DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR PERFLUOROALKYLS

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

December, 2014

Peer reviewers for the third pre-public comment draft of the Toxicological Profile for Perfluoroalkyls were:

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Comments provided by Peer Reviewer #1:

General Comments

COMMENT 1 (MRLs for PFOA and PFOS): The studies were conducted and supported by manufacturers and commercial users of PFOA and PFOS. While there is no basis for it, some readers of the Profile may be skeptical of data from a source with a vested interest in the results. Use of additional data sources would alleviate this potentially negative perception.

RESPONSE 1: *Although the Butenhoff et al. (2002) and Seacat et al. (2002) studies were conducted at 3M, the studies were published in a peer-reviewed journal (Toxicological Sciences) and are supported by findings in laboratory animal studies conducted at other facilities.*

COMMENT 2 (MRLs for PFOA and PFOS): The data come from a relatively small number of animals and doses. There were a control group and three test doses, with four or fewer animals per dose for PFOA and four or fewer animals per sex per dose for PFOS. For PFOA there were only two animals that completed the six-month exposure regime at the highest dosage – 30/20 mg/kg/day; one animals that started the 30/20 dosage died during the study, possibly due to toxicity of the initial 30 mg/kg/day dosage, and three animals were removed from the study before six months. This raises the possibility that the two animals who completed the 30/20 dosage at the 20 mg/kg/day level were impaired by exposure to the initial 30 mg/kg/day dosage and that their elevated liver weights are compromised. Another possibility is that these two animals were less sensitive than the other four animals that dropped out of the high-dose test group. The high-dose result is thereby suspect. The low and intermediate dosages for PFOA produced average serum concentrations that were similar: 77 and 86 μg/mL. Thus it appears that the PFOA data used to determine the BMDL for PFOA (Appendix A) were sparse and the range of exposure values was narrow. For PFOS, the distribution of serum concentration values is less constricted than for PFOA, but there is an issue of how well they represent the exposure to PFOS over the study period.

RESPONSE 2: *Due to the early toxicity observed in the high-dose PFOA group (30/20 mg/kg/day), the BMD modeling was repeated excluding the high-dose group. The resultant BMDL values were slightly lower, but did not result in a change in the MRL.*

COMMENT 3 (MRLs for PFOA and PFOS): Markedly different dose ranges were studied. For PFOA, dosages were 3, 10, and 30/20 mg/kg/day; for PFOS, 0.03, 0.15, and 0.75 mg/kg/day. Systemic exposures for the two compounds were similar, however, because the elimination clearance value for PFOS is about one-sixth that for PFOA (1.4 vs. 8.9 mL/kg/day, Chang 2012 and Butenhoff 2004) and the PFOA animals were at steady state while the PFOS animals were accumulating PFOS throughout the study. The serum concentrations of PFOA and PFOS used in the BMD estimations were similar and ranged over 77–158 μg/mL for PFOA (Table 2-1) and 15.8–173 μg/mL for PFOS (Table 2-3). The serum concentrations for PFOA are averages of values measured at bi-weekly intervals from 6 weeks to the end of the study and likely represent steady-state concentrations due to the relatively short half-life of PFOA compared with PFOS in the monkey. The PFOA concentrations thus represent the systemic exposure of the animals over the final 4.5 months of the study. In contrast, the PFOS serum concentrations are the end-of-study, six-month concentrations. The half-life for PFOS in monkey is long and PFOS serum concentrations were rising throughout the exposure period, especially for the two lower dosages (Fig. 1, Seacat 2002). To make the PFOS serum concentrations representative of the average systemic exposure over the six-month exposure period, as they are for PFOA, it would make sense to integrate the serum

concentration-time profiles in Fig. 1 (Seacat 2002) and divide by the length of the exposure period to give an average serum concentration. This would likely reduce the POD value determined for PFOS.

RESPONSE 3: *Using the data in Figure 1 of the Seacat et al. (2002) paper, ATSDR calculated a time-weighted average (TWA) serum PFOS concentration for each dose group. The BMD modeling was repeated using the TWA serum concentrations. The corrected serum PFOS levels resulted in lower BMDL values and decreased the NOAEL for the female monkeys, resulting in a decrease in the MRL from* $5x10^{-5}$ *to* $3x10^{-5}$ *mg/kg/day.*

COMMENT 4 (MRLs for PFOA and PFOS): The dynamic range of the adverse effect (increase in liver weight) was relatively small and it showed considerable animal-to-animal variability as reflected in the magnitudes of the standard deviations of the mean weights, Tables 2-1 and 2-3. For PFOA, only the highest dose showed a relative mean liver weight that differed from the control. For PFOS, only the highest dose showed a difference in liver weight (absolute and relative; male and female) from the control (Table A-13). The measured serum concentrations also showed considerable variability. For example for PFOA the average serum concentration for the 10 mg/kg/day dosage was 86±33 μg/mL with a range for the 70 measured concentrations (every two weeks starting at Week 6) of 10–180 μg/mL. Fig. 1 in Seacat (2002) shows that the serum concentration of PFOS continuously increased over the six-month exposure period, especially for the 0.03 and 0.15 mg/kg/d dosages. In addition, there is a weak relationship between the daily dosage of the test substances and their measured serum concentrations, especially for PFOA, Table 2-1. The liver weight at six months in the test animals was compared with the liver weight in the controls; while this is a necessary study design, the starting liver weights of the animals are not known, which adds uncertainty to the apparent magnitude of the putative increase in liver weight. I.E., it was tacitly assumed that all study groups had the same average liver weight at the start of the exposure and would have had the same average liver weight at the end of the exposure if there had been no exposure to perfluoroalkyls. But the inter-animal variability in the liver weights suggests that this assumption is problematic. Taken together, these characteristics of the dose-response data instill a lack of confidence in their capacity to support a valid MRL.

RESPONSE 4: *Although there is some degree of uncertainty in using the absolute liver weights due to the assumption that starting liver weights were same across groups, ATSDR believes that it is the better metric for perfluoroalkyl liver toxicity due to the observed decreases in body weight. Additionally, the lowest POD for PFOA was for increased absolute liver weight and the lowest PODs for PFOS were the same for absolute and relative liver weight increases.*

COMMENT 5 (MRLs for PFOA and PFOS): The adverse effect quantified was an increase in liver weight; both absolute and relative (to body weight) liver weights were determined after six months of exposure to the test substances. While the Profile indicates that this adverse effect occurs at or below exposures that produce other adverse effects, there are data to suggest that there may be more sensitive adverse effects. Epidemiological studies (Post, G. B., Cohn, P. D., & Cooper, K. R. (2012). Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. Environmental Research, 116, 93-117) have found associations between PFOA and PFOS serum concentrations and a number of potential or actual adverse effects; e.g., bladder and kidney cancer, elevated serum cholesterol, elevated serum uric, incidence of thyroid disease, and altered levels of reproductive hormones. Serum concentration-response relationships have apparently been found with significant adverse effects associated with serum concentrations as low as 10 ng/mL. If this degree of sensitivity is correct, then there are adverse effects that are more sensitive to perfluoroalkyls than is liver weight increase. Furthermore, healthy adult humans and animals may be less sensitive to perfluoroalkyls than are individuals with particular pre-existing conditions and disease states. Also 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 MRL based upon it.

