EPA Response to External Peer Review Comments on
EPA Draft Documents:

Health Effects Support Document for Perfluorooctanoic Acid (PFOA)

and

Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)

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DISCLAIMER

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Contents

1. Introduction

In 2014, Versar, Inc., under contract to the U.S. Environmental Protection Agency (EPA), Office of Water,[1] conducted an independent, scientific peer review of EPA's draft documents, *Health Effects Document for Perfluorooctanoic Acid* (PFOA) (USEPA 2014a) and *Health Effects Document for Perfluorooctane Sulfonate* (PFOS) (USEPA 2014b). The draft documents and charge questions were prepared by EPA to ultimately develop drinking water health advisories for the chemicals PFOA and PFOS.[2] The goal of the peer review was to ensure that EPA's interpretations of toxicological studies and their conclusions were reasonable, sound, and consistent with the underlying science, and that, as a whole, the documents were clear and scientifically credible. This report describes the external peer review process and provides the peer reviewers' final comments and recommendations (verbatim) and EPA's responses.

External Peer Review Process

EPA followed the process recommended in its 2013 guidance, Conflict of Interest (COI) Review Process for Contractor-Managed Peer Review[3], for the draft health effects documents for PFOA and PFOS. On February 28, 2014 EPA published a Federal Register notice[4] calling for public nominations of experts to serve on the panel peer review. The August 2014 draft health effects documents were made public and interested parties were able to submit comments on the draft documents. The contractor (Versar) developed a preliminary list of peer reviewers based on the public nominations and application of traditional peer reviewer identification techniques (e.g., literature searches). On April 30, 2014 EPA published a second notice[5] to announce and request public comment on a preliminary list of peer reviewers. Following closure of the comment period, Versar identified a proposed final peer review panel and consulted with EPA Science Advisor designees on June 20, 2014. On July 10, 2014 EPA published a third notice[6] announcing the final peer reviewers, meeting logistics, and registration instructions. Public comments received during the comment period were provided to the peer reviewers for their consideration during their review of the health effects documents prior to their panel meeting on August 21-22, 2014.

The purpose of the peer review was to provide a documented, independent, and critical review of the draft health effects documents, and identify any necessary improvements to the documents prior to being finalized and published. In assembling these peer reviewers and coordinating the peer review, Versar was charged with evaluating the qualifications of peer review candidates, conducting a thorough COI screening process, independently selecting the peer reviewers, consulting with EPA Science Advisor designees on the proposed final panel, distributing review materials, maintaining

[1] Task Order No. 4-10, Contract No. EP-C-07-059, with a period of performance from September 2011 through September 2012.

[2] These chemicals are listed on EPA's third Contaminant Candidate List (CCL) (2009) and on the draft fourth CCL (2015) and are currently being monitored under the third Unregulated Contaminant Monitoring Rule (UCMR3). Results from UCMR3 can be examined as they become available at the following website: http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/.

[3] http://www.epa.gov/osa/conflicts-interest-review-process-contractor-managed-peer-reviews-epa-highly-influential

[4] http://www.gpo.gov/fdsys/pkg/FR-2014-02-28/pdf/2014-04455.pdf

[5] http://www.gpo.gov/fdsys/pkg/FR-2014-04-30/pdf/2014-09888.pdf

[6] http://www.gpo.gov/fdsys/pkg/FR-2014-07-10/pdf/2014-16176.pdf

contact with the peer reviewers, organizing and hosting the public peer review meeting, and developing a final peer review report.

EPA reviewed the qualifications of the candidates proposed by Versar and verified that the range of the candidates' qualifications met the technical selection criteria. Versar then contracted with the following reviewers to perform the review:

- James V. Bruckner, Ph.D.; University of Georgia, Athens, Georgia
- Deborah A. Cory-Slechta, Ph.D.; University of Rochester School of Medicine and Dentistry, Rochester, New York
- Jamie C. DeWitt, Ph.D.; East Carolina University, Greenville, North Carolina
- Jeffrey W. Fisher, Ph.D.; U.S. Food and Drug Administration, Jefferson, Alaska
- William L. Hayton, Ph.D.; The Ohio State University, Columbus, Ohio
- Matthew P. Longnecker, Sc.D, M.D.; National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina
- Angela L. Slitt, Ph.D.; University of Rhode Island, Kingston, Rhode Island

Versar distributed EPA's draft PFOA and PFOS documents and 12 charge questions (see below) to the peer reviewers, who were asked to evaluate the scientific and technical merit of the draft documents and provide responses to the 12 charge questions. This effort included evaluating the appropriateness of the quality, accuracy, and relevance of the data in the documents. Peer reviewers were not charged with making any regulatory recommendations or reaching consensus in either their written comments or public deliberations. In addition to being provided the draft documents and charge questions, comments submitted to EPA's public docket during the 60-day public comment period and a summary of those comments developed by Versar were provided to the peer reviewers ahead of the meeting for their consideration.

Technical Charge to External Peer Reviewers

The peer reviewers were asked to evaluate the scientific and technical merit of the draft documents and provide their responses to the following charge questions.

1. Please comment on the strengths, weaknesses, and characterization of the studies selected as key for quantification.
2. Please provide citations (and, where possible, pdfs or hard copies) for any references you suggest EPA consider adding to the document. Describe where you suggest these references be incorporated.
3. The OW concluded that the human epidemiology data for PFOS/PFOA do not provide adequate quantifiable dose-response information for use as the basis of a candidate RfD because of uncertainty regarding the routes, levels and timing of exposures plus the confounding influences of other PFCs present in serum. Please comment of the OW characterization of the data.
4. Please comment on the transparency and characterization of the epidemiological data.
5. The OW has concluded that the cancer classifications for PFOA and PFOS are most consistent with respective classifications of suggestive evidence for carcinogenicity as described the EPA Guidelines for Carcinogen Risk Assessment (pp. 2-56, 2-57). Please comment on the strengths and weaknesses of this classification.

6. Significant interspecies differences in pharmacokinetics exist for both PFOA and PFOS. Adjusting for interspecies differences was an important step in developing candidate RfDs given the totality of the human and animal data. Please comment on the strengths and weaknesses of the pharmacokinetic model adjustments to accommodate the impact of albumin binding and renal tubule transporters in determining average serum values.
7. Table 5-5 in the PFOA document and Table 5-7 in PFOS document list the parameters used for the ORD pharmacokinetic models that provide the final serum and AUC values for calculating the internal dose point of departure for the RfD calculation. Please comment on the strengths and weaknesses of the selected parameters.
8. The volume of distribution (Vd) and half-life values are critical in the derivation of the interspecies uncertainty factor applied in derivation of candidate RfDs from a NOAEL, LOAEL or a BMDL. The available data for both values are provided in Section 3.5.2 and 3.5.3 of both documents. Please comment the strengths and weaknesses of the values selected.
9. A variety of endpoints and studies were used to compare points of departure and the resultant RfDs for both PFOA and PFOS. In addition, comparisons were provided across RfD outcomes based on the model outputs compared to those for the NOAEL, LOAEL and BMDL points of departure. The range of candidate RfDs derived from the different points of departure is fairly narrow. Please comment on the strengths, weaknesses and transparency of this analysis.
10. The RfDs for PFOS and PFOA are derived from the modeled steady state serum concentrations and their association with effects that include short term and longer term exposures with associated diverse effects. The studies considered included effects due to exposure durations that ranged from 11 to 182 days, and occur at comparable human equivalent dose (HED) levels. The current, draft RfDs do not include an uncertainty factor for study duration because of the apparent concordance HEDs despite duration differences. Given this pattern of response, is it appropriate to conclude that the candidate RfDs are applicable to both short-term and lifetime exposures?
11. In addition to using the average serum values from animal studies to calculate internal doses for humans, the animal to human extrapolation can be accomplished by dividing animal average serum values by the human to animal clearance ratios to project a human average serum point of departure in units of mg/L serum. Please provide recommendations for applying uncertainty factors to the extrapolated average human serum values to determine serum-based thresholds that are protective for humans. A NOAEL expressed in average human serum units would be useful in interpreting NHANES population monitoring data.
12. Please describe any suggestions you have for improving the clarity, organization, and/or transparency of the draft documents.

2. EPA Responses to Peer Reviewer General Impressions

Bruckner Comments

PFOA-specific Comments

COMMENT 1: This is one of the most comprehensive Health Effects Documents I have reviewed. The clarity and accuracy of accounts of pertinent research reports/publications are excellent. It is obvious considerable time and efforts were devoted to its composition. If anything, the amount of detail is so great that it is difficult to distill the mass of information on each topic and capture its "essence". This is likely the result of directions the authors were given for writing the document. Some topics in the Hazard Identification section do have summarizing sentences, in which the key/critical studies and their finding(s) are integrated and conclusions reached. It would be very helpful to devote much more attention to this for more topics, perhaps as an addition to Section 4.4 Hazard Characterization.

RESPONSE: In response to this and other similar comments, EPA rewrote major sections of both health effects support documents (HESDs) (in 2014 referred to as the draft health effects documents) to enhance readability, clarity, and transparency (USEPA 2016a, 2016b). As requested by the peer reviewers, a summary section was added to the toxicokinetic section (section 2.6.4 for PFOA and 2.6 for PFOS) and at the end of the epidemiology sections for both the noncancer (sections 3.1.1.12 for PFOA and 3.1.1.8 for PFOS) and cancer endpoints (section 3.1.2.1 for both PFOA and PFOS). Introductions were added at the beginning of the animal toxicity data portion of the report (section 3.2) to inform the reader regarding the material included. An independent summary of the animal toxicology was not prepared to avoid making the subsequent synthesis (section 3.4) of hazard repetitive.

COMMENT 2: I do have a real problem with the scientific basis and soundness of certain conclusions in the document. The primary effect of PFOA in different species is increased absolute and/or relative liver weight. These are quite modest, reversible, non-specific effects that usually are not considered toxicologically significant. Livers of mice and rats dosed with PFOA typically exhibited hypertrophy characterized by increased peroxisomes, numerous mitochondria, reduced rough endoplasmic reticulum (RER), proliferation of smooth endoplasmic reticulum (SER), and increased autophagosomes or lipid-like droplets. Such morphological changes, particularly those in RER and SER, are manifestations of microsomal enzyme induction. This is considered adaptive, rather than adverse. Hall et al. (2012) points out that activation of a battery of genes involved in xenobiotic metabolism and transport serve to maintain homeostasis by enhancing the systemic elimination of the foreign chemical. Although PFOA is very poorly metabolized, it does persistently induce microsomal enzymes and the accompanying hepatocellular morphological changes. Upregulation of genes responsible for biliary excretion may be beneficial, since excretion of bilirubin, bile acids and conjugates of toxic chemicals/metabolites would be enhanced.

There are substantial qualitative and quantitative differences in responses of rodents and humans to PPARα activation. Therefore, many of the PFOA-induced alterations in lipid metabolism/homeostasis and associated biological processes in mice will be absent or an order of magnitude less pronounced at comparable doses in humans. Many of PFOA's effects on the liver of rodents are dependent on PPARα activation, though some effects appear to be PPARα-independent. Studies in PPARα-knockout mice show activation of other nuclear receptors by PFOA, including PXR, CAR, LXRA and FXR. Bjork et al. (2011) observed markedly lower transcriptional responses of PPARα, PXR, CAR and FXR to PFOA in cultured human than in cultured rat hepatocytes. These more subtle effects lead the investigators to conclude the changes in human cells reflected an adaptive metabolic remodeling rather than overt metabolic dysregulation, or disorder occurring in rat cells. The PFOA document's authors should go into detail discussing and summarizing the relative toxicological significance of non-PPARα effects in rodents versus humans.

RESPONSE: Increased liver weight is acknowledged as a common finding following exposure to PFOA and PFOS in the final HESDs documents (USEPA 2016a, 2016b). Liver weight was not considered as adverse in the absence of other effects as defined by Hall et al. (2012). See also EPA response to Charge Question 1.

The NOAELs and LOAELs that were originally based on increased liver weight were revised so that the LOAELs based on liver effects in the final HESDs reflect a liver endpoint that meets the Hall et al. (2012) criteria for adversity. The revised assessment provides detailed discussions of peroxisome proliferator-activated receptor (PPAR) receptors, the impact of their activation, and associated cellular responses in the final HESDs. Information on other activated receptors such as constitutive androstane receptor (CAR), pregnane X receptor (PXR), and farnesoid receptor (FXR) were also included where appropriate.

COMMENT 3: It is important to recognize that clearly adverse effects of PFOA are seen. Loveless et al. (2008), Cui et al. (2009) and others have seen focal necrosis and degenerative changes in the liver of mice and rats given relatively high doses of PFOA, as well as modest elevations in serum (hepatic) enzyme activities. Wolf et al. (2008a) observed a variety of degenerative structural changes in the liver of PPARα-null mice dosed with PFOA. Sakr et al. (2007a,b) and Olsen and Zobel (2007) reported associations between serum PFOA levels and slightly elevated serum enzyme activities in some occupationally-exposed populations. The increases in enzymes may have been attributable to factors other than PFOA. In light of the foregoing, it would be preferable to utilize hepatic morphological changes in rodents and/or elevated serum enzymes as the critical effect(s), rather than increased liver weight. These are clearly adverse effects seen in both rodents and humans.

An international panel of experts (Hall et al., 2012) opined that an increase in liver weight of ≤ 150%, at doses of chemical that do not produce structural or biochemical evidence of hepatocellular damage, would not be considered adverse. Absolute and relative liver weights were not increased as much as 50% by most PFOA doses in the majority rodent and monkey studies. Perkins et al. (2004), for example, reported dose-dependent increases in liver/body weight in rats fed 1, 10, 30 and 100 ppm PFOA for 13 weeks of 0, 10, 30, and 41%, respectively. Butenhoff et al. (2002) measured increases of 17, 21 and 37.5% and relative liver weight in monkeys given 3, 10 or 30/20 mg PFOA/kg/day for 26 weeks, respectively. Liver hypertrophy of this magnitude does not warrant such a low RfD. By adhering to EPA policies of calculating a $BMDL_{10}$ and using multiple UFs, regardless of the (lack of) severity of the critical effect and relatively low level of concern about other potential health effects, the end result is a vanishingly low RfD (i.e. 0.00002 mg/kg/day). A great deal of time and effort were spent on the PFOA hazard assessment, toxicokinetic modeling and extrapolations, dose metric and POD considerations, etc. Despite all of these scientifically-credible exercises and deliberations, the end result (RfD) seems to this reviewer to have been preordained--to be extremely low.

RESPONSE: NOAELs and LOAELs that were originally based on increased liver weight were revised so that the effects noted at the LOAEL are endpoints that met the Hall et al. (2012) criteria for adversity. In the summary of the studies that provide dose response (Tables 4-1 and 4-2 in the final HESDs), liver weight, hypertrophy, and similar effects are acknowledged when part of the spectrum of effects at the LOAEL dose, if they accompany the effect identified as adverse. Because the numbers of animals in some of the studies were low, hepatic necrosis is noted as an effect when it exceeded the incidence in the controls and showed a relationship to dose.

COMMENT 4: Logic expressed on page 5-6, in support of use of liver weight gain as a critical effect and biomarker of loss of hepatocellular homeostasis seems flawed. As pointed out in the second paragraph, liver weight changes were not observed in PFOA-treated mice with a humanized PPARα receptor. It is noted that changes in gene products that modulate lipid metabolism do occur in these mice. EPA argues that this supports adoption of increased liver weight as a biomarker/critical effect. It has not been established that these changes in gene expression are adverse, or whether they are sufficient in magnitude to significantly alter lipid metabolism. It would be expected that repeated dosing with enough of a molecule (i.e., PFOA) that

resembles a fatty acid would affect expression of such genes. Reversible changes in total cholesterol, bile acids, bilirubin, etc. have been observed. It has not been established, however, whether mild fluctuations in these indices are detrimental. No increases in mortality from cerebrovascular disease or ischemic heart disease have been found in PFOA-exposed humans. How then does the concurrence of alteration of expression of such genes and of liver weight gain support the latter as toxicologically-significant effect that should be prevented by setting the RfD low enough?

RESPONSE: Based on feedback received from the peer review panel, the critical endpoint selected to serve as the POD for the reference dose (RfD) for PFOA (and PFOS) is no longer increased liver weight. The critical study and endpoint for the derivation of the RfD for PFOA are based on reduced ossification of the proximal phalanges and accelerated puberty in males observed in the Lau et al. (2006) study. For PFOA, the candidate RfDs developed for consideration were based on multiple adverse effects resulting from short-term and long-term exposures and fell within a narrow dose range. Increased liver weight is acknowledged as a common finding following exposure to PFOA (and PFOS) in the revised documents. However, it is not considered as adverse in the absence of other effects as defined by Hall et al. (2012). The NOAELs and LOAELs that were originally based on increased liver weight were revised so that the LOAELs based on liver effects in the final HESDs reflect a liver endpoint that meets the Hall et al. (2012) criteria for adversity.

In a human health context, the associations observed between exposure to PFOA and cholesterol and serum lipids observed in the epidemiology studies are well accepted as risk factors for cardiovascular disease. It is important to note that several of the animal studies published after the completion of the peer review drafts and included in the final HESDs for PFOA and PFOS (USEPA 2016a, 2016b) show that dietary fat is an important variable influencing the presence of fat accumulation in the liver and insulin resistance. Diet is a variable that was not considered in many of the epidemiology studies. The available information from these additional animal studies, taken together with the observed effects on cholesterol in the epidemiology studies, provide support for the identification of hazard for these effects.

PFOS-specific Comments

COMMENT 5: This Health Effects Document, like that for PFOA, is quite comprehensive. Its descriptions of the many studies of PFOS are clear, quite complete, and apparently quite accurate. As with the PFOA document, so much detail is given about many studies in the Hazard Identification section, that is difficult to compare study designs/dosage regimens/species/indices/findings/etc. and to draw conclusions. The summary tables for single and multiple studies, however, are quite helpful in this regard. It would also be very useful to have more summary statements or paragraphs at the end of each topic. These should address the scientific importance of findings, their relevance to humans; and their impact on the weight of evidence on an issue.

RESPONSE: See the response to Bruckner General Impression Comment 1 above. The correlation between the epidemiology and the animal toxicology results are integrated in the hazard synthesis to reduce redundancy between an independent summary of animal toxicology and the subsequent synthesis of hazard.

COMMENT 6: The hazard characterization section (4.4) is, for the most part, inclusive and balanced in its presentation and integration of findings of the more important studies in each subject area. This is true for both non-cancer and cancer effects in humans and animals. It concerns me, however, that the document's authors do not focus in the remainder of the document on science (i.e., the candidate critical effects and their relevance to human health), but merely choose the most sensitive end-points and stress how similar the RfDs are after dosimetry modeling estimates and adjustments. I am not sure how this similarity of derived points of departure and other values, calculated from dissimilar endpoints, supports or validates the final RfD.

RESPONSE: The peer reviewed version of the HESD was largely focused on comparing the outcomes from use of NOAEL/LOAEL, lower 95th percentile confidence bound benchmark doses (BMDLs), and the HEDs derived from the average serum levels projected by the EPA toxicokinetic model. That exercise demonstrated

that the modeled results were comparable to the outcomes from using the more conventional approaches (i.e., NOAEL/LOAEL and BMD modeling). Based on the feedback received from the peer reviewer panel, the revised HESD presents the results from the toxicokinetic model in developing candidate RfD values. Accordingly, there is now considerably more text that compares the modeled outcomes to the effect doses seen across the spectrum of studies that provide information on dose and response, but lack serum information for modeling. For PFOA, multiple studies were modeled to derive average serum values and from these results candidate RfDs were quantified. The RfD selected is based on developmental effects (reduced ossification and accelerated puberty in males) resulting from gestational and lactational exposures.

The selected RfD for PFOA is supported by the longer-term RfD for effects on the response of the immune system (DeWitt et al. 2008) to external challenges as observed following the short-term exposures to mature rats and the effects on kidney weight observed at the time of sacrifice in the F1 males from the Butenhoff et al. (2004a) study. Support for the selected RfD is also provided by other key studies with NOAELs and LOAELs similar to those used for quantification, but lacking serum data that could be used for modeling. There were effects on liver weight and hepatic hypertrophy in the Perkins et al. (2004) and DeWitt et al. (2008) studies that were modeled but not considered in the derivation of the RfD because of a lack of data to demonstrate adversity, as determined by the Hall et al. (2012) criteria.

The RfD for PFOS is supported by the 0.00002 mg/kg/day value derived from the LOAEL for the same effect in the one-generation Luebker et al. (2005a) study and the 0.00003 mg/kg/day value for neonatal neurodevelopmental effects in the Butenhoff et al. (2009) study. The RfD is protective of the most sensitive populations (i.e., developing fetus and nursing infant) and the general population. The rationale for selection of the developmental endpoint has been revised and support for each of the modeled endpoints from the studies with NOAELs and/or LOAELs is part of the discussion that accompanies the RfD derivation.

COMMENT 7: I recommend that an additional section be written, in which the primary adverse effects of PFOS are discussed-- in terms of their relative toxicological significance, their apparent mechanism(s), their relevance to humans, their likelihood in realistic exposure scenarios, and implications of altered experimental indices to actual organ dysfunction.

RESPONSE: In the revised HESDs, an integrated summary of the effects of PFOS on humans and animals is included. Mode of action information presented in the documents include data demonstrating involvement of receptor activation (e.g., PPARα) and gap junction communications (both involve proteins) plus oxidative damage. The implications of these mode of actions to human relevance are also discussed. The sources of exposure to PFOS for humans (diet, dusts, indoor air, etc.) are included in the Health Advisory documents (USEPA 2016c, 2016d) that accompanies the HESD.

COMMENT 8: I am quite concerned about the increased rat pup mortality in several studies at relatively low maternal doses, but not about reversible liver weight changes or centrilobular hypertrophy. Is the decreased pup survival in several studies at relatively low maternal doses of PFOS relevant to humans?-- Is the dose-response curve steep, as suggested by Luebker et al. (2005a), such that there would be less concern about sub-threshold doses? -- What is the most likely mode of action (pulmonary surfactant or maturation, dietary, hormonal)? -- Is decreased survival PPARα-related? -- Is the mechanism in rats relevant to other species? -- Does pup mortality occur in other species at comparable doses? -- Might there be a dose-dependent alteration of maternal-fetal partitioning of PFOS?

RESPONSE: Yes, decreased pup survival is an endpoint of concern for humans. In the case of PFOS, the selected RfD applies to low birth weight. Effects on increased pup mortality occurred at doses greater than the values for these body weight effects. The toxicokinetic studies show high levels of PFOS in the lung in early life (e.g., Borg et al. 2010). Pup deaths occurred in both rat and mouse studies at comparable doses (Grasty et al. 2003; Lau et al. 2003; Luebker et al. 2005a, 2005b; Yahia et al. 2008; Abbott et al. 2009). Several of these studies suggest involvement of the lung in mortality, but the data of Grasty et al. (2003) do

not fully support lung surfactant as a cause of death, and this potential mode of action is discussed in more detail in the HESD. The observed decreased survival cannot be fully explained by the role of PPARα. The study by Abbott et al. (2009) evaluated the role of PPAR in mortality, the authors reported early mortality in both wild type and knock out PPARα mice. The effects on the wild type were impacted to a greater extent than the knock out but survival was decreased for both. The lowest LOAEL for effects on survival was 0.8 mg/kg/day from the Luebker et al. 2005a one-generation study that was quantified and included among the candidate RfDs. The slope associated with the response is curvilinear, in other words the slope is low at the next two highest doses and then increases steeply at the following two doses. Additional data are needed before questions regarding mechanism and dose-response can be resolved with regard to pup/fetal mortality.

Cory-Slechta Comments

COMMENT 1: Both documents, although the PFOA document to some degree more than PFOS, overall are more of a tabulation of studies than a critical review of studies from which a rationale is presented for a choice of studies to model and from which to derive associated RfDs. The Executive summaries are too abbreviated and do not include sufficient rationale, description and detail to provide the reader with an understanding of how decisions described in Chapter 5 were made. Since in some cases, this will be the only sections read, they could provide a more informative summary.

RESPONSE: The HESDs for both PFOA and PFOS were extensively revised to present a more in-depth analysis of the human epidemiology data, integrated summary of the animal and human evidence, and rationale for selection of the critical studies for quantitative analysis and selection of the RfDs.

COMMENT 2: The Executive Summaries of both documents detail the available human and animal data and describe the basis for the RfD and studies supporting that derivation. It would be very helpful to provide a section up front that describes all of the parameters of the literature search, including the years that are included in the document review, as well as descriptions of criteria for studies that were included vs. those that were excluded. In addition, it should be indicated whether there was a criterion that studies be peer-reviewed. This is particularly important given the voluminous size of the data base that has accumulated for these two chemicals. Given that revisions will be done and that such documents do not get updated with any frequency, it would be good to attempt to include as much of the new pertinent literature as possible.

RESPONSE: The revised documents include a description of the literature search strategy and search terms used (see Appendix A). The forward of the HESD lists the criteria that were applied in deciding which of the multiple studies reviewed would be included in the final report. Although most of the studies came from peer reviewed journals, some are reports of primary research provided to the EPA Office of Pollution Protection and Toxics. Several of those were published in the peer reviewed literature. The current document includes citations to the unpublished and published reports. It was also updated to include studies recommended by peer reviewers and in public comment.

COMMENT 3: The section on Toxicokinetics in the documents present studies in detail, but no real conclusions; this is true of most of the sections in these documents. Chapters 3 and 4 in particular read like tabulations of studies rather than critical reviews and because of that, the documents seem disjointed and Section 5, i.e., derivation of values, tend to be difficult to read through and require constant searching back to the original chapters in which they are described. It is critical to identify the strengths and weaknesses of the various studies, and which were given weight to use in the final determinations. It would be helpful if Sections 3, 4 and 5 included an introductory paragraph describing the goal of the chapter, and that each ends with an overall summary with conclusions. The tables in these chapters also would benefit from the inclusion of additional information that ultimately permits comparisons within the Table and does not require continually returning to the text to recall the species, sample sizes, etc.

RESPONSE: EPA rewrote major sections of both HESDs to enhance readability, clarity, and transparency (USEPA 2016a, 2016b). As requested by the peer reviewers, a summary section was added to the toxicokinetic section (section 2.6.4 for PFOA and 2.6 for PFOS) and at the end of the epidemiology sections for both the noncancer (sections 3.1.1.12 for PFOA and 3.1.1.8 for PFOS) and cancer endpoints (section 3.1.2.1 for both PFOA and PFOS). Introductions were added at the beginning of the animal toxicity data portion of the reports (section 3.2) to inform the reader regarding the material included.

A major difference between the peer reviewed and final drafts is the reliance on the modeled, average-serum data for quantification, an approach that was supported by the peer reviewers and an expansion of the discussion of both the strengths and weaknesses, as well as the similarities and differences across the studies that provided dose-response information.

The quantification section is more compressed and more fluid because the now published toxicokinetic model is included in section 2.6.1 for PFOA and 2.5.1 for PFOS with the information on other toxicokinetic models. This facilitates a more streamlined presentation of the average serum and human equivalent doses and a better discussion of the similarities between the effects and critical doses from the modeled studies compared to the studies with dose-response but lacking in serum measurements. Species and effects information are now included in each of the model summary tables so that the reader does not have refer to earlier summary tables to retrieve that information.

COMMENT 4: In the sections on Hazard Identification, it is useful that studies are summarized by target organ, but there are almost no conclusions and no discussions of strengths or weaknesses of studies and therefore their use or not in future decisions. In fact, one is left with the impression that all studies are equal, especially in the section describing human studies. Within Chapter 4, the sub-sections entitled "evaluative and integrative" are actually neither. Data are presented simply as positive or negative with no real discussion of the strengths and limitations and what was concluded overall. For this reason, Chapter 5 is also lacking. It provides very little in the way of rationale and conclusions. Thus, the transparency of the process is really insufficient.

RESPONSE: The HESDs for both PFOA and PFOS were extensively revised to present a more in-depth analysis of the human epidemiology data, integrated summary of the animal and human evidence, and rationale for selection of the critical studies for quantitative analysis and selection of the RfDs.

DeWitt Comments

COMMENT 1: The information presented throughout the documents appears to be accurate (with one minor exception noted in Table 1 of these comments) and is presented clearly. For PFOA, a reference dose (RfD) of 0.00002 mg/kg/day was determined and evidence of carcinogenicity is considered suggestive with a human equivalent dose (HED) of 0.58 mg/kg/day. The RfD was based on changes in liver weight reported as a common denominator in four rodent (three rat and one mouse) studies and carcinogenicity was based on a limited number of epidemiology studies linking kidney and testicular tumors with exposure and evidence of tumor induction in the liver, testes, and pancreas (the "tumor triad") in rats. For PFOS, a RfD of 0.00003 mg/kg/day was determined and evidence of carcinogenicity is considered suggestive but with insufficient evidence to determine human carcinogenic potential. The RfD was based on developmental neurotoxicity and changes in liver weight.

