immunotoxicity of PFNA in a mouse model is described in two papers by Rockwell et al. (2013 and 2017). While the relevance of these two papers to immunotoxicity is of questionable value as they

present findings after a single high dose that produces profound weight loss in the mice, they may be relevant to the section on body weight effects in experimental animal models exposed to PFNA.

- Rockwell CE et al. 2013. Acute immunotoxic effects of perfluorononanoic acid (PFNA) in C57BL/6 mice. Clinical and Experimental Pharmacology. Suppl 4:S4-002.
- Rockwell CE et al. 2017. Persistent alterations in immune cell populations and function from a single dose of perfluorononanoic acid (PFNA) in C57BL/6 mice. Food and Chemical Toxicology. 100:24-33.

Corsini et al. 2011 and 2012 are papers that address potential mechanism of immunotoxicity in vitro. These papers would be relevant to the section on cellular mechanisms of toxicity, both for the section on PPARα-dependent and the section on PPARα-independent mechanisms.

- Corsini E et al. 2011. In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). Toxicology and Applied Pharmacology. 250:108-116.
- Corsini E et al. 2012. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). Toxicology and Applied Pharmacology. 258:248-255.

RESPONSE: *The Jiang et al. (2012, 2013, 2016) studies were added to the developmental effects section of Chapter 2. The Benninghoff et al. (2011, 2012) studies examining carcinogenicity in rainbow trout were added to Section 2.20.6—cancer mechanisms of action; the Williams (2012) paper was not added because it was a review of the data presented in the Benninghoff et al. (2011, 2012) papers. The Rockwell et al. (2013, 2017) studies were added to Section 2.14, Immune effects. The results of the Corsini et al. (2011, 2012) studies, as reviewed by Corsini et al. (2014), are noted in Section 2.20.4—immune mechanisms of action.*

QUESTION: Are the LSE tables and figures complete and self-explanatory? Does the "Users Guide" explain clearly how to use them? Are exposure levels (units, dose) accurately presented for the route of exposure? Please offer suggestions to improve the effectiveness of the LSE tables and figures and the "User's Guide."

COMMENT: The tables, figures, and “User’s Guide” are mostly helpful and effective.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

COMMENT: Seriousness of body weight gain and loss is confusing to interpret in the LSE tables. For example, on Page 29 Table 2-1, weight loss for 1-2 days after exposure (Kennedy et al., 1986 study) is listed as a serious effect and 7% weight loss by exposure day five (also Kennedy et al., 1986) is listed as a less serious effect? On Page 37 Table 2-3, 33% reduced maternal body weight gain is listed as a serious effect but there were no alterations in fetal body weight (Stales et al., 1984). On page 38 Table 2-3, decreased body weight gain is listed as a serious weight loss (Loveless et al., 2006). Other inconsistencies with body weight loss and gain are noted throughout the LSE tables for all compounds and it is not clear why some body weight gains/losses are considered more or less serious.

RESPONSE: *The body weight effect reported in the Kennedy et al. (1986) 2-week study was revised to indicate that rats at 84 mg/m^3 weighed 7% less than controls. It is unclear from the paper whether this reflects a body weight loss or a decrease in body weight gain. ATSDR considers decreases in body weight of >10% to be an adverse effect; if the decrease is >20% or is a weight loss, the dose is*

categorized as a serious LOAEL. Categorizing the adversity of decreases in maternal body weight is irrespective of fetal effects.

QUESTION: Have the major limitations of the studies been adequately and accurately discussed? How might discussions be changed to improve or more accurately reflect the proper interpretation of the studies?

COMMENT: This is a particularly positive aspect of the profile, especially in the Appendices.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?

COMMENT: This is a particularly positive aspect of the profile, especially in the Appendices

RESPONSE: *No revisions were suggested.*

QUESTION: Have "bottom-line" statements been made regarding the relevance of the endpoint for human health?