RESPONSE 5: *ATSDR applied an uncertainty factor of 10 to account for possible increased sensitivity in individuals with pre-existing conditions and disease states and an uncertainty factor of 3 to account for the lack of studies examining developmental and immunological endpoints in monkeys. Although there are limitations in the epidemiology database, the available data do not suggest that developmental effects would occur at lower serum concentrations than those associated with increases in serum cholesterol or uric acid levels. The available data on bladder and kidney cancer, thyroid disease, and altered reproductive hormone levels are inadequate for establishing a causal association with perfluoroalkyl exposure.*

COMMENT 6 (MRLs for PFOA and PFOS): Other approaches/data that might be used to estimate an MRL are the following:

- On p. 35 of the Profile, "The lowest LOAEL for developmental effects in mice (0.01 mg/kg/day; Hines et al. 2009)". CLmouse is about 6.6 mL/kg/d (Lou I 2009), which gives an estimated Css of 1.5 µg/mL (= 10 µg/kg/d ÷ 6.6 mL/kg/d). But Hines' mice were not at steady state since they were dosed daily for 17 days and the $t_{1/2,mouse}$ is 19 days. A considerably lower dose rate would have produced the 1.5 µg/mL concentration at steady state. Also, the NOAEL is unknown; 0.01 mg/kg/day was the lowest dose used. A lower dosage, say 0.025 mg/kg/day administered until steady-state to the dams prior to mating, and continued throughout gestation, could reasonably be expected to provide a PFOA systemic exposure to the fetus similar to that of Hines study. The corresponding serum C_{ss} would have been 375 ng/mL and division by an uncertainty factor of 90 would give a steady-state serum concentration of 4 ng/mL. This would convert to an HED of 0.34×10^{-6} mg/kg/d, which is about one sixtieth of the HED determined from the monkey liver weight gain data. Macon 2011 also found impaired offspring mammary gland development at 0.01 mg/kg/d during gestation in CD-1 mouse.

- Epidemiological studies (reviewed in GB Post et al., 2012) find associations between several adverse health effects and serum PFOA or PFOS concentrations in the 5–50 ng/mL range. While the epidemiological studies are cross-sectional and a cause-effect relationship cannot be assumed, the consistency of findings among studies for some adverse effects and support from controlled animal studies argue for consideration of the epidemiological data. Using a value of say 20 ng/mL and applying an uncertainty factor of 10 for human variability gives an estimated safe steady-state serum concentration of 2 ng/mL, which would lead to an HED that was less than 1% of that determined in the Profile.

RESPONSE 6: *ATSDR considered the alternative approaches suggested by the Reviewer. Regarding epidemiology studies, the strong evidence is for an association between serum PFOA or PFOS levels and increases in serum cholesterol levels; a causal relationship has not been firmly established. Although some studies have found dose-response relationships, there are a number of inconsistencies across studies; several studies of highly exposed subjects have not found significant associations between serum PFOA or PFOS levels and serum cholesterol levels (Emmett et al. 2006a; Olsen and Zobel 2007; Olsen et al. 2000; Wang et al. 2012). Unlike most of the laboratory animal studies, humans have been exposed to multiple perfluoroalkyl compounds and possible interactions (particularly dose additivity) between the compounds and serum cholesterol levels have not been fully evaluated. One study found a 20–30% attenuation of the serum cholesterol association when both PFOA and PFOS were considered (Steenland et al. 2009b). Lastly, the mechanism of action for the increased serum cholesterol levels has not been elucidated and reverse causality has not been ruled out.*

Many of the health effects observed in rodents, particularly those observed at the lowest doses, result from the activation of PPARα and species differences in the response to PPARα agonists are known. Because rodents are the most sensitive species and humans are much less responsive, basing an MRL on a rodent study could result in an overly conservative value.

COMMENT 7 (MRLs for PFOA and PFOS): Finally it is worrisome that the MRLs of $2x10^{-5}$ mg/kg/day for PFOA and $5x10^{-5}$ mg/kg/day for PFOS would lead to average steady-state serum concentrations of 235 and 625 ng/mL, respectively, in humans. While these concentrations are a small fraction of those found in some exposed workers and members of communities with contaminated water supplies, they are also large relative to concentrations measured or inferred in some animal and epidemiological studies that report associations with adverse effects. While healthy adults may not be adversely affected by serum concentrations in the hundreds of ng/mL, it is quite possible that such concentrations may cause serious adverse effects to the fetus, to children during neonatal and childhood development, and to adults with particular disease states or conditions. In summary, this reviewer is uncomfortable with use of such a narrow data base as provided by single studies for PFOA and PFOS in adult Cynomolgus monkey. In addition to adjustment of the PFOS serum concentrations to reflect the average over the exposure period, other data that permit quantification of the adverse effect – exposure relationship should be included in the Profile and used to estimate MRL values. A weight-of-evidence approach with several independently derived MRLs should be considered to arrive at defensible MRL values.

RESPONSE 7: *Most of the available animal studies have not measured serum PFOA or PFOS concentrations; with few exceptions, the available studies reported effects at 1,000 ng/mL, which is much higher than the human serum concentration that would result from exposure at the MRL. Effects observed at lower concentrations (immunotoxicity in mice or mammary gland alterations in mice) may not be relevant to humans since the mechanisms involve PPARα activation. As noted in the response to COMMENT 6, the available human data are not adequate to establish dose-response relationships or causal associations for adverse health effects. As noted in the response to COMMENT 5, ATSDR has used an uncertainty factor of 10 to account for human variability including increased sensitivity due to pre-existing conditions or stage of development. The available epidemiology data do not suggest that the fetus is unusually sensitive to the toxicity of perfluoroalkyls; additionally, the limited data available on children do not suggest increased sensitivity.*

COMMENT 8 (MRLs for PFOA and PFOS): This reviewer applauds the "… use [of] the serum concentration as an internal dosimetric and the assumption that a serum concentration level that would result in an effect in monkeys would also result in an effect in humans". This is a sound approach to making sense of dose-response relationships among species.

RESPONSE 8: *No revision is suggested.*

COMMENT 9 (MRLs for PFOA and PFOS): Calculation of the MRL was difficult to follow, but appears to have involved the following for PFOA:

a. Determination of $BMDL_{RD10\%}$ = 21.5 µg/mL from benchmark dose modeling of the absolute liver weights of Cynomolgus monkeys administered 0, 3, 10 or 30/20 mg/kg/d. This was taken as the point of departure (POD) for calculation of the HED.
b. The POD was multiplied by the elimination rate constant (ke) and the volume of distribution (V) for human; this estimates the intake rate of PFOA in humans that would produce a steady-state

serum concentration of 21.5 µg/mL.
HED = 21.5 µg/mL x 4.95E-4 d^{-1} x 200 mL/kg = 2.13 µg/kg/day.

c. The HED was divided by a safety factor of 90 to give the MRL.
MRL = 2.13 µg/kg/day /90 = 0.0237 µg/kg/day
This value was rounded off and expressed in mg/kg/day: $2x10^{-5}$ mg/kg/day.

This approach indicates that a steady-state serum concentration of 21.5 µg/mL PFOA is the threshold concentration above which there is a "significant" increase in absolute liver weight in the monkey, that this threshold concentration in humans would be an acceptable upper limit, and that from what we know about PFOA pharmacokinetics in humans an average daily intake of $2x10^{-5}$ mg/kg/day should be acceptable with a safety factor of 90 making allowance for monkey – human differences in sensitivity, for inter-individual differences among humans, and for the possibility that data are lacking for developmental and immune system effects in monkeys that might have led to a lower POD.

RESPONSE 9: *The description of the derivation of the MRL has been revised to clarify the approach.*

COMMENT 10 (MRLs for PFOA and PFOS): For PFOS, BMD modeling found BMDL values for male monkeys, but not for females. Instead of using BMD modeling to identify a serum concentration for the POD, the female NOAEL serum concentration was used for the POD. This was the lowest serum concentration for which liver weights (both absolute and relative) were not statistically different from the control liver weights, using $p<0.01$ (Table A-13). The absolute liver weights at the 13.2 and 66.8 µg/mL serum concentrations were very close (56.8 vs. 57.0 g) and given the use of $p<0.01$ for the significance test, it is difficult to distinguish whether 66.8 µg/mL is superior to 13.2 µg/mL as the NOAEL and from that the POD. BMDLs for the males range from 24.8 to 116.4 µg/mL for the various modeling approaches that were used. The small numbers of animals used, the fact that serum PFOS concentrations were rising throughout the six month period but only the end-of-study values were used, and the apparently flat dose-response relationship altogether point to a considerable degree of uncertainty in the 66.8 µg/mL value for the POD. Calculations as described above for PFOA produce an MRL value for PFOS of $5x10^{-5}$ mg/kg/day.