While the carcinogenicity assessment seems appropriate for the two compounds given the limitations of the data sets, changes in liver weight as a basis of both of the RfDs is questionable in terms of its significance to exposed humans. Exposure to these agents increases liver weight and hepatocellular hypertrophy in rodents (and the definition of these endpoints as "adverse" or "toxic" also is contentious); this has been demonstrated across various rodent strains and under myriad exposure paradigms. However, there is no consensus in the scientific community regarding the mechanism by which exposure to these compounds increases liver weight

and induces hepatocellular hypertrophy in rodents and whether any of the putative mechanisms are sufficient to induce hepatotoxicity in exposed humans. Proposed mechanisms include peroxisome proliferator activated receptor alpha (PPARα) activation, activation of other nuclear receptors, peroxisome proliferation (which may or may not be dependent on PPARα activation), and oxidative stress. Humans can certainly respond to PPARα agonists (i.e., fibrate drugs are used as hypolipidemic agents) and a handful of epidemiological studies of highly exposed human populations have reported associations between PFOA/PFOS and alterations in liver enzymes, but the clinical relevance of the changes to the liver enzymes reported for these studies is uncertain. These liver-related changes in humans generally occur at higher doses than required to induce changes in the livers of rodents, which occurs at relatively lower doses than other observed effects. Therefore, a critical endpoint that occurs at very low doses in rodents, has no agreed upon mechanism that may or may not be relevant in humans at relatively high doses, may not be the best choice for the basis of a RfD. Liver weight change has been reported to occur in several species, including non-human primates, and at low doses, it may be an adaptive response and not a toxicological response. While this response may be protective of human health because it is common following low dose exposure to PFOA or PFOS, other endpoints may be more relevant to humans, especially endocrine system effects, including changes to thyroid hormones and mammary gland development, and immune system effects. Endocrine and immune system effects have been reported in exposed humans, suggesting that such endpoints may operate via a mechanism that is more relevant to humans than mechanisms related to changes in liver weights.

RESPONSE: Based on peer review comments, EPA examined a multitude of effects observed in the available animal studies. For PFOA, EPA modeled data from six studies for effects on development (delayed ossification, accelerated puberty, pup body weight, adult body and kidney weight), liver, and immune system. For PFOS, EPA modeled data from six studies for effects on development (pup body weight, neurodevelopment, pup survival) and liver. For both PFOA and PFOS, the RfDs based on multiple adverse effects resulting from short-term and longer-term exposures fall within a narrow dose range. The HESDs also describe available data on other endpoints (e.g., endocrine system and mammary gland development). EPA selected the most sensitive RfDs based on developmental effects so that they are protective for the general population and sensitive life stages.

COMMENT 2: In addition, the one developmental neurotoxicity study used, in part, for the PFOS RfD is only weakly supported by additional studies in rodents or other species and is based on behavioral responses that could be influenced by factors other than direct effects on the nervous system. Additional confirmatory studies are necessary for this observation to be considered a critical effect of PFOS exposure.

RESPONSE: The developmental neurotoxicity study by Butenhoff et al. (2009) was retained as one of the studies for dose-response quantification but it was not selected as the critical study for derivation of the RfD for PFOS. The Butenhoff et al. (2009) study and neurotoxicity endpoints are supported by studies by Long et al. (2013) demonstrating effects on special learning and memory in mature mice and Wang et al. (2015) showing increased water maze escape latency in prenatally exposed rats.

COMMENT 3: Finally, while well-written overall, the documents lacked an overall critical analysis or depth required of a risk assessment. Why specific studies were included or not should be better explicated in the text.

RESPONSE: The HESDs for both PFOA and PFOS were extensively revised to present summary sections to the toxicokinetic chapter and the epidemiology sections, a more in-depth analysis of the human epidemiology data for the identification of hazard, more detailed tables summarizing the results of the epidemiology studies, an integrated summary of the animal and human evidence, and a rationale for selecting the critical studies for quantitative analysis and selection of the RfDs.

Fisher Comments

COMMENT 1: The document was well written in terms of balance and presenting information. Summary statements are needed for chapters; a synthesis/analyses of the data are needed in some cases. A more critical evaluation of the human and non-human responses to PFOA/PFOS is required to justify not using human or non-human primate data. A rationale for the modeling approaches is needed given the more recent PBPK models that are available.

RESPONSE: The documents have expanded the literature used in the analysis to include papers recommended by the peer reviewers and many identified by the literature searches conducted during the post peer review period.

Summary sections were added to the toxicokinetic chapter and the epidemiology sections to assist the reader. New tables summarizing the results of the epidemiology studies were also developed. The original epidemiology summary tables were expanded significantly as recommended by the peer reviewers and are presented in Appendix B. The synthesis section was revised to better integrate findings between the human epidemiology studies and controlled animal studies. Taken together, the weight of evidence for human studies supports the conclusion that PFOA and PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an Integrated Risk Information System (IRIS) assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda. That effort may further evaluate and consider these human epidemiology studies.

The current HESDs describe why EPA selected the PK model, in addition to a description of the model and results. EPA notes that the final HESDs utilized a peer-reviewed and updated model (Wambaugh et al. 2013), which was revised as suggested by the peer reviewers.

Hayton Comments

COMMENT 1: The literature that pertains to the health effects of PFOA and PFOS is large and presents a major challenge to accurately summarize and analyze it and develop an RfD for PFOA and PFOS. Reported health effects in animals and humans, sometimes contradictory, include exposure-associated changes in serum cholesterol, lipids, uric acid, and thyroid hormones, obesity-related metabolism, immune system function, and effects on reproduction, development of the mammary gland, the nervous system, and behavior. Target organ effects (e.g., liver, kidney) have been reported, as well as associations of PFOA and PFOS exposure with testicular, prostate and kidney cancer. Studies in several laboratory animal species have added the complications of interspecies comparisons and extrapolation of findings to humans. In humans, there have been a Phase I clinical trial of PFOA, and epidemiological studies of populations exposed to PFOA and PFOS occupationally and in communities with and without water supplies contaminated with PFOA and PFOS. The draft documents have accurately presented in summary form the results of many animal and human studies and used pharmacokinetic methods to link PFOA and PFOS exposure rates to internal dose metrics such as serum concentration. While the overall effort is commendable, there are two issues that the draft documents raise: 1) the literature cited does not include many apparently relevant published works. The cut-off date for cited literature was early 2013 (this should be indicated in the documents), but commenter's noted a number of pertinent publications in 2011 and 2012 that were not cited, and there have appeared several highly pertinent papers since the cut-off date, and 2) while the descriptions of individual studies are generally clear and accurate, there is a lack of independent, critical analysis of the studies and a lack of synthesis of results from multiple studies common to a particular health effect.

RESPONSE: Papers recommended by the peer reviewers and from the public comments were retrieved and reviewed for inclusion in the revised documents. As noted in an added literature search strategy appendix,

bimonthly literature searches have been ongoing since 2009. Each identified study and any additional relevant literature published post peer review were reviewed and considered for inclusion. The papers evaluated for inclusion are documented in Appendix B. The evaluation of the epidemiology data was significantly revised and more detailed study summary tables were added. The original summary tables are now included in Appendix B. In addition, the HESDs for both PFOA and PFOS were extensively revised to include tabular presentation of the study details that inform an in-depth analysis of the human epidemiology data, introductory and summary sections for the human and animal health effects information, and an integrated summary of the animal and human evidence.

Longnecker Comments

COMMENT 1: The PFOA and PFOS documents achieve the goal of identifying RfDs that are well founded. My main criticism is that the rationale for not using the human data to provide a POD needs to be strengthened.

For example, in the PFOA document, on page 5-19, first paragraph below the table, it says "human data … lack the exposure information for dose-response modeling." This statement is logically inconsistent with techniques that were used to estimate HED on the basis of serum concentration, as given on page 5-17, near the bottom. Or, in some cases, such as in the C8 study, the exposure estimates that were calculated based on water district were sufficiently good that a dose-response analysis would be possible. In other words, because many human studies have serum concentration of PFOA or reasonable estimated exposure values, the corresponding HED could be estimated, and hence the dose-response could be modeled. Granted, some assumptions would be needed, but the methods could be serviceable (see response to item 3 below). (Some of the above also applies to pages 5-1 and 5-2). More compelling arguments for not basing the POD on human data are, e.g., that: 1) the low probability that humans are 1,000 times more sensitive to PFOA than other species (the number is based on the last column in table 5-9 compared with PFOA values in the C8 study and background exposed populations), especially given the relatively tight agreement between LOAEL (average serum concentration basis) among other species, 2) the possibility that the observed associations in humans were due to unmeasured confounding factors or reverse causality, and 3) other weaknesses in the epidemiologic data such as inconsistent results across studies (selected outcomes), unreplicated findings,or associations with clinical chemistry results for which corresponding adverse clinical correlates (i.e., morbidity) are not clearly established.

RESPONSE: The HESDs for both PFOA and PFOS have been extensively revised to present a more in-depth analysis of the human epidemiology data, including a more robust discussion of the data that supports the conclusion that there is evidence of an association between exposure to these chemicals and human health hazard. For a few of the outcomes (e.g., serum lipids [PFOS and PFOA], effects on fertility and fecundity [PFOS], and pregnancy-induced hypertension [PFOA]), the associations are particularly strong and fairly consistent. There still remains some uncertainty related to other observed associations, as for various endpoints the data for both PFOA and PFOS are mixed (i.e., some studies show positive associations with the serum PFOA or PFOS value, while others do not).

EPA has limited information to allow estimates of human serum concentrations for a specific, known pathway, such as drinking water. However, EPA does not have sufficient exposure information to attribute the serum concentrations observed in biomonitoring and other studies to specific exposures (time, route, and magnitude) in such a way that would allow dose-response modeling. The serum level at which the effects were first manifested and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contributed to serum PFOS values come from derivatives or precursors that break down metabolically to PFOA and PFOS. These compounds can originate from the diet and materials used in the home; thus, there is potential for confounding in the C8 studies where the drinking water PFOA was considered to be the primary medium of exposure and for PFOS precursors where degradation produces amines that could contribute to the effects observed. Additionally,

most of the subjects of the epidemiology studies have many PFAS and/or other contaminants in their blood. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal studies. The documents have also been revised to include a discussion of the potential confounding of serum PFOA and PFOS concentrations by low glomerular filtration and the initiation or cessation of menstruation (a route of excretion for females) is included in the discussion of the epidemiology.

Taken together, the weight of evidence for human studies supports the conclusion that PFOA and PFOS exposure is a human health hazard. At this time, for the development of the RfD in support of the development of a drinking water health advisory for PFOA and PFOS, EPA's Office of Water (OW) concludes that the human studies are adequate for qualitative use in hazard identification and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda. That effort may further evaluate and consider these human epidemiology studies.

Slitt Comments

COMMENT 1: The documents provide a very thorough evaluation of PFOA and PFOS studies. It is logically organized, presenting findings in a way that the reader can understand the findings related to human, monkey, and rodents. The organization of the document makes allows the reader to easily find information about each species within the subchapters and summarizes key points in table form. PFOA is a well-studied compound, with a substantial amount of toxicokinetic and endpoint studies in rodents. Mechanistic data describing the role of membrane transporters to understand gender differences in PFOA elimination in rats is fairly well written. Little data exist regarding contribution of membrane transporters to PFOS disposition and elimination. The documents thoroughly describe species differences in PPAR-alpha signaling that might contribute to observed endpoints in rats, but not humans or monkeys. Overall, both documents are very thorough are provide a reliable basis for PFOS and PFOA evaluation.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

COMMENT 2: For PFOA toxicokinetics, mechanisms of PFOA transport are important for understanding species differences in response to PFOA exposure, with focus placed on kidney. Figure 3-2 in the PFOA document does not adequately present the localization of renal transporters with relationship to their contribution to the urine compartment or renal reabsorption. A very nice diagram showing the subcellular localization of renal transporters presented by Klaassen and Aleksunes (Pharmacol Rev. 2010 Mar;62(1):1-96) clearly depicts the contribution of various transporters to filtrate or blood. This is an easier diagram to put PFOA elimination into context than the one presented. Contribution of membrane transporters to species differences in PFOA excretion Section 3 (specifically 3.4.1) would be put in better context if a table could be generated to compare Km and Vmax values for PFOA for various transporters, with specific focus on species information for OATps and OATs. Data regarding information on contribution for OATps in liver accumulation of PFOS and PFOA is lacking, with specific regard to species differences. As PFOS is a likely candidate for hepatic uptake transport, understanding a mechanism to explain species differences in hepatic effects possibly due to difference in hepatic exposure is critical. Understanding impact species specific regulation of OATp expression in liver (e.g. whether species difference in PPAR-alpha signaling contributes) is also important in putting rodent distribution data into context.

RESPONSE: EPA agrees that additional studies are needed to improve the understanding of uptake and transport of PFOA and PFOS. OW appreciates the recommendation of the Klaassen and Aleksunes publication on transporters (*Pharmacological Reviews*. 2010 Mar;62(1):1-96). The original figure in the document compiled findings from multiple papers. The integrated diagram for the kidney transporter has replaced the original (Figure 2-2).

COMMENT 3: Increased liver weight is considered to be a critical effect, but how increased liver weight relates to the observed human and monkey health effects needs to be further explained. In layman terms, if someone is walking around with an increased liver weight, is he or she at risk for disease? Will his/her life span be shortened? To increase transparency of the document, a more comprehensive explanation is needed to justify why increased liver weight should be considered as a critical endpoint for human health.

RESPONSE: As a result of multiple comments from peer reviewers on this topic, liver weight is no longer the critical endpoint. In the revised assessment, liver weight was not considered adverse unless accompanied by other hepatic effects, such as necrosis, fibrosis, and/or inflammation as defined by Hall et al. (2012). As a result, the NOAELs and LOAELs in these studies (i.e., previously based liver weight and associated hypertrophy) have also changed. The PODs for both PFOA and PFOS are based on developmental endpoints in the final documents.

COMMENT 4: Use of humanized PPARα mice are a sexy tool to delineate species differences in effects associated with peroxisome proliferation. For transparency, the document should acknowledge the limitations of that model. Specifically, lack of response may not necessarily correlate to a lack of response for human PPARα because of species differences in binding to DNA elements (e.g. a human receptor may have lower binding capacity to mouse DNA due to structural differences and species differences in co-activator/co-repressor interactions). Wording in the documents using these mice should acknowledge this limitation.

RESPONSE: Studies of mice with the humanized PPARα are included in the HESD to demonstrate that there are liver responses to PFOA that are independent of PPARα activation. No weight is given to whether or not a response was lacking in either the PPARα-null or hPPARα animals because there were effects in each group that could be relevant to humans for these chemicals based on their physical (negative charge nonaromatic) and protein binding properties.

COMMENT 5: The documents often have redundancy in information, especially in regard to hormone effects (there are very similar write ups in sections about effects on thyroid hormone) and metabolic/cardiovascular disease risk factors (e.g. lipid endpoints).

RESPONSE: For the sake of completeness and transparency in the final HESDs, EPA has described the available epidemiological evidence for each study by health endpoint. Many of the epidemiology studies analyzed potential associations between PFOA and/or PFOS and multiple health endpoints. By organizing the effects assessment by health endpoints (rather than by individual study descriptions), the reader can get a better sense of the weight of evidence supporting the potential associations between PFOA and/or PFOS and each health endpoint.

3. EPA Responses to Peer Reviewer Comments on Charge Questions

Charge Question 1: Studies Used for Quantification

Please comment on the strengths, weaknesses, and characterization of the studies selected as key for quantification.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: The document's authors have done a good job describing and integrating the findings of the numerous studies in which liver weight gain was observed. Although there is a consensus about the effect and the dosage required to elicit it in different species, this reviewer does not believe it should be utilized, as described above. There are several clearly adverse effects such as elevated serum (hepatic) enzyme activities, focal hepatocellular necrosis, bile duct degeneration and fibrosis, etc. These effects are generally seen in response to relatively high PFOA doses, so the PODs will be higher than with liver weight increase. Alternatively, a human endpoint such as elevated serum cholesterol could be considered. See responses to Charge Question 3.

RESPONSE: EPA re-evaluated the outcomes related to PFOA exposure based on peer review comments, and selected an endpoint that reflects adverse effects in the developing fetus and newborn as the most sensitive endpoint to serve as the basis for the derivation of the RfD. The POD for PFOA is based on low birth weight (Lau et al. 2006). Increased liver weight is acknowledged as a common finding, but not considered adverse in the absence of other effects as defined by Hall et al. (2012).

PFOS-specific Comments

COMMENT 2: There have been a substantial number of well-conducted toxicological studies of PFOS. My major concern, as expressed above, is its potential to cause adverse effects in children. Other than that, PFOS doesn't appear to produce effects other than those anticipated from a repetitive, cumulative dose of an 8-carbon fatty acid.

RESPONSE: EPA re-evaluated the outcomes related to PFOS exposure based on peer review comments, and selected an endpoint that reflects adverse effects in the developing fetus and newborn as the most sensitive endpoint to serve as the basis for the derivation of the RfD. The POD for PFOS is based on decreased pup body weight in a two-generation rat study (Luebker et al. 2005b). The sensitive endpoint of body weight changes in pups is protective of other offspring effects such as decreased survival or alterations in glucose homeostasis manifested later in life.

Cory-Slechta Comments

COMMENT 1: In general, it appears that, at least with respect to the animal studies, the choices are appropriate both in the case of PFOA and PFOS. The derivation of the RfDs/RfCs are based on studies of sufficient strength, duration and represent the most sensitive endpoints.

RESPONSE: EPA re-evaluated the outcomes related to PFOA and PFOS exposures based on peer review comments, and selected endpoints that reflects adverse effects on the developing fetus and newborn as the most sensitive endpoint to serve as the basis for the derivation of the RfDs for both chemicals.

COMMENT 2: Having said that, in both documents, the reader is forced to that conclusion with no real assistance from the text itself. There is virtually no discussion of the strengths and weaknesses of the studies overall. Human study outcomes for the most part are simply enumerated, although an occasional statement will be made about a limitation (usually) of one of those studies. There is no discussion in the human studies of the power to detect effects, the sample sizes, etc. Much weight seems to be given to occupational studies in some cases, being used to essentially dismiss effects in a community cohort as the same effect was not seen in occupationally exposed workers, when in fact finding effects in a population with seemingly longer, albeit lower exposure levels actually makes the outcome more robust. Also, population studies with smaller sample sizes that nevertheless find significant effects are in fact more compelling and suggest robust effects which can be detected even with a small sample size. This deficiency is manifest in statements such as those in the PFOS document (p. 5-1) that 'in most cases the findings are suggestive and not conclusive of an effect'.

RESPONSE: The human epidemiology section was substantially rewritten with study type, sample size, and serum levels added for each study where these data were available. Tables were expanded for each major endpoint to summarize the studies described in the text. An overall summary and conclusion was added at the end of the human epidemiology section. In the revised HESD, effects from human studies are used qualitatively as a line of evidence to support the assessment.

COMMENT 3: There is a bit more discussion of the animal studies in both documents, at least with respect to methods, but as with the human studies, there is little text addressing which studies represent stronger studies or what the weaknesses are. From these increase liver weight has been chosen as the endpoint from which to derive RfDs. This reviewer does not have an issue with that choice, as while it has been described as adaptive by some, it represents a response to an involuntary exposure with a direction of effect that is potentially associated with adverse consequences. The fact that it is reversible when exposure ends seems irrelevant as reversal of exposure is not happening in the human environment.

RESPONSE: Based on peer review comments, liver weight is no longer used as the critical effect. For both chemicals, the RfD is now based on developmental effects, as suggested by others peer reviewers. Additional text was added describing the studies chosen for modeling and selection of the RfD. This discussion includes strengths and weaknesses of the human epidemiology data, the strengths of the animal studies selected for quantitation, and the support for the PODs from studies with dose-response that lacked serum information.

DeWitt Comments

COMMENT 1: *Strengths:* The studies selected as key for quantification were generally well-conducted studies, employing a range of doses and sample sizes large enough for detecting statistical differences. Additionally, the doses associated with LOAELs for the identified critical endpoints were not associated with signs of overt or systemic toxicity in the animal models and nearly all of the studies measured serum and/or tissue concentrations of the parent compounds.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

COMMENT 2: *Weaknesses:* No obvious experimental design weaknesses were noted in any of the studies selected as key for quantification.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

COMMENT 3: *Characterization:* The studies selected as key for quantification for PFOA are all rodent studies while at least one study selected for PFOS quantification includes a non-human primate study. It is therefore surprising that the PFOA database does not include, as a study key for quantification, the Butenhoff et al. 2002 study of non-human primates. Additionally variability in putative mechanisms among species was

not adequately addressed in the characterization of the selective studies, although all of the selective studies were descriptive and not mechanistic.

RESPONSE: The data from the monkey study (Butenhoff et al. 2002, 2004b) were not used because of the small number of animals evaluated and the wide variability in the responses among the individual animals. For example, succinate dehydrogenase activity was highly variable in the six animals given 3 mg/kg/day despite this group having the most consistent liver PFOA concentrations. In addition, although serum steady-state had been attained by 4–6 weeks of dosing, liver PFOA levels ranged from 11.3–18.5, 6.29–21.9, and 16–83.3 µg/g tissue in the 3 (n=4), 10 (n=3) and 20 (n=2) mg/kg/day groups, respectively.

Fisher Comments

COMMENT 1: PFOA and PFOS: Data bases are massive and both need to be updated. Several human studies and a few non-human primate toxicity studies are available. The authors need to explain why these studies are not adequate for causality (dose-response).

RESPONSE: A substantial number of human epidemiology studies were added to both documents. The human epidemiology section was substantially rewritten with study type, sample size, and serum levels added for each study where these data were available. Tables were expanded for each major endpoint to summarize the studies described in the text.

EPA is unaware of non-human primate studies other than those included in the HESD. The studies are described in the HESD, but not used in the derivation of candidate RfDs. The effects observed in Seacat et al. (2002) were significant only at the high dose where there was also mortality. Additionally, for both PFOS and PFOA, the liver effects observed in monkeys were inadequate to establish adversity following the Hall et al. (2012) criteria, as recommended by the peer reviewers.

Hayton Comments

COMMENT 1: An advantage to assessment of health effects for both PFOA and PFOS is the large amount of published work that informs the topic. While the draft health-effects documents have summarized the results of many pertinent studies, the literature reviewed was not comprehensive, which projects an appearance of weakness. The documents do not state whether the intention was to include all relevant health-effects literature, or to be selective and summarize those studies judged to be most relevant. Such a statement at the beginning of the documents would be helpful; a cut-off date for the literature review would also frame expectations of readers. If the intention was to be selective, a description of selection criteria would help allay concerns of readers about papers that were not included. If the intention was to comprehensively review all the PFOA and PFOS health-effects literature, then it appears that more work should be done to include omitted works. Public comments list a number of works to consider for inclusion.

RESPONSE: One of the challenges inherent in conducting theses assessment was the wealth of experimental data published before and during their development. A background section has been added to both PFOA and PFOS HESDs to provide a synopsis of the approach used in identifying and selecting the publications reflected in the final assessment. The criteria used to evaluate each study and to select those for inclusion is provided in the background section of each document. Additionally, a more detailed description of the literature search strategy, including dates of the literature search and search strings used, was added as Appendix A in both documents.

The studies included in the final HESDs are those determined to provide the most current and comprehensive description of the toxicological properties of PFOA and PFOS and the risk they pose to humans exposed to them in their drinking water. EPA has added Appendix B to both PFOA and PFOS HESDs, which

summarizes the studies evaluated for inclusion in the HESD as a result of the peer review and in the time following the peer review and identifies those selected for inclusion in the final assessment.

COMMENT 2: A general, albeit minor weakness of the literature is that PFOA and PFOS serum concentrations in control animals were not measured for many studies – they were likely non-zero and, since there is no information on how high they were, it is possible that baseline health-effects metrics were affected and that dose-response relationships were affected, especially in the low dose range. It is perhaps worthwhile to mention this shortcoming somewhere in the health effects documents.

RESPONSE: In each case where serum information was available, it was reported in the draft document and used in the development of the Wambaugh et al. (2013) toxicokinetic model applied in the derivation of the RfD. The literature review was updated since the peer review draft was developed and some of the newer studies also include serum data. In such cases, the serum information is included with the study description. For PFOA, only one study in the toxicokinetic section had serum information for the controls. The control levels were identified as below the level of detection. In PFOS, some of the control animal serum levels are given in the toxicokinetic chapter; these are either below the LOQ or are orders of magnitude lower than those of dosed animals.

Longnecker Comments

COMMENT 1: EPA may want to consider the article by AP Hall et al. 2012, about liver hypertrophy. The defense of increased liver weight as the POD (or a POD) could be strengthened by evaluating the evidence in the context of Hall's Figure 9, where evidence regarding hepatoxicity and toxic mechanisms are also considered. In this case, the possibility of an unknown mechanism exists that could be relevant to humans, and long-term exposure could have effects that have not yet been detected. See Hall page 986, where it defines adverse as: "…affects [response] to an additional environmental challenge". Thus, an adverse effect, via an unknown mechanism, by this definition is possible and has not been studied in animals or humans.

RESPONSE: The reference has been added to both HESDs and used in determining whether or not effects on the liver can be considered as adverse. For the studies where liver effects were the only effects observed, the LOAEL was assigned based on effects characterized as adverse by the Hall criteria. Where there was increased liver weight, with or without hypertrophy, those effects are acknowledged in Tables 5-1 and 5-2.

COMMENT 2: While AP Hall's article is not all that supportive of using increased liver weight as a point of departure (unless certain criteria are met), they are focused on animal studies, especially those done in rodents. If increased relative liver weight were to occur in a human population, I suspect that it would be considered an adverse outcome, whether or not there was evidence of hepatotoxicity or a specific mechanism. Note also that for PFOA, in monkeys, there was an increase in relative liver weight with chronic exposure (PFOA document, page 4-66), so increase in liver weight in the animal experiments may be relevant to humans.

RESPONSE: The POD for both PFOA and PFOS was altered so that liver weight alone is no longer the endpoint of concern. Increased liver weight is acknowledged as a common finding but not considered adverse in the absence of other effects as defined by Hall et al. (2012). EPA reevaluated all studies reporting presence of increased liver weight for other adverse effects using the Hall criteria. The RfD for PFOA is based on reduced ossification in males and females and accelerated puberty in males (Lau et al. 2006). The RfD for PFOS is based on decreased pup body weight (Luebker et al. 2005b) in rats over two generations.

COMMENT 3: An additional comment of relevance here pertains to whether the human data support hepatoxicity. While there are studies that report elevated liver function tests in subjects with higher serum concentrations of perfluorakyl substances, these elevations do not clearly support the presence of toxicity.

Again, AP Hall's discussion of what constitutes evidence of hepatoxicity is relevant here, and takes into account the number of LFTs elevated, the specific LFTs involved, and the magnitude of their elevation.

RESPONSE: A better summary of the human data is provided in both documents detailing the strengths and weaknesses of the data set and support provided for hazards identified in animal studies. Although serum levels of alanine aminotransferase (ALT) were increased in several of the human studies, the ALT increased were not accompanied by other indices (e.g., increased AST, lactic dehydrogenase [LDH]) that would clearly identify the presence of liver damage.

COMMENT 4: Finally, as discussed at the meeting, for the PFOA document on page 5-23 ("RfD Selection"), and the PFOS document on page 5-26 ("RfD selection"), I suggest minor editorial changes to deemphasize the "consistency of response" point and instead focus a little more on how the RfD is robust to choice of POD endpoints. If the selection of RfD does not hinge on increased liver weight as a POD, it will be more defendable.

RESPONSE: The POD for both PFOA and PFOS was changed such that liver weight is no longer the endpoint of concern. The critical effect selected as the basis for determining the POD for PFOA is reduced ossification and accelerated puberty in male mice (Lau et al. 2006). The critical effect selected as the basis for determining the POD for PFOS is decreased pup body weight (Luebker et al. 2005b) in rats over two generations. Increased liver weight is acknowledged as a common finding but not considered adverse in the absence of other effects, as defined by Hall et al. (2012).

As an initial step in the dose-response assessment, EPA identified a suite of animal studies with NOAELs and/or LOAELs that identified them as potential candidates for development of candidate RfD for PFOA and PFOS (e.g., identified low dose, adverse effects). These studies included short-term, subchronic, and chronic exposures, including developmental and reproductive toxicity studies. The available studies evaluated endpoints including liver effects (weight changes with histopathology), body weight changes in adults and offspring, and developmental effects (developmental neurotoxicity, altered puberty, survival), and immune effects. The candidate studies were selected based on their NOAEL and/or LOAEL values and use of a control and two or more doses. From these studies, those that presented serum data amenable for modeling (i.e., determination of human equivalent doses) were selected for dose-response analysis.