COMMENT: Limitations identified for the epidemiology database were lack of exposure doses associated with adverse health outcomes. An additional limitation was lack of information on interactions among compounds. Limitations highlighted for the experimental animal database included toxicokinetic variability among species and relevance of PPARα-dependent effects in experimental animal models to humans. The bottom line statements in the profile related to these limitations are associated with the uncertainties that arise from both epidemiological and experimental animal study databases as well as the lack of toxicokinetic models in humans. The limitations and uncertainties associated with them have been clearly stated, defined, and managed via the use of appropriate uncertainty factors.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: The conclusions regarding limitations in the epidemiological and experimental animal model databases are mostly appropriate. The issue of PPARα relevance to humans is without a doubt a major point of uncertainty concerning a major mechanism by which these compounds are thought to work. What is missing from the profile is a small section on PPARα agonists used pharmacologically. For example, the U.S. EPA health effects document on PFOA provides a small discussion of a paper (Rigden et al., 2015) that considered PFOA along with fenofibrate, a fibrate drug that is used for hyperlipidemia. Humans are responsive to these drugs, but serum cholesterol increases following PFAS exposure. Rodent responses to PFASs are as we would expect from PPARα agonists, so it's puzzling that humans do not respond similarly. Therefore, PPARα agonism may be relevant to humans, but likely there are additional mechanisms in humans that supersede effects from PPAR agonism. Therefore, that a PPARα mechanism is not *relevant* to humans is not really correct, but that rodents appear to have a

stronger PPARα agonist response than do humans is "more" correct. It therefore is suggested that this conclusion be adjusted somewhat.

- Rigden M, et al. 2015. Assessment of urinary metabolite excretion after rat acute exposure to perfluorooctanoic acid and other peroxisomal proliferators. Archives of Environmental Contamination and Toxicology. 68:148-158.

RESPONSE: *A note was added to Section 2.9, Hepatic Effects, regarding the hypolipidemic effects observed in humans experimentally exposed to PFOA (MacPherson et al. 2011) or to other PPARα agonists, such as fibrates. The Rigden et al. (2015) study was not added because the study did not produce the lipidemic effects that have been observed in most studies involving exposure to perfluoroalkyls in rodents.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT: The discussion on dose-response issues is adequate.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the animal data been used to draw support for any known human effects? If so, critique the validity of the support.

COMMENT: The animal data have been used appropriately to draw support for known human effects.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: The sections on mechanisms of action are very well done. As mentioned previously, the discussion of PPARα agonism in humans could be expanded with inclusion of a study of fenofibrate (Rigden M, et al. 2015. Assessment of urinary metabolite excretion after rat acute exposure to perfluorooctanoic acid and other peroxisomal proliferators. Archives of Environmental Contamination and Toxicology. 68:148-158.). See fourth bullet point in previous section.

RESPONSE: *A note regarding human exposure to fibrates was added to the discussion of hepatic effects. The Rigden et al. (2015) study was not added since it did not observe the characteristic liver lipid effects.*

QUESTION: Are the hazard identifications clear and justifiable based on ATSDR's SR [systematic review] process? (In other words, if you follow ATSDR's SR protocol from start to finish, would you come to the same hazard identification conclusions?) If not, discuss where in the process there was a deviation from the protocol.

COMMENT: The hazard identification/systematic review information was clear and justifiable and would allow for the same hazard identification conclusions if followed by another entity.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the selection of endpoints that was carried forward through the SR process? If not, please indicate which endpoints you think should or should not have been included and why.

COMMENT: The endpoints that were carried forward and the process used to evaluate them appears to be scientifically justified and the process was transparent.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the SR framework as presented in Appendix B? Are there any steps that need to be revised? Please offer any suggestions to improve the utility, effectiveness, or clarity of the SR Framework.

COMMENT: Relying only on "laboratory mammals" may ignore studies done in other vertebrate species that are translationally relevant to humans for a variety of reasons. Five studies done in non-mammalian species were identified in a previous section of this review (three with a chicken model and two with a trout model) and these studies do have translational relevance to humans for the health effects that they examined. Further, having no specific search term for "in vitro" or "ex vivo" leaves out studies that are potentially relevant for understanding mechanisms of action (two of which were identified in a previous section). Some of the in vitro and ex vivo studies are likely to get picked up under some of the other search terms, but studies of relevant health effects in non-mammalian species would not. It therefore is recommended that a section on non-mammalian species be included, at least to locate papers that could be screened for potential relevance to human health.