RESPONSE 10: *As noted in the response to COMMENT 3, TWA serum concentrations were calculated for PFOS and these data were used for BMD modeling. The female NOAEL was selected as the basis for the MRL because it was the lowest POD.*

COMMENT 11 (MRLs for PFOA and PFOS): For Tables A-8 and A-20 it seems that the D_{SS} values should be the MRL values that were determined for PFOA and PFOS; i.e., 2×10^{-5} and 5×10^{-5} mg/kg/day. It is unclear where the D_{SS} values of 4×10^{-5} for both compounds come from. Footnote ***c*** indicates that the values were calculated from Eq. A-5, which would require a C_{ss} value. As noted in the accompanying review, the footnotes indicate that the D_{SS}, C_{ss}, and B_{SS} values all come from each other but that seems not possible as one of the values has to come from outside this loop.

RESPONSE 11: *Tables A-8 and A-20 were revised and the D_{ss}, B_{ss}, and C_{ss} values have been deleted.*

Annotations and Comments on the Toxicological Profile

COMMENT 1 (page 5, line 31): What about ref. that showed reproductive effects at low levels of exposure?

RESPONSE 1: *This section of the Public Health Statement discuses developmental effects. Using a weight-of-evidence approach, ATSDR concluded that the available human data do not suggest a relationship between PFOA/PFOS exposure and most developmental effects. The Reviewer did not specify what reproductive effects were observed at low exposure levels or provide a citation. A limited number of studies have examined potential effects of perfluoroalkyl exposure on the onset of puberty. These studies have found conflicting results precluding a weight-of-evidence determination for this end point. ATSDR believes these data support the statement in the Public Health Statement, and thus, no changes were made in response to this comment.*

COMMENT 2 (page 10, lines 12-13): ? seems that there must be some data – what does it mean to "be established"

RESPONSE 2: *ATSDR has changed the wording to suggest that levels vary significantly depending upon whether or not a local point source exists*

COMMENT 3: Worthwhile to indicate foods for which high concentrations occur?

RESPONSE 3: *No changes were made since there is little consistency in which foods show the highest values.*

COMMENT 4 (page 10, line 28): mammals?

RESPONSE 4: *The suggested revision was made.*

COMMENT 5 (page 11, lines 16-17): Checked Trudel; these are accurate. At steady state, Cserum would be 375 ng/mL for PFOS and 553 ng/mL for PFOA, using total body clearance values [mL/kg/day] of 0.080 and 0.085 for PFOS and PFOA, respectively.

RESPONSE 5: *No revisions were suggested.*

COMMENT 6 (page 11, lines 19-20): True but misleading as the CL in humans is about 1% of that in mouse and monkey, and even less in rat, so the Css, serum values in humans and animals would be much closer than would the intake rates.

RESPONSE 6: *This statement is providing information on a predicted intake for humans; it is not comparing the associated serum level to animal levels.*

COMMENT 7 (page 11, line 21): True that doses administered in lab animals are <u>usually</u> in the mg/kg/day range, but Hines et al. (2009) found an LOAEL of 0.01 mg/kg/day for increased weight gain, and increased serum leptin and insulin in adulthood in CD-1 mice exposed for 17 days during gestation, with no NOAEL identified. So effects have been reported in the mcg/kg/day range. Since CL values for PFOS and PFOA are about 100-fold higher in mice than humans, the <u>effective</u> exposures (Css serum) are not so different as ng/kg/d vs. mg/kg/d.

RESPONSE 7: *The intent of this paragraph is to discuss background exposure levels in humans; the comparison with the animal doses was deleted.*

COMMENT 8 (page 12, line 5): Insert "and"

RESPONSE 8: *The suggested revision was made.*

COMMENT 9 (page 12, line 6): Insert space

RESPONSE 9: *The suggested revision was made.*

COMMENT 10 (page 12, lines 24-25): If "human data" refers to serum concentrations, are these limitations? Serum concentrations are superior metrics of exposure compared with intake estimates, which are based on the large uncertainties associated with for example drinking water concentration and intake, and workplace exposures based upon job classification. In addition, serum concentrations have the advantage of integrating exposure from all sources. Given the long half-life, serum levels may not fluctuate much day-to-day even though intake rates per day may show considerable day-today variability. For epidemiology studies, measured serum concentrations would generally be superior measures of exposure than would be estimates of intake via inhalation and oral routes. Also, serum concentrations can be used to estimate intake rates via all routes, simply multiplying them by the CL value (IE "reverse toxicokinetics").

RESPONSE 10: *The statement was revised to indicate that most studies lack exposure monitoring data but used serum perfluoroalkyl levels as a biomarker of exposure.*

COMMENT 11 (page 12, line 26): It could be argued that "monitoring data" are surrogates of exposure and that serum levels are not biomarkers, but direct measures of exposure.

RESPONSE 11: *No revisions were suggested.*

COMMENT 12 (page 14, line 10): Suggest citing the longitudinal studies here.

RESPONSE 12: *The longitudinal studies are cited in the next two sentences.*

COMMENT 13 (page 14, line 26): should be "mechanisms"

RESPONSE 13: *The suggested revision was made.*

COMMENT 14 (page 16, line 16): A recent review (Ellen T. Chang, Hans-Olov Adami, Paolo Boffetta, Philip Cole, Thomas B. Starr, and Jack S. Mandel, A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans. Crit Rev Toxicol, 2014; 44(S1): 1–81.) found that the epidemiologic evidence did not support the hypothesis of a causal association between PFOA or PFOS exposure and cancer. This review examined critically 18 epidemiologic studies.

RESPONSE 14: *ATSDR has reviewed the Chang et al. (2014) paper and identified two recent papers that were not in the profile; these studies were evaluated and added to the profile.*

COMMENT 15 (page 16, line 23): Obesity/metabolic effects from gestational exposure of mice, and in humans have been reported Hines 2009; Halldorsson 2012. (Halldorsson, T.I., Rytter, D., Haug, L.S., Bech, B.H., Danielsen, I., Becher, G., Henriksen, T.B., Olsen, S.F. 2012. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ. Health Perspect. 120, 668-73)

RESPONSE 15: *The Hines et al. (2009) and Halldorsson et al. (2012) studies examining increases in body weight in mice and humans, respectively, are cited in the profile.*

COMMENT 16 (page 19, line 23): Macon 2011 found mammary gland development effects at 0.01 mg/kg PFOA to dam on GD 10-17. Not cited. (Macon, M.B., Villanueva, L.R., Tatum-Gibbs, K., Zehr, R.D., Strynar, M.J., Stanko, J.P., White, S.S., Helfant, L., Fenton, S.E. 2011. Prenatal perfluorooctanoic acid exposure in CD-1 mice: low dose developmental effects and internal dosimetry. Toxicol. Sci. 122, 134-45).

RESPONSE 16: *The Macon et al. (2011) study was added to the profile.*

COMMENT 17 (page 20, line 3): Should be deleted; redundant to say increased gain.

RESPONSE 17: *Body weight gain is the effect examined; an increase in body weight gain indicates that the exposed animal gained more weight than the controls. No changes were made to the profile.*

COMMENT 18 (page 21, line 30): Also cite Macon 2011.

RESPONSE 18: *The mammary gland effects observed in the Macon et al. (2011) study are considered developmental effects; the citation for this study was added to the developmental effects discussion.*

COMMENT 19 (page 21, line 34): Dosing rate?

RESPONSE 19: *This section is intended to be a hazard identification discussion; no doses were included. Dosing information is provided in Chapter 3.*

COMMENT 20 (page 24, line 13): There has been a Phase I clinical trial of the ammonium salt of PFOA in humans (Macpherson M., Bissett D., Tait B., Samuel L.M., MacDonald J., Barnett A.L., Wolf C.R., Elcombe C.R., Jeynes-Ellis A., Evans T.R.J. A first-in-human phase I clinical trial of CXR1002 in patients with advanced cancer. ASCO Annual Meeting 3-7 June 2011. J Clin Oncol 29: suppl; abstr 3063 (2011)). 36 patients were treated across 10 dosages (50-1200 mg/week).

RESPONSE 20: *This study has not been published and is only available as a meeting abstract; thus, it was not added to the profile.*

COMMENT 21 (page 24, lines 16-17): Since these compounds are not metabolized, the serum concentrations should be considered more than a biomarker – they represent the best available quantitative metric of systemic exposure available.