For both PFOA and PFOS, the candidate serum-derived RfDs represent multiple adverse effects resulting from short-term and longer-term studies with exposures that fall within a narrow dose range. They are supported by the NOAELs and LOAELs from other studies with dose-response that lacked the serum information needed for modeling. EPA selected the most sensitive RfDs for PFOA and PFOS based on developmental effects that are protective for the general population and sensitive life stages.

COMMENT 5: Transparency might be increased by saying why (more clearly, or more clearly by implicit reasoning) the Macon et al. 2011 study, in which the LOAEL was 0.01 mg PFOA/kg from GD10 to GD17, based on delayed mammary gland development, was not considered as a POD, and why the Hines et al. 2009 study, in which the LOAEL was 0.01 mg PFOA/kg from GD1 to GD17, based on various outcomes, was not considered as a POD. The PFOS studies with low LOAELs were considered in the dose-response assessment (no suggestions for improvement there).

RESPONSE: The data from the Macon et al. (2011) and Hines et al. (2009) studies are included in the PFOA HESD in sections 3.2.7 and 3.3.3, respectively.

A number of studies have focused on mammary gland development in animals (dams and female offspring) following exposure to PFOA and are described in the HESD for PFOA. Researchers focused on effects resulting from indirect exposure of offspring via treatment of pregnant animals and/or direct exposure of peripubertal animals starting about the time of weaning. These studies show effects on mammary gland

morphology (branching and bud growth) in both dams and pups at low doses. Studies with higher doses demonstrated that exposed neonate pups showed no significant difference in body weight compared to controls despite the fact that there were differences in the gland duct structure. Thus, indicating that the function of maternal milk delivery was not impacted by the structure. In another study, Tucker et al. (2015) demonstrated that a dose-response for developmental mammary gland effects varies by more than an order of magnitude depending on the strain of mouse studied. Increased discussion of mammary gland development and rationale for not selecting this endpoint as the critical effect was added to the document (see section 4.1.1 of PFOA).

Slitt Comments

COMMENT 1: My response is basically the same as my General Impressions above.

RESPONSE: See response to General Impressions comments 1-5.

Charge Question 2: Additional References

Please provide citations (and, where possible, pdfs or hard copies) for any references you suggest EPA consider adding to the document. Describe where you suggest these references be incorporated.

References recommended by the peer reviewers and public, along with publications collected from the ongoing literature searches after peer review, were evaluated for inclusion based on selection criteria described in Appendix A. Date of publication and whether or not the publication provided new toxicity information or support for hazard identification or dose response was considered. The appendix documenting literature considered and decisions relative to their inclusion is in the final document. Many of the recommended studies concern liver pathophysiology in general but did not evaluate liver effects as a result of PFOA or PFOS exposure and thus were not included (Ipeki et al. 2003, Morfrad et al. 2003, Oh et al. 2008, Delgado 2008, Wieckowska et al. 2008, Kunde et al. 2005, Lizardi-Cervere et al. 2006, Amarapurka et al. 2006, Chen et al. 2006, Fracanzani et al. 2008, Sorrentino et al. 2004, Uslusoy et al. 2009, Allen et al. 2004).

Bruckner Comments

COMMENT 1: PFOA-specific comments

Fabrega, F. et al. (2014). PBPK modeling for PFOS and PFOA: Validation with human experimental data. Toxicol. Lett. On line. (Hard copy available)

Stahl, T., Mattern D and Brunn, H. (2011). Toxicology of perfluorinated compounds. Environ. Sci. Europe 23: 38-60.

Hall, A. P., et al. 2012. Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes-Conclusions from the 3rd International ESTP Expert Workshop. Toxicol. Pathol. 40: 971-994.

Bjork, J. A., Butenhoff, J. L., and Wallace, K. B. 2011. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rat hepatocytes. Toxicology 228: 8-17.

RESPONSE: Hall et al. (2012) and Fàbrega et al. (2014) were added to both final HESDs. The Stahl et al. (2011) paper is a review paper that does not include primary data, therefore it was not included. Bjork et al. (2011) is an in vitro study of nuclear receptors related to PPAR, CAR, FXR, etc. in rats and humans with findings that are mostly duplicative of those from other studies already included in the HESD.

Papers that were not included were those that failed to meet the selection criteria established for the updates to the draft document as follows (described in Appendix B):

- The study examines a toxicity endpoint or population that had not been examined by studies already present in the draft assessment.
- Aspects of the study design, such as the size of the population exposed or quantification approach, make it superior to key studies already included in the draft document.
- The data contribute substantially to the weight of evidence for any of the toxicity endpoints covered by the draft document.
- There are elements of the study design that merit its inclusion in the draft assessment based on its contribution to the mode of action or the quantification approach.
- The study elucidates the mode of action for any toxicity endpoint or toxicokinetic property associated with PFOS exposure.
- The observed effects differ from those in other studies with comparable protocols.
- The data are relevant to drinking water exposures and to the U.S. population.

COMMENT 2: PFOS-specific comments

No additional references were located.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Cory-Slechta Comments

COMMENT 1: For both PFOA and PFOS, the document should include a description of the process through which studies were identified and how they were processed for inclusion or not. It is not clear what the exact dates of the studies examined included, i.e., what the cut-off date was for these studies. This makes it difficult to evaluate whether there are missing studies. That said, this reviewer is not aware of any specific omissions in the peer-reviewed literature other than those that were discussed at the face-to-face meeting.

RESPONSE: One of the challenges inherent in conducting these assessments was the wealth of experimental data published before and during their development. A background section has been added to both PFOA and PFOS HESDs to provide a synopsis of the approach used in identifying and selecting the publications reflected in the final assessment. The criteria used to evaluate each study and to select those for inclusion is provided in the background section of each document. Additionally, EPA added a description of the literature search strategy, including dates of the literature search and search strings used, to Appendix A in both documents.

The studies included in the final HESDs were determined to provide the most current and comprehensive description of the toxicological properties of PFOA and PFOS and the risk they pose to humans exposed in their drinking water. EPA added Appendix B to both PFOA and PFOS HESDs, which summarizes the studies evaluated for inclusion in the HESD as a result of the peer review and in the time following the peer review. It also identifies those selected for inclusion in the final assessment.

DeWitt Comments

COMMENT 1: Granum, B., Haug, L.S., Namork, E., et al. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol. 10:373–379; Looker, C., Luster, M.I., Calafat, A.M., et al. 2014. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol. Sci. 138:76–88.

Any time the Grandjean et al. (2012) findings related to PFAS and vaccine responses are discussed, these references could/should be discussed as well as they report related findings in human populations. Although they also are confounded by multiple PFAS (as was the Grandjean et al. study), they lend additional support to immunotoxicity as an endpoint worthy of consideration. However, it is noted that these references were published after the cutoff date for consideration for inclusion in the document.

RESPONSE: Both the Granum et al. 2013 and Looker et al. 2014 studies were added to both HESDs. A summary and conclusions write-up was added to the epidemiology section, which discusses the immune function-related findings together.

COMMENT 2: Lopez-Espinosa, M.J., et al. 2012. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ.Health Perspect. 120:1036–1041. This study is missing from the discussion of thyroid hormone disruption. It reports a positive correlation between hypothyroidism and PFOA in children from the C8 population aged 1-17.

RESPONSE: Lopez-Espinosa et al. 2012 was added to the HESD for PFOA. The PFOA HESD describes results from the C8 study on hypothyroidism.

COMMENT 3: Corsini E., et al. 2011. In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). Toxicol. Appl. Pharmacol. 250:108-116. Corsini E. et al. 2012. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). Toxicol. Appl. Pharmacol. 258:248-255.These studies are in vitro/ex vivo studies of human-derived cells that provide evidence that in vitro measures of immunocompetence in mice may be relevant to the human experience.

RESPONSE: EPA reviewed these studies but did not include them in the HESD for PFOA or PFOS because both papers were reviews of the literature, rather than primary reports from the individual studies, and failed the criteria for inclusion on the basis that they would not substantially alter the weight of evidence conclusion for the immunotoxicity findings. The HESDs for both PFOA and PFOS describe available data on the immunotoxicity of these chemicals.

Fisher Comments

COMMENT 1: For completeness sake, at least, please update lab animal studies conducted since 2012.

RESPONSE: A literature search was conducted on a bimonthly bases between 2009 through 2015. An updated description of the literature search strategy, including dates of searches and search strings used, was added as Appendix A in the documents.

Hayton Comments

COMMENT 1: A review of PFOA health-effects literature (GB Post et al. (2012) Environ. Res. 116: 93-117) provides an excellent, in-depth discussion of many issues covered in the PFOA health effects document. Consider citing this review in the document.

RESPONSE: EPA reviewed the paper, and has included a brief description of the type of review presented by Post et al. 2012 in Appendix B of the HESD for PFOA. Many of the papers referenced in Post et al. 2012 study are included in the HESD.

COMMENT 2: The literature on PFOA and PFOS toxicokinetics (Section 3) has been comprehensively covered in the health effects documents, with the notable omission of Wambaugh et al., Dosimetric

Anchoring of In Vivo and In Vitro Studies for Perfluorooctanoate and Perfluorooctanesulfonate. Toxicol. Sci. 136:308-327, 2013. This paper informed a significant part of the health effects documents.

RESPONSE: This citation (Wambaugh et al. 2013) was added.

COMMENT 3: Commenters have suggested a number of references to consider with regard to Section 4 Hazard Identification. Many recent publications report on toxicity associated with PFOA/PFOS exposure. For the Dose-Response Assessment (Section 5) it is desirable to focus on those toxicities that have occurred at the lowest PFOA/PFOS exposures. For PFOA, the literature that is used in Section 5 to determine an RfD was published prior to 2009 (Tables 5-8 – 5-11). The benchmark response chosen based on the Section 4 literature was a 10% increase in liver weight, which was the biological response that occurred at the lowest PFOA exposure; it was acknowledged that this response "… is a biomarker for systemic exposure in rodents, rather than a biomarker of adversity …" (p. 5-6). More recent studies of hazard have identified potential adverse effects that result from, or are associated with, PFOA exposures that are lower than the LOAEL for a 10% increase in liver weight. For example, adverse effects on fetal, neonatal and early childhood stages of development may occur at lower exposures than does liver weight gain, which suffers in addition from not being a biomarker of adversity, and which therefore raises a question about the validity of any RfD based upon it. Macon et al. 2011 reported an LOAEL for delayed mammary gland development of 0.01 mg/kg administered to pregnant CD-1 mice during GD10 – GD17. As this relatively brief exposure was well below that required for steady state, it is possible that had the dams been at steady state at the time of conception (about 9 weeks of exposure) a much lower LOAEL may have been observed; i.e., a much lower dose rate at steady state would have produced the same exposure to the fetal pups as did the 0.01 mg/kg administered to the dams during GD10 – GD17. The steady state situation is more relevant to adverse effects in humans than is a brief exposure.

RESPONSE: The POD for both PFOA and PFOS was changed such that liver weight is no longer the critical endpoint. PFOA is based on reduced ossification of the proximal phalanges (forelimb and hindlimb) in male and female pups and accelerated (4 days earlier than controls) puberty in male pups of dams exposed to PFOA gestationally and lactationally (Lau et al. 2006). PFOS is based on decreased pup body weight (Luebker et al. 2005b) in rats over two generations. Increased liver weight is acknowledged as a common finding, but not considered adverse in the absence of other effects as defined by Hall et al. (2012). Reasons for not using delayed mammary gland development are described in the HESDs and include lack of consistent scoring, no effects on body weight of pups nursing from affected dams, and no differences in response to lactational challenge. A discussion of steady state was added to section 4.

One of the challenges inherent in conducting these assessments was the wealth of experimental data published before and during their development. A background section was added to both PFOA and PFOS HESDs to provide a synopsis of the approach used in identifying and selecting the publications reflected in the final assessment. The criteria used to evaluate each study and to select those for inclusion is provided in the background section of each document. Additionally, a detailed description of the literature search strategy, including dates of the literature search and search strings used, was added to Appendix A in both documents.

The studies included in the final HESDs were determined to provide the most current and comprehensive description of the toxicological properties of PFOA and PFOS and the risk they pose to humans exposed in their drinking water. EPA added Appendix B to both PFOA and PFOS HESDs, which summarizes the studies evaluated for inclusion in the HESD as a result of the peer reviewers and public in the time following the peer review. It also identifies those selected for inclusion in the final assessment.

Longnecker Comments

COMMENT 1: I suggest you include the following citation and include a discussion of the evidence presented:

Paula I. Johnson, Patrice Sutton, Dylan S. Atchley, Erica Koustas, Juleen Lam, Saunak Sen, Karen A. Robinson, Daniel A. Axelrad, and Tracey J. Woodruff. The Navigation Guide—Evidence-Based Medicine Meets Environmental Health: Systematic Review of Human Evidence for PFOA Effects on Fetal Growth. Environ Health Perspect; DOI:10.1289/ehp.1307893 (in press and available through the journal's website).

Based on the meta-analysis in this paper, the evidence that PFOA is associated with lower birthweight is consistent. Thus, the rationale for not basing the POD on the human data needs to be strengthened, as noted above. The Johnson et al. report could be discussed in the section on anthropometric endpoints that begins on p 4-22.

RESPONSE: This study (Johnson et al. 2014) and the other reports from the Navigation Guide projects related to PFOA are now included in the HESD for PFOA. In addition, the PODs for both PFOA and PFOS are now based on developmental effects observed in animal studies, with human study results described qualitatively and used to support conclusions.

COMMENT 2: The relationship between birthweight and PFOA or PFOS may be confounded because glomerular filtration (and hence excretion of the compounds) is proportional to birthweight, as discussed in:

Morken NH, Travlos GS, Wilson RE, Eggesbø M, Longnecker MP. Maternal glomerular filtration rate in pregnancy and fetal size. PLoS One. 2014 Jul 8;9(7):e101897

RESPONSE: It has been suggested that low glomerular filtration rate (GFR) can affect birth weight (Morken et al. 2014). Verner et al. (2015) conducted a meta-analysis based on physiologically-based pharmacokinetic model (PBPK) simulations and found that some of the association reported between PFOA and birth weight is attributable to GFR and that the actual association may be closer to a 7 gram reduction (95% CI [-8, -6]). Verner et al. (2015) showed that in individuals with low GFR there are increased levels of serum PFOA and lower birth weights. Although some uncertainty exists in the interpretation of the observed association between PFOA and birth weight given the potential impact of low GFR, the available information indicate that the association between PFOA exposure and birth weight cannot be ruled out. In humans with low GFR (which includes women with pregnancy-induced hypertension or preeclampsia) the impact on body weight is likely due to a combination of the low GFR and the serum PFOA. The Morken et al. (2014) study was added to both of the final HESDs along with the subsequent Verner et al. (2015) paper on the same topic.

COMMENT 3: In the PFOA document, on page 4-18, you might want to also cite:

Taylor KW, Hoffman K, Thayer KA, Daniels JL. Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). Environ Health Perspect. 2014 Feb;122(2): 145-50.

The Taylor et al., like the Knox et al. report (already cited in the PFOA document) is from a large-cross sectional study. Both studies, in their discussion sections, note that the association of PFOA or PFOS concentration in serum with age at menopause could be expected because postmenopausal women have lost a route of excretion for the compound and will have higher serum concentrations on that basis. It would be worth noting this possible explanation in the PFOA document on page 4-18, and in the PFOS document on page 4-8.

RESPONSE: This study (Taylor et al. 2014) was added to the HESD for PFOA; menstruation as a route for excretion is covered by additional studies published after work on the 2013 peer review draft was completed.

COMMENT 4: Additional data are available on the potential carcinogenicity of PFOA:

Steenland K, Woskie S. Cohort mortality study of workers exposed to perfluorooctanoic acid. Am J Epidemiol. 2012;176(10):909-17.

Barry V, Winquist A, Steenland K. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ Health Perspect. 2013;121(11-12):1313-8

Hall AP et al. Toxicol Pathol 2012:40:971-94. (About liver hypertrophy.)

The Steenland and Barry reports could be discussed in Section 4.1.2, on pages 4-28 and 4-29, respectively.

RESPONSE: These three studies were added to the HESD for PFOA.

Slitt Comments

COMMENT 1: Evidence is presented for PFOA and PFOS as substrates for the related OATp1d1 in zebra fish. Establishing whether PFOS is an OATp transporter substrate is needed to better understand PFOS accumulation in liver. This study suggests that it might be. The following finding should be included in the PFOS document in Section 3.2.3 and the PFOA document in Section 3.0:

Popovic M, Zaja R, Fent K, Smital T. Toxicol Appl Pharmacol. 2014 Interaction of environmental contaminants with zebrafish organic anion transporting polypeptide, OATp1d1 (Slco1d1).

RESPONSE: This paper was not included because the literature EPA included for the transporters was limited to mammalian species.

COMMENT 2: This publication presents the finding that PFOS inhibits Pgp, Mrp1, and Mrp4 activity. The following finding should be included in the PFOS document in Section 3.2.3 and the PFOA document in Section 3.0:

Dankers AC1, Roelofs MJ, Piersma AH, Sweep FC, Russel FG, van den Berg M, van Duursen MB, Masereeuw R. Toxicol Sci. 2013 Dec;136(2):382-91. Endocrine disruptors differentially target ATP-binding cassette transporters in the blood-testis barrier and affect Leydig cell testosterone secretion in vitro.

RESPONSE: EPA reviewed this study but did not include it in the HESD for PFOS because it was not a study of PFOS per se, it was a study to determine whether the assay specified would be a reliable tool for identifying endocrine disruption. PFOS was one of a group of chemicals used to evaluate ATP-binding cassette transporters as a tool for identifying endocrine disrupters. See Appendix B in the final report.

COMMENT 3: PFOS induced ABC transporters in grey mullets.

de Cerio OD1, Bilbao E, Cajaraville MP, Cancio I. Gene. 2012 Apr 25;498(1):50-8. Regulation of xenobiotic transporter genes in liver and brain of juvenile thicklip grey mullets (Chelon labrosus) after exposure to Prestige-like fuel oil and to perfluorooctane sulfonate.

RESPONSE: EPA did not retrieve this paper because the literature EPA included was limited to mammalian studies.

COMMENT 4: These are new publications regarding epidemiology findings for PFOS and PFOA exposure and serum lipids:

Fitz-Simon N, Fletcher T, Luster MI, Steenland K, Calafat AM, Kato K, Armstrong B. Epidemiology. 2013 Jul;24(4):569-76. doi: 10.1097/EDE.0b013e31829443ee. Erratum in: Epidemiology. 2013 Nov;24(6):941.

Starling AP, Engel SM, Whitworth KW, Richardson DB, Stuebe AM, Daniels JL, Haug LS, Eggesbø M, Becher G, Sabaredzovic A, Thomsen C, Wilson RE, Travlos GS, Hoppin JA, Baird DD, Longnecker MP. Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study. Environ Int. 2014 Jan;62:104-12.

Fu Y, Wang T, Fu Q, Wang P, Lu Y. Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Ecotoxicol Environ Saf. 2014 Aug;106:246-52.

RESPONSE: Fitz-Simon et al. 2013 and Starling et al. 2014 were added to the final PFOS document. Fu et al. 2014 was not added to either HESD because it is a study of serum lipids relative to serum levels of several perfluorocarboxylates among the study population. Other studies demonstrate that the serum PFOA branched chain isomers are higher among Chinese populations compared to U.S. populations because of differences in the process used to manufacture PFOA. The Fu et al. 2014 study did not have a meaningful impact on the conclusions related to serum lipids for PFOS.

COMMENT 5: These are publications regarding PFOS exposure and hepatic steatosis:

Lv Z, Li G, Li Y, Ying C, Chen J, Chen T, Wei J, Lin Y, Jiang Y, Wang Y, Shu B, Xu B, Xu S. Glucose and lipid homeostasis in adult rat is impaired by early-life exposure to perfluorooctane sulfonate. Environ Toxicol. 2013 Sep;28(9):532-42. doi: 10.1002/tox.20747. Epub 2011 Aug 24. PMID: 23983163 Select item 22484034

Wan HT, Zhao YG, Wei X, Hui KY, Giesy JP, Wong CK. PFOS-induced hepatic steatosis, the mechanistic actions on β-oxidation and lipid transport. Biochim Biophys Acta. 2012 Jul;1820(7):1092-101. doi: 10.1016/j.bbagen.2012.03.010. Epub 2012 Mar 28. PMID: 22484034 [PubMed - indexed for MEDLINE] Free Article

Bijland S, Rensen PC, Pieterman EJ, Maas AC, van der Hoorn JW, van Erk MJ, Havekes LM, Willems van Dijk K, Chang SC, Ehresman DJ, Butenhoff JL, Princen HM. Perfluoroalkyl sulfonates cause alkyl chain length-dependent hepatic steatosis and hypolipidemia mainly by impairing lipoprotein production in APOE*3-Leiden CETP mice. Toxicol Sci. 2011 Sep;123(1):290-303. doi: 10.1093/toxsci/kfr142. Epub 2011 Jun 24.

RESPONSE: All three references were added to and described in the final HESD for PFOS.

Charge Question 3: Use of Epidemiology Data

The OW concluded that the human epidemiology data for PFOS/PFOA do not provide adequate quantifiable dose-response information for use as the basis of a candidate RfD because of uncertainty regarding the routes, levels and timing of exposures plus the confounding influences of other PFCs present in serum. Please comment of the OW characterization of the data.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: The document's authors have done a good job summarizing and accurately characterizing the epidemiology literature for various endpoints in Section 4.4 - Hazard Characterization. It is true there are a number of confounding factors that make estimation of PFOA exposures difficult. The EPA might consider, however, utilization of reverse dosimetry modeling. There is a reasonable body of data on serum PFOA levels, which could be used to estimate a range of PFOA exposures that would result in such internal doses.

RESPONSE: EPA did not use a reverse dosimetry modeling approach for this effort but rather relied on animal data and the peer reviewed PK model to develop candidate RfDs for PFOA and PFOS. The available epidemiology studies for PFOA and PFOS provide evidence of hazard from exposure to these chemicals. The HESDs for both PFOA and PFOS have been extensively revised to present a more in-depth analysis of the human epidemiology data, including a more robust discussion of the data that supports the conclusion that there is evidence of an association between exposure to these chemicals and human health hazard. For a few of the outcomes (e.g., serum lipids [PFOS and PFOA], effects on fertility and fecundity [PFOS], and pregnancy-induced hypertension [PFOA]), the associations are particularly strong and fairly consistent. There still remains some uncertainty related to other observed associations, as for various endpoints the data for both PFOA and PFOS are mixed (i.e., some studies show positive associations with the serum PFAS value while others do not).

Although mean serum values are presented in the human studies, actual estimates of PFOA and PFOS exposure (i.e., doses/duration) are not currently available. Thus, the serum level at which the effects were first manifested and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contribute to serum PFOA and PFOS values come from PFOS derivatives or precursors that break down metabolically to PFOA or PFOS. These compounds might originate from PFOA or PFOS in diet and precursor materials used in the home, which creates potential for confounding by the metabolites of the chemicals esterified to the carboxylate or sulfonate functional group. Additionally, most of the subjects of the epidemiology studies have many PFASs and/or other contaminants in their blood. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal studies. Adjusting for the other PFAS from the acid and sulfonate families is complicated because of the many commonalities in their effects, a factor often acknowledged in the epidemiology studies.

Taken together, the weight of evidence for human studies supports the conclusion that PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda. That effort may further evaluate and consider these human epidemiology studies.

PFOS-specific Comments

COMMENT 2: I agree that human epidemiology data do not provide an adequate basis for calculation of a RfD or RfC. A reverse dosimetry modeling approach, however, could be used to estimate a range of PFOS exposures that could have resulted in measured body burdens. The human data might then be utilized in the risk assessment.

RESPONSE: EPA concluded that the human studies are adequate for use qualitatively in the identification hazard, but not quantitatively at this time given the limitations described above (see more detailed response to Dr. Bruckner's comment on PFOA, directly above). EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda. That effort may further evaluate and consider these human epidemiology studies.

Cory-Slechta Comments

COMMENT 1: It is not clear that such an assertion should be used in the construction of this document. It is not clear why the route of exposure should be raised to a concern in the calculations, in fact in the human environment, there are exposures from multiple routes, no doubt and thus this is consistent with human environmental exposures. Further, if there is data on serum levels, it should reflect that cumulative exposure across exposure routes. Indeed, at the end, the goal is to arrive at an RfD based on serum levels. There is, moreover, no guarantee that there is no contamination in studies in animals from food, glassware etc.

Furthermore, in many epidemiological studies in which mixed exposures are the norm, controlling for other exposures is utilized to address this concern and to therefore make conclusions about individual exposures. In point of fact, in every single human study, there will invariably be other exposures and not a single exposure, and thus this strategy essentially says that no human studies can ever be used for any risk assessments. The stated rationales for not using human data based on these statements is not adequate. This is why it is important as well to evaluate the strengths and weaknesses of each of the studies in terms of whether appropriate controlling for other known exposures was carried out and sample sizes sufficient etc. to arrive at some conclusions with respect to their ultimate usability in constructing RfDs.

RESPONSE: EPA agrees that the human epidemiology studies provide valuable information on adverse effects resulting from exposure to PFOA and PFOS. The HESDs for both PFOA and PFOS have been extensively revised to present a more in-depth analysis of the human epidemiology data, including a more robust discussion of the data that support the conclusion that there is evidence of an association between exposure to these chemicals and human health hazard. For a few of the outcomes (e.g., serum lipids [PFOS and PFOA], effects on fertility and fecundity [PFOS], and pregnancy-induced hypertension [PFOA]), the associations are particularly strong and fairly consistent. There still remains some uncertainty related to other observed associations, as for various endpoints the data for both PFOA and PFOS are mixed (i.e., some studies show positive associations with the serum PFAS value while others do not).

Although mean serum values are presented in the human studies, actual estimates of PFOA and PFOS exposure (i.e., doses/duration) are not currently available. Thus, the serum level at which the effects were first manifest and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contribute to serum PFOA and PFOS values come from PFOS derivatives or precursors that break down metabolically to PFOA or PFOS. These compounds might originate from PFOA or PFOS in diet and precursor materials used in the home, which creates potential for confounding by the metabolites of the chemicals esterified to the carboxylate or sulfonate functional group. Additionally, most of the subjects of the epidemiology studies have many PFASs and/or other contaminants in their blood. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal

studies. Adjusting for the other PFAS from the acid and sulfonate families is complicated because of the many commonalities in their effects, a factor often acknowledged in the epidemiology studies.

Taken together, the weight of evidence for human studies supports the conclusion that PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda.

DeWitt Comments

COMMENT 1: While the OW characterization of the epidemiological data for PFOA/PFOS is, technically, true, it also is somewhat misguided. Almost any epidemiological database will contain uncertainty regarding the routes, levels, and timing of exposures and will have confounding influences of other compounds. Very few epidemiological studies are free from these uncertainties, but when similar observations and conclusions are reached from multiple studies with these types of uncertainties, the database becomes useful for determining a candidate RfD or other value relevant to human health. What is particularly valuable about the PFOA/PFOS database is that it is relatively extensive in that it includes data not only from occupationally-exposed humans, but from people highly exposed to environmental concentrations of PFOA/PFOS and from people in the general population who have detectable concentrations of these compounds. Additionally, for establishing an RfD, do all of these uncertainties need to be absent? In other words, do animal studies used to derive RfDs lack these uncertainties?

RESPONSE: EPA did not use a reverse dosimetry modeling approach for this effort, but rather relied on animal data and the peer reviewed PK model to develop candidate RfDs for PFOA and PFOS. The available epidemiology studies for PFOA and PFOS provide evidence of hazard from exposure to these chemicals.

The HESDs for both PFOA and PFOS have been extensively revised to present a more in-depth analysis of the human epidemiology data, including a more robust discussion of the data that supports the conclusion that there is evidence of an association between exposure to these chemicals and human health hazard. For a few of the outcomes (e.g., serum lipids [PFOS and PFOA], effects on fertility and fecundity [PFOS], and pregnancy-induced hypertension [PFOA]), the associations are particularly strong and fairly consistent. There still remains some uncertainty related to other observed associations, as for various endpoints the data for both PFOA and PFOS are mixed (i.e., some studies show positive associations with the serum PFAS value while others do not).

Although mean serum values are presented in the human studies, actual estimates of PFOA and PFOS exposure (i.e., doses/duration) are not currently available. Thus, the serum level at which the effects were first manifest and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contribute to serum PFOA and PFOS values come from PFOS derivatives or precursors that break down metabolically to PFOA or PFOS. These compounds might originate from PFOA or PFOS in diet and precursor materials used in the home, which creates potential for confounding by the metabolites of the chemicals esterified to the carboxylate or sulfonate functional group. Additionally, most of the subjects of the epidemiology studies have many PFASs and/or other contaminants in their blood. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal studies. Adjusting for the other PFAS from the acid and sulfonate families is complicated because of the many commonalities in their effects, a factor often acknowledged in the epidemiology studies. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal studies.