RESPONSE: *In general, the literature search strategy used for toxicological profiles is designed to identify studies that are relevant to the profile. Relevant* in vitro *and* ex vivo *studies are typically identified using this search strategy. Non-mammalian species are not usually included in the profile due to the question of relevance to humans. In specific cases, such as for perfluoroalkyls, non-mammalian data are included. The recommended studies on chickens and rainbow trout were added to the profile.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: Not ALL PFASs fail to undergo metabolism; some precursor compounds that may enter the environment can degrade/metabolize to other PFASs. It may be important to note in section 3.1.3 that no metabolism has been reported for the PFASs included in the profile, but metabolism may be possible for other types of PFASs.

RESPONSE: *The suggested revision was made.*

QUESTION: Have the major organs, tissues, etc. in which the substance is stored been identified? If not, suggest ways to improve the text.

COMMENT: The text appears to be sufficiently extensive.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all applicable metabolic parameters been presented? Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT: If it is assumed that all of the PFASs included in the profile fail to undergo metabolic alteration, then the first question is not applicable. Otherwise, the sections describing the various PBPK models is among the most extensive currently available. Again, commendations to the authors of the profile to distilling a highly complex topic into fairly understandable summaries.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? What other observations should be made?

COMMENT: Section 3.1.6, which addresses the animal-to-human extrapolations for toxicokinetics, only addresses PFOA and PFOS, likely as PBPK models have only been developed for PFOA and PFOS. It may be helpful to include a sentence about whether these models would or could be appropriate – or not – for other PFASs.

RESPONSE: *As PBPK models are not discussed in Section 3.1.6, ATSDR assumes that the reader is referring to Section 3.1.5. A statement was added that PBPK models were not identified for other perfluoroalkyls and that given identified toxicokinetic differences between perfluoroalkyl compounds, the PFOA and PFOS models may not be appropriate for other compounds.*

QUESTION: Is there an adequate discussion of the relevance of animal toxicokinetic information for humans? If not, please explain.

COMMENT: In section 3.1.6, it is noted that "Using an internal dose metric such as serum perfluoroalkyl concentration and PBPK models that can account for these toxicokinetic differences can decrease the uncertainty in extrapolating from animals to humans." It would help in explaining what specific uncertainties could be decreased – just those associated with serum concentrations?

RESPONSE: *The text was revised to indicate that PBPK models could account for the species differences in elimination rates.*

QUESTION: If applicable, is there a discussion of the toxicokinetics of different forms of the substance (e.g., inorganic vs. organic mercury)?

COMMENT: No/not applicable as there do not appear to be PBPK models for PFASs beyond those developed for PFOA and PFOS.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?

COMMENT: As pregnancy-induced hypertension/pre-eclampsia is one of the health links associated with PFAS exposure, it may be helpful to include mention of this link and its potential to impact development in section 3.2. No discussion of developmental effects on the immune system or kidneys were included in this section. Evidence for immune effects (i.e., suppression of vaccine responses and increases in asthma) and renal dysfunction do exist in children and these studies are already included in the profile.

RESPONSE: *Adverse renal outcomes was added to the list of health effects that have been examined in children. A discussion of immune effects in children (altered antibody response to vaccines and asthma risk) was added to Section 3.2. A statement regarding hypertension/pre-eclampsia was also added.*

QUESTION: Are there any general issues relevant to child health that have not been discussed in the profile and should be?

COMMENT: The profile appears to be complete, but the section on children and other populations should contain a broader summary, even if information has been presented elsewhere.

RESPONSE: *See response to previous comment.*

QUESTION: If you answer yes to either of the above questions, please provide any relevant references.