RESPONSE 21: *ATSDR considers them to be biomarkers of exposure because they are not actual exposure measurements.*

COMMENT 22 (page 24, line 22): Obesity following prenatal exposure?

RESPONSE 22: *Human data examining the possible association between maternal perfluoroalkyl serum levels and obesity is limited to four studies (Andersen et al. 2010, 2013; Halldorsson et al. 2012; Maisonet et al. 21012). Studies of infants (<2 years of age) have found inconsistent results (Andersen et al. 2010; Maisonet et al. 2012). Data on older children or adults come from one study each. ATSDR does not believe that there is sufficient evidence to establish a relationship between perfluoroalkyl exposure and this effect.*

COMMENT 23 (page 24, line 33): These are very low serum concentrations, approaching levels observed in the general population. 15 ng/mL would be the steady-state concentration that resulted from a daily oral intake of 1.27 ng/kg/day using a CL value of PFOA of 0.085 mL/kg/day.

RESPONSE 23: *No revision is suggested.*

COMMENT 24 (page 25, line 11): Also very low serum concentrations. A PFOA serum concentration of 5 ng/mL would be produced by a daily intake of 0.4 ng/kg/day, far below the rates of Trudel et al. quoted earlier.

RESPONSE 24: *The primary bases of the intakes estimated by Trudel et al. (2008) are the levels of PFOA in environmental media; they are not estimated based on serum PFOA levels.*

COMMENT 25 (page 25, line 20-22): This tends to cast doubt on the validity of the findings. Serum levels are arguably a much better metric of exposure than are exposure concentration or doses. As noted on the next page, marked interspecies PK differences makes extrapolation of animal tox results "highly uncertain". Serum concentration provides an integrated exposure from all routes, and because of the long half-life, it would not exhibit day-to-day fluctuations in response to fluctuations in daily intake.

RESPONSE 25: *The text was revised to indicate that most studies provided serum perfluoroalkyl levels.*

COMMENT 26 (page 25, lines 33-34): Disagree. This reviewer's opinion is that the epidemiologic studies are more certain than are animal studies with regard to quantification of adverse health effects in humans with systemic exposure to perfluoroalkyls.

RESPONSE 26: *For the reasons outlined in the profile and in the responses to General COMMENT 6, ATSDR does not believe that the available epidemiology studies would be a stronger basis for MRLs for PFOA and PFOS.*

COMMENT 27 (page 26, lines 4-6): Right, and this is why measured human serum concentration is useful; it obviates any need to account for interspecies PK differences and any animal-human differences in MOA or pharmacodynamics.

RESPONSE 27: *ATSDR believes that the uncertainty associated with MRLs based on nonhuman primate data is lower than the certainty associated with basing the MRLs on the available epidemiology data.*

COMMENT 28 (page 26, line 10): Other than in rat?

RESPONSE 28: *Sex differences have also been observed in mice and hamsters.*

COMMENT 29 (page 26, line 16): Consider citing Wambaugh et al. here. (Wambaugh, J.F., Setzer, R.W., Pitruzzello, A.M., Liu, J., Reif, D.M., Kleinstreuer, N.C., ... & Lau, C. (2013). Dosimetric Anchoring of In Vivo and In Vitro Studies for Perfluorooctanoate and Perfluorooctanesulfonate. Toxicological Sciences, 136(2), 308-327.)

RESPONSE 29: *The Wambaugh et al. (2013) paper compares the tissue concentrations associated with non-immunological effects estimated from in vivo exposures to those found in in vitro studies. The study found that in vitro studies could be used to identify tissue levels resulting in an adverse effect, but could not predict the effect. ATSDR does not believe that the results of this study are relevant to the profile and they were not added.*

COMMENT 30 (page 27, line 6): Should be noted that in exposures less than 3.3 half-lives, the body levels are below steady-state levels. The consequence is that the nominal dose rate to produce the associated adverse effect is higher than the dose rate that would produce the adverse effect under steady-state conditions. If acute duration studies are to be interpreted in terms of consequences to chronically exposed humans, this time course to steady state should be part of the consideration.

RESPONSE 30: *ATSDR only uses acute exposure studies to derive acute-duration MRLs (up to 14 days of exposure).*

COMMENT 31 (page 27, line 10): Using this as an example, the mouse half-life for PFOA is 19 days and 63 days (3.3 t1/2) would be required for steady state. The average serum concentration over 10 days would only average about 15% of the steady-state exposure. IE, a dose rate of 0.15 mg/kg/day for 10 days at steady-state would produce a similar systemic exposure as would 1 mg/kg/day for 10 days.

RESPONSE 31: *See the response to COMMENT 30.*

COMMENT 32 (page 27, line 22): Macon 2011 reported significantly stunted mammary epithelial growth in offspring of mice treated with 0.01 mg/kg/d on GD 10-17 with no LOAEL observed. Css would be 0.01 / 6 mL/kg/d = 1700 ng/mL. Cserum at 7d would be 378 ng/mL and the average concentration over 7d would be half that or 190 ng/mL, a concentration within an order of magnitude of a relatively large population of exposed individuals.

RESPONSE 32: *The Macon et al. (2011) study provided serum PFOA levels for the offspring, but did not provide maternal levels. An issue to consider when comparing the serum levels in the mouse offspring with those of humans is that the effects on the mammary gland are likely due to PPARα activation and may not be a relevant effect for humans.*

COMMENT 33 (page 27, line 33): Suggest inclusion of the associated measured serum concentrations (21 – 66 ng/mL), Table 5, White 2011b.

RESPONSE 33: *To be consistent with the rest of the discussion of animal data, the serum concentrations were not added.*

COMMENT 34 (page 28, line 13): Male and female?

RESPONSE 34: *The text in this paragraph was revised to make it consistent with the other discussions in this section; sex information was added to the revised text.*

COMMENT 35 (page 28, line 14): Should state the dose rate here.

RESPONSE 35: *As noted in the previous response, this paragraph was extensively revised; the dose information for the 3M (1983) study was added.*

COMMENT 36 (page 29, line 18): Species?

RESPONSE 36: *It was noted that the immune effects were in mice.*

COMMENT 37 (page 29, line 27): But Table 3 (Seacat 2002) reports significantly depressed cholesterol at 0.03 mg/kg/d on Days 62 and 182.

RESPONSE 37: *Even though the values were significantly lower than controls, they were not significantly different from pre-treatment levels; additionally, there were no significant alterations in the 0.15 mg/kg/day group. Thus, the alterations in the 0.03 mg/kg/day group were not considered biologically relevant.*

COMMENT 38 (page 31, line 2): Should be “were”.

RESPONSE 38: *The suggested revision was made.*

COMMENT 39 (page 32, line 3): Figure 3- series show some exceptions: For Intermediate duration, monkey shows greatest sensitivity to PFOA for adverse developmental effects (~0.001 mg/kg/d). Also for intermediate duration, monkey shows greatest sensitivity to PFOS for adverse immunological effects (~0.001 mg/kg/d).

RESPONSE 39: *There are no monkey studies reporting reproductive or developmental effects following exposure to PFOA or PFOS. ATSDR believes the Reviewer is referring to studies marked as "90m" and "101m" in Figure 3-3, these are mouse studies; "k" is used to indicate monkey studies.*

COMMENT 40 (page 32, lines 13): Fig. 3-4 shows developmental and immunological LOAELs for intermediate PFOS exposure of monkey and Fig. 3-3 shows developmental LOAEL for intermediate PFOA exposure of monkey.

RESPONSE 40: *See the response to COMMENT 39, "m" is used to indicate mouse studies and "k" is used to indicate monkey studies. The lowest LOAEL values for developmental and immunological effects were identified in mouse studies.*

COMMENT 41 (page 32, lines 13-14): Fig. 3- series seems to indicate that developmental effects for monkey are considerably more sensitive than are hepatic effects (0.001 mg/kg/d vs. 0.1-1 mg/kg/d)

RESPONSE 41: *See the response to COMMENTS 39 and 40.*

COMMENT 42 (page 32, line 15): ?? dose rates or serum concentrations?