While animal studies are designed to address some of these uncertainties, these studies also have related uncertainties. EPA has addressed these uncertainties in the adoption of standard uncertainty factors (USEPA 2002).

Taken together, the weight of evidence for human studies supports the conclusion that PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda.

COMMENT 2: What is missing from the OW characterization of the epidemiological data is a thorough evaluation concerning hepatotoxicity and developmental toxicity reported in human populations and how these endpoints are relevant to or related to animal studies.

RESPONSE: EPA has added text describing how the epidemiology studies are used qualitatively as additional lines of support to the RfD in both the final HESDs and HAs. Similarities in endpoints observed in the human and animal studies are integrated in the synthesis and evaluation section (section 3.4 in each document).

Fisher Comments

COMMENT 1: The use of non-human and human data is very important for interpreting exposure extrapolations from rats. I am not an epidemiologist so I cannot comment with authority on the epidemiology data for dose-response. Justify why human data are not suitable for use in the analysis of the health hazards of PFOA and PFOS.

RESPONSE: EPA did not use a reverse dosimetry modeling approach for this effort, but rather relied on animal data and the peer reviewed PK model to develop candidate RfDs for PFOA and PFOS. The available epidemiology studies for PFOA and PFOS provide evidence of hazard from exposure to these chemicals. The HESDs for both PFOA and PFOS have been extensively revised to present a more in-depth analysis of the human epidemiology data, including a more robust discussion of the data that support the conclusion that there is evidence of an association between exposure to these chemicals and human health hazard. For a few of the outcomes (e.g., serum lipids [PFOS and PFOA], effects on fertility and fecundity [PFOS], and pregnancy-induced hypertension [PFOA]), the associations are particularly strong and fairly consistent. There still remains some uncertainty related to other observed associations, as for various endpoints the data for both PFOA and PFOS are mixed (i.e., some studies show positive associations with the serum PFAS value while others do not).

Although mean serum values are presented in the human studies, actual estimates of PFOA and PFOS exposure (i.e., doses/duration) are not currently available. Thus, the serum level at which the effects were first manifest and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contribute to serum PFOA and PFOS values come from PFOS derivatives or precursors that break down metabolically to PFOA or PFOS. These compounds might originate from PFOA or PFOS in diet and precursor materials used in the home, which creates potential for confounding by the metabolites of the chemicals esterified to the carboxylate or sulfonate functional group. Additionally, most of the subjects of the epidemiology studies have many PFASs and/or other contaminants in their blood. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal studies. Adjusting for the other PFAS from the acid and sulfonate families is complicated because of the many commonalities in their effects, a factor often acknowledged in the epidemiology studies.

Taken together, the weight of evidence for human studies supports the conclusion that PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda.

Hayton Comments

COMMENT 1: There are a number of epidemiological studies that have been based on large numbers of subjects chronically exposed (over decades in some studies) to the subject compounds over a broad range of intakes. Steady state serum concentrations have also been available for quantification of the systemic exposure. While the route, levels and timing of the exposures may have been uncertain, the long half-lives of PFOA and PFOS in humans and the long periods of exposure to them indicate that 1) subject serum concentrations were generally at steady state, and 2) daily fluctuations in the amount and timing of the exposure would not produce much day-to-day fluctuation in the serum concentration of PFOA/PFOS. These consequences of the long exposure period and long half-life indicate that variability in the route and level of exposure would not have led to a measured serum concentration that was unrepresentative of the subjects' long-term average serum concentration. The serum concentration then should be relatively stable over time and it should reflect an integrated measure of the individual's exposure to PFOA and PFOS.

The serum concentration is a quantitative measure of systemic exposure to the subject chemicals, and is arguably a better metric of exposure than are intake rate. The over-all rate of intake (R) that produces a particular steady state serum concentration (Css) can readily be calculated from the clearance (CL) of the chemicals, which is about 0.08 mL/d/kg body weight: R = Css x CL. The calculated rate of intake would represent all intake routes.

Confounding influences of other PFCs and indeed other chemicals and life-style factors such as smoking, diet, alcohol use, etc. would have to be considered, as is generally the case with epidemiological studies. Methodology exists for dealing with such influences.

Thus it appears that the epidemiological results should be used in the RfD determination. Their strength is that uncertainties associated with extrapolation from laboratory animal studies are avoided. Health effects that are positively associated with serum PFOA/PFOS concentration and that are observed in large populations of subjects should seriously be considered as potentially arising from PFOA/PFOS exposure. If mode of action studies in lab animals or in vitro studies support a cause-effect relationship, then the threshold serum concentration could inform the calculation of the RfD.

RESPONSE: Calculations to predict the steady-state concentration (C_{ss}) for each of the average serum values used in quantification were included in the final report.

EPA did not use a reverse dosimetry modeling approach for this effort, but rather relied on animal data and the peer reviewed PK model to develop candidate RfDs for PFOA and PFOS. The available epidemiology studies for PFOA and PFOS provide evidence of hazard from exposure to these chemicals. The HESDs for both PFOA and PFOS have been extensively revised to present a more in-depth analysis of the human epidemiology data, including a more robust discussion of the data that support the conclusion that there is evidence of an association between exposure to these chemicals and human health hazard. For a few of the outcomes (e.g., serum lipids [PFOS and PFOA], effects on fertility and fecundity [PFOS], and pregnancy-induced hypertension [PFOA]), the associations are particularly strong and fairly consistent. There still remains some uncertainty related to other observed associations, as for various endpoints the data for both PFOA and PFOS are mixed (i.e., some studies show positive associations with the serum PFAS value while others do not).

Although mean serum values are presented in the human studies, actual estimates of PFOA and PFOS exposure (i.e., doses/duration) are not currently available. Thus, the serum level at which the effects were first manifest and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contribute to serum PFOA and PFOS values come from PFOS derivatives or precursors that break down metabolically to PFOA or PFOS. These compounds might originate from PFOA or PFOS in diet and precursor materials used in the home, which creates potential for confounding by the metabolites of the chemicals esterified to the carboxylate or sulfonate functional group. Additionally, most of the subjects of the epidemiology studies have many PFAS and/or other contaminants in their blood. Although the study designs adjust for other potential toxicants as confounding factors, their presence constitutes a level of uncertainty that is usually absent in the animal studies. Adjusting for the other PFAS from the acid and sulfonate families is complicated because of the many commonalities in their effects, a factor often acknowledged in the epidemiology studies.

Taken together, the weight of evidence for human studies supports the conclusion that PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda.

Longnecker Comments

COMMENT 1: As noted in the General Impressions section above, the human studies with data on plasma or serum concentrations of PFOA and PFOS, especially for several categories of such levels, could be used to estimate dose-response information. However, there are other reasons why the human data may not be useful for setting the RfD (see above). Either PK or PBPK models might be useful for estimating the dose that human are exposed to; an advantage of a PBPK model is that it could incorporate information about routes and timing of exposure. Estimates of the contribution of various routes are available (e.g., Haug et al. 2011; Lorber & Egeghy 2011), and exposure trends could be assumed and evaluated in sensitivity analyses. Some occupational studies had data that allowed an estimate of serum levels, or measured them directly. Several reports show estimated exposure based on serum concentrations of PFOA or PFOS (Locissano et al. 2013; Lorber & Egeghy 2011; Thompson et al. 2010). With respect to confounding, the assessment of how likely this is could be informed by: 1) the correlation of serum concentration of PFOA, PFOS, and other compounds of this type in a particular study population (or in a series of studies), and 2) whether the other compound(s) has been associated with the particular outcome being considered. If the correlation is low or the other compound has not been associated with the outcome, concern about confounding may not be strongly justified. Without additional consideration of data that address these points, it may be premature to assume confounding would be a problem.

RESPONSE: A variable portion of the exposures to the general population comes from household and workplace sources (e.g., carpets, furnishings) that contain precursors that degrade metabolically and abiotically to PFOA or PFOS, especially from household dusts and ambient air. These exposures would contribute to the concentrations of serum PFOA and PFOS. Telomere alcohol PFOA derivatives and PFOS/A derivatives that break down metabolically to PFOA and PFOS after consumption are a potential source in addition to direct exposure to PFOA and PFOS. These derivatives can be metabolized and form not only PFOA and PFOS, but other chemically reactive metabolites. Thus, the potential for results to be confounded by the other metabolites adds uncertainty in the observed associations between serum PFOA and health effects. These compounds can originate from the diet and materials used in the home; thus, there is potential for confounding in the C8 studies where the drinking water PFOA was considered to be the primary medium of exposure and for PFOS precursors where degradation produces amines that could contribute to the effects observed. In contrast, in the animal studies, test organisms were dosed with either PFOS or PFOA and potential confounding with other metabolites is reduced.

Taken together, the weight of evidence for human studies supports the conclusion that PFOA and PFOS exposure is a human health hazard. At this time, for the development of the RfD in support of the development of a drinking water health advisory for PFOA and PFOS, OW concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals. EPA plans to begin another effort to determine the range of perfluoroalkyl compounds for which an IRIS assessment is needed, as indicated in the 2015 IRIS Multi Year Agenda. That effort may further evaluate and consider these human epidemiology studies.

Slitt Comments

COMMENT 1: *Strengths of the studies*: Several studies, which all demonstrate a positive association between serum PFOA and/or PFOS and cholesterol or LDL levels are based on drinking water as a route of exposure. These studies are in agreement with Nelson et al., 2010, which was analyzing data from the 2003-4 NHANES study. Steenland et al., 2009 (*Environ Health Perspect*. Jul 2009; 117(7): 1083–1088) as part of the C8 Health Project collected data on 69,030 subjects with findings that serum PFOA was higher for males, those consuming local vegetables, and those using well water rather than public water, and *lower for those using bottled water.* The estimated response rate for participants >20 years of age was 81% and mean serum PFOA concentration was 83 ng/l. Subjects were eligible to participate in the C8 Health Project if they had consumed drinking water for at least one year before 3 December 2004 supplied by Little Hocking Water Association (Ohio), City of Belpre (Ohio), Tuppers Plains Chester Water District (Ohio), Village of Pomeroy (Ohio), Lubeck Public Service District (West Virginia), Mason County Public Service District (West Virginia), or private water sources within these areas that were contaminated with PFOA. Subjects were also eligible if they could document that they had either worked in a contaminated water district or went to school there for at least one year. From this population, which the route of exposure is considered to be primarily via drinking water, serum lipids were analyzed with regard to PFOA levels and a positive correlation was observed for all serum lipids except HDL. Frisbee further characterized this cohort, analyzing 12, 476 children and adolescents included in the C8 Health Project, finding an increase in total cholesterol.

A recent epidemiology study (Fitz-Simon et al., 2013), not included in the current documents, described positive associations between PFOA and PFOS in serum LDL cholesterol. This study examined a study population that consisted of 560 adults living in parts of Ohio and West Virginia where public drinking water had been contaminated with PFOA. They had participated in a cross-sectional study in 2005-2006, and were followed up in 2010, by which time exposure to PFOA had been substantially reduced. Overall, the findings demonstrate a positive association between serum PFOA and PFOS levels and serum and LDL cholesterol.

RESPONSE: Fitz-Simon et al. 2013 was added to the document. EPA agrees that the human data for PFOA and PFOS indicate that exposure to these chemicals can impact serum lipids. The human studies are now summarized in the HESD and reported for different endpoints, including reproductive and developmental endpoints. The strongest associations are related to serum lipids with increased total cholesterol and high density lipoproteins (HDLs).

COMMENT 2: *Weaknesses:* The studies did not appear to analyze PFOS or PFOA levels in drinking water from the participants analyzed and did not analyze data based on the length of exposure.

RESPONSE: The studies of the C8 community included information on the concentrations in drinking water for the impacted public water systems. Concentrations varied temporally within systems and between systems based on the information available.

Charge Question 4: Characterization of Epidemiology Data

Please comment on the transparency and characterization of the epidemiological data.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: See comments above.

RESPONSE: See corresponding responses above.

PFOS-specific Comments

COMMENT 2: The document's authors have done a good job describing and summarizing the designs and findings of the epidemiology studies.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Cory-Slechta Comments

COMMENT 1: The PFOA document in particular and to some extent the PFOS document present all of the epidemiological studies but do not actually evaluate them; there is not a consistent indication of individual strengths and limitations of the studies, failures or not to adequately control potential confounding variables. Furthermore, there is no 'power analysis' type of evaluation, i.e., some of these studies included very small sample sizes and thus their power to actually detect effects may be limited, and yet they all appear to be weighted basically the same, i.e., studies with very small sample sizes with obviously extremely limited power to detect any effects appear to be considered the same as those with extremely large sample sizes. Studies with small sample sizes that nevertheless do find an effect of PFOA or PFOS actually suggest a robust type of effect.

RESPONSE: The human epidemiology section in both of the final HESDs has been substantially rewritten to include details on study type, sample size, and serum levels where these data were available. Tables were expanded for each major endpoint to summarize the studies described in the text as recommended by the peer reviewers and are now included in Appendix B. Additional, more detailed tables were added to sections 3.1.1 and 3.1.2. They include quantitative information from the studies. An overall summary and conclusion for the cancer and noncancer endpoints was added at the end of the epidemiology section (sections 3.1.2.1 and 3.1.1.12 for PFOA and 3.1.2.1 and 3.1.1.8 for PFOS).

COMMENT 2: The discarding of positive associations in human epidemiological studies because they do not produce frank clinical disease seems inappropriate and inconsistent with other EPA documents. For example, p. 4-3 in the PFOS document states that only a small number of ALT values were outside the normal range making the results difficult to interpret in terms of health. Physiological changes that are moving in the wrong direction, even if sub-clinical at the time, are still adverse effects. Are actual clinical diagnoses required for an adverse effect? This is especially the case given that the ranges of normal across populations are extremely broad.

RESPONSE: The reviewer is correct in that associations in the absence of clinical disease should not be ignored. In the HESDs, EPA described these studies and used them as another line of evidence to support the finding from animal studies.

COMMENT 3: The latter also raises the question of the cumulative toxicity of PFOA and PFOS and whether any consideration is being given to this.

RESPONSE: Cumulative exposure and toxicity was taken into consideration when calculating the drinking water health advisory values. Because of the similar toxicological effects at similar concentrations (the RfD for PFOA and PFOS are both 0.00002 mg/kg/day), where PFOA and PFOS co-occur at the same time and location at a drinking water source, EPA recommends that the health advisory guideline be applied as the sum of the concentrations (i.e., additive lifetime health advisory for [PFOA] + [PFOS] = 0.07 μg/L).

COMMENT 4: Another such example is in the PFOS document, where it actually refers to a statistically significant, but not toxicologically significant effect (p.4-38); what does that mean? Also, p. 5-4 appears to dismiss any changes in thyroid function since no evidence of clinical hypothyroidism actually occurred. This whole approach with the human studies seems quite inconsistent with the reliance on increased liver weight in the absence of clinical pathology as the endpoint in the human studies.

RESPONSE: The sections on thyroid effects in humans were completely revised and new data were added. The statement about a toxicological significance referenced in the comments is no longer in the document. The revisions to the thyroid epidemiology data conclude that "generally null associations were found between PFOA and TSH in people who have not been diagnosed with thyroid disease." The conclusions of the C8 Panel and other human studies are described in the HESDs and used as a line of evidence supporting the assessment.

DeWitt Comments

COMMENT 1: It is not obviously or abundantly clear how the OW characterized the epidemiological data for either PFOA or PFOS. The studies were well-described, but the contribution of particular studies to the overall assessment was not. The results of studies described in the hazard characterization section (4.4) need to be better characterized. For example, in the PFOA risk assessment:

COMMENT 1a: An increase in serum lipids associated with PFOA/PFOS exposure in humans is discussed as a risk factor for cardiovascular disease in humans; however, no evidence of increased cardiovascular disease has been observed in human populations as related to either chemical. Additionally, serum lipids typically are decreased in animal models after PFOA/PFOS exposure, which is thought to be associated with/typical of exposure to agents that activate PPARα. If humans are known to respond to PPARα activators (i.e., fibrate drugs), why would the results between humans and animal models be discordant? This should be discussed.

RESPONSE: The most consistent response observed in the epidemiology studies related to serum lipids is a positive association in serum total cholesterol. The epidemiology data for PFOA showed a weak association with increased LDL cholesterol, but no association for HDL cholesterol. For PFOS, when there was an association with LDL and HDL, it was positive for both lipoprotein complexes. The only available studies in animals evaluating serum cholesterol for PFOA and PFOS show a decrease. There are also animal data comparing the effect of PFOA and PFOS to the fibrate Wy 14,643 on cellular histological and biochemical changes and gene activation in the liver. These studies demonstrate that the cellular histological and biochemical changes and patterns of gene activation share some commonalities but also have distinct differences. In humans, treatment with fibrates usually results in a decrease in serum LDL cholesterol and increase in HDL cholesterol (Staels et al. 1998). The animal data do not provide an explanation for the differences in the observations of increased total cholesterol in humans. The mode of action for the observed effects on PFAS impact on serum lipids in humans is not completely understood and rodents may be impacted differently compared to humans by PPARα stimulation when it comes to lipid metabolism. The HESDs describe how PPAR stimulation is involved with lipid metabolism.

COMMENT 1b: Several epidemiological studies reporting changes in liver enzymes clearly state that the clinical relevance of the changes in enzymes is unknown. Therefore, stating that the human studies "suggest effects on the liver as indicated by increases in liver enzymes" amounts to a mischaracterization of the data.

RESPONSE: There is indirect evidence of an effect on the liver in humans, as indicated by changes in several enzymes that are biomarkers of liver damage. The human epidemiology studies have been revised to further describe these studies. The human epidemiology studies varied in the enzymes biomarkers they evaluated and the results differed across studies; this is described in the HESDs. In the case of PFOA, an association of serum PFOA concentration with elevations in serum levels of ALT and gamma-glutamyl transpeptidase (GGT) was consistently observed in occupational, highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential to affect liver function. For PFOS, there was a slight positive association between serum PFOS levels and increased serum ALT values. The association between PFOS levels and increased serum GGT levels was less defined and overall did not appear to be affected.

Very few of the animal studies examined these liver enzymes other than Seacat et al. 2002, Thomford 2002/Butenhoff et al. 2012[7], where there was a significant increase in ALT but not AST at some doses. Neither GGT or LDH were evaluated in the animal studies.

COMMENT 1c: No direct evidence of hepatotoxicity has been reported in epidemiological studies. This should be discussed.

RESPONSE: Considering ALT and GGT results, effects on the liver are suggested; however, hepatotoxicity was not reported in epidemiological studies. In an epidemiology study of highly exposed members of a general population and based on collected serum, information that would inform a diagnosis of hepatotoxicity is unlikely to be available unless medical records were obtained for the individual subjects.

COMMENT 1d: More in-depth characterizations are needed for the additional sections of the hazard characterization, with the exception of the thyroid section, which was well-described.

RESPONSE: The synthesis and evaluation sections were updated in both documents to better characterize human and animal findings. The revised sections include a comparison between the outcomes from the epidemiology as they compare with the data from the animal studies to emphasize the consistencies and inconsistencies between findings. The characterization of each of the responses observed in the epidemiology studies is covered in the new summary sections for the noncancer and cancer epidemiology findings and integrated with the findings from the animal studies in the synthesis of hazard section 3.4.

COMMENT 2: For example, in the PFOS risk assessment: Similarly to the PFOA risk assessment, the hazard characterization section needs to better discussion differences and similarities between effects reported in humans and effects reported in animal models.

RESPONSE: The synthesis and evaluation sections were updated in both documents to better characterize human and animal findings for both cancer and noncancer. The revised sections include a comparison between the outcomes from the epidemiology as they compare with the data from the animal studies to emphasize the consistencies and inconsistencies between findings.

[7] Thomford (2002) is unpublished, but it contains the raw data. Butenhoff et al. (2012) is the published study.

Fisher Comments

COMMENT 1: I am not an epidemiologist, but it appears to be adequate. Better characterization of the pros and cons of the human analyses and interpretation of the outcomes would be helpful.

RESPONSE: The human epidemiology section was substantially rewritten with study type, sample size, and serum levels added for each study where these data were available. Tables for each major endpoint were expanded to summarize the studies described in the text. An overall summary and conclusion was added at the end of the epidemiology section.

Hayton Comments

COMMENT 1: The characterization of the individual epidemiological studies presented seems to be adequate. Public comments have identified the need to distinguish positive and negative associations with statistical significance, which seems to be a fair criticism. As noted in the response to Question 2, there are relevant studies that have not been described in the health-effects documents that ought to be considered and this includes some epidemiological studies. Most of the cited epidemiological studies have focused on healthy adults – workers exposed occupationally, residents of communities with or without contaminated water. These populations might be expected to be less sensitive to adverse effects than would early life stages and particular disease populations. Studies of potentially more sensitive populations would be desirable. The Frisbee et al. (2010) study of children 1-11.9 years and adolescents 12-17.9 years showed significant positive associations with serum lipid levels. Studies such as this one would be informative.

RESPONSE: The human epidemiology section was substantially rewritten with study type, sample size, and serum levels added for each study where these data were available. EPA reviewed the Frisbee et al. (2010) study and added it to both the HESD for PFOA and PFOS. See Appendix B for a list of the epidemiology studies that were retrieved, reviewed, and included in the revised HESDs.

Longnecker Comments

COMMENT 1: Please see the long paragraph above, under General Impressions, and some of the comments in response to item #2 above. Another point that the authors may want to consider is that studies that examine external exposure in relation to health outcomes may have special advantages in the case of PFOA and PFOS. While in general it is considered best to have a measure of exposure that is based on a biomarker of internal exposure, this may be problematic for several outcomes for PFOA and PFOS, because of the possibility of confounding or reverse causality that would not be an issue if an external estimate of exposure were used. For example, in Steenland K, Zhao L, Winquist A., a cohort study of workers exposed to PFOA., (Occup Environ Med. 2014 Jun;71 Suppl 1:A55), when an external estimate of exposure was used for the Washington Works employees, no association with elevated cholesterol was found. The Viera et al. (2013) results are based on external estimates of exposure, whereas the similar study by Barry et al. (2013) are based on serum levels or estimates based on serum levels. The fact that association with kidney cancer is present in the Viera study decreases concern that the association was due to reverse causality. Steenland et al. 2012 used an external estimate of exposure to study cancer mortality and also found an association with kidney cancer. Lundin et al. (external estimate of exposure) had no cases of kidney cancer, though their study was also small.

RESPONSE: In the revisions to the HESDs, EPA discussed cases where associations can partially be due to other factors such as BMI, age and diet for elevated cholesterol, and low GFR for low birth weight. The studies specified in the comment (Barry et al. 2013; Lundin et al. 2009; Steenland et al. 2015; Vieira et al. 2013) are all included in the document, as well as a discussion of the links between PFOA and kidney cancer.

Slitt Comments

COMMENT 1: The epidemiology data is well described and a thorough read. The data would be put in better context for the reader if there are average serum concentrations or ranges for the studies summarized in tables in addition to other key pieces of information.

RESPONSE: The human epidemiology section in both documents was substantially revised with study type, sample size, and serum levels added for each study where these data were available. Tables were expanded for each major endpoint to summarize the studies described in the text. An overall summary and conclusion section was added at the end of the epidemiology section in each document for both the noncancer and cancer endpoints. There are two sets of epidemiology tables in the final document. Detailed tables presenting quantitative data from the individual studies are in sections 3.1.1 and 3.1.2 and the updated original summary tables are now included with Appendix B.

COMMENT 2: A recent publication should be included in the document for consideration. Fitz-Simon N, Fletcher T, Luster MI, Steenland K, Calafat AM, Kato K, Armstrong B. Epidemiology. 2013 Jul;24(4):569-76. doi: 10.1097/EDE.0b013e31829443ee.

RESPONSE: This study (Fitz-Simon et al. 2013) was added to both of the final HESDs.

Charge Question 5: Cancer Classifications

The OW has concluded that the cancer classifications for PFOA and PFOS are most consistent with respective classifications of suggestive evidence for carcinogenicity as described the EPA Guidelines for Carcinogen Risk Assessment (pp. 2-56, 2-57). Please comment on the strengths and weaknesses of this classification.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: I agree with EPA's choice of "Suggestive Evidence for Carcinogenicity." Epidemiological findings in occupationally-exposed and general populations to date are equivocal. Increases in Leydig cell tumors and liver adenomas have been reported in high-dose male rats. Increased incidences of pancreatic cell hyperplasia/adenomas and ovarian stromal hyperplasia/adenoma have been observed in female rats. More studies are necessary to confirm/expand these findings, and to assess carcinogenic potential in other species. Most mutagenicity and genotoxicity assays have been negative. Thus, there is some, but not undue cause for concern about the human carcinogenic potential of PFOA.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

PFOS-specific Comments

COMMENT 2: The document's authors have adequately and convincingly presented evidence for classifying PFOS as "suggestive of carcinogenicity."

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Cory-Slechta Comments

COMMENT 1: The classification of both PFOA and PFOS evidence for carcinogenicity as suggestive seems consistent with the clear limitations in the available data bases. In addition, the animal studies are limited to one species and mutagenicity does not occur in response to PFOA.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

DeWitt Comments

COMMENT 1: This classification is appropriate for both PFOA and PFOS given the epidemiological evidence, which is somewhat limited for PFOA and quite limited for PFOS. For PFOA, there is an association between kidney and testicular cancer, but there are limited data in animal models for these cancers and there is uncertainty that the mechanism of PFOA-induced carcinogenicity in animal models is applicable to humans. Studies of PFOS have the same limitations, but epidemiological studies have failed to find an association between PFOS exposure and cancer.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Fisher Comments

COMMENT 1: I did not review the cancer studies for PFOA and PFOS.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Hayton Comments

COMMENT 1: The classification of "suggestive" is not unreasonable. The epidemiological studies, while showing apparent associations between PFOA exposure and cancer incidence in testicle and kidney as well as other tissues, do not provide a cause-effect relationship. However, they certainly do raise a concern about the carcinogenicity of the subject substances. Studies in animals have demonstrated conclusively that PFOA causes liver cancer in rats but the MOA that involves PPAR activation is absent in humans and it has been concluded that PFOA and PFOS cannot be carcinogenic in humans via this mechanism.

An EPA SAB panel (2006) consideration of this question resulted in a majority of the panel members favoring a classification of "likely to be carcinogenic" for PFOA. Board members acknowledged the PPAR MOA argument against causation of cancer in humans, but also found evidence that liver cancer in rats administered PFOA may also have had a MOA independent of PPAR activation. Recent epidemiological studies have added to the weight of evidence for an association between PFOA/PFOS exposure and cancer. Therefore a classification of "likely" is also not unreasonable to this reviewer. Lacking expertise in the nuances of applying the EPA's classification scheme, it is difficult for this reviewer to argue in favor of either "suggestive" or "likely".

RESPONSE: Under EPA's *Guidelines for Carcinogen Risk Assessment* (USEPA 2005) there is *suggestive evidence of carcinogenic potential* of PFOA in humans. The bioassay findings for Leydig Cell testicular tumors in rats combined with the C8 Panel finding of a probable link to testicular and renal tumors among the members of the C8 Health Project support this conclusion.

In June 2014, 20 experts met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of PFOA, among other chemicals. Although the assessments have not yet been published (to be published in volume 110 of the IARC monographs), the expert findings from this meeting are available in a peer-reviewed publication (Benbrahim-Tallaa et al. 2014) and their determination is available on the IARC website. The working group classified PFOA as *possibly carcinogenic to humans (Group 2B)* and considered the evidence regarding mechanisms of PFOA-associated carcinogenesis to be moderate. This assessment did not lead to a change in the overall classification of PFOA by IARC.

With regard to mode of action, please see section 3.4.3 in the final HESD for PFOA.

Longnecker Comments

COMMENT 1: The classification as "suggestive evidence for carcinogenicity" for both PFOA and PFOS is consistent with the guidelines put forth in the EPA Guidelines for Carcinogen Risk Assessment (2005). There are few pertinent data, including some suggestive but weak human evidence. There is clearly not enough evidence to classify these agents as likely human carcinogens.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Slitt Comments

COMMENT 1: Overall, the assessments for each PFOS and PFOA appear to be consistent with the EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a). Strengths: Both classifications use evidence from human studies as guidance.

COMMENT 1a: PFOS: The limited data that exist regarding PFOS and cancer were presented, the classification for PFOS under the EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a) is currently consistent with the suggestive evidence of carcinogenic potential descriptor. This assessment is reasonable, given that it is based on two studies that show a slight increase in adenomas that occurred in males and females.