COMMENT: The relevant references already are included in the profile, but a recent review published in June of 2017 summarizes the developmental effects of PFASs and may be helpful to include in the profile.

- Rappazzo KM et al. 2017. Exposure to perfluorinated alkyl substances and health outcomes in children: A systematic review of the epidemiological literature. International Journal of Environmental Research and Public Health. 14:691.

RESPONSE: *The Rappazzo et al. (2017) paper discussing associations between perfluoroalkyl exposure and childhood health outcomes was not added to the profile because it is a review of health effects data. The studies cited in this paper are discussed in the profile.*

QUESTION: Is there a discussion of populations at higher risk because of biological differences that make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?

COMMENT: Given the abundance of data on the ability of PFASs to impact the immune system, are the authors aware of any studies to suggest that populations with certain immune diseases or conditions may be at higher risk of increased susceptibility? Overall, the paragraph of susceptible subpopulations seems cursory. Is this because of lack of data? If so, perhaps the authors should indicate the identification of especially susceptible subpopulations is limited by the database.

RESPONSE: *ATSDR did not identify studies examining adult populations with increased susceptibility of perfluoroalkyl toxicity; a statement to this effect was added to the profile. The text was also revised to*

include a statement that immunocompromised individuals may be at an increased risk of perfluoroalkyl-induced immunotoxicity.

QUESTION: Are the biomarkers of exposure specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

COMMENT: Serum concentrations for specific PFASs seems like a pretty specific biomarker of exposure.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there valid tests to measure the biomarker of exposure? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.

COMMENT: While not addressed in this section of the document (but addressed in the section on data needs for analytical methods), there are woefully few academic and government laboratories and likely even fewer for-profit/clinical laboratories that are appropriately trained in the methodology necessary for measuring PFASs in biological fluids. Would it be possible to include a statement about existing standard methods for evaluating PFAS in biological fluids, if any have been developed? Or perhaps reference the following paper:

- Reiner JL et al. 2009. Analysis of PFOA in dosed CD1 mice part 1: Methods development for the analysis of tissues and fluids from pregnant and lactating mice and their pups. Reproductive Toxicology. 27:365-372.

RESPONSE: *Analytical methods are not discussed in the profile, with the exception of a presentation of limits of detection for environmental and biological media (Section 5.5) and discussion of data needs (Section 6.2). The Reiner et al. (2009) study was added to the data needs discussion.*

QUESTION: Are the biomarkers of effect specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

COMMENT: There appear to be no biomarkers of effect specific for PFASs.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there valid tests to measure the biomarker of effect? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.

COMMENT: Not applicable.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? If not, please clarify and add additional references.

COMMENT: At least one study has evaluated the interactions of PFOS with TCDD, although the title, abstract, and introduction make it difficult to discern that this is indeed a focus of the paper. A paper on the ability of PFOS to act as a chemosensitizer in zebrafish embryos also has been published. These papers are listed below:

- Hu Wy et al. 2003. Alterations in cell membrane properties caused by perfluorinated compounds. Comparative Biochemistry and Physiology Part C. 135:77-88.
- Keiter S et al. 2016. Does perfluorooctane sulfonate (PFOS) act as chemosensitizer in zebrafish embryos? Science of the Total Environment. 548-549:317-324.

RESPONSE: *The Hu et al. (2003) study was not added to the profile because the study examined the interaction between PFOS and TCDD on TCDD-induced EROD activity. Similarly, the Keiter et al. (2016) study examined the underlying mechanisms of how PFOS increases the toxicity of other compounds. The discussion in Section 3.4, Interactions with Other Chemicals, is limited to compounds that influence the toxicity or toxicokinetics of perfluoroalkyls.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? If not, please clarify and provide any appropriate references.

COMMENT: Not applicable as currently written. Please see previous point.

RESPONSE: *As noted in the response to previous response, the studies identified by the Reviewer did not examine the influence of other chemicals on the toxicity of perfluoroalkyls and were not added to the profile.*

Chapter 4. Chemical and Physical Information

QUESTION: Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables? Please provide appropriate references for your additions or changes.