RESPONSE 42: *The dose levels associated with the immune effects are discussed in prior paragraphs of this discussion; since these effects are not likely to be relevant to humans, the doses were not listed here.*

COMMENT 43 (page 32, line 16): Would not the relevance be a reduction in immune response to infection and immunization that leads to a possible increase in morbidity and mortality due to infection?

RESPONSE 43: *Since the effects most likely involve activation of PPARα and humans are much less responsive to PPARα agonists than mice, the immunological effects were not considered to be particularly relevant to humans.*

COMMENT 44 (page 32, lines 20-24): Agree.

RESPONSE 44: *No suggested revisions.*

COMMENT 45 (page 33, line 4): not in Eq. 2-1; should indicate that it was used to calculate the ke value.

RESPONSE 45: *The $t_{1/2}$ definition was deleted from the text; k_e was defined earlier in the sentence.*

COMMENT 46 (page 33, lines 10-11): Suggest restating this: "The average serum levels of PFOA measured every 2 weeks starting at week 6 are summarized in Table 2-1."

RESPONSE 46: *A footnote was added to Table 2-1 indicating that the serum PFOA levels were measured every 2 weeks starting at week 6.*

COMMENT 47 (page 34, Table 2-1): Suggest using a footnote to indicate that these are the average of values measured every 2 weeks, starting with the 6 wk values. Also suggest including the +/- SD values, the range of values, and the number of values that each average represents as shown in the text of Butenhoff 2002, p. 250.

RESPONSE 47: *The suggested footnote was added to Table 2-1. Additionally, the standard deviation and range of values were added to the table.*

COMMENT 48 (page 35, line 9): "as" should be "at".

RESPONSE 48: *The suggested revision was made.*

COMMENT 49 (page 35, line 9): Just the absolute liver wts. The relative weights were only increased at the highest dosage.

RESPONSE 49: *The sentence states that the absolute liver weights were increased at all of the dose levels.*

COMMENT 50 (page 35, lines 15-16): CLmouse is about 6.6 mL/kg/d (Lou I 2009), which gives an estimated Css of 1.5 mcg/mL. But Hines mice were not at steady state since they were dosed daily for 17 days and the t1/2mouse is 19 days. A considerably lower dose rate would have produced the 1.5 mcg/mL concentration at steady state. Also, the NOAEL is unknown; 0.01 mg/kg/day was the lowest dose used. These data suggest that the MRL of $2x10^{-5}$ could be ~50X too large. Macon 2011 also found impaired offspring mammary gland development at 0.01 mg/kg/d during gestation in CD-1 mouse

RESPONSE 50: *The Reviewer is comparing the MRL to an estimated serum concentration in mice. The available data on PFOA provide strong support that mice are more sensitive than humans due to toxicokinetic differences between the species.*

COMMENT 51 (page 35, line 22): This dosage would generate an average Css of 235 ng/mL in humans: 20 ng/kg/d ÷ 0.085 mL/kg/d = 235 ng/mL

RESPONSE 51: *No revisions were suggested.*

COMMENT 52 (page 35, line 24): Issues w/ Seacat: 1) KPFOS for dosing was 87% pure with contamination by lower chain length homologues of PFOA. 2). The capsules had large inter-capsule variability and the intermediate dose content was only 72% of the nominal content. No corrections were made to the nominal dosages.

RESPONSE 52: *Table 2-3 was revised to indicate that nominal doses were reported; a footnote indicates that the purity was 86.8% and reports the percent of the target dose contained in the capsules. Because the serum concentrations, rather than the doses, were used to derive the MRLs, ATSDR does not consider the lack of actual doses to be a major concern.*

COMMENT 53 (page 35, line 25): Much lower than for PFOA.

RESPONSE 53: *No revisions were suggested.*

COMMENT 54 (page 37, Table 2-3): These are concentrations at the 6 mo point. For the lower doses (.03, 0.15) serum concentration increased linearly while at the high dose it plateaued at ~110 days. Might be more reasonable to integrate the concentrations and divide by the exposure time to obtain the average concentration over the study period. Note that in Table 2-1 the PFOA concentrations are averages of values measured starting at 6 weeks and for every 2 weeks thereafter until the end of the exposure period.

RESPONSE 54: *In response to previous comments, ATSDR calculated the TWA serum concentrations using data provided in Figure 1 of the Seacat et al. (2002) paper. The TWA data were presented in Table 2-3.*

COMMENT 55 (page 37, Table 2-3): Weak dose-response relationships. Small dynamic range and the t=0 weights for the test groups are unknown so the extent of weight gain is not well known. Also very small numbers of animals and substantial variability.

RESPONSE 55: *ATSDR agrees with the Reviewer that the study demonstrated a weak dose-response and a small number of animals were tested.*

COMMENT 56 (page 40, lines 7-8): 4.63E-03 / 90 = 5.14E-05.

RESPONSE 56: *The MRL was rounded to 1 significant figure; thus, it was reported as $5x10^{-5}$ mg/kg/day. Note that when the TWA serum concentrations were used to calculate the MRL, the value was changed to $3x10^{-5}$ mg/kg/day.*

COMMENT 57 (page 41, lines 13-19): Might consider putting this into a Table format.

RESPONSE 57: *The text was revised to include a one column list of the compounds.*

COMMENT 58 (page 41, line 30): Somewhere a discussion of time to SS is needed. IE chronic exposure in humans is higher for a given dosage than for acute and intermediate, as well as longer.

RESPONSE 58: *Section 3.4.4 includes a discussion of time to steady state.*

COMMENT 59 (page 42, lines 31-32): Not clear that this is a limitation when serum concentrations are available. Serum concentrations integrate systemic exposure from all sources/routes and are superior to intake amounts as a metric of systemic dosimetry.

RESPONSE 59: *The text was revised to indicate that many of the studies provided serum perfluoroalkyl levels.*

COMMENT 60 (page 44, line 4): ? Significance – does this indicate: asphyxiation or what?

RESPONSE 60: *The significance of this finding is not known.*

COMMENT 61 (page 49, line 23): This is a good example of the stability of serum PFOA concentration over time at steady state, which makes it a good metric for systemic exposure to PFOA in humans.

RESPONSE 61: *No revisions were suggested.*

COMMENT 62 (page 55, line 20): That's HUGE! Is it actually 1.97 with CI 1.23-2.98?

RESPONSE 62: *The investigators reported the SMR as a percentage rather than a ratio; the ratio would be 1.97.*

COMMENT 63 (page 55, line 22): Seems to be a typo; Steenland's SMR was 1.90 so Leonard's is probably 1.97.

RESPONSE 63: *The text was revised to indicate that the SMR should be 1.90.*

COMMENT 64 (page 58, line 12): pregnancy?

RESPONSE 64: *The suggested revision was made.*

COMMENT 65 (page 59, line 30): Decimal points omitted; should be 1.85 0.95-3.23.

RESPONSE 65: *The SMRs were reported as percentage rather than as a ratio; the values are correct as reported.*

COMMENT 66 (page 61, line 18): Workers

RESPONSE 66: *The suggested revision was made.*

COMMENT 67 (page 64, line 15): Insert "at" after occurred.

RESPONSE 67: *The suggested revision was made.*

COMMENT 68 (page 65, line 12): It would be helpful to include serum concentrations of perfluoroalkyls; controls had an average total concentration of 72.7 ng/mL while asthmatics average was 115 ng/mL (Dong, Table 2).

RESPONSE 68: *Section 3.2 is a high-level discussion on the available data on the health effects of perfluoroalkyl exposure focusing on a weight-of-evidence evaluation of whether the effects does or does*

not occur in humans and animals. Thus, in-depth discussion of a single study (such as providing a table of serum levels of the seven perfluoroalkyls examined) would be beyond the scope of this section.

COMMENT 69 (page 67, line 19): Seems that measured levels would be more reliable than would levels predicted from addresses.

RESPONSE 69: *No revision suggested.*

COMMENT 70 (page 67, line 20): Should include the range of levels and or the median levels.

RESPONSE 70: *The suggested revision was made.*

COMMENT 71 (page 67, line 24): Should indicate the median concentration for this decile.