COMMENT 1b: PFOA: There is conflicting evidence regarding PFOA exposure and cancer risk. However, several human studies have found associations between PFOA exposure and elevation of cancer of the bladder and kidney. This is also supported by a chronic bioassay in rats, which demonstrated that PFOA was tumorigenic.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Charge Question 6: Use of Pharmacokinetic Model

Significant interspecies differences in pharmacokinetics exist for both PFOA and PFOS. Adjusting for interspecies differences was an important step in developing candidate RfDs given the totality of the human and animal data. Please comment on the strengths and weaknesses of the pharmacokinetic model adjustments to accommodate the impact of albumin binding and renal tubule transporters in determining average serum values.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: The adjustments made to accommodate the influence of albumin binding and saturable renal tubular resorption of PFOA seem reasonable. I would defer, however, to someone with more experience in providing for these processes in PBPK models.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

PFOS-specific Comments

COMMENT 2: The PBPK model adjustments to estimate human equivalent doses appear to be appropriate. I defer to someone more qualified on the subject.

RESPONSE: Comment is acknowledged; EPA notes that the model used was not physiologically based, but rather an empirical model with physiological (i.e., saturable resorption in the kidney proximal tubules) motivation.

Cory-Slechta Comments

COMMENT 1: This falls outside my area of expertise and therefore no significant comments are provided. However, at the face-to-face meeting there was significant discussion regarding the modeling, including clarifications from Dr. Wambaugh that were found by those panel members with expertise to clarify these issues and these particular issues were considered adequately addressed.

RESPONSE: The clarifying information provided by Dr. Wambaugh at the meeting was used to update the modeling sections of both HESDs.

DeWitt Comments

COMMENT 1: Several PK models have been reported in the literature for these compounds and are relatively well described in the documents. The documents assert that the existing PK models do not consider the impact of renal tubule transporters and albumin binding; while, many of the existing models appropriately predict serum concentrations in humans and other species, but they are mostly based on empirical models. Please explain the weaknesses of such empirical models.

RESPONSE: EPA chose to use mathematical models of pharmacokinetics to allow extrapolation within species. Extrapolation assumes that the model accurately captures the relevant phenomena in a way that is applicable to both the calibration data and the new situation for which predictions (extrapolations) are made. In this document, EPA performed within species extrapolation between different dose regimens using a model that was empirically calibrated to PK data for the relevant species. This makes the assumption that the empirical calibration has captured the biological aspects for that species. EPA did not extrapolate across

species because there is currently no model available that explains the cross-species differences in clearance (or half-lives) of the chemicals.

COMMENT 2: Additionally, numerous studies for both compounds report serum and tissue concentrations in humans and other species, which can be compared to existing models. Both documents present a revised model that amounts to a reanalysis of data from studies that report serum concentrations. A more thorough discussion of the improvements made by the reanalysis is needed to better understand if the improved model adequately estimates or predicts the clearance rate and other parameters for which confidence is low. Alternatively, the publication (Wambaugh et al., 2013) that thoroughly describes the reanalysis could be referenced.

RESPONSE: A description of the publication (Wambaugh et al. 2013) was added to section 2 of both HESDs with the description of the other available models.

Fisher Comments

COMMENT 1: Serum protein binding: Both PFOA and PFOS are highly bound in serum proteins across species, thus model adjustments seem trivial for interspecies extrapolation. Steady-state conditions can be assumed to estimate the free fraction (e.g., 2% based on paper by Han et al., 2005 for humans). I did not find a discussion about the half-life of serum proteins, which may have some influence on the 'apparent' serum half-life of PFOA and PFOS. The estimated fraction of free PFOA or PFOS is important for describing urinary and fecal elimination in rats (and other species) and the plasma concentrations of total PFOA and PFOS. Thus, the model predicts total PFOA and PFOS in serum or plasma, but the free fraction estimates drive the gradual clearance of total BPA from plasma or serum by describing clearance of free.

RESPONSE: The reviewer is correct that the chemicals are highly bound and EPA's model incorporates this. The Andersen et al. (2006) model does include binding of PFOA and PFOS when predicting urinary elimination. Fecal elimination is not included in that model. The analysis of the available pharmacokinetic (PK) studies estimated that both chemicals were highly bound across species. The half-life of human serum albumin is long (16–18 days) and levels remain stable except in extreme malnutrition or those with infections, burns or severe injuries. The binding of PFOA to albumin is much greater than that to other serum proteins (see section 2 in the PFOA HESD). No data were identified that examined PFOS binding to other serum proteins.

COMMENT 2: Renal reabsorption: The renal reabsorption hypothesis involving species specific and sometimes gender specific transporters to describe the pharmacokinetic data represents sound judgment. This departure from normal allometric scaling is suggestive of active transport processes. Few PBPK models explicitly describe transporters with drugs or chemicals, although the field is moving in this direction. Thus, the approach used for PFOA and PFOS is adequate, that is, a hypothesis was evaluated by employing empirical PK-based kinetic analyses. Because the mechanistic details are missing for each species/gender, scaling of this biological phenomenon is not possible at this time. This is not a weakness, but represents the state of the science.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Hayton Comments

COMMENT 1: A very important strength of the documents is the attempt to deal with the interspecies differences in pharmacokinetics so that adverse effects across species are compared on the basis of internal, systemic exposure to PFOA and PFOS, instead of basing comparisons on the administered mg/kg dosages. PFOA and PFOS have complicated pharmacokinetics that have proven difficult to model. While a relatively

simple one-compartment model appears adequate to analyze single, low doses, this model fails when it is extended to higher doses and repeated doses. Nonlinearities appear associated with saturable plasma protein binding and with saturation of transporters thought to be involved in the reabsorption of the compounds from renal filtrate.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

COMMENT 2: A weakness of the pharmacokinetic model adjustments is the lack of robustness of the models. Despite the extensive efforts of talented pharmacokineticists, development of a model that scales across species and handles a range of dosages and a variety of administration routes has proven elusive. The two compartment model of Andersen et al. (2006) has formed the basis of the model used in the draft documents. The model incorporates saturable resorption of PFOA and PFOS from renal tubular filtrate. While protein binding is known to be saturable (fraction free increases with concentration), the model uses a species-specific but constant free fraction. Model parameter values for mouse, rat and monkey were used to predict reasonably well measured serum concentrations after a fixed daily dosing regimen, Tables 5-6 – 5-8 for PFOA and 5-8 and 5-10 for PFOS. This agreement between predicted and measured serum concentrations gives confidence that the model-calculated AUC values and final serum concentrations associated with adverse health effects (or in the case of liver weight, biological marker of exposure) are realistic and a basis for estimation of RfD. While the model used appears adequate for the purpose, the model parameters that were used have some markedly non-physiological values. (Information subsequently provided at the reviewers meeting explained some of the departure from expected physiological values, as discussed in a following section.)

RESPONSE: The model used (Andersen et al. 2006) has been demonstrated to be robust across doses, as in Wambaugh et al. (2013) in which good predictive agreement was found across multiple dose regimens in differing studies. This is described in both the PFOA and the PFOS HESDs. When sufficient data exist to allow calibration to specific species, the same model structure, albeit with different parameters, was also shown to be robust in Wambaugh et al. (2013). However, EPA agrees with the reviewer that a unified model would eventually be ideal when the state of the science permits it.

In the PFOA HESD, EPA states that "in this case, an oral dosing version of the original model introduced by Andersen et al. (2006) and summarized early in section 2.6.1 was selected for having the fewest number of parameters that would need to be estimated. A unique feature of the Wambaugh et al. approach was to use a single model for all species in the toxicological studies to examine the consistency in the average serum values associated with effects and with no effects from nine animal studies of PFOA." A similar discussion appears in the PFOS HESD.

The "non-identifiability" of some parameters is discussed below. For some species the PK data are limited such that parameters may take extreme values and still be consistent with the available data. It is for this reason that protein binding and other potentially non-linear processes have not yet been specifically modeled – there are insufficient data to inform such models. The general, non-linear form of the Andersen et al. (2006) model is assumed to capture all relevant non-linearity for which there is evidence.

Longnecker Comments

COMMENT 1: For PFOA and PFOA, the MCMC model results (predicted final serum value) were compared to the measured final serum values, and the agreement was fairly good. For PFOS, the MCMC model results were compared to those from Loccisano et al. (2012b) and were found to be similar, which is also reassuring. Because the PBPK models of PFOA and PFOS are empirical, and have been shown to give results that agree reasonably well with observed data, the adjustments to accommodate the impact of albumin binding and renal tubule transporters are not critical. More data on albumin binding and renal tubule

transporters might allow improved understanding of the pharmacokinetics of these compounds, but may not necessarily cause substantial improvements in the empirical predictions from current models.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Slitt Comments

COMMENT 1: The current weakness of the models is that data on species differences in PFOA and PFOA for various key transporters is limited and the document is also using mRNA data for various transport proteins to explain gender differences in urinary elimination. First, with regard to PFOS accumulation in the liver compartment, it is necessary to compare affinity of human versus rat for OATp mediated transport. This alone is tricky because of species differences in OATps. If PFOS-induced liver effects are related to PFOS accumulation in liver, it is would be helpful to understand whether a lower affinity of human OATp1b1 and 1b3 compared to rat OATp1a1 predicts lower hepatic PFOS accumulation. More is known about PFOA, but a similar argument can be made for PFOA. In addition, more comprehensive, controlled assessment of renal transporter affinity for PFOA and PFOS is needed to better model the species difference in urinary elimination.

RESPONSE: Agreed, but to EPA's knowledge at present this type of information does not exist. EPA agrees that further refinement of the model would eventually be ideal when the state of the science permits it.

COMMENT 2: The document often speculates about PFOA or PFOS regulation of transporter expression, but some papers cited (Cheng and Klaasen) do not have enough data at the protein level to support whether these differences in transporter expression are the drivers of toxicokinetic differences between males and females.

RESPONSE: There are a number of studies that demonstrate an impact of hormones on renal resorption for PFOA transporters in rats that elucidate their impact on renal excretion and appear to relate to transporter expression. See section 2.5.1 of the HESD.

Charge Question 7: Selected Parameters of Pharmacokinetic Model

Table 5-5 in the PFOA document and Table 5-7 in PFOS document list the parameters used for the ORD pharmacokinetic models that provide the final serum and AUC values for calculating the internal dose point of departure for the RfD calculation. Please comment on the strengths and weaknesses of the selected parameters.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: Despite the complexities and unknowns involved in plasma protein binding and renal tubular functions (i.e., glomerular filtration, basolateral tubular excretion and resorption, and apical tubular excretion and resorption), it is necessary to: (a) simply model only for saturable tubular resorption; and (b) use a range, or distribution of parameter values consistent with existing kinetic data. Unfortunately, optimization sometimes results in selection of physiological parameters that are not biologically-realistic, or plausible.

RESPONSE: The Andersen et al. (2006) model was selected because it was the simplest model that described the non-linear serum kinetics as a function of dose. Saturable resorption from the kidney is a likely hypothesis for the mechanism, but it is not unique. The difficulty interpreting the values of the model are a limitation of the available PK data, not the model itself. "Optimization" was not performed, rather a formal Bayesian statistical analysis was performed, which generated all possible model parameterizations that were consistent with the available data. Since some (but notably not all) parameter combinations are not consistent with the physiology of saturable renal resorption, this reflects uncertainty with respect to that hypothesis. This uncertainty has been propagated into the predicted serum concentrations for the animal studies.

PFOS-specific Comments

COMMENT 2: The parameters used in the modeling are biologically plausible.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Cory-Slechta Comments

COMMENT 1: This falls outside my area of expertise and therefore no significant comments are provided. However, at the face-to-face meeting there was significant discussion regarding the modeling, including clarifications from Dr. Wambaugh that were found by those panel members with expertise to clarify these issues and these particular issues were considered adequately addressed.

RESPONSE: The model descriptions in the HESD capture the clarifications described to the panel members. In particular, the HESD emphasizes that a single PK model was used to reanalyze all available data. The tables containing the new PK parameter estimates have been retitled "Pharmacokinetic parameters from Wambaugh et al. (2013) meta-analysis of literature data" to further indicate that this reanalysis occurred.

COMMENT 2: One unclear component of Table 5-7 in the PFOA document is the column labeled Species/Strain Used for prediction, which in every case is the same as the column labeled Species/Strain and is not otherwise adequately explained.

RESPONSE: This information is provided to be consistent with other tables in which the strain used for determining pharmacokinetics could have differed from the strain used in the toxicity study.

DeWitt Comments

COMMENT 1: It is not clear that the parameters in Table 5-5 in the PFOA document and Table 5-7 in the PFOS document are from the Andersen et al. 2006 PK model or if they are parameters used in the reanalysis of the data. This needs to be better explained in both documents. Additionally, all of the units in the tables need to be explained and re-checked for accuracy.

RESPONSE: The methods used in model development have been better described to note that this model is a reanalysis of existing data. Units have been checked and corrected where needed.

Fisher Comments

COMMENT 1: The authors should entertain the calculation of data derived AUC (e.g. Table 5-6) to compare to the model derived AUC, just as was done with measurement of total PFOA in serum. This works for the animal studies. The choice of using the empirical model over the more recent physiological models may be a weakness and our understanding of transporters advance. The evolution of chemical-specific PBPK models for use in risk assessment and regulatory applications has repeated itself several times. This is, the first empirical non-physiological model(s) or PBPK models contain hypotheses generating ideas and later models test some of these hypotheses, especially if additional experimental data become available. In the case of PFOA and PFOS, the EPA selected not to use the most recent PBPK models for PFOA and PFOS, but instead use a computational empirical based model (Andersen et al. 2006) that was the first attempt to quantitatively interpret the kinetics of PFOA and PFOS across species of laboratory animals. The authors did publish their model (Wambaugh et al., 2013). The authors chose not to use a human model because a lack of information for Bayesian analyses. The justification for their extrapolation methods should be stated and the published reference for the model should be cited.

RESPONSE: A comparison of predicted versus data-derived area under the plasma concentration curve (AUC) is an excellent research idea for further evaluating the modeling described in Wambaugh et al. (2013). At this time the serum time course data for the studies in Table 4-6 have not been collected and organized in a manner that would allow this analysis to be performed. Further, many of the studies only reported a final serum concentration, which would not allow AUC to be calculated in a meaningful way.

A physiologically-based PK (PBPK) model for PFAS would be preferable because it would allow extrapolation between species, provide better estimates of chemical-specific parameters, and allow estimation of chemical concentration in the specific tissues for which toxicity is observed. However, as Dr. Fisher noted earlier (see comment from Fisher above), the state of the science is not yet developed such that extrapolation between species is possible. Calibrating a model developed in animal to humans mitigates the benefits of a PBPK model because there are multiple ways a model might be calibrated and only considering one would understate the uncertainty associated with the use of the model. Further, data for chemical-specific partitioning into tissues are extremely limited. They exist only for PFOA from the single-dose Kemper (2003) rat study and for PFOS for a feed study (i.e., greater dose uncertainty). Wambaugh et al. (2013) found that due to the dose selection of the Kemper (2003) study, the non-linear PK of PFOA was not present. If any portion of the non-linear PK for PFAS is due to tissue distribution (e.g., binding or transport) then these processes would be missed by the Kemper (2003) data set. Given the limitations of the available data for estimating parameters, the simpler Andersen et al. (2006) empirical PK model was preferable.

COMMENT 2: Model parameter distributions (Bayesian analyses) appear to be biologically implausible in some cases, covering many orders of magnitude. The authors should discuss this issue and check the units of model parameters in Tables.

RESPONSE: EPA believes that the model parameter estimates are correct reflections of the available data. The Bayesian analysis attempted to assess what ranges of parameter values would be consistent with the

available PK data. A wide distribution for a parameter value indicates that there is uncertainty about that value. There are at least two sources of large uncertainty: (1) the data are not informative about the particular parameter, for instance, in the case of species where only single dose PK studies are available, it is hard to characterize the approach to steady-state, and (2) the model is insufficient to describe the data. Because the species for which there are large amounts of PK data lead to parameter estimates with minimal uncertainty, for example PFOA in female CD1 mice, EPA believes that the uncertainties are more associated with the available data. EPA's analysis of the available PK data attributed any indeterminacy of parameter estimates to uncertainty, however, if there were intra-species variability with respect to key parameters, the variability would also be represented by the range of parameter estimates. It is for this reason that the range of values consistent with the data was used to predict PK. Thus, model parameter estimates are correct. Further data collection would be required to determine whether the ranges of parameter values reflected uncertainty or biological variability.

COMMENT 3: Both model parameters tables need to include a description of what the parameter represents and cite a figure. The figures showing the Andersen et al. 2006 model do not show all the model parameters and have different nomenclature. The Andersen et al. 2006 paper is a critical paper offering a quantitative explanation for the PFOA and PFOS kinetic data sets.

RESPONSE: The first sentence of the captions was changed to: "Means and 95% confidence interval from Bayesian meta-analysis of PK datasets available in peer-reviewed scientific literature are reported (Wambaugh et al. 2013)."

Hayton Comments

COMMENT 1: In the "Pharmacokinetic Model Approach" sections of the documents, it is not made sufficiently clear that the parameter values in Table 5-5 (PFOA) and Table 5-7 (PFOS) were from re-fitting the published data, rather than using parameter values from the original literature reports.

RESPONSE: The title of these tables has been changed from "Pharmacokinetic parameters used in the Andersen et al. (2006) model" to "Pharmacokinetic parameters from Wambaugh et al. (2013) meta-analysis of literature data."

COMMENT 2: PFOA Table 5-5, p. 5-12

- Body Weight and Cardiac Output values are reasonable and typical.
- k_a values for mouse and monkey seem extremely large; absorption half-lives would be on the order of 10 seconds, which is physiologically unrealistic. All of an oral dose would be absorbed within a minute, mimicking a rapid i.v. bolus dose. Serum concentration-time profiles may not be sensitive to these values, however so they are not disconcerting for the intended use of the models. The rat values appear reasonable.
- V_{cc} values appear reasonable. The total steady-state volume of distribution value [$V_{ss} = V_{cc}$ x (1 + $R_{v2:v1}$)] compares favorably with one-compartment V_d values for CD1 mouse, but V_{ss} values for the other columns (species) appear too large, due to the large $R_{v2:v1}$ values.
- k_{12} values vary a lot across the columns, suggesting that k_{12} may be highly correlated with another parameter (e.g., $R_{v2:v1}$).
- $R_{v2:v1}$ values also vary a lot across the columns.
- T_{maxc} values are consistent across the columns; expressed in Gm/hr, they seem very large. For example, 2032 Gm/hr (4.91 moles x 414 Gm/mole) for the CD1 mouse. Even on a kg body weight basis could mouse renal tubules resorb 2 kg PFOA per hour? This maximum rate of resorption must far exceed the rate of filtration of PFOA at the glomerulus. (Clarification at the reviewers meeting explained this apparent departure from physiological reality. The units had been mis-specified in

Tables 5-5 and 5-7. They were in fact micromole per hour and micromolar for T_{maxc} and k_T instead of molar based. Thus T_{maxc} mouse value was 2 mg/hr, which is physiologically plausible.)

- k_T values are the concentration in glomerular filtrate that half saturates the resorption transporters. Expressed in mg/mL, they seem large, much larger than the urine concentration that would be expected; e.g., for CD1 mouse, k_T is 15 mg/mL where free serum concentrations (Free x C_{serum}) would be about 0.3 µg/mL with 10 mg/kg in the mouse. So the transporter would not become saturated except at extreme doses. The value used by Andersen et al. (2006) for monkey was 0.00001 mg/mL. Unit specified in Tables 5-5 and 5-7 should be µM, not M.
- Free fraction values measured in vitro are 0.01 or less at low PFOA serum concentrations (Table 3-1). The Free values for rat seem much higher than the measured values.
- Q_{filc} is defined as a fraction of blood flow (renal or cardiac output?) to the filtrate (bottom of p. 5-11) but has units of flow in Table 5-5.
- V_{filc} values are much smaller than the 0.01 L value used by Andersen et al. (2006), although Andersen et al. state that the model output is insensitive to this parameter and that their value was assumed.

RESPONSE: Comments are acknowledged. The secondary compartment appeared to be statistically-non identifiable for most species, that is, there were insufficient data available to estimate the volume of the second compartment. However, since the compartment was identifiable for the data-rich CD1 mouse, EPA believes that it is relevant to the PK and so that compartment was included when modeling all species. For those species where the second compartment was uncertain, the quantitative uncertainty about that compartment was propagated to the predictions. The reviewer is correct that the units are misreported for Qfilc – that value is a unitless fraction of the cardiac output. This has been corrected in the HESD. Qfil has units of L/h but is not reported in the table.

COMMENT 3: PFOS Table 5-7, p. 5-15

- Body Weight and Cardiac Output values are reasonable and typical.
- k_a values for female mouse and monkey seem extremely large – see comment above for PFOA.
- V_{cc} values appear reasonable. See comment above for PFOA.
- k_{12} values vary a lot across the columns, suggesting that k_{12} may be highly correlated with another parameter.
- $R_{v2:v1}$ values appear reasonable and consistent with other reports of V_{ss} values for PFOS.
- T_{maxc} values are highly variable across the columns and seem much higher than physiological reality would allow. See comment above for PFOA.
- k_T values are physiologically unrealistic and highly variable across columns. See comment above for PFOA.
- Free fraction values have been measured in vitro and are 0.01 or less at low PFOS serum concentrations (Table 3-1, p. 3-3). The Free values in Table 5-7 are consistent with the measured values.
- Q_{filc} is defined as a fraction of blood flow (renal or cardiac output?) to the filtrate (bottom of p. 5-14) but has units of flow in Table 5-7.
- V_{filc} values are much smaller than the 0.01 L value used by Andersen et al. (2006), although Andersen et al. state that the model output is insensitive to this parameter and that their value was assumed.

RESPONSE: The reviewer is correct that the units are misreported for Qfilc – that value is a unitless fraction of the cardiac output. This has been corrected in the HESD. Qfil has units of L/h but is not reported in the table.

COMMENT 4: While the parameter values for the pharmacokinetic models predict reasonable serum concentrations that generally agree with measured values (Tables 5-6 – 5-8 for PFOA and Tables 5-8 and 5-10 for PFOS), their high interspecies variability suggest that the models may be unreliable for prediction of internal exposures after other intake regimens and during a depuration phase.

RESPONSE: EPA believes that an empirical PK model structure including saturable resorption is appropriate for the female, CD1 mouse, which has been extensively studied with respect to saturable resorption (Lou et al. 2009). EPA's modeling assumes that this structure is appropriate for all animal species that EPA analyzed. There is evidence for this in the scientific literature: Andersen et al. (2006) developed the saturable resorption model structure for monkey, Lou et al. (2009) used it for CD1 mouse, and Loccisano et al. (2011, 2012a) applied a physiologically-based model with a similar saturable resorption term to humans and rats. If this model is applicable across species, then, for those species where some parameters have broad uncertainties, uncertainty in the parameter values reflect the inadequacy of the PK data for that species. EPA believes that differing ranges in parameter certainty are an artifact of the available scientific data and do not reflect variation in the overall mechanics of PFOA/PFOS PK between species. For this reason, EPA made serum predictions using the full range of parameter values consistent with the data for each species. If the uncertain parameters had significant impact on the serum concentration, then species where the parameter values were more uncertain had predictions with wider ranges of uncertainty.

Longnecker Comments

COMMENT 1: Please see the answer to the previous question.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Slitt Comments

COMMENT 1: The parameters included appear to be appropriate, but this lies outside of my area of expertise.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Charge Question 8: Volume of Distribution and Half-life Values

The volume of distribution (Vd) and half-life values are critical in the derivation of the interspecies uncertainty factor applied in derivation of candidate RfDs from a NOAEL, LOAEL or a BMDL. The available data for both values are provided in Section 3.5.2 and 3.5.3 of both documents. Please comment the strengths and weaknesses of the values selected.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: The adult male rat data of Kemper (2003), from which the rat half-life and clearance (CL) were obtained, appear to be solid. It is reasonable to select the human half-life of 2.3 years reported by Bartell et al. (2010), as their study population included equal numbers of males and females. Division of the rat CL by the human CL to yield a value of 219 is fine. I did not examine the publication of Bartell et al. (2010) to evaluate their data or methodology used to derive a human half-life of 2.3 years. Therefore, I am uncertain about its accuracy.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

PFOS-specific Comments

COMMENT 2: I would again defer to someone with more expertise.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Cory-Slechta Comments

COMMENT 1: This falls outside my area of expertise and therefore no significant comments are provided. However, at the face-to-face meeting there was significant discussion regarding the modeling, including clarifications from Dr. Wambaugh that were found by those panel members with expertise to clarify these issues and these particular issues were considered adequately addressed.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

DeWitt Comments

COMMENT 1: While the overview of the individual studies that calculated Vd and half-life for each compound was detailed and complete, the rationale and analysis concerning *why* particular values were selected were insufficient. Additionally, as addressed in Charge Question 6, the rate of clearance/elimination likely contributes to the differences in half-life that are not associated with differences in the Vd. Therefore, a 3-fold uncertainty factor for species differences in pharmacodynamics (UF_A) was utilized for both compounds. What was the justification for using a UF_A of 3? The section on UF application needs a more thorough discussion regarding the choice of this value given differences in clearance. If the section on model adjustment (a suggestion in Charge Question 6) is better described, this comment may no longer be applicable.

RESPONSE: Sections 2.6.2 and 2.6.3 for PFOA and 2.5.2 and 2.5.3 for PFOS of the HESDs provide the available data on half-life and volume of distribution. In some cases there is only one option for a value such as the human half-life for PFOS. In cases where there was more than one option the reason for the one used is identified. The animal half-lives that were used in the derivation of UF_A in the peer review draft that

presented a comparison between the derivation of potentials RfDs using a clearance ratio between humans and animals to quantify UF_A were removed based on peer reviewer recommendations that supported the toxicokinetic model average serum approach over the clearance ratio approach.

The interspecies UF represents differences between animals and humans with regard to toxicokinetics and toxicodynamics. In cases where the POD for RfD quantification is the product of toxicokinetic modeling, the toxicokinetic portion of the interspecies UF is not applied. In the absence of data regarding toxicodynamic differences between species the toxicodynamic portion of the UF is retained. The toxicodynamic factor accounts for differences in the way the chemical interacts with tissues in the animals versus humans. The UF applied to account for toxicodynamics in such circumstances is 3 (see section 4.4.5.3 in EPA's document *A Review of the Reference Dose Reference Concentrations Processes.*[8]

Fisher Comments

COMMENT 1: The use of this non-compartmental method should be justified. Why not use a PBPK model? Assuming steady state in the humans does allow for calculation of a human equivalent serum concentration associated with a laboratory animal concentration. In what region of the exposure-dose range would nonlinearity occur in humans? Some type of discussion is needed about the assumptions of this methodology and why it was used. I would like to see statements about if the NOAEL, LOAEL, and a BMDL doses are in the linear range for kinetics.

RESPONSE: The non-linear PK of PFOA/PFOS leads to more rapid clearance at high doses in lab animals. More rapid clearance would lead to lower plasma concentrations for the same exposure. The only PK information directly measured for humans is the serum half-life. The only available PBPK models do not have the ability to correctly predict the human serum half-life when extrapolated to humans. Thus, there is no way to confirm that any available PBPK model accurately reflects any non-linearities that might occur in humans. It appears prudent to take a more parsimonious approach, and use a one compartment PK model in which an estimated volume of distribution that is largely consistent with the animal data and a measured elimination rate are the only two parameters.

COMMENT 2: The authors should use the Bayesian analysis for animal studies to inform the UF. Use percentiles to explore Vd and half-life to support UF values. I did not see any attempt to use distribution information generated from the model beyond the central tendency or mean values. Please state why this is the case. It seems that the distribution information generated from the Bayesian analysis could be used to support UF development.

RESPONSE: The specifics on how to replace uncertainty factors with Bayesian analysis are a matter of ongoing discussion for all chemicals, not just PFOA and PFOS. Since there are no agreed upon guidelines for the new approach recommended by the peer reviewer, EPA used the current Agency approach for determining UF in this assessment.

Hayton Comments

PFOA-specific Comments

COMMENT 1: For male rat, the Kemper (2003) study appears to be the best source of pharmacokinetic parameter values, which were obtained by a model-independent analysis of serum concentration-time data from rats that were dosed by oral gavage at dosages of 0.1, 1.0, 5.0 and 25 mg/kg. In addition, there was a 1.0 mg/kg dosage administered intravenously, and a 0.1 mg/kg oral gavage dose with an extended sampling

[8] https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

time. Each treatment used four animals. The CL and $t_{1/2}$ values appeared to be independent of dosage and route of administration. It would therefore be reasonable to average all 6 mean values for each parameter to give an over-all mean of 24 determinations. The average (n=24) values for male rat were CL = 0.0209 L/kg/d and $t_{1/2}$ = 7.83 d. These values can be used to calculate a V_d value ($t_{1/2}$ x CL / ln 2), which is 0.236 L/kg.

RESPONSE: The Kemper (2003) data were used in the PFOA model of the Sprague-Dawley rat. Comment is acknowledged; no formal response or action is necessary.

COMMENT 2: It is not apparent on p. 5-20 why a V_d value of 0.17 was used with half-life to calculate CL_{rat} when Kemper (2003) reported CL values and not $t_{1/2}$ values. (At the peer review meeting, it was clarified that the data of Kemper (2003) were re-analyzed and as a result the parameter values in the health effects documents differ somewhat from those published with the data in the original reports.)