COMMENT: This section appears to be correct and complete.

RESPONSE: *No revisions were suggested.*

QUESTION: Is information provided on the various forms of the substance? If not, please explain.

COMMENT: Available information on each of the PFASs included in the profile appears to be complete.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and

technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: This section appears to be complete.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: This section appears to be complete.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: This section appears to be complete.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: Although described in a later section of Chapter 5, firefighters are not mentioned in the overview section, 5.1. It is recommended that firefighting is mentioned in the paragraph of highly exposed populations on Page 525 Lines 6-18.

RESPONSE: *The suggested revision was made.*

Chapter 6. Adequacy of the Database

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note where the text shows bias.

COMMENT: The data needs assessment is neutral and non-judgmental.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the identified data needs? If not, please explain your response and support your conclusions with appropriate references.

COMMENT: Note that on Page 613 Lines 33-34, the text notes that animal studies have not evaluated immune effects following perinatal exposure. At least one study has evaluated developmental toxicity following PFOA exposure (Hu et al., 2010) and one has evaluated developmental toxicity following PFOS exposure (Keil DE et al., 2008); both are already in the profile.

RESPONSE: *The text was revised to indicate that data are available for PFOA and PFOS but are lacking for other perfluoroalkyls.*

QUESTION: Does the text indicate whether any information on the data need(s) exist(s)?

COMMENT: Section 6.3 indicates ongoing studies identified in NIH reporter (2017). Several other studies are ongoing according to NIH reporter:

- Braun J, Brown University, Early life perfluoroalkyl substance exposure and obesity: Mechanisms and phenotyping. NIEHS.
- Kissling GE, NIEHS, Identifying and evaluating sources of variability in rodent studies, NIEHS.

RESPONSE: *The Braun and Kissling studies were added to Table 6-1.*

QUESTION: Does the text adequately justify why further development of the data need(s) would be desirable; or, conversely, justify the "inappropriateness" of developing the data need(s) at present? If not, how can this justification be improved.

COMMENT: The justifications are more than adequate

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of other regulations or guidelines that may be appropriate for the table? If so, please provide a copy of the reference.

COMMENT: Other than regulations or guidelines for individual states within the U.S., this table appears to be complete.

RESPONSE: *ATSDR does not typically include state regulations or guidelines in Table 7-1.*

Chapter 8. References

QUESTION: Are there additional references that provide new data or are there better studies than those already in the text? If so, please provide a copy of each additional reference.

COMMENT: Additional references have been recommended in previous comments.

RESPONSE: *See responses to specific comments regarding recommended studies.*

Specific Comments

COMMENT: Figures 2-1 through 2-3 are a great way to illustrate the total number of human and animal studies for PFOA, PFOS, and other PFASs. However, a few aspect of each figure are confusing, including:

- Each figure contains an asterisk indicating "Includes studies discussed in Chapter 2." Is this for the overall figure or does the asterisk refer to specific types of studies?
- Each figure contains an asterisk indicating a total number of studies, but when the total number of studies is added for each figure, it exceeds the value indicated in the asterisk. For example, in Figure 2-1, the sum of studies in the bars = 475, but the asterisk indicates a total of 271 studies. The asterisk also indicates that most animal studies examined multiple endpoints, which would mean that the values in the bars doesn't reflect the number of *independent* studies, which is somewhat confusing. Perhaps the note can be clarified that numbers do not reflect independent studies, the total represents the number of independent studies, and the bars represent studies evaluating that endpoint.
- Each title contains a header indicating that more studies evaluated health effects in humans than animals, but the sum of values for human studies (light blue) is less than the sum of values for animal studies (dark blue). This may be a problem associated with the previous comment.
- Similar issues are noted in Figures 6-1 through 6-3, where it appears as if the number of animal studies (dark blue) exceeds the number of human studies (light blue), but the text indicates that more human than animal studies for PFASs exist.