RESPONSE 71: *The suggested revision was made.*

COMMENT 72 (page 80, line 15): Should be "Considerably"

RESPONSE 72: *The suggested revision was made.*

COMMENT 73 (page 82, line 22): Does this information support reverse causality? Perhaps this should be explicitly stated.

RESPONSE 73: *A statement was added that the results suggest that it was not due to reverse causality.*

COMMENT 74 (page 82, line 29): This reviewer would say that they speculated and further that it is highly speculative. Renal clearance of PFOA is likely less a function of GFR than it is of the activity of active transport systems for reabsorption in the proximal tubule or of the free fraction of PFOA in plasma, with the concentration in filtrate equal to the concentration free in plasma water.

RESPONSE 74: *The text was revised to indicate that Watkins et al. (2013) suggested that the association was due to reverse causation.*

COMMENT 75 (page 82, line 34): Should be "physiologically".

RESPONSE 75: *The suggested revision was made.*

COMMENT 76 (page 84, line 22): Suggest "Serum levels of TSH were not correlated with those of PFOA …"

RESPONSE 76: *The text was revised to indicate that levels of TSH were not correlated with PFOA levels.*

COMMENT 77 (page 93, line 20): was

RESPONSE 77: *The suggested revision was made.*

COMMENT 78 (page 94, line 21): Wording suggests that PFOA is immunotoxic – based on its effects in humans via epidemiological data?

RESPONSE 78: *The sentence was revised to indicate that the available data do not suggest that PFOA is immunotoxic in rats and monkeys.*

COMMENT 79 (page 96, line 24): Should this be kg?

RESPONSE 79: *The suggested revision was made.*

COMMENT 80 (page 96, line 25): This dosage would produce a steady state serum concentration of about 33 ng/mL using 5 mL/kg/day for the mouse PFOS clearance. The average serum concentration over the 28 day exposure would be about 7 ng/mL, using a ke value of 0.0189 d^{-1} ($T_{1/2}$ = 36 days).

RESPONSE 80: *No revisions were suggested.*

COMMENT 81 (page 96, line 27): This corresponds to a steady-state serum concentration of 0.025 / 5 = 5 mcg/mL. Time to 90% steady state for PFOS in mouse is about 120 d so the average serum concentration during the exposure period was about 780 ng/mL.

RESPONSE 81: *No revisions were suggested.*

COMMENT 82 (page 106, Table 3.6): 2004

RESPONSE 82: *The suggested revision was made.*

COMMENT 83 (page 136, line 17): This seems important given the short exposure and 95% mortality.

RESPONSE 83: *No revisions were suggested.*

COMMENT 84 (page 141, line 17): Should be “were”.

RESPONSE 84: *The suggested revision was made.*

COMMENT 85 (page 142, line 10): or “altered rates were not detected in males”?

RESPONSE 85: *The statement was revised to “no significant increases in cancer rates were found in males.”*

COMMENT 86 (page 146, line 23): area

RESPONSE 86: *The suggested revision was made.*

COMMENT 87 (page 155, line 11): delete "a".

RESPONSE 87: *The suggested revision was made.*

COMMENT 88 (page 155, line 13): "elimination half-time" should be "elimination rate constant".

RESPONSE 88: *The suggested revision was made.*

COMMENT 89 (page 155, line 17): k should be k_e, same as in Eq. 3-1.

RESPONSE 89: *The suggested revision was made.*

COMMENT 90 (page 155, line 19: Should be elimination rate constant and k should be k_e.

RESPONSE 90: *The suggested revision was made.*

COMMENT 91 (page 155, line 20): In females, males, females and males combined?

RESPONSE 91: *The sentence was revised: "The time to peak concentrations of ^{14}C in plasma occurred at approximately 1.1 hour (range 0.6–1.5 hours) in female rats and 10 hours (range 7–15 hours) in male rats following single oral doses ranging from 0.1 to 25 mg/kg mg ammonium PFOA/kg (Kemper 2003)."*

COMMENT 92 (page 155, line 32): Suggest changing "Elimination kinetics" to "Plasma concentration-time profiles"

RESPONSE 92: *The suggested revision was made.*

COMMENT 93 (page 157, line 14): Seems to be inconsistent with above statements that they do not attach to or enter cellular components.

RESPONSE 93: *The text was revised to indicate that a ratio of whole blood:serum perfluoroalkyl of one-half corresponds to volume displacement by red blood cells, suggesting that they do not enter the cellular components of blood. A similar ratio was found in animal studies. The ratio of red blood cell:plasma did not change between 24 and 48 hours post exposure, suggesting that there was no selective retention of PFOA by red blood cells.*

COMMENT 94 (page 158, line 10): Should be "exhibits".

RESPONSE 94: *The suggested revision was made.*

COMMENT 95 (page 158, line 15): Suggest replacement of "relative to" with "compared with"

RESPONSE 95: *The suggested revision was made.*

COMMENT 96 (page 158, line 22): Insert comma after lung.

RESPONSE 96: *The suggested revision was made.*

COMMENT 97 (page 167, line 27): No mention of nursing infant serum:mother serum ratio. Fromme 2010 reported that serum concentrations in breast-fed infants are higher than the serum concentrations in their mothers. Post et al. (2012) concluded that exposure of a nursing mother to PFOA in drinking water results in a higher PFOA exposure and serum level in the breast-fed infant than in the mother. Similarly, PFOA exposures and serum levels in infants fed with formula prepared with contaminated water are also greater than in adults using the same water source. Thus infants can receive a higher systemic exposure than adults for a particular environmental concentration.

RESPONSE 97: *The suggested revision has been made. Text has been revised to state: Serum concentrations in breast-fed infants can be higher than maternal levels (Fromme et al. 2010; Post et al. 2012).*

COMMENT 98 (page 172, Table 3-13): The values in this table should be reconciled with the values listed in Table A-8, p. A-18.

RESPONSE 98: *A typographical error in Table 3-13 has been corrected. The serum half-time for PFOA is 1,387 days (not 1,187 days as reported in Draft 3). The values in Table 3-13 come directly from Harada et al. (2007b), whereas values in Table A-8 were rounded to two significant figures for use in the derivation of the MRL.*

COMMENT 99 (page 172, Table 3-13): There are no robust direct measurements of the V value in humans. While 300 mL/kg is credible, Thompson's (Thompson, J., Lorber, M., Toms, L. M. L., Kato, K., Calafat, A. M., & Mueller, J. F. (2010). Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. Environment International, 36(4), 390-397) values are well reasoned and compound specific, with 170 mL/kg for PFOA and 230 mL/kg for PFOS. Use of these values gives smaller values for total clearance. Also note that Table A-8 (p. A-18) lists V values of 0.2 L/kg for both PFOA and PFOS in humans.

RESPONSE 99: *The text and table have not been revised for the following reasons. Table 3-13 is intended to present the estimates reported by Harrada et al. (2007b) and the value for the volume of distribution, as weak supported as it is, comes directly from Harrada et al. 2007b). ATSDR considered data from several studies of nonhuman primates in selecting a value of 0.2 L/kg for use in deriving the MRL (Butenhoff et al. 2004c; Chang et al. 2012; Harada et al. 2005a). As noted by the Reviewer, this value yields lower predicted clearance and higher serum levels for a given intake rate and is therefore more conservative than the value selected by Harrada et al. (2007b).*

COMMENT 100 (page 173, line 31): Insert "of"

RESPONSE 100: *The suggested revision was made.*

COMMENT 101 (page 180, line 14): For consistency in Eq. 3-4, the units for V should be mL/kg. It would also be helpful to state where human V values were from.

RESPONSE 101: *The text has been revised to indicate that the renal clearances reported in Harrada et al. (2005a) were renal plasma clearances (i.e., mL plasma/ day), and that the parameter V was the plasma volume, which was estimated to be 4.3% of body weight (ICRP 1981).*

COMMENT 102 (page 187, line 30): Other possibilities are 1) that renal tubular reabsorption is carrier-mediated, saturable, and therefore less effective at higher doses that saturate the reabsorption process (as described on p. 215), and 2) that plasma protein binding is saturable and the higher plasma concentrations after higher doses have a higher free fraction and therefore a higher CL.