RESPONSE: The HESDs were revised to further emphasize that all data were reanalyzed using a single model by Wambaugh et al. (2013), and that these new parameter estimates were used for comparing across toxicological studies.

COMMENT 3: The CL_{human} value was taken to be 0.00014 L/kg/d. There are no direct measurements of this parameter. Thompson et al. (2010) assumed that the intake rate of PFOA for subjects using PFOA-contaminated water was 91% of the PFOA in 1.4 L/d of water. This intake rate was used along with a PFOA half-life of 2.3 years to calculate a V_d value of 0.17 L/kg. This is the same value that was used in the health effects document for the rat (p. 5-20). The V_d values available in mouse, rat and monkey are about 0.2 L/kg, so the $V_{d,human}$ set at 0.17 L/kg is not unreasonable but it lacks the certainty of the rat V_d value.

RESPONSE: Since volume of distribution is best determined experimentally, using known, controlled doses, human data would be required to attain the accuracy of the estimates for animal species. No such published human data are currently available. In silico methods for predicting volume of distribution have not yet been developed for perfluorinated compounds.

COMMENT 4: The health effects document used a $t_{1/2}$ for PFOA in human of 839.5 d (2.3 years), which seems to be toward the low end of the range of values that have been reported. Along with Vd = 0.17 L/kg one arrives at CLhuman = ln 2 x 0.17 / 839.5 = 0.00014 L/kg/d.

RESPONSE: The Bartell et al. (2010) half-life represents an estimate corresponding to the U.S. general population rather than the occupational populations as reported in studies, such as Olsen et al. (2007). It was derived using the declines in serum values among members of a highly exposed population following a change in residence that lowered the ongoing exposures. The Health Advisory guidelines apply to members of the general population exposed to a chemical through their drinking water. Accordingly, the Bartell et al. (2010) estimate was used rather than one based on occupationally exposed cohorts. The recent NHANES data demonstrate that serum levels are declining among the general population. This strengthens the decision to utilize the Bartell et al. (2010) half-life.

COMMENT 5: The ratio CL_{rat} / CL_{human} calculated using the mean CL_{rat} from Kemper (2003) would be 0.0209 / 0.00014 = 149, which is about twice the value calculated on p. 5-21. This difference arises from the calculation of CL_{rat} using the $V_{d,human}$ and a half life of 11.5 d instead of using the CL_{rat} directly from Kemper (2003). The mean half life from Kemper (2003) was 7.8 d.

RESPONSE: The Kemper (2003) study used a non-compartmental analysis of PK. A reanalysis of this data was done by Wambaugh et al. (2013) using a consistent PK model for all species that included a saturable resorption mechanism, which results in CL changing with concentration in blood.

COMMENT 6: The CL_{mouse} / CL_{human} ratio is accurate, using Lou et al. (2009) data. A calculation for monkey is not shown.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

PFOS-specific Comments

COMMENT 7: Chang et al. (2012) appears to be the best source of pharmacokinetic parameter values for mouse, rat and monkey. Butenhoff and Chang (2007) is given as the reference for a 48-day half-life in rat; this is a final report, internal to 3M. The Chang paper gives half-life values for male and female Sprague-Dawley rat at 2 mg/kg and 15 mg/kg. The average V_d for the four groups of three/group was 0.71 L/kg. This is higher than the 0.23 L/kg value used in the draft document (p. 5-23). The 0.71 L/kg value is also higher than values for mouse, monkey and human, which are closer to the 0.23 L/kg value used in the draft document. The draft document ought to acknowledge this difference; it may be that the value in the 3M report is lower than the published value; Chang was a co-author for both sources. The Chang et al. (2012) paper gives CL_{rat} values that are 0.0051 L/h/kg for female (similar for 2 and 15 mg/kg doses) and for males, 0.022 and 0.011 L/h/kg for the 2 and 15 mg/kg doses. A single average value for CL_{rat} would be 0.011 L/h/kg, about 3 times the value used for the UF_A calculation in the draft document. The male value is about 2-3 times the female value and it may be appropriate to calculate a different UF_A value for each sex. Using the single CL_{rat} averaged across two doses and both sexes (0.011 L/H/kg) would give a CL_{rat} / CL_{human} ratio of 0.011 / 0.000081 = 135 and a UF_A = 407, substantially higher than the value of 123 in Table 5-15.

RESPONSE: With respect to volume of distribution, EPA's reanalysis of the Chang et al. (2012) data suggest a range of primary compartment volumes of distribution between 0.264 – 0.637 L/kg across species. For the male Sprague-Dawley rat, EPA estimates a volume of distribution of 0.637 (95% credible interval 0.593–0.68) L/kg, and 0.535 (0.49–0.581) L/kg for female. These values are not that dissimilar from the original Chang analysis that did not account for non-linear PK. Further, EPA notes that in EPA's analysis the volume of distribution for monkeys is 0.303 L/kg, which is closer to the value of 0.23 L/kg that Thompson et al. (2010) inferred for humans and was used for EPA's extrapolation to humans.

With respect to clearance EPA is unsure why the reviewer believes that a "value for CL_{rat} would be 0.011 L/h/kg, about 3 times the value used for the UF_A calculation in the draft document." Because the extrapolation was made on the basis of toxicological study serum concentration to human serum concentration, the animal clearances, including differences between male and female rats, did not factor into the UF_A calculation. A UF_A = 3 was used to account for pharmacodynamics differences. The only clearance used for determining the RFD was 0.000081 L/kg bw/day, as derived from the Olsen et al. (2007) half-life of 5.4 years and the volume of distribution inferred by Thompson et al. (2010).

COMMENT 8: The UF_A values calculated for mouse and monkey appear to be in line with the literature values for PFOS CL values in these species.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Longnecker Comments

COMMENT 1: The PBPK model of Loccisano et al. 2013 (for humans) can be used to calculate a volume of distribution for PFOA of 177 ml/kg, which is very close to the value of 170 ml/kg based on Thompson et al.'s 2010 one-compartment model. For PFOS, the corresponding value from the PBPK is 280 ml/kg, compared with the value of 230 ml/kg used in the Health Effects Document. This 22% difference could have an impact on some calculations. (Note: the PBPK model-based volumes of distribution were calculated by Marc-Andre Verner of the University of Montreal. He had calculated these values in the course of a separate project.)

RESPONSE: A volume of distribution can be calculated using a PBPK model by summing the tissue volumes weighted by the partition coefficients, as in Verner et al. (2015, EHP) which used the Loccisano et al. (2011, 2012a, 2012b, 2013) PBPK models. For PFOA, the Loccisano et al. (2011, 2012a, 2012b, 2013) model partition coefficients are derived from a single dose rat study (Kemper 2003) for which the tissue data is unpublished. The serum data from Kemper (2003) was shown by Wambaugh et al. (2013) to have been collected at doses too low to explore saturable resorption or other non-linear aspects of the pharmacokinetics. For PFOS partition coefficients, Loccisano et al. (2011, 2012a, 2012b, 2013) used data from male C57Bl/6 mice, which is similarly unpublished and was not available for analysis by Wambaugh et al. (2013). The PFOS data is referenced as DePierre (2009) through personal communication to authors (Loccisano et al. 2012a). Since the Thompson et al. (2010) volume of distribution was estimated for humans, while the Loccisano et al. (2011, 2012a, 2012b, 2013) model value is derived from unpublished data for rats and mice, EPA concluded that the values are consistent but that the choice of the human-derived value is more relevant. EPA further notes that Verner et al. (2015) used "volumes of distribution of 170 mL/kg of body weight for PFOA and 230 mL/kg of body weight for PFOS as estimated by Thompson et al. (2010)."

COMMENT 2: For humans, the half-life data all depend on the assumption that ongoing exposure is negligible compared to baseline exposure, a reasonable assumption in most of the populations used to estimate half-life. While the Seals et al. (2011) gave estimates that were slightly different for PFOA in some cases, the methods employed in this study were not as strong as for Bartell et al. (2010) or the Burris et al. studies (2000; 2002). The agreement within species for the half-life estimates for PFOS are reassuring. The animal data on the half-life of PFOA are relatively sparse (2 rat studies that agreed reasonably well, 1 mouse study, 1 monkey study). For PFOA, the UF_As and RfD that were calculated based on the half-lives (expressed as clearance) would not have been substantially altered by alternate choices for specific values. The same is true for PFOS.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Slitt Comments

COMMENT 1: Strengths of the available data is that for the several species thorough evaluated, the half-life values are very consistent. For example, the several human studies cited report a range in calculated PFOS half-life in humans to be 4.1-8.67 years, two studies putting monkeys at 110-132 days, and rat generally has a narrow range with 3 out of 4 values provided ranging from 39.8-48.2 days for PFOS. An inconsistency is the Chang et al., 2012 describing a half-life of females of 66.7 days when in general female rodents may have faster elimination of PFOS.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Charge Question 9: Candidate RfDs

A variety of endpoints and studies were used to compare points of departure and the resultant RfDs for both PFOA and PFOS. In addition, comparisons were provided across RfD outcomes based on the model outputs compared to those for the NOAEL, LOAEL and BMDL points of departure. The range of candidate RfDs derived from the different points of departure is fairly narrow. Please comment on the strengths, weaknesses and transparency of this analysis.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: The procedure used to calculate PODs adheres to standard EPA guidelines and policy. The presentation of their derivation is clear, concise and transparent. It is certainly interesting that the range of PODs and resulting candidate RfDs is so narrow. Nevertheless, as discussed previously, I do not agree with their selection.

RESPONSE: Dr. Bruckner's objection to the selection of the candidate RfDs applied to the original selection of increased liver weight and hypertrophy as the critical effects for some of the studies quantified in the draft health effects documents. EPA subsequently followed the recommendations of the peer review panel in revising the HESDs and only used liver effects that were characterized by Hall et al. (2012) as adverse when identifying the LOAEL in those studies. In cases where liver effects are nonadverse, but accompanied the adverse effects, they are listed along with the adverse effect in the summary of effects for the LOAEL dose (Tables 4-1 and 4-2).

PFOS-specific Comments

COMMENT 2: See my comments under General Impressions.

RESPONSE: See previous response to comments.

Cory-Slechta Comments

COMMENT 1: While it is the case for both PFOA and PFOS that values from different points of departure are fairly narrow, the transparency of the analyses in neither case is clear. There is no rationale described even as to why these analyses were done on all of the studies, what was the primary study and how others related to that etc., i.e., this presentation does not follow the typical presentation format of IRIS documents in either its presentation of rationales and strategies, nor in the conclusions that it reaches. In both cases, it is only the single sentence indicating that modeling from one particular study will be protective of effects at other studies using higher exposures. This section in both documents needs introductory paragraphs that describe the specific strategy, choices of studies and the rationales for those choices.

RESPONSE: For both final HESDs, EPA added text describing the studies chosen for modeling and selection of the RfD. The studies selected as key for quantification were generally well-conducted studies, evaluating a duration of ≥7 weeks for those other than the developmental studies and the DeWitt et al. (2009) immunological study, use of a control, employing a range of doses and sample sizes large enough for detecting statistical differences, and with serum data amenable for modeling that showed the most sensitive effects following exposure to PFOA and PFOS. Additionally, the doses associated with LOAELs for the identified critical endpoints were not associated with clinical signs of overt toxicity in the animal models and nearly all of the studies measured serum and/or tissue concentrations of the parent compounds.

COMMENT 2: As noted in response to Charge Question 3, the rationale for discarding the human epidemiological studies is not sufficient and requires rationale other than that stated and therefore, the question of using the human data remains open. As noted in response to Charge Question 1, in this reviewer's opinion, the increased liver weight can be justified as a departure point for assessment of RfDs, but as discussed at the face-to-face meeting, additional text supporting this choice is needed.

RESPONSE: EPA utilized the human epidemiology studies as a line of evidence in this assessment, including a discussion of strengths and weaknesses of the human epidemiology data and strengths of the animal studies relevant to quantitation. Also, EPA followed the recommendations of the peer review panel in revising the HESDs and only used liver effects that were characterized by Hall et al. (2012) as adverse when identifying the LOAEL in those studies. In cases where liver effects are nonadverse, but accompanied the adverse effects, they are listed along with the adverse effect in the summary of effects for the LOAEL dose (Tables 4-1 and 4-2).

DeWitt Comments

COMMENT 1: This particular section contained inadequate detail on why particular studies were or were not chosen. For example, immunotoxicity as an endpoint was not chosen for PFOS, based on "in vitro measures of immunocompetence on mice may not be relevant to the human experience and limited human data from epidemiology studies are inconclusive regarding the immunotoxicity of PFOS in humans"; however, the breadth of data from in vitro/ex vivo immunotoxicity studies for PFOS were not thoroughly discussed (please see Charge Question #2 for two additional in vitro studies).

RESPONSE: The synthesis and evaluation section of both documents was revised to better integrate the human and animal findings suggestive of immunotoxicity. Discussion and presentation of the in vitro/ex vivo studies was expanded.

COMMENT 2: For both compounds, an increase in absolute liver weight was selected as an endpoint as it was a common effect [sic] in both short and long term studies. However, the toxicological relevance of an increase in absolute liver weight was not discussed other than to indicate that it was a sign of altered homeostasis. Further, the co-occurrence of increases in absolute liver weight with other toxicologically-relevant endpoints (i.e., immunotoxicity and/or reproductive/developmental toxicity) is not a toxicologically valid justification for the use of liver weight as an endpoint for an RfD. Therefore, the analysis was not sufficiently transparent to deduce its relative strengths and weaknesses. Certainly, choosing an endpoint that occurs across species and occurs at relatively low doses will likely be protective of exposed humans; however, will it be a defensible endpoint? As currently written, the choice of this endpoint for an RfD is not adequately defended.

RESPONSE: EPA reevaluated the outcomes related to PFOA exposure based on peer review comments, and selected an endpoint that reflects adverse effects in the developing fetus and newborn as the most sensitive endpoint to serve as the basis for the derivation of the RfD. The POD for both PFOA and PFOS was altered so that liver weight alone is no longer the endpoint of concern. Increased liver weight is acknowledged as a common finding but not considered adverse in the absence of other effects as defined by Hall et al. (2012). EPA reevaluated all studies reporting presence of increased liver weight for other adverse effects using the Hall criteria. The RfD for PFOA is based on reduced ossification in males and females and accelerated puberty in males (Lau et al. 2006). The RfD for PFOS is based on decreased pup body weight (Luebker et al. 2005b) in rats over two generations.

Fisher Comments

COMMENT 1: I did not review the toxicity data.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Hayton Comments

COMMENT 1: For PFOA, a 10% increase in liver weight was selected as the metric for effect, which was "… not made based on toxicity but on the desire to find a common denominator against which to evaluate dose-response across studies and justified by the fact that other adverse effects accompanied the LOAEL for increased liver weight in some cases." The lowest serum concentration associated with an increase in liver weight was calculated for female mouse to be 20.33 mg/L (p. 5-16, PFOA document). These data are referenced to DeWitt (2008); this paper has only summary information on liver weights, all of which exceeded 20% weight gain, going as high as 70%; and it is not apparent in PFOA document how these liver weight gains were used to estimate an LOAEL for 10% liver weight gain.

RESPONSE: EPA has re-evaluated the outcomes related to PFOA exposure based on peer review comments, and selected an endpoint that reflects adverse effects in the developing fetus and newborn as the most sensitive endpoint to serve as the basis for the derivation of the RfD. The PODs for both PFOA and PFOS were altered so that liver weight alone is no longer the endpoint of concern. Increased liver weight is acknowledged as a common finding but not considered adverse in the absence of other effects as defined by Hall et al. (2012). EPA reevaluated all studies reporting presence of increased liver weight for other adverse effects using the Hall criteria. The RfD for PFOA is based on reduced ossification in males and females and accelerated puberty in males (Lau et al. 2006). The RfD for PFOS is based on decreased pup body weight (Luebker et al. 2005b) in rats over two generations.

COMMENT 2: Many of the animal studies of hazard assessment were conducted under conditions where the duration of the exposure was relatively short compared with the half-life, and steady state had not been achieved. It is not apparent how the NOAEL and LOAEL values from such studies were adjusted to account for the non-steady state situation. For example, the 20.33 mg/L PFOA concentration associated with a 10% increase in liver weight (Table 5-9) emanated from a 15 day drinking water exposure to 0.94 mg/kg/day that resulted in an average serum exposure of 20.33 mg/L (0 – 29.7 mg/L over 15 d, Tables 5-7, 5-9). For a fixed daily dose, the time to 90% steady state for mouse would be about 63 days (3.3 x half life, which was 19 days), and after 15 days the serum concentration would only be about 15% of its steady-state value. This seems to suggest that the RfD would have been over-estimated by a factor of 7, since the 0.94 mg/kg/day at steady state would have produced a serum concentration of about 150 mg/L, not 20.33 mg/L. This analysis is based upon the behavior expected from one-compartment model pharmacokinetics. As discussed on p.5-9 of the PFOA document, the steady-state serum concentration of PFOA is achieved in a much shorter time than one-compartment model kinetics would predict. Whether the target-site steady-state concentration of PFOA also occurs in a much shorter time than one-compartment model kinetics would predict (3.3 x half-life) is apparently unknown.

RESPONSE: Lou et al. (2009) demonstrated that the serum concentration of PFOA does reach steady-state faster than 3.3 x half-life. The Andersen et al. (2006) saturable resorption mechanism for PK hypothesizes that the half-life depends upon the concentration of PFOA or PFOS in the kidney filtrate. The clearance is therefore not constant: at low concentrations the chemical is readily resorbed back into the body leading to a long-half-life, while at higher concentrations (more typically generated by animal studies) the half-life is actually shorter. This leads to a rapid approach to steady-state as the result of high dose regimens, followed by a longer half-life for chemical elimination. For this reason the RfD would not be an overestimation because the approach to steady-state for PFOA and PFOS is more rapid than would be calculated using half-lives.

EPA added a table comparing the average serum concentration to an estimate of steady-state for each endpoint used to determine the RfD. In the case of the DeWitt et al. (2008) study referred to by the reviewer, the LOAEL of 3.75 mg/kg/day was estimated to produce a serum concentration that was ~74% of steady-state in 15 days.

Longnecker Comments

COMMENT 1: This part of the document seemed especially strong and transparent. The agreement between methods was reassuring. The weaknesses and assumptions were well discussed. Please see the minor editorial comment on this issue given for Charge Question 1, above.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Slitt Comments

COMMENT 1: The RfD Point of Departure was based on animal studies that include monkey and rat.

RESPONSE: In the revisions to the document, the data from the monkeys for PFOS and PFOA were modeled and average serum levels were determined. However, in neither case was the study considered as a candidate RfD. In the case of PFOS, the effect level was the highest dose and associated with the deaths of some of the animals. In the case of PFOA the high dose was adjusted part way through the study because of frank toxicity. In both cases the number of monkeys per sex were low (≤6) and the liver effects that were quantified in the peer review draft did not qualify as adverse under the Hall et al. (2012) criteria.

Charge Question 10: Duration

The RfDs for PFOS and PFOA are derived from the modeled steady state serum concentrations and their association with effects that include short term and longer term exposures with associated diverse effects. The studies considered included effects due to exposure durations that ranged from 11 to 182 days, and occur at comparable human equivalent dose (HED) levels. The current, draft RfDs do not include an uncertainty factor for study duration because of the apparent concordance HEDs despite duration differences. Given this pattern of response, is it appropriate to conclude that the candidate RfDs are applicable to both short-term and lifetime exposures?

Bruckner Comments

PFOA-specific Comments

COMMENT 1: I do not believe it is appropriate to conclude that the candidate RfDs are applicable to both short-term and lifetime exposures. Steady-state is apparently achieved in monkeys within 4 - 6 weeks (Butenhoff et al., 2002). Steady-state likely takes considerably longer in humans. Thus, RfDs for shorter periods of exposure should be based upon results of studies of similar duration.

RESPONSE: The selected RfDs for PFOA and PFOS were revised based on peer review comments and now are based on developmental effects on the developing fetus and offspring resulting from exposures that occur during gestation and lactation (see section 4.1.2 in the PFOA HESD and 4.1.1 in the PFOS HESD). These developmental endpoints are the most protective for the population at large and are effects that can carry lifetime consequences for a less than lifetime exposure. Developmental toxicity endpoints (following less than chronic exposures during a defined period of gestation or lactation) can be analyzed in both acute and chronic exposure scenarios. Because the developing organism is changing rapidly and is vulnerable at a number of various stages in development, a single exposure at a critical time in development can produce an adverse effect (USEPA 1991). Additionally, PFOA and PFOS are extremely persistent in both the human body and the environment; thus, even a short-term exposure results in a body burden that persists for years and can increase with additional exposures.

Because the critical effects identified for both PFOA and PFOS are developmental endpoints and can potentially result from a short term exposure during a critical period of development, the lifetime drinking water health advisories are applicable to both chronic and short-term risk assessment scenarios; e.g., weeks to months, including during pregnancy and lactation.

PFOS-specific Comments

COMMENT 2: I do not believe the candidate RfDs, as calculated, are applicable to different durations of exposure.

RESPONSE: Peer reviewers were in agreement that the serum values in some of the studies were not at steady state. As a result, the percent of steady state was determined for each of the modeled serum values using the Wambaugh et al. 2013 model, and considered during the application of uncertainty factors for a duration adjustment. For PFOA, EPA added a duration adjustment of 10 to DeWitt et al. (2008) because the study was a 15 day study for an immunological effects that could occur across more than once across a lifetime exposure. In the case of PFOS an uncertainty factor to address duration was not applied to Seacat et al. (2003) because there were chronic exposure data for the same endpoint (Thomford 2002/Butenhoff et al. 2012) that demonstrated that the serum levels had decreased to 50%. Thus, protecting for the exposure that was associated with the subchronic exposure that lead to a higher serum level would protect the lower serum levels observed later in life. The dietary dose associated with the LOAEL was the same for both studies. An

uncertainty factor of 1 was used for the Luebker (2005a, 2005b) because EPA did not use one for an exposure associated with a sensitive life stage.

Cory-Slechta Comments

COMMENT 1: While initially believing that it was appropriate conclusion for PFOA and PFOS, based on the correspondences in RfDs across short and longer term exposure, discussion at the face-to-face meeting made clear that this approach is not reasonable and requires additional consideration.

RESPONSE: The final RfDs for PFOA and PFOS were revised based on peer review comments and now are based on developmental effects on the developing fetus resulting from exposures that occur during gestation and lactation (see section 4.1.2 in the PFOA HESD and 4.1.1 in the PFOS HESD). These developmental endpoints are the most protective for the population at large and are effects that can carry lifetime consequences for a less than lifetime exposure. Consistent with EPA policy, developmental toxicity endpoints (following less than chronic exposures during a defined period of gestation or lactation) can be analyzed in both acute and chronic exposure scenarios (USEPA 1991). Because the developing organism is changing rapidly and is vulnerable at a number of various stages in development, a single exposure at a critical time in development can produce an adverse effect (USEPA 1991). Additionally, PFOA and PFOS are extremely persistent in both the human body and the environment; thus, even a short-term exposure results in a body burden that persists for years and can increase with additional exposures.

Because the critical effects identified for both PFOA and PFOS are developmental endpoints and can potentially result from a short term exposure during a critical period of development, the lifetime drinking water health advisories are applicable to both chronic and short-term risk assessment scenarios; e.g., weeks to months, including during pregnancy and lactation.

DeWitt Comments

COMMENT 1: This approach may be appropriate given the relative similarity of serum concentrations attained regardless of study duration, i.e., steady state in serum is attained after a relatively short period of exposure. This appears to be consistent across studies with various species of animal models. However, the document authors might need to reconsider given what we may or may not know about liver hypertrophy. In the Hall et al. (2012) paper on liver hypertrophy (discussed during the public meeting), increase in liver weight is an adaptive response that may not be adverse UNLESS weight increases >150% over a three month or longer period may. Following this large and prolonged increase in weight, the end result may be a hepatocarcinogenic response. However, none of the studies contained in the documents indicate that longer term exposures increase liver weight to this degree.

RESPONSE: EPA adjusted the NOAELs and LOAELs for all the studies in the peer review draft that had used liver weight and hypertrophy as critical effects to correspond to the Hall et al. (2012) criteria for adverse liver effects.

The final RfDs for PFOA and PFOS were revised based on peer review comments and now are based on developmental effects on the developing fetus and offspring resulting from exposures that occur during gestation and lactation (see section 4.1.2 in the PFOA HESD and 4.1.1 in PFOS HESD). These developmental endpoints are the most protective for the population at large and are effects that can carry lifetime consequences for a less than lifetime exposure. Because the developing organism is changing rapidly and is vulnerable at a number of various stages in development, a single exposure at a critical time in development can produce an adverse effect (USEPA 1991). Because the critical effects identified for both PFOA and PFOS are developmental endpoints and can potentially result from a short term exposure during a

critical period of development, the lifetime drinking water health advisories are applicable to both chronic and short-term risk assessment scenarios; e.g., weeks to months, including during pregnancy and lactation.

Fisher Comments

COMMENT 1: The departure from K=CXT (Haber's law) should be based on the toxicity endpoints of concern and what is known about dose-exposure kinetics/responses for these chemicals and other chemicals that target the same endpoint, not that the HED values are comparable. The NAS AEGL committee only considered primary irritation for inhaled chemicals as an endpoint that was independent of duration of exposure. There is an SOP if needed for reference.

RESPONSE: EPA's statement that the HEDs were consistent across studies is equivalent to saying that the average serum concentrations were equivalent across studies. Haber's law relates effect to the product of concentration and duration. For the HEDs, EPA considered state conditions, determining the human dose that would produce a steady-state serum concentration equivalent to the average serum concentration in each study. In other words, EPA calculated an HED that is expected to produce human serum concentrations equal to the concentrations that the modeling has predicted to consistently produce adverse effects in toxicological studies.

COMMENT 2: The time to steady state should be included in a table for the lab animals. Toxicity studies conducted for less than 30 days (perhaps?) are not at steady state for the pharmacokinetics of PFOA. Thus the measured serum levels would be different than at steady state. The shorter the duration of the toxicity test, the more impact this could have on extrapolation to chronic exposures in humans. My personal preference would be to use PBPK models for all species and consider only long term exposures for extrapolation to humans.

RESPONSE: In response to peer review comments, EPA calculated the fraction of steady-state achieved for all the studies used for extrapolation to human chronic exposures and included this as an additional table. Because the PK of PFOS is believed to be non-linear, there is no unique value for the time to steady-state for a species. The time to steady-state depends on the dose regimen (e.g., magnitude and spacing of doses). Under the assumption of constant infusion dosing, an analytic solution exists for the Andersen et al. (2006) model that allows the steady-state concentration to be predicted for a given dose rate. The average serum concentration during a given study were compared to steady-state, indicating that for most studies the average serum concentration was between 36–96% (mean 75%) for PFOA and 9–69% (mean 31%) for PFOS of the ideal steady-state. Thus, for PFOA, the toxicity studies appear to be appropriate for informing steady-state human conditions. The studies that have C_{ss} values less than 80% are mostly developmental studies that represent a sensitive life stage where effects occur at serum concentrations well below the predicted steady state concentration yet have lifetime consequences. That is the situation for most of the studies that were quantified for PFOS.

Hayton Comments

COMMENT 1: This depends in part on how quickly the PFOA/PFOS concentrations at sites of toxicity come to steady state. Since the Vd for these chemicals is small (~ 0.3 L/kg) it seems likely that the concentrations in tissues rise in pseudo equilibrium with the rise in serum concentration. That said, the half-lives are relatively long due to the very small clearance ($t_{1/2} = \ln 2 \times V_d / CL$). If one-compartment kinetics apply, then a guideline for time to 90% steady state is 3.3 $t_{1/2}$. For studies that expose animals for a period of time shorter than 3.3 $t_{1/2}$, the serum concentration would not be at steady state and the internal systemic exposure (serum concentration) would be less than what it would be if the exposure were longer than 3.3 $t_{1/2}$. This effect would seem to lead to overestimation of the intake rate that was associated with a particular internal exposure and associated biological endpoint. For example, the $t_{1/2}$ of PFOS in mouse is about 36

days and 3.3 $t_{1/2}$ is 120 days. Consider a 28-day exposure using a fixed daily dose that produced an LOAEL of "X" mg/kg/day. On Day 28, the body level would only be 42% of the steady state level, and the average body level over the 28-day period would be about 21% (approximating the increase as linear and not exponential). The true LOAEL would be 0.21 "X" mg/kg/day; i.e., intake of 0.21 "X" mg/kg/day would produce a body level at steady state that was the same as the average body level produced by X mg/kg/day administered over 28 days. The time to 90% steady state for a fixed intake rate is quite long; from the literature in the health effects documents, the times in the following table were calculated. From this line of reasoning, exposure times less than two half-lives begin to significantly overestimate intake rates associated with particular endpoints. This analysis is based upon the behavior expected from one-compartment model pharmacokinetics. As discussed on p.5-9 of the PFOA document, the steady-state serum concentration of PFOA is achieved in a much shorter time than one-compartment model kinetics would predict. Whether the target-site steady-state concentration of PFOA also occurs in a much shorter time than one-compartment model kinetics would predict (3.3 x half life) is apparently unknown.