RESPONSE: *The number of studies in the footnote refers to all studies discussed in Chapter 2; the figures were revised to clarify this point. As noted in the footnote, many studies, particularly animal studies, examined multiple endpoints; thus, totaling the number of studies in the bars would exceed the total number of studies. Additionally, a statement is made in the table header that the counts represent studies examining the endpoint.*

COMMENT (page 1, line 38): Change perfluoroalkyls substances to perfluoroalkyl substances.

RESPONSE: *This change was made throughout the profile.*

COMMENT (page 2, line 33): "eastern Ohio" should really be "southeastern Ohio" as the C8 science panel study of this area encompassed only the southeastern portion of Ohio abutting the Ohio River near Parkersburg, WV.

RESPONSE: *The suggested revision was made.*

COMMENT (page 15, line 5): Missing "in" between "PFOA" and "wild-type."

RESPONSE: *The suggested revision was made.*

COMMENT (page 112, line 3): Change "animal" to "animals."

RESPONSE: *The suggested revision was made.*

COMMENT (page 33, Figure 2-5): Change "PFA" in title to "perfluoroalkyls."

RESPONSE: *The suggested revision was made.*

COMMENT (page 135, lines 4-5): The phrase "in a capsule" seems out of place and makes it seem like the monkeys were dosed inside of some sort of capsule. Maybe re-write to "Cynomologus monkeys were dosed with 0, 0.03, 0.15, or 0.75 mg/kg/day of PFOS (potassium salt) formulated as a capsule fed to the monkeys for 26 weeks; comprehensive hematological tests during the study revealed a significant effect of a 9% decrease in hemoglobin in 0.75 mg/kg/day males at termination (Seacat et al. 2002)."

RESPONSE: *The text was revised to indicate that it was administered via a capsule.*

COMMENT (page 141, line 24): Same issue as previous comment. "…in a capsule" placement makes it seem as if monkeys were exposed while they were inside of a capsule.

RESPONSE: *See previous response.*

COMMENT (page 165, line 29): In discussing the Hall et al. (2012) report on hepatomegaly, should this sentence end "…are not considered adverse OR relevant for human risk assessment" rather than "…are not considered adverse AND relevant for risk assessment?"

RESPONSE: *The suggested revision was made.*

COMMENT (page 180, line 31): Wording for feeding PFOA to monkeys via capsules is appropriate here!

RESPONSE: *No revisions were suggested.*

COMMENT (pages 189-197): Page numbers missing from header.

RESPONSE: *This has been corrected.*

COMMENT (page 206, line 34): Placement of "in a capsule" subject confusion again (will not make any further notations of this editorial suggestion).

RESPONSE: *This has been revised throughout the profile.*

COMMENT (page 262, line 19): Recommend changing "…the number of thymic and splenic cells" to "…the number of thymic and splenic lymphocytes."

RESPONSE: *The suggested revision was made.*

COMMENT (page 269, line 6): Recommend changing "splenocytes and thymocytes" to "splenic and thymic lymphocytes." Recommend making this change elsewhere in the immune section.

RESPONSE: *The suggested revision was made.*

COMMENT (page 506, line 17): The phrase "unchanged PFOA and PFOS" is used in a section to discuss biomarkers of exposure in children. PFOA and PFOS are not metabolized, so the word "unchanged" is unnecessary and potentially confusing as it implies that "changed" PFOA and PFOS are measurable.

RESPONSE: *The word "unchanged" was deleted.*

COMMENT (page 611, line 8): Insert "a" between "and" and "chronic-duration."

RESPONSE: *The suggested revision was made.*

COMMENT (page 612, line 23): Remove the word "were."

RESPONSE: *The suggested revision was made.*

COMMENT (page 612, line 25): Insert "of" between "number" and "chronic."

RESPONSE: *The suggested revision was made.*

COMMENT (page 614, lines 25-26): Remove the second "is" from the sentence "…effects is also is needed."

RESPONSE: *The suggested revision was made.*

COMMENT (page 614, line 30): Insert "be" between "would" and "useful."

RESPONSE: *The suggested revision was made.*