RESPONSE 102: *The suggested revision has been made.*

COMMENT 103 (page 196, line 25): PFOS?

RESPONSE 103: *The suggested revision was made.*

COMMENT 104 (page 197, line 13): pup

RESPONSE 104: *The suggested revision was made.*

COMMENT 105 (page 201, line 23): Agree – excellent point. It has been argued that more relevant than the target organ dose (AUCorgan) is the free concentration in the plasma, which at steady state is the best estimate of the active concentration at the site of toxicity (action). Thus, knowledge of CL, free fraction in plasma, and dose rate are all that is required to calculate the steady state free concentration, which obviates the need for complex PBPK models. It might be worthwhile to include this line of thinking in the Profile so that readers have a simpler alterative to the PBPK modeling approach that is useable by those who are specially trained and who are relatively limited in number.

RESPONSE 105: *The suggested revision has been made.*

COMMENT 106 (page 205, line 17): Agree – this reviewer cannot see the value of this feature.

RESPONSE 106: *No revisions were suggested.*

COMMENT 107 (page 205, line 21): particular

RESPONSE 107: *The suggested revision was made.*

COMMENT 108 (page 205, line 23): Agree, but saturable plasma protein binding is known for PFOS and PFOA and without basing distribution kinetics on the free concentration, it is not possible for concentration-dependent free fraction to be modeled.

RESPONSE 108: *The suggested revision was made.*

COMMENT 109 (page 206, lines 10-13): It would be interesting to know about the dissociation kinetics of plasma protein bound perfluoroalkyls, particularly to know if equilibrium is rapid compared with capillary transit time. Consider adding this to Section 3.12.2 Identification of Data Needs.

RESPONSE 109: *The suggested revision was made.*

COMMENT 110 (page 210, line 17): receptor?

RESPONSE 110: *The suggested revision was made.*

COMMENT 111 (page 212, line 34): Dosage?

RESPONSE 111: *The doses of 1, 3, or 10 mg/kg/day were added.*

COMMENT 112 (page 214, line 15): Yes. To illustrate this point it might be worthwhile to include in the Profile a table of PK parameter values for the heavily studied species (CL, V, $t_{1/2}$) along with time to steady state and the dosage that would provide the same Css. Suggested table is presented below:

	CL [mL/d/kg]		Vd [mL/kg]		t1/2 [d]		Time to 90%steady state [d]		Dose rate for Css = 100 ng/mL [µg/kg/day]	
Species	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
Mouse	6.6	5	180	265	19	36	63	120	0.66	0.50
Rat - Male	23	16	273	947	8.4	40	28	92	2.3	1.6
Rat - Female	776	5.2	150	476	0.13	66	0.43	218	77.6	0.54
Monkey	6.3	1.4	190	238	27	121	89	400	0.63	0.14
Human	0.085	0.08	170	230	1378	2000	12.5 yr	18 yr	0.0085	0.0080

RESPONSE 112: *No change has been made to the text. While ATSDR agrees with the Reviewer that such a tabular presentation would be valuable, selection of best or consensus values for each of the parameters for each species would be difficult (see Tables 3-14 and 3-15). For this reason, approximate relative differences in times to steady state are given in the current text to illustrate some outcomes of the large interspecies differences in kinetics.*

COMMENT 113 (page 223, line 30): long

RESPONSE 113: *The suggested revision was made.*

COMMENT 114 (page 232, line 13): Insert "near" after "living".

RESPONSE 114: *The suggested revision was made.*

COMMENT 115 (page 234, line 18): Agree – this would be important.

RESPONSE 115: *No revisions were suggested.*

COMMENT 116 (page 234, line 26): Yes – good suggestions.

RESPONSE 116: *No revisions were suggested.*

COMMENT 117 (page 241, line 6): Is this a suggested data need? If so, it should be more explicitly stated.

RESPONSE 117: *The text was revised to indicate that additional data are needed.*

COMMENT 118 (page 241, line 13): miniscule systemic clearance

RESPONSE 118: *The text was revised to delete the word "lengthy."*

COMMENT 119 (page 241, line 21): Insert "than adults" after "compounds".

RESPONSE 119: *The suggested revision was made.*

COMMENT 120 (page 254, line 4): Should replace "Above these values" with "At temperature above the Krafft point, the apparent solubility …

RESPONSE 120: *The suggested revision was made.*

COMMENT 121 (page 262, lines 31-34): True that micelle may suppress ionization via micellarization of nonionized form, but unclear how this would enhance volatilization.

RESPONSE 121: *No changes made. The neutral species is likely to volatilize, while the ionized species will not.*

COMMENT 122 (page 264, line 3): Worthwhile to indicate foods in which very high concentrations were found? Where from: environment of packaging?

RESPONSE 122: *No changes were made since there was little consistency in which foods show the highest values and there was some uncertainty in the analytical method which measured the highest value.*

COMMENT 123 (page 265, line 22): This would only give a steady-state serum concentration of about 30 ng/mL. Yet reported serum concentrations in some workers are much higher; exposures therefore seem to be understated.

RESPONSE 123: *These values seem to be for workers like groundkeepers etc. rather than workers directly involved in production processes; therefore, the sentence was deleted.*

COMMENT 124 (page 327, line 34): I would add that information about self-association in water would be useful. The carboxylate ion could conceivably complex with itself, or at sub-CMC there may be dimers, trimers, etc. If that happens, it could add concentration-dependence to absorption and perhaps other PK events.

RESPONSE 124: *ATSDR has added a sentence suggesting that information regarding association of these species in water would be helpful.*

COMMENT 125 (page 342, line 9): Seems very high; would produce a steady-state serum concentration of 235 ng/mL.

RESPONSE 125: *This concentration is several orders of magnitude lower than levels that have been associated with adverse effects in laboratory animal studies. The epidemiology data are not adequate to allow for identifying serum concentrations which are likely to result in health effects. Although several studies have reported significant associations between low serum concentrations (15-20 ng/mL) of PFOA or PFOS and alterations in serum cholesterol or uric acid levels, these studies involve exposure to multiple perfluoroalkyl compounds and the PFOA and PFOS serum levels account for only a small amount of the variance in serum cholesterol or uric acid levels. For example, the percentage of the variance in uric acid attributable to PFOA was only 1% in a study of communities living in an area with high levels of PFOA in the drinking water (Steenland et al. 2010b). Additionally, for both serum cholesterol and uric acid levels, the dose-response curve appears to attenuate at higher exposures and there are studies of workers with high serum PFOA concentrations that have not found significant associations.*

COMMENT 126 (page A-3, line 31): no" should be "not due to" ?

RESPONSE 126: *The study found no food consumption to low food consumption; no changes were made.*

COMMENT 127 (page A-4, Table A-1): Measured biweekly starting at 6 weeks.

RESPONSE 127: *The table was revised to indicate that serum PFOA levels were measured every 2 weeks beginning at study week 6.*

COMMENT 128 (page A-17, line 20): R_{CD} is also the reciprocal of the systemic clearance.

RESPONSE 128: *The equation and discussion of R_{CD} were deleted from the text.*

COMMENT 129 (page A-18, Table A-8): Should indicate "For Humans".

RESPONSE 129: *The suggested revision was made.*

COMMENT 130 (page A-18, Table A-8): Why is this not 2 x exp-5 as indicated on p. A-3 which was derived from the monkey study? Rather than have been calculated from Eq. A-5, this value seems to be the starting point for calc. of Css and Bss.

RESPONSE 130: *This table has been revised and the steady state concentrations were deleted.*

COMMENT 131 (page A-18, Table A-8): The units for R_{CD} are (kg x day / L); i.e., the inverse of the units for systemic clearance.

RESPONSE 131: *R_{CD} was deleted from Table A-8.*

COMMENT 132 (page A-18, Table A-8): This is circular: Css gives Dss which gives Bss which gives Css.

RESPONSE 132: *As noted above, Table A-8 was revised; the values for C_{ss}, D_{ss}, and B_{ss} have been deleted.*

COMMENT 133 (page A-20, line 11): Assuming steady state, using CLmouse = 6.6 mL/kg/d, Css = 0.01 / 6.6 = 1.5 mcg/mL. This is substantially lower than the PODs from monkey shown above.