	CL		**Vd**		**$t_{1/2}$**		**Time to 90% steady state**	
	[mL/d/kg]		**[mL/kg]**		**[d]**		**[d]**	
Species	**PFOA**	**PFOS**	**PFOA**	**PFOS**	**PFOA**	**PFOS**	**PFOA**	**PFOS**
Mouse	6.6	5	180	265	19	36	63	120
Rat - Male	23	16	273	947	8.4	40	28	92
Rat - Female	776	5.2	150	476	0.13	66	0.43	218
Monkey	6.3	1.4	190	238	27	121	89	400
Human	0.085	0.08	170	230	1378	2000	12.5 yr	18 yr

RESPONSE: EPA calculated the fraction of steady-state achieved for all the studies used for extrapolation to human chronic exposures and included this as an additional table. The average serum concentrations during a given study were compared to steady-state, indicating that for most studies the average serum concentration was between 36–96% (mean 75%) for PFOA and 9–69% (mean 31%) for PFOS of the ideal steady-state. See the response to the above question for a full discussion.

However, the final RfDs for PFOA and PFOS are based on developmental effects on the developing fetus resulting from exposures that occur during gestation and lactation. These developmental endpoints are the most protective for the population at large and are effects that can carry lifetime consequences for a less than lifetime exposure. Because the critical effects identified for both PFOA and PFOS are developmental endpoints and can potentially result from a short term exposure during a critical period of development, the lifetime drinking water health advisories are applicable to both chronic and short-term risk assessment scenarios; e.g., weeks to months, including during pregnancy and lactation.

COMMENT 2: In addition, this line of reasoning may be incorrect if the assumption of one-compartment kinetics is incorrect. For multi-compartment models the serum concentration and target organ/tissue could come to their pseudo steady state levels relatively quickly while slowly equilibrating (deep) sites slowly approached steady state. Simulation with PBPK models for PFOS and PFOA may help answer this question.

Associated with the uncertainty introduced by exposures that were shorter than the time to achieve steady-state concentration at the target site is the exposure time required for the adverse effect to be expressed. While some adverse effects may occur immediately and directly in proportion to the concentration of PFOA or PFOS at the target site, other adverse effects may be slow to become manifest. These "indirect adverse response" behaviors are well known in the drug action arena; e.g., certain antidepressant drugs require several weeks of exposure to the target site before the effect of the drug appears. This lag time is not associated with pharmacokinetics (time to steady state) but with indirect-response pharmacodynamics. It

could be argued that uncertainty factors are needed for both pharmacokinetics (pre-steady state condition) and pharmacodynamics (or toxicodynamics) to account for possible indirect response behavior.

RESPONSE: Because the PK of PFOS is believed to be non-linear, there is no unique value for the time to steady-state for a species. The time to steady-state depends on the dose regimen (e.g., magnitude and spacing of doses). The average serum concentrations during a given study were compared to steady-state, indicating that for most studies the average serum concentration was between 36–96% (mean 75%) for PFOA and 9–69% (mean 31%) for PFOS of the ideal steady-state. EPA calculated the fraction of steady-state achieved for all the studies used for extrapolation to human chronic exposures and included this as an additional table. See the response to the Fisher's comment above for a full discussion.

Longnecker Comments

COMMENT 1: EPA might want to consider using an uncertainty factor for duration, for two reasons. First, the monkey data for PFOS used for the point of departure were from a study where the duration of exposure was relatively short-term relative to the half-life, and it appeared that duration of dose affected liver and other adverse outcomes detected at higher doses, and no monkey data were used in the POD for PFOA. Second, questions raised by Drs. Hayton and Fisher at the peer-review meeting made me less comfortable with the calculations that used average serum concentration derived from the AUC and duration of dosing to compare with humans, who are more likely to be near steady-state.

RESPONSE: The monkey studies are no longer used for the quantification of the RfD. In the case of PFOS, the effect level was the highest dose and associated with the deaths of some of the animals. In the case of PFOA the high dose was adjusted part way through the study because of frank toxicity. In both cases the number of monkeys per sex were low (≤6) and the liver effects that were quantified in the peer review draft did not qualify as adverse under the Hall et al. (2012) criteria. EPA no longer considers use of points of departure associated with death of the animals as appropriate for RfD derivation, especially when there are data that identify points of exposure that associated with effects earlier in the spectrum of adversity than death.

Slitt Comments

COMMENT 1: Yes, but this lies outside of my area of expertise.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Charge Question 11: Interspecies Uncertainty Factor

In addition to using the average serum values from animal studies to calculate internal doses for humans, the animal to human extrapolation can be accomplished by dividing animal average serum values by the human to animal clearance ratios to project a human average serum point of departure in units of mg/L serum. Please provide recommendations for applying uncertainty factors to the extrapolated average human serum values to determine serum-based thresholds that are protective for humans. A NOAEL expressed in average human serum units would be useful in interpreting NHANES population monitoring data.

Bruckner Comments

PFOA-specific Comments

COMMENT 1: No comment.

RESPONSE: No response necessary.

PFOS-specific Comments

COMMENT 2: No comment.

RESPONSE: No response necessary.

Cory-Slechta Comments

COMMENT 1: In initial response to charge questions, I found it difficult to understand specifically what this charge question was asking for a response to: Does this refer to the data in Table 5-10 for PFOA? Wouldn't you include animal to human UF values at the least. Since the data for the studies listed in the Table is not clear as to their duration (columns are needed for this information, or add to the Study box), it is not clear whether a UF for study duration is warranted. It is not clear how sex differences are being accommodated in any of these.

At the face-to-face meeting, however, with some additional input from EPA, it was clear to all that there was no need to do such derivations from animal to human, which could instead be derived directly from the human data and thus presumably this is no longer an issue.

RESPONSE: The starting point for derivation of the RfD is an HED derived from the modeled average serum value for the NOAEL and/or LOAEL. The use of a pharmacokinetically-derived HED based from the animal studies reduces the interspecies UF from a 10 to a 3 according to EPA policies. Per the USEPA (2002) report *Review of the Reference Dose and Reference Concentration Processes* the 10-fold intraspecies factor accounts for both toxicokinetics and toxicodynamics (section 4.4.5.3). In the absence of data, each component receives an uncertainty value of $10^{1/2}$. The 3-fold UF that was applied for interspecies differences accounts for pharmacodynamics differences between animals and humans.

DeWitt Comments

COMMENT 1: Would this approach take into account differences between animal studies that have a defined exposure duration and data from NHANES, where exposure duration is assumed to be continuous (although it may not be), if exposure duration does not appear significantly impact serum concentrations?

Additionally, how would the half-life estimations from the Seals et al. (2011) study, which contained two half-life estimations based on concentration and time, impact this approach?

RESPONSE: EPA calculated the fraction of steady-state achieved by the average serum concentration for the animal toxicity studies used to inform the HEDs. For PFOA, the studies are generally close to achieving steady-state, making the comparison to steady-state conditions easily reconcilable. Seals et al. (2011) found that individuals with higher estimated exposures had lower estimated half-lives (high clearance). The difference between the half-lives for higher exposed (2.9 years) and lower exposed (8.5 years) groups was roughly 3-fold. If the longer half-life was used, a lower HED would be estimated because the clearance would have been slower.

Seals et al. (2011) suggested that, if their assumptions were correct, a simple first order elimination model might not be appropriate for PFOA given that the rate of elimination appeared to be influenced by both concentration and time. There was a difference in the clearance for the two locations even though the range of years elapsed since relocation was the same for both communities. The authors identified three potential limitations of their analysis: the cross-sectional design, the assumption that exposure was uniform within a water district, and a potential bias introduced by exclusion of individuals with serum values <15 ng/mL. EPA chose to use the Bartell et al. (2010) half-life derived from the decline in serum values for individuals who had moved away from the C8 high exposure area because they have the closest correlation with the general population members whose exposures are declining due to the phase out of production of PFOA and PFOS.

EPA used the half-life for Bartell et al. (2010) as the one that is most relevant to the general populations because it was derived using the declines in serum values among members of a highly exposed population following a change in residence that lowered the ongoing exposures. The NHANES data demonstrate that serum levels in the U.S. population are also declining.

Fisher Comments

COMMENT 1: Again, is the system linear in the exposure/dose ranges of interest? I would try to determine an UF by exploring a range of predicted human serum levels. Attempt to use 5,50, and 95% for animal serum concentrations with a 5,50, and 95% CL values in the animals and for the human perhaps use two CL values representing a high and low. The idea is to use as much information as you can to determine the possible range of values. This will help guide the selection of uncertainty values.

RESPONSE: Unfortunately, the data necessary to inform the linearity of pharmacokinetics in humans are lacking. The affinity for PFOS and PFOA for the relevant transporters and the expression levels of those transporters would need to be included in a model that also included any endogenous substrates for those transporters that have sufficiently high concentration to produce competitive inhibition. These data and models are not yet available. The Seals et al. (2011) study did find preliminary evidence for differing half-lives in humans. There are some data for organic anion transporter (OAT) and organic anion transporting polypeptide (OATp) kinetics from ex vivo studies but a lack of information that applies to other transporters known to function in the kidney.

Hayton Comments

COMMENT 1: This calculation is equivalent to dividing the animal dosage by the CLhuman, assuming that the animal serum concentration is at steady state ($C_{ss,animal}$) maintained by a constant dose rate (DR).

$$C_{ss,animal} / CL_{human} \div CL_{animal} = CL_{animal} * C_{ss,animal} / CL_{human} = DR / CL_{human}$$

This calculation would give the steady-state serum concentration in human that would be produced by the animal dose rate. (I will have to study this to understand the question; the calculation does not make sense to me.)

At the peer review meeting, the aim of this calculation was clarified. Authors desired a way to calculate a steady-state serum concentration ($C_{ss,human}$) that would result from the human equivalent dose rate (HED) administered until steady state. The appropriate calculation would be:

$$C_{ss,human} = HED / CL_{human}$$

RESPONSE: In response to Dr. Haydon's suggestions, the percent of steady state (%C_{ss}) was determined for each of the average serum values. The need for the UF_S was determined based on the percent steady state result. If the %C_{ss} was 80% or greater no duration was applied. If the effect was one that could only occur during a sensitive life stage (e.g., pregnancy/lactation) and the serum represented that life stage in the animals, no UF was applied. If the endpoint was one that could occur across the lifetime, a full 10-fold factor for UF was applied unless serum data supported a lower data derived UF.

Longnecker Comments

COMMENT 1: The proposed division by animal clearance ratios does not make sense to me. The average serum values from animal studies is already taking pharmacokinetic variability in blood levels during the observation period into account, and human blood levels will be relatively constant. Thus, it would make sense to directly compare the POD estimated average serum concentrations from animal models to the blood levels in NHANES. With respect to uncertainty factors that would be need consideration for this approach, it seems that UF_H, UF_L (For LOAEL and HED_{LOAEL}), UF_D, and the component of UF_A that takes pharmacodynamics into account would all still be applicable.

RESPONSE: An estimate of human clearance (based upon serum half-life and estimated volume of distribution from epidemiological studies) was used to determine the human dose that would produce a given serum concentration. The extrapolation is necessary to relate serum concentrations to potential exposures (e.g., drinking water concentration). This extrapolation did not involve a ratio between the human and the animal clearance as had been done for the UF_A in the NOAEL/LOAEL and BMDL derivations in the peer reviewed documents. Derivations derived from those endpoints are not included in the final documents.

The final documents retain the UF_A for toxicodynamics and apply UF_D and UF_L using agency guidelines that recommend a 10-fold factor as a default. There are two places where other than a 10-fold factor was used. In the first situation (Luebker et al. 2005a) a 3-fold factor was applied for the UF_L because the Luebker et al. (2005b) two-generation study showed that the difference between the NOAEL and LOAEL supported a value of 3. The one-generation Luebker et al. (2005a) study lacked a NOAEL. The model only applied to the toxicokinetic portion of UF_A. In the second case (Seacat et al. 2002), a UF_D of one was applied because the serum values measured in the rats that were maintained until the end of their life were half of those values seen at the end of the subchronic duration. As a result, a potential RfD that protected at that early stage would also protect over a lifetime. The chosen RfD is the value from the two-generation Luebker et al. (2005b) study not the one-generation (2005a) study.

Slitt Comments

COMMENT 1: This is outside of my area of expertise.

RESPONSE: Comment is acknowledged; no formal response or action is necessary.

Charge Question 12: Other Suggestions

Please describe any suggestions you have for improving the clarity, organization, and/or transparency of the draft documents.

Bruckner Comments

PFOA and PFOS-specific Comments

COMMENT 1: See specific observations.

RESPONSE: Specific observations are addressed as noted in the following section.

Cory-Slechta Comments

COMMENT 1: While the EPA authors are aware of updates in the IRIS process, it might be very helpful to look at some of the new documents coming through that process for guidance as to the levels of critique and evaluation that are now included in these documents. They also include an introductory chapter focused specifically on the literature searches and literature that is included vs. excluded.

RESPONSE: A description of the literature search was added as an appendix to the document and the criteria used for selecting papers during development of the documents is included in background section. A second appendix provides a list of the papers recommended by the peer reviewers and those from literature search conducted between August 2014 and December 2015 that were retrieved and evaluated for inclusion in the revised HESDs.

COMMENT 2: The Executive summary does not provide sufficient rationale and descriptions to lead a reader through the steps to what is concluded and reads more like an abstract than an Executive Summary. Since this may be the only section read by many reviewers, it is important that it provide a succinct journey through the process. Here again, the new IRIS documents could provide a useful template.

RESPONSE: The executive summary in each of the HESDs was revised to reflect changes made to the documents.

COMMENT 3: Chapters 3, 4 and 5 could each benefit from an opening paragraph describing what the section's goals are, and integration and conclusion sections at the end that establish the basis for the presentation in Chapter 5. Currently the Hazard Identification studies generally treat all studies as of equal strength/power, which is certainly not the case. These chapters should present that kind of critical and transparent assessment as it ultimately serves as the basis for decisions that are made.

RESPONSE: In section 3, an overall summary and conclusions section was added after the human epidemiology section for noncancer and cancer endpoints. Introductory statements were added at the beginning of each major section in the animal study portion of section 3, and the synthesis and evaluation of the combined human and animal studies were revised in response to the comments received. A summary was also added to the section on toxicokinetics.

COMMENT 4: The inclusion of sections on in vitro data did not ultimately seem particularly relevant in the outcome for these compounds and could be significantly shortened to add more to Chapter 4 on study strengths and weaknesses. However, where pertinent, it would probably be more useful to break that section up and insert test where it follows an in vivo discussion.

RESPONSE: For PFOA and PFOS, there are a large number of published papers on mechanism, some linked to topics with in vivo data and others not linked. There are cases where some of the data are included with the in vivo topic (e.g., neurotoxicity) or when there are both in vivo and in-vitro components of the same study.

COMMENT 5: Tables could be considerably improved and made far more useful to the reader for comparative assessments. As of now, they require going back and forth to the text to capture additional details of the studies, e.g., sample sizes, species etc. and could benefit the reader significantly with those additions. For the human assessments, it is equally important to include these details in the chapter as well as a column of study strengths and limitations.

RESPONSE: The tables in section 3.1 were completely revised to include more of the data from the published papers. The original tables in the human epidemiology section were expanded to include study type, sample size, and serum levels as recommended by the peer reviewers. They are now in Appendix B.

In the section on quantification, tables that identify each of the studies modeled also identify the species and critical effects for the study, reducing the need to consult earlier portions of the document to find that information.

COMMENT 6: While charge questions ask whether the appropriate studies were chosen as key studies, this reviewer does not remember that that term was even used in the documents, certainly no explicit mention was made as to which studies were considered key studies. This would seem to be a section that should be included in Chapter 4 more explicitly. Chapter 5 of both documents, more so PFOA, are confusing as almost all studies are subjected to modeling, for reasons that are never presented in sufficient detail and simply followed by statements that a selected study (not really well presented in Chapter 4 as a selected study) will protect against other adverse effects.

RESPONSE: The synthesis section in section 3 is revised. The studies that identified a NOAEL or LOAEL in section 4 are the initial group of key studies. Only a subset of those studies had the serum information needed to pharmacokinetically model dose response. They are the ones that provided the average serum results used in the determination of potential RfDs. The use of serum, rather than dose, permits consideration of the nonlinear toxicokinetics exhibited by both PFOA and PFOS. The study, species, and effects are now given in each of the tables that present the average serum results for the NOAELs and LOAELs, the Human Equivalent Doses, and the PODs for the RfD.

DeWitt Comments

COMMENT 1: The documents lack a critical analysis of differences between findings of epidemiological studies and findings of animal models. As stated in the comments to Charge Question #3, what is particularly valuable about the PFOA/PFOS database is that it is relatively extensive in that it includes data not only from occupationally-exposed humans, but from people highly exposed to environmental concentrations of PFOA/PFOS, and from people in the general population who have detectable concentrations of these compounds.

Critical to this analysis is a discussion of concordance and lack of concordance between human data and animal model data. For example, immunotoxicological findings appear to be consistent between humans and rodent models whereas serum lipids are not. How do these differences impact the overall confidence in the database and derivation of the RfD?

RESPONSE: The human epidemiology tables were expanded for each major endpoint to summarize the data for the studies described in the text. An overall summary and conclusion was added at the end of the epidemiology section for the cancer and noncancer endpoints. The epidemiology tables from the peer review

draft were revised to include details on study type, sample size, and serum levels where those data were available. Those tables are now in Appendix B of the final report. Section 3.4 in each of the final HESDs provides a synthesis of the human and animal data (i.e., discussion of concordance and lack of concordance between human data and animal model data) for each of the key effects.

COMMENT 2: All of the sections related to the PK models developed by ORD need additional information for clarity and transparency. As written, it is not clear that the PK values presented throughout the document actually represent a reanalysis of existing data from studies that reported serum concentrations. The Wambaugh et al. (2013) study could be referenced to shorten this exercise as this publication provides details on the reanalysis of existing data.

RESPONSE: The serum data presented with the description of the studies are the data reported by the authors of the referenced papers for both the epidemiology and animal toxicity studies. They are not a reanalysis of the original data. The revised HESDs provide the details of the data used to develop the toxicokinetic model that identified average serum from the animal data from which to derive the HED published in Wambaugh et al. (2013). Information from other published phamacokinetic or toxicokinetic models is also provided. As a result, the quantification of dose response in section 4 to arrive at the RfD is much more focused on the application of the model results in obtaining average serum values, the human equivalent dose, and the RfD.

COMMENT 3: Justifications for choosing or not choosing particular values or endpoints needs to be more thoroughly detailed throughout both documents, especially for endpoints that appear to occur in both experimental animal models and exposed humans (i.e., thyroid hormone disruption and immunotoxicity).

RESPONSE: The dose-response assessment (section 4) of each of the HESDs was substantially revised to improve clarity and justification of EPA's endpoint selection. The studies were selected because of the data they provided, including having dose-response information that identified a NOAEL and LOAEL, or a LOAEL without a NOAEL and for the final document, serum information that could be used to determine the average serum associated with the NOAEL or LOAEL plus consideration of study quality.

Despite the breadth of the available data, the critical effects from studies with dose response fell into five broad categories for both chemicals: those linked to liver, kidney, reproductive/developmental, immunological, and/or neurological effects for both chemicals. Support for the liver, kidney, developmental and immunological effects from the epidemiology data are moderate to strong.

The candidate RfDs that cover the spectrum of critical effects differ by less than an order of magnitude for each chemical. EPA made the choice among the candidate RfDs for each chemical based on the endpoint and the exposure conditions associated with the effect (e.g., a sensitive life stage) as described in both the HESD and the companion Health Advisory documents.

Fisher Comments

COMMENT 1: These documents represent an enormous undertaking to describe studies with PFOA and PFOS. Keep the same writing style for reporting studies. This was very good. A synthesis of the most important studies is needed and some statements about why other studies are not used by EPA. It is easy to get lost in the document because of its size, but if there was an analysis or synthesis section for the key toxicity studies and another for PK modeling rationale, it would help readers.

RESPONSE: The synthesis part in section 3 and the discussion of relevant studies in section 4 was revised extensively to focus on key studies and better describe why certain ones were chosen for modeling. The effects observed at the LOAEL in the animal studies are included in Tables 4-1 and 4-2 and are carried over to the tables that include the modeling and quantification results so that the reader does not have to refer back

to the earlier summary tables for the species and effects associated with the each average serum, HED, and/or potential RfD entries.

Hayton Comments

COMMENT 1: It would be helpful to use one set of units for test article amount and concentration. The draft documents use ng/mL, μg/mL, μg/L, ppb, ppm, and μM for PFOA/PFOS concentration in water, diet, and serum. It would be more straightforward to use one concentration term, preferably ng/mL, and perhaps μg/mL in addition as necessary. But making comparisons among ng/mL, ppm, and μM is a distraction.

RESPONSE: The units reported for the animals studies are those used by the authors. The important variable is the dose which is usually given in units of mg/kg/day. In some cases a paper does not present dose. In those situations, EPA (1988) conventions for converting concentration in drinking water or diet to dose were applied.

COMMENT 2: In Section 3 of both documents, it would be helpful to include a summary table of primary pharmacokinetic parameter values for the species included in this section. Tables 3-17 – 3-20 in the PFOS draft document are a good start. In the PFOA document, Table 3-23 lacks CL values, and Tables 3-24 and 3-25 lack V_d values. For the pharmacokinetic model analyses presented, primary parameters values could be limited to CL, Vd_{ss}, and half life (see table in response to question 10). The CL and V_{dss} values should be normalized to body weight. Where there are multiple models for a species, there should be separate entries for each study. Where there are multiple dosages for a species, there should be separate entries for each dosage. For the PBPK models, V_{dss} values are not available and therefore should not be included. Such a table would be helpful to show consistency or lack thereof among studies and would facilitate selection of the best available values for CL and V_{dss} for use in a human PK model that would predict steady-state serum concentration from intake (dosing) rate and, conversely, predict intake rate from steady-state serum concentration. These predictions are probably the primary reason to include a pharmacokinetics section in the documents.

RESPONSE: EPA provided this information in tables, where data were available. For Table 2-24, clearance was not given in the paper with the other parameters; it was stated that clearance was optimized. Also EPA does not have V_d values from Kemper (2003) to add to Tables 2-26 and 2-27. Extensive details of the Wambaugh et al. (2013) model were added in tables and include model parameters for mice, rats, and monkeys and output by dose for predicted average serum and AUC.

COMMENT 3: The pharmacokinetic sections of both documents lack example graphs of serum concentration-time data on semilog coordinates for PFOA and PFOS. Inclusion of a few representative graphs would help the reader evaluate the consistency of the data used to generate the pharmacokinetic parameter values, and where model-based equations have been fitted to the data, the scatter of the measured concentrations around the model-predicted line would be informative as to the goodness of fit and the validity of the model and its parameters.

RESPONSE: While EPA agrees that graphical representation is often useful with data evaluation, EPA elected not to generate graphs from the published tabular data for distribution and excretion results. The data were utilized in the development of the PK models used to estimate average serum.

Longnecker Comments

COMMENT 1: I can see advantages to treating this more like a systematic review of the literature, where the specific search algorithm for included articles is laid out, as are the range of dates of publication to be considered, and any other selection criteria applied for articles considered. In these documents, while the review of earlier literature appears to be comprehensive, after some point there must have been some decision making about which of the more recent articles to include.

The EPA has many guidelines about how data like these are to be evaluated, yet in the document few, if any, references to these guidelines were cited. Because so many guidelines exist, it could help readers if the authors cited specific places in critical documents that provide guidance for specific decisions.

RESPONSE: A description of the criteria used to evaluate each study is included in the background in each document. The literature search strategy is now included as Appendix A.

Slitt Comments

COMMENT 1: The document reads very well. Although not included in the RfD determination, including a table of the observed human effects along with serum concentrations in Section 5.0 would put Tables 5-2 and 5-3 into context. Some sort of layman explanation to help understand why only non-human exposures are being included would be helpful to the general public.

RESPONSE: Human serum levels are included in the summary of epidemiology in section 3 of the HESDs for comparison to the tables of animal data and the animal serum information used in quantification. The description of the use of human data qualitatively, as an additional line of evidence in the derivation of the RfD, has been added.

EPA Responses to Specific Editorial and Technical Comments

Bruckner Comments

PFOA-specific Comments

Page	Paragraph	Comment or Question	Response
3-11	5, lines 7-11	It is stated that the PFOA concentration in bile increased by a factor of 12.5 with the increase in PFOA dose from 12.5 to 25 umol/kg in wild-type mice and 19.5 in PPARα-null mice. These factors should be 2.8 for wild-type and 6.1 for PPARα-null mice. The document's authors may want to rethink their interpretation of the data. The results for the wild-type mice do suggest saturation of transport from liver to bile ducts, but the PPARα-null results do not, indicating a role for PPARα in this process. In contrast to the foregoing, the findings of Lou et al. (2009) (p. 3-11, pgr. 2) indicate their highest dose of PFOA is cleared from the blood of mice more rapidly than lower doses, suggesting saturation of hepatic and/or renal reuptake transporters. What is the relative importance of biliary and renal elimination of PFOA?	Bile numbers have been corrected. EPA agrees with the reviewer's interpretation. Text has been changed to reflect non-saturation in PPARα-null mice and suggestion of PPARα-mediated clearance.
3-12	3, lines 2-4	It should also be stated that upregulation of MRP3&4 and the OATs may be <u>beneficial</u>, due to increased biliary excretion of bile acids, bilirubin, conjugated metabolites of toxic chemicals, etc.	Sentence inserted.
3-14	1 & 2	It might be stated that the findings of Hinderliter (2004) support those of Han (2003), in regards to development of female rats.	That these studies support each other has been added.
3-14& 3-15		It is problematic to try to compare values in Table 3-14 with values referred to at the end of the second paragraph on p. 3-17. Whole pup and pup serum PFOA levels decrease between PND 1&18 for each dosage in the table. It would be preferable to include another table showing the PFOA levels with body weight taken into account. Table 3 – 14 and other tables should include the species in the title. It would also be helpful to include some details of the experimental protocol in the footnotes.	Species name was added to the table titles for Tables 2-11 to 2-15. An additional table was not added as data are presented as published. Details of the experimental protocol in the footnotes of tables were not added because this information is clearly laid out in nearby paragraphs. Also, this has not been done for any of the other tables in the document.

Page	Paragraph	Comment or Question	Response
3-20		It would be useful at the end of this section (Distribution During Pregnancy and Lactation) to summarize the primary findings, or conclusions that can be drawn from the data that were presented.	An overall summary has been added at the end of the Toxicokinetic section, which includes distribution during pregnancy and lactation.
3-23	4, line 2	It should be emphasized that urinary excretion of PFOA was substantially higher in female than male rats.	Changed.
3-28	2, line 4	Replace "receptors" with "transporters".	Done.
3-28	6	Did 10 uM PFOA inhbit PAH and estrone uptake to a greater extent than 100 uM PFOA?	The % uptake at each concentration has been added.
3-29	3 & 4	It is not clear what Yang et al. (2009) concluded about the role of OATp1a1 in the uptake of PFOA from glomerular filtrate.	As stated in the paragraph, levels are much higher in male rats than females, which would favor resorption.
3-32	3 & 4	These two summary paragraphs are very helpful.	Comment noted.
3-37	1, line 1	Should "adsorption" be "absorption"?	Changed.
4-7 & 4-9	Tables 4-1 & 4-2	Tables 4-1 and 4-2 are quite helpful in integrating the results of studies of occupationally-exposed populations.	Comment noted; these tables have been extensively revised.
4-13		A concluding sentence should be added to summarize the findings of a lack of association of PFOA with diabetes, metabolic syndrome, etc.	Sentence added.
4-32		The NOAEL and/or LOAEL for this study should be stated at the end of the paragraph.	These have been added before reference to the immunological endpoints.
4-34	2	Is the LOAEL for liver effects 1 ppm in the study of Loveless et al. (2008)?	The LOAEL is 10 mg/kg/day based on increased liver weight, hypertrophy, and necrosis. This has been added.
4-38	1	Include the meaning of the abbreviation "mPPARα".	Added.
4-39		Inclusion of the table for Minata et al. (2010) would be useful to help readers better comprehend the study findings.	EPA agrees that tables are often useful in presenting data, however, an additional table was not added as the results are described with a clear NOAEL/LOAEL statement.
4-40		A table of short-term LOAELs and NOAELs should be added here or in Section 5.	This is included in section 4.
4-47	2	It is hard to believe, judging from the slight difference in mean values and their standard deviations, that absolute and relative liver weights are significantly higher than controls in the 1 mg/kg/day group.	Data are presented as published.
4-67	2, line 5	Insert "absolute" before "liver weight".	Done.