RESPONSE 133: *As noted in the response to several other comments, mice are likely to be more sensitive to the toxicity of perfluoroalkyls than monkeys or humans. Most of the effects observed at low concentrations in mice are due to an interaction between perfluoroalkyl and the PPARα receptor; monkeys and humans are less responsive to PPARα agonists than rodents.*

COMMENT 134 (page A-22, line 31): Seacat et al. state (Discussion): "The cause of death or morbidity for the two males terminated on days 155 and 179, respectively, cannot be equivocally determined from the available information." They go on to describe PFOS-associated mortality in Rhesus monkeys that had received a total dose of PFOS about twice that of the Cynomolgus monkeys that died. It is not clear that the deaths "did not appear to be related to dosing with PFOS".

RESPONSE 134: *The text was revised to note that the cause of deaths were not determined and the results of histological and clinical chemistry evaluations suggest that the probably causes were pulmonary inflammation and hyperkalemia.*

COMMENT 135 (page A-38, Table A-20): The comments attached to Table A-8 also apply here.

RESPONSE 135: *The revisions made to Table A-8 were also made to Table A-20.*

Charge Questions and Responses

GENERAL QUESTION

QUESTION 1: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?

COMMENT: The following publications are not cited in the Profile and may report relevant data:

Barry V, Darrow LA, Klein M, Winquist A, Steenland K. 2014. Early life perfluorooctanoic acid (PFOA) exposure and overweight and obesity risk in adulthood in a community with elevated exposure. Environ Res. Apr 14;132C:62-69.

Fujii Y., Yan, J., Harada, K.H., Hitomi, T., Yang, H., Wang, P., Koizumi, A. 2012. Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. Chemosphere 86, 315-21.

Liu, J., Li, J. Zhao, Y., Wang, X., Zhang, L., Wu, Y. 2010. The occurrence of perfluorinated alkyl compounds in human milk from different regions of China. Environ. Int. 36, 433-438.

Llorca, M., Farré, M., Picó, Y., Teijón, M.L., Alvarez, J.G., Barceló, D. 2010. Infant exposure of perfluorinated compounds: levels in breast milk and commercial baby food. Environ. Int. 36: 584-592.

Macon, M.B., Villanueva, L.R., Tatum-Gibbs, K., Zehr, R.D., Strynar, M.J., Stanko, J.P., White, S.S., Helfant, L., Fenton, S.E. 2011. Prenatal perfluorooctanoic acid exposure in CD-1 mice: low dose developmental effects and internal dosimetry. Toxicol. Sci. 122, 134-45.

Mondal, D., Weldon, R.H., Armstrong, B.G., Gibson, L.J., Lopez-Espinosa, M.J., Shin, H.M., Fletcher, T. 2014. Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. Environ. Health Perspect. 122, 187-92.

Palkar, P.S., Anderson, C.R., Ferry, C.H., Gonzalez, F.J., Peters, J.M. 2010. Effect of prenatal peroxisome proliferator-activated receptor alpha (PPARalpha) agonism on postnatal development. Toxicology 276, 79-84.

Post, G.B., Cohn, P.D., and Cooper, K.R. 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. Environ. Res. 116, 93-117.

RESPONSE: *The Barry et al. (2014), Fujii et al. (2012), Liu et al. (2010), Llorca et al. (2010), Macon et al. (2011), and Mondal et al. (2014) studies were added to the profile. The Post et al. (2012) paper is a review article; this paper was evaluated to identify additionally papers to add to the profile. The Palkar et al. (2010) paper was not added to the profile because it does not involve exposure to a perfluoroalkyl compound.*

QUESTION 2: Are there any general issues relevant to child health that have not been discussed in the profile and should be?

COMMENT: Child development effects may be among the most sensitive to perfluoroalkyls exposure. Compared with adults, many unique developmental events must occur in a highly orchestrated time series. These events may be very sensitive to perfluoroalkyls exposure, but only during a relatively short time frame; if the event is interrupted, there may be downstream knock-on effects that permanently affect the function of neurological, behavioral, hormonal, etc. systems. Consequences of these impairments may not occur until much later in life, and they may be extremely difficult to discover in animal studies.

For a given level of exposure to perfluoroalkyls in the environment children tend to receive a greater exposure than do adults due to hand-to-mouth ingestion, breast milk transfer to infants, dermal exposure to carpets and fabrics treated with perfluoroalkyls. Section 3.2.2.6 Developmental Effects does not convey sufficiently the concepts of critical time points for susceptibility to perfluoroalkyls exposure, although Section 3.7 covers this concept, and the difficulty of discovering effects on subtle but important developmental pathways. Admittedly the potential for adverse effects on a number of developmental pathways has not been investigated and more studies in this area are needed. Section 3.2.2.6 does summarize a large amount of data that have been published on developmental effects, but much of the data involves effects on birth weight, length of gestation and other markers that are readily quantifiable but generally inadequate to uncover subtle alterations of behavior, susceptibility to disease later in life, immune function impairment, and so forth.

Section 3.7 captures in the introductory material (in bold type) the concept of critical time periods of exposure and the potential for latency in the manifestation of adverse effects. It also well summarizes the large amount of data on serum perfluoroalkyls associations with a number of clinical chemistry values, cognitive function, incidence of ADHD

Section 6.6 presents the extensive amount of published data on occurrence and concentrations of perfluoroalkyls in blood serum from children, and in breast milk and considers the transfer of perfluoroalkyls to infants via breast milk. It might be worthwhile to include observations that exposure of a nursing mother to PFOA in drinking water results in a higher PFOA exposure and serum level in the breast-fed infant than in the mother. Also that PFOA exposures and serum levels in infants fed with formula prepared with PFOA-contaminated water are greater than in adults using the same water source (Post et al. 2012).

RESPONSE: *There are no data to support the statement that infants fed with formula prepared with PFOA-contaminated water are greater than in adults using the same water source. The Reviewer cites this statement to Post et al. (2012). Post et al. (2012) predicts that the infants would have higher serum levels based on a higher water intake to body weight ratio. The factors that could lead to higher intakes in children and infants have been added to the profile in Section 6.6.*

CHAPTER 1 – PUBLIC HEALTH STATEMENT

QUESTION 1: Does the chapter present the important information in a non-technical style suitable for the average citizen?

COMMENT: Yes

RESPONSE: *No revision suggested.*

QUESTION 2: In your opinion, do the answers to the questions adequately address the concerns of the lay public? Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text? Please note sections that are weak and suggest ways to improve them.

COMMENT: Yes

RESPONSE: *No revision suggested.*

QUESTION 3: Are scientific terms used that are too technical or that require additional explanation?

COMMENT: No – this section uses appropriate non-technical language. The Glossary is a useful resource in this regard as well.

RESPONSE: *No revision suggested.*

CHAPTER 2 – RELEVANCE TO PUBLIC HEALTH

QUESTION 1: Do you agree with those effects known to occur in humans as reported in the text?

COMMENT: These aspects have been well covered in the Profile.

RESPONSE: *No revision suggested.*

QUESTION 2: Are the effects only observed in animals likely to be of concern to humans? Why or why not?

COMMENT: Yes

RESPONSE: *No revision suggested.*

QUESTION 3: Have exposure conditions been adequately described?

COMMENT: Except as noted in marginal comments in the accompanying review, exposure conditions have been described as reported in the original publications. For animal studies these have mostly been the administered dosage; e.g., mg/kg body weight/day. To make interspecies comparisons, however, these dosages do not account for interspecies differences in pharmacokinetics, which are large. Also confounding interspecies comparisons using administered dosage is the fraction of steady state that has been achieved over the study period. For a given duration of dosing, say six months, some species may be at steady state for most of the exposure period when another species is accumulating test substance over the exposure period. Because of the dose-dependence of the pharmacokinetics, different dosages may have different times to steady state. Since relevant pharmacokinetic information is available for the commonly used species (see Table at end of this document), it is technically possible to convert the administered dosages to steady-state serum concentrations and if necessary to adjust the concentrations to an average concentration that the animal experienced during