Page	Paragraph	Comment or Question	Response
4-69	1, lines 1 & 2	It might be worthwhile to point out that the actual study by Butenhoff et al. was conducted prior to 2004.	Done.
4-73		A summary sentence (or two) should be added at the end of the Mutagenicity and Genotoxicity section.	Done.
4-83		A summary paragraph should be included at the end of the Immunotoxicity section.	An introductory paragraph has been added at the beginning of the section.
4-101	1, line 14	Insert the word "some" before "occupational studies". In order to present a more balanced perspective of findings in occupational studies, the following sentences could be added at the end of the paragraph: "Olson and Zobel (2007) examined groups of male workers at 3 fluorochemical production facilities. Serum PFOA concentrations were not associated with total cholesterol, LDL or HDL in workers at these facilities."	This paragraph has been re-written along with the revised epidemiological data such that this revision is no longer relevant.
4-102	4	It should be stated that the increases in serum enzyme activity in workers were quite modest/small. The following sentence should be added at the end of the paragraph: "Emmett et al. (2006), however, found no association between serum PFOA and liver or renal enzymes".	See above comment.
4-103	2, line 2	Change "apoptotic or necrotic damage of" to "apoptosis or necrosis of". Apoptosis and necrosis are types of cell death, not damage/injury.	Done.
4-103	3, line 1	It is true that PFOA may interfere with the biliary excretion of other compounds that are transported by the same transporters. Upregulation of the genes for these transporters, however, may be beneficial in that the excretion of bile acids, bilirubin and conjugates of toxic chemicals/metabolites may be hastened.	This has been revised.
4-103	4, line 2	I would avoid the word "critical" until the section on Dose-Response Assessment.	Done.
4-103	4	Increases in absolute and relative liver weights were dose-dependent (Cui et al., 2009; Elcombe et al., 2010; Wolf et al., 2008a)	Done.

Page	Paragraph	Comment or Question	Response
4-103	5	It is important to distinguish between effects of PFOA on rough and smooth endoplasmic reticulum (RER and SER). RER content was diminished, but there was a proliferation of SER.	Distinction added.
4-104	2, line 5	This last line should be amended to read "that PFOA has some effects of unknown toxicological significance that appear to be independent of PPARα activation.	Done.
4-104	4, line 3-5	The meaning of the sentence is not clear. Has something been omitted?	This paragraph has been revised.
4-105	3, line 3	Add "of offspring" between "abilities" and "at 6 and 18". Include Fei and Olsen's (2011) finding of no association between prenatal PFOA exposure and behavioral or coordination problems in children at age 7.	Changes made. Sentence added.
4-109	3	The species (i.e., mice) studied by White et al. (2009) and by Wolf et al. (2007) should be stated.	Paragraph no longer present in revised document.
4-111	4, line 2	Replace "examine" with "determine whether there was".	Paragraph no longer present in revised document.
4-112	2, lines 1 & 2	The first sentence is misleading and should be rewritten. Butenhoff et al. (2012) did not see a significant increase in liver adenomas or carcinomas. Biegel et al. (2001) reported an increased incidence of hepatic adenoma but not carcinoma.	This section has been extensively revised.
4-112	2, line 13	What is hepatic cystoid degeneration?	Definition added.
4-114	2, line 3	Insert "decreased" before "apoptosis".	Paragraph revised such that comment no longer relevant.
4-115	5, line 2	What is meant by "PRAR exposures"?	This has been changed to PPAR activation.
4-116		There is no mention of PFOA-induced changes in expression of genes (e.g., cell cycle control, peroxisomes biogenesis, inflammation, etc.) that are PRARα-dependent. There is no mention of the role of PRARα or peroxisomes in oxidative injury and carcinogenesis.	These are discussed in the MOA for liver tumors on the previous pages. The mechanism for PFOA-induced Leydig cell tumors has not been fully elucidated.
4-120	1, lines 11 & 12	Insert "these" between "that" and "hormones".	Done.

Page	Paragraph	Comment or Question	Response
4-121	3	It would be helpful to give the PFOA dosages of White et al. (2007) and one or two other studies, so the reader will have some idea of the magnitude of PFOA exposure required to alter mammary gland development.	Doses added.
5-1		RfD: Omit the word "wealth" from the bullet pertaining to epidemiology studies. There have been relatively few epidemiology studies of PFOA-exposed populations.	This section extensively revised; comment no longer relevant.
5-2	1, lines 2-6	Another obvious point should be made here, mainly that occupational exposures result in much higher plasma PFOA levels and body burdens than do environmental exposures. Thus, it would be anticipated that adverse effects would be more apparent in PFOA facility workers.	Serum levels have been added.
5-2	1, line 5	Include the words "in some instances" between the words "shown" and "between". Otherwise, it appears from this paragraph the serum PFOA concentrations are consistently/usually associated with the various maladies.	This section extensively revised; comment no longer relevant.
5-2	3, line 8	Insert "failure to attain" between the words "with" and "developmental".	Done.
5-7	2, line 4	Insert the word "rodent" between "between" and "species"	This section extensively revised; comment no longer relevant.
5-19	1, line 1	Insert "from some studies" between "data" and "have".	Done.

PFOS-specific Comments

Page	Paragraph	Comment or Question	Response
3-2	5, lines 2 & 3	It is stated here that "the ratio of PFOS identified in serum and liver tissue are similar". Do the authors mean that PFOS concentrations in the serum and liver are similar?	The reviewer is correct: "ratio" has been changed to "concentration".
3-2	6	How does PFOS distribute between plasma lipoproteins and proteins/albumin?	Sentence changed to state that incubation was with separate protein fractions.
3-5	1, lines 9& 10	How much lower were milk PFOS levels than serum levels?	Deleted reference to serum levels since these were not measured in the study. Added mean milk and hepatic levels.
3-7	1, line 2	Oral and gavage are redundant.	Deleted oral.
3-16	Figure 3-1	This figure nicely illustrates relative PFOS levels in dams and fetuses/pups over time.	Comment is acknowledged; no formal response or action is necessary.

Page	Paragraph	Comment or Question	Response
3-19	1,line 3	Insert “groups” between “day” and “on”.	Done.
3-21	1, line 10	Substitute “longer” for “slower”.	Done.
3-23	2	It is not clear who conducted the human PBPK modeling nor which model they used.	Reference added to line 3: Loccisano et al. 2011.
4-21	2, lines 1-3	What did the 2nd monkey die from?	It is stated in the following sentence that the cause of death was unknown.
4-26	3, line 3	The word “concentrations” should be replaced by “doses”.	Done.
4-39	1	Does an increase in motor activity on PND 17, but no such effect on PND 13, 21 or 61, constitute a toxicologically-significant effect?	In the analysis by EPA, this is considered a toxicologically significant effect.
4-56	2, lines 1 & 2	It is stated that “taken together, these studies suggest a PPARα-independent mechanism…” Of the studies reviewed to this point in the document, only that of Abbott et al. (2009) supports this premise. Qazi et al. (2009), Rosen et al. (2010) and other groups of investigators have reported other PPARα-independent effects of PFOS.	“Taken together, these studies…” has been replaced with “The studies by Abbott et al. (2009) and Rosen et al. (2010)…”
4-60	2, lines 15-17	Is oxidative damage likely to be operative to a significant extent at lower PFOS doses?	No data were available.
4-61	2, line 4	What is meant by “The concentration…”?	The concentration used in the culture; this has been added.
4-61	4, line 2	Change “dose of exposure is” to “levels of exposure are”.	Done.
4-62	1, lines 2 & 3	What did Olsen et al. (2003) find correlation between?	This has been revised to note correlation between serum and hepatic levels.
4-62	3, lines 4 & 5	Identify the species (i.e., rat) studied by Chang et al. (2009) and Stein et al. (2012).	Done: added rat and human, respectively.
4-62	5	The liver of rats and monkeys was examined for histopathological changes, but the histological changes should not be considered lesions nor pathological.	Changed to microscopic lesions.
4-68	4, lines 5 & 6	The elevated incidence of hepatocellular adenomas/ carcinomas was almost entirely due to adenomas. Only 1 of 60 high-dose female rats exhibited carcinoma.	No changes made. Increased adenomas in females is already stated in the sentence.

Page	Paragraph	Comment or Question	Response
4-69	5, lines 3 & 4	It is stated here that there was no increase in hepatocellular proliferation detected in the subchronic study of Seacat et al. (2003). It is stated previously on page 4-69 that "the data for PFOS are adequate to support some but not all key events…" I assume that cell proliferation is thought to be a missing event. Seacat et al. (2003) reported that the average hepatocyte proliferation index was not increased, but that some animals exhibited mild increases. It is clear in the current document that PFOS is not as potent a PPARα inducer as PFOA.	Comment is acknowledged; no formal response or action is necessary.
5-4	2 & 5, line 7	Again the terms "histopathological" and "lesions" are misnomers.	Changed to microscopic lesions.
5-4	3, line 9	What is meant by a "biologically significant decrease in survival" at 0.8 mg/kg?	This section was revised with better wording.

Cory-Slechta Comments

PFOA-specific Comments

Page	Paragraph	Comment or Question	Response
Chapter 5		The text of Chapter 5 in the PFOA document (and other places) continues to state that a 10% increase in liver weight would not be an adverse effect, but merely a denominator for loss of homeostasis. On what basis was this conclusion derived? What is the support for this statement? It appears that benchmark dosing was applied to studies that had liver weight as the common denominator, but does this accommodate the lowest NOAELS and LOAELS observed for any endpoint in the long duration studies? Use of just studies with the common denominator because they provide replication ignores the fact that some other effect may occur at lower levels but simply hasn't been evaluated in as many studies as focused on PPARa-based targets. If this isn't the case, then the text should clearly address this.	The POD for PFOA has been changed to be based on low birth weight, developmental delays, reduced body weight, and increased kidney weight in mice and rats (Lau et al. 2006, Butenhoff et al. 2004a). Increased liver weight is acknowledged as a common finding but not considered adverse in the absence of other effects as defined by Hall et al. (2012).
5-7	2	States that the $BMDL_{10}$ values all fall below the experimental LOAELs. So, what does that mean, is there some conclusion that is supposed to be reached from this? IF so, please state it.	This is no longer relevant since the BMD analysis is not used in the RfD determination.
5-13	1	States "Generally these values were similar." What does similar mean? What is acceptable in this context?	The sentence has been deleted.

Page	Paragraph	Comment or Question	Response
5-16	3	States that the half-life value Bartell et al. (2010) was sued for half-life because it seemed more relevant to scenarios where exposure result from ingestion of contaminated drinking water by members of the general population. This rationale does not appear to consider the potential different strengths and weaknesses of the other potential studies. Is it necessarily the case that general population is more important than occupational studies? The rationale needs to be described in greater detail. Virtually no rationale is provided for the choice of the Thompson et al. (2010) study for a volume of distribution value.	The Bartell et al. (2010) half-life represents an estimate corresponding to the U.S. general population rather than the occupational populations as reported in studies, such as Olsen et al. (2007). It was derived using the declines in serum values among members of a highly exposed population following a change in residence that lowered the ongoing exposures. The Health Advisory guidelines apply to members of the general population exposed to a chemical through their drinking water. Accordingly, the Bartell et al. (2010) estimate was used rather than one based on occupationally exposed cohorts. The recent NHANES data demonstrate that serum levels are declining among the general population. This strengthens the decision to utilize the Bartell et al. (2010) half-life. With regard to the volume of distribution for PFOA, none of the available studies provide data for calibration of volume of distribution of PFOA in humans. However, several researchers have attempted to characterize PFOA exposure and intake in humans (Thompson et al. 2010; Lorber and Egeghy 2011) through pharmacokinetic modeling. In the models, volume of distribution was defined as the total amount of PFOA in the body divided by the blood or serum concentration. Both research groups defined a volume of distribution for humans using a simple, single compartment, first-order pharmacokinetic model (Thompson et al. 2010; Lorber and Egeghy 2011). The models developed were designed to estimate intakes of PFOA by young children and adults and the general population. In both models, the volume of distribution was calibrated using human serum concentration and exposure data from NHANES, and it was assumed that most PFOA intake was from contaminated drinking water. Thus, the value for volume of distribution was calibrated so that model prediction of elevated blood levels of PFOA matched those seen in the study population.

Page	Paragraph	Comment or Question	Response
5-16	3		Thompson et al. (2010) used a single compartment, first-order pharmacokinetic model to predict PFOS concentration in blood serum as a function of dose, elimination rate, and volume of distribution. The volume of distribution was first obtained for PFOA by calibrating human serum and exposure data. The volume of distribution for PFOS (230 mL/kg) was adjusted from the calibrated PFOA data by 35% in accordance with the differences in PFOA and PFOS volumes of distribution calculated by Andersen et al. (2006), the study used by Wambaugh et al. (2013) in the development of the model utilized in the determination of the RfD for PFOA.

PFOS-specific Comments

Page	Paragraph	Comment or Question	Response
1-1	4	There are similar concerns for the PFOS document. Loose terminology should be eliminated, e.g., what is a 'finding of note' as used in the executive summary for PFOS.	The Executive Summary has been revised; comment no longer relevant.
Executive summary		The PFOS executive summary is of limited utility; for many readers this may be as much of the document as they read; as currently written it is not clear or transparent nor does it sufficiently explain how it arrived at an RfD.	The Executive Summary has been revised; comment no longer relevant.
3-3	Table 3-1	Couldn't a sentence essentially substitute for Table 3-1; it really isn't useful.	Possibly, but table has been retained in the document.
3-25	1	Loose terminology should be eliminated, e.g., what is "generally good"	The word "generally" has been deleted.
3-26	Figure 3-7	Figure 3-7 has no explanation of what is the black vs. gray line.	This is indicated in the figure caption.
All tables		There is a need to improve all of the tables; they should always include study name/year, sample size and exposure duration information on them; this would make all of the comparisons easier to evaluate and not require the reader to continue to go back and forth to the text.	All tables have references footnoted at bottom of table. All of the epidemiology tables have been changed to include study name, sample size, and exposure duration, if provided.

Page	Paragraph	Comment or Question	Response
4-4	Table 4-1	For example, table 4-1 has only study name and year, but what really matters is also exposure duration and sample sizes, because the comparisons of outcomes in the Table depend upon the power of the study to detect effects at the very least.	All tables of human epidemiology data have been revised to include type of study, sample size, serum levels, and outcomes.
4-9	Table 4-2	The same comment applies to Table 4-2 and any others with this intended purpose.	All tables of human epidemiology data have been revised to include type of study, sample size, serum levels, and outcomes.
4-11	Table 4-3	Table 4-3 needs sample sizes, exposure duration etc.	All tables of human epidemiology data have been revised to include type of study, sample size, serum levels, and outcomes.
4-24	Table 4-7	Tables that summarize a significant amount of data from a single study (e.g., 4-7) should include the study authors and year in the Table title so it doesn't have to be searched for.	This information is given directly below each table.
		In several instances in the PFOS document, adverse effects early that appear to be reversed at a later age are discounted with the suggestion that they therefore do not matter; given our increasing understanding of the importance of early changes in terms of epigenetic changes, this is no longer appropriate and in fact, misleading.	EPA has attempted to revise all incidences of this language to note reversibility, but not discount the effect.
5-16	Table 5-8	What do the parentheses signify?	Numbers in parentheses indicate standard deviation as noted below the table.
5-17	Table 5-9	What do the parentheses signify?	Numbers in parentheses indicate standard deviation as noted below the table.

DeWitt Comments

PFOA-specific Comments

Page	Paragraph	Comment or Question	Response
4-102	2 & 3	DeWitt et al. 2009 also included data on triglyceride levels in C57BL/6 mice exposed to PFOA for 15 days; triglyceride levels were dose-responsively decreased.	A sentence containing these data has been added.

PFOS-specific Comments

No specific observations.

Fisher Comments

No specific observations.

Hayton Comments

PFOA-specific Comments

Page	Paragraph	Comment or Question	Response
1-2	Last, line 5	Delete "in"; should read "… in rats was analyzed …"	Paragraph has been revised such that this comment is no longer relevant.
3-2	1, lines 6-8	Assumption that fecal excretion represented unabsorbed PFOA is problematic; suggest rephrasing this sentence.	The phrase "and did not include biliary loss" has been added to this sentence.
3-3	Table 3-1	Protein binding is important for PK modeling, where the fraction unbound (fup) is the important parameter, not the fraction bound. Suggest listing fup values rather than percent bound.	Data are as presented in the reference. The study author did not include fup values.
3-6	Last, line 3	"concentration" should be "dose rate"	Changed.
3-8	2, line 4	In addition to liver, kidney, and blood, other tissues are prominent. E.G., Table 42 of Kemper shows that in male at 1 mg/kg, t=Tmax, GI tract, GI contents, muscle, bone and skin contained a greater percentage of dose than did the kidney.	Sentence changed to note other tissues.
3-8	2, line 8	"Blood to kidney" should be "kidney to blood"	Sentence was revised.
3-8	2, line 10-11	In Kemper, Tables 44-45, blood to kidney ratios are not 10 or higher in males.	Sentence changed to state blood levels were 10-fold or higher than kidney levels.
3-8	2	This paragraph reports both percent of dose found in tissues, and concentrations found in tissues. But Tables 3-4 and 3-5 present only the former. When presenting tissue concentrations, please make it clear that those data are not shown.	Changed to note distribution in tissues.
3-18	Last, line 3	"were" is repeated.	Deleted.
3-19	1, line 1	Technically incorrect to say that the level peaked at PND7; that was the earliest sample time. The peak may have occurred before PND7.	Changed to "at or before".
3-19	Table 3-15	The last dose was on GD17; strange that at 1 and 3 mg/kg the serum concentration increases from PND7 to PND14.	Comment noted.
3-22	4	Last sentence is garbled.	The paragraph has been revised.

Page	Paragraph	Comment or Question	Response
3-22	4, 5	Agree that biliary elimination is possible, but it could be that chloestyramine binds PFOA and PFOS in the GI tract lumen after they passively diffuse from the blood to the gut. There seems to be no direct evidence of biliary elimination, e.g., bile collected from treated animals.	These two paragraphs have been revised with reference to elimination in bile noted as possible.
3-23	Last, line 4	Should be Table 3-18.	Table numbers corrected.
3-34	Last, line 9	Should be "nonlinear least squares"	Corrected.
3-35	Table 3-23	Column 2, "Adsorption" should be "Absorption"	Corrected.
3-38 3-38	2 Figure 3-7	The arrow from Gut to Liver appears to point in the wrong direction; it should represent biliary excretion of PFOA from Liver to Gut.	The figure is as presented in the reference, Loccisano et al. 2011. Absorption from the gut was included in the model, but possible biliary elimination to the gut was not included.
3-43	Last line	"… indicating the absence of active excretion in human kidneys." This does not follow from the observation of renal clearance being about 0.001% of GFR. A plasma free fraction of 0.001 would account for the CLr being 0.1% of GFR, and passive tubular reabsorption would make it 0.001% of GFR since urine flow is about 1% of GFR. Other scenarios are possible that do not invoke the absence or presence of active excretion.	Phrase has been deleted.
3-44	Table 3-24	Should report all data values with three significant figures. For example, Lambda z values have only one sig. fig., while $T_{1/2}$ values have 5-6.	All values are as presented by the study author.
3-46	2	This reviewer does not follow the derivation and use of a value for volume of distribution with regard to intake rate and serum concentration of PFOA. If the subjects were at steady state, the body burden would have to be known. At steady state, the serum concentration would be independent of the volume of distribution, so any V value ought to match the intake rate to the steady state serum concentration.	The description of the calibration of volume of distribution is as given by the study authors.
4-9	1	Log transformed concentration was 1.51 and 1.48 ng/mL – are these the logarithms? IE, are the actual concentrations 10^1.51 = 32 and 10^1.48 = 30 ng/mL?	Reported as log PFOA concentration in the paper. It was not clear if actual concentrations are 32 and 30 ng/mL, so left this as stated in the reference.
4-20	2, line 8	Anderson here is spelled Andersen in the reference list.	Corrected.
4-30	1, line 9	prostrate should be prostate.	Corrected.

Page	Paragraph	Comment or Question	Response
4-31	4, line 10	decreased should be decrease.	Corrected.
4-112	1	It would be helpful to restate the serum concentrations for the Eriksen and Vieira studies, or refer reader to p. 4-29 where they are provided.	Serum levels have been added to this section.
4-112	1, line 9	Delete "for".	Paragraph was revised; no longer relevant.
4-112	2, line 12	Delete "were".	Paragraph was revised; no longer relevant.
4-118	4	Delete "of actions" after MOAs	Done.
4-120	3	The broad range of half lives could also be due to person-to-person variability in the free fraction of PFOA in serum (fup). This is the case for highly bound drugs; e.g., warfarin.	"…and binding.." has been added to the sentence.
5-1	3	Pharmacokinetic is misspelled.	This section has been completely revised; comment no longer relevant.
5-1	5	Disagree – exposure assessment based on the human data is feasible. In fact, the serum concentrations are a better measure of exposure than are intake measures as they reflect all intake pathways and eliminate bioavailability and pharmacokinetic influences on internal exposure.	This section has been completely revised; comment no longer relevant.
5-12	Last	Table numbers should be 5-6, 5-7, and 5-8.	Corrected.

PFOS-specific Comments

Page	Paragraph	Comment or Question	Response
3-5	3	The low CSF : serum concentration ratio could also be due to an export transporter that pumps PFOS out of the CSF and/or to extensive serum protein binding, where only the free serum concentration of PFOS is in equilibrium with the free PFOS concentration in the CSF.	Comment is acknowledged; no formal response or action is necessary.
3-22	2	The free fraction used for the model is much larger than that determined experimentally, Table 3-1; this should be pointed out in the text.	The text accurately states what is used in the model; Table 2-1 represents in vitro data so is not directly applicable.
3-22 3-23	2 Figure 3-5	The arrow from Gut to Liver appears to point in the wrong direction; it should represent biliary excretion of PFOS from Liver to Gut.	Figure is copied from Loccisano et al. 2011.
3-24	4	Anderson should be Andersen.	A search and replace was done for the entire document.
4-26	4	"concentrations" should be "dosages".	Changed.

Page	Paragraph	Comment or Question	Response
5.2	3	Should note for many of these studies, that steady state may not have been achieved due to the long half-life of PFOS. Half-life values from Section 3 are: mouse, 37 days; rat male, 40 days and female 64 days; monkey, 120 days. Using a one-compartment PK model, the time to 90% steady state is 3.3 half lives.	Sentence added: It is noted, however, that in some of these studies, steady states of PFOS may not have been achieved due to the long half-life of PFOS in animal models (see discussion of steady state in section 4.1.1.1).
5-5	3	The NOAEL for liver effects in rats of 0.072 mg/kg/day is not consistent with p. 5.4, para. 2, which states that lesions of the liver were observed in male rats after 104 weeks at this dosage.	This sentence has been removed in the revision.
5-7	2	For female rat, the PFOS half life is about 60 d and the period of gestation is about 20 d or one-third of a half life. If PFOS is administered to the dam only during gestation at a fixed daily dose, the serum concentration of PFOS would rise from 0 to 21% of the steady-state serum concentration that the fixed dose rate would produce at steady state. The exposure of the fetus during gestation would average only about 10% of the exposure that would have occurred if the dam had received PFOS for 4 half-lives (240 days) prior to mating. BMDs based on such a fixed dose could be elevated by as much as a factor of 10 compared with the steady state situation. Steady state would be the relevant situation for humans. For the Luebker study (Table 5-3) the serum concentration during gestation would have increased from about 38% to 50% of the eventual steady state concentration.	A discussion of steady state has been added to section 4.1.1.1. It is noted that "the average serum values from the studies that do not approach steady state have lower average serum LOAELs for endpoints of toxicological concern. Thus, the data do not appear to indicate increasing sensitivity as steady-state is approached. If anything, the average serum values appear to be more protective than serum concentrations at steady state."

Longnecker Comments

PFOA-specific Comments

Page	Paragraph	Comment or Question	Response
3-28	1st complete	Should the end of the sentence be "increase the transporters" rather than "increase the receptors"?	Done.
3-30	2nd complete	L 3, would insert "transfected" between "OAT3" and "cells"	Done.
3-39	1st complete	Next to last sentence: I doubt that Olsen assumed the major source of exposure was drinking water in the occupational study	Agreed, the sentence has been deleted.
3-41	4th complete	In the first formula listed, the plus sign should be an equal sign	Corrected.

Page	Paragraph	Comment or Question	Response
4-9	1st complete	L 3 from bottom: the values of 1.51 and 1.48 given are probably better described as geometric means.	This section has been completely revised; comment no longer relevant.
4-16	2nd complete	L 3 from bottom: would insert "draw" after "blood"	Done.
4-21	2nd complete	L 5: the value of 6.78 ug/L is a water level, not a serum level; this issue recurs on P 4-23, paragraph at bottom	This section has been completely revised; comment no longer relevant.
4-30	1st complete	L 8: should read "exposure categories" rather than "cancer categories"?	Done.
4-37	Table	Would note dose of PFOA somewhere in table or footnote	10 mg/kg has been added.
4-55	Last para	L 3: should the ">" be a "<"?	Done.
4-79	Last para	Last sentence: should "50 and 25" be "50 and 250"?	Done.
4-80	1st complete	The last sentence does not accurately describe the table. E.G., the CD4+CD8+ cells decreased at the 47.21 mg/kg/d dose	Decrease for CD4+CD8+ cells has been added.
4-82	Next to last para	Last sentence: the 37.5 mg/kg/dose is not mentioned earlier, so this is a little confusing.	Deleted; changed to note three highest dose groups.
4-85	Last para	L 2: should "0.5" be "0.05"?; Same issue for L 5.	Yes, corrected.
4-89	4th para	How long were the animals dosed?	Added "for 7 days".
4-110	3rd complete para	L 5: should "serum" be "blood"?	Yes, changed.
4-113	3rd complete para	L 1: insert "in" before "liver cells"	Done.
5-4	Last para	Were the criteria for inclusion in Table 5.2 the same as for Table 5.1?	This section has been revised and reason for including studies on the tables is given.
5-12	Para below table	8-6, 8-7, and 8-8 should be 5-6, 5-7, and 5-8	Corrected.
5-16	Last line	I do not see in the Thompson et al. (2010) study any mention of using exposure data from NHANES to calibrate the volume of distribution. Other sources of data were used, where the water had been contaminated.	This was described in section 2.5.3.
5-17	1st formula	"/day" should be deleted from "0.17 $L/kg_{bw}/day$"	Done.
5-20	Table 5-12	The first three values in the UF_{total} column need to be corrected; they should be 21900, 219000, and 21900	The UFa derived from clearance ratios has been deleted; comment no longer relevant.
5-21	Paragraph above table	Last sentence: UF_L should be UF_H	This section has been deleted; comment no longer relevant.
5-21	Last sentence	UD_S should be UF_S	This has been deleted; comment no longer relevant

Page	Paragraph	Comment or Question	Response
5-27	Calculations	The text says the body weight conversions should be based on the ¾ power. If so, the HED formulas are incorrect, and the HED should be 1.99 x 0.0254 = 0.0506, the dosimetric adjustment factor should be 0.0254, and the CSF should be 1.57. All the figures here should be checked as should the paragraph on P 5-28. The HED is 2,530-fold greater than the RfD, not 29,000.	Correct as written since the calculation is for a DAF which uses inverse of BW3/4 resulting in BW1/4. The sentence comparing HED to RfD has been deleted; comment no longer relevant.

PFOS-specific Comments

Page	Paragraph	Comment or Question	Response
1-1	2nd	1st sentence: would revise for clarity. Do you mean uncertainties exist about whether PFOS-induced peroxisome proliferation is involved in causing PFOS-induced hepatic lesions?	Sentence has been revised for clarity by adding "hepatic lesions induced by PFOS".
1-1	3rd	1st sentence: would revise for clarity; the occupational studies were done at PFOS production plants, but to my knowledge there are no residential populations that have been studied for health effects who lived near PFOS production plants. (Mid-Ohio valley factory was a source of PFOA.) In the 2nd sentence, I do not believe that exposure was mainly through contaminated drinking water in any of these studies.	First sentence has been revised to note that the population lived near a PFOA plant. Second sentence has been deleted.
4-66	2nd	The earlier summary of the Bloom et al. study (P 4-10) said the results were not statistically significant, whereas here the interpretation appears to be that the study found an association. The interpretation does not seem consistent across the two sections.	This paragraph has been revised to more accurately describe the data presented in the epidemiology section.
5-17	Below table	L 3: the word "terminal" should be deleted from this sentence	Done.
5-20	1st formula	The "/day" should come out of "0.23 L/kg bw/day"	Done.
5-26	L 2 from bottom	This should be 35 ug/L not 35 mg/L	Done.

Slitt Comments

No specific observations.

4. References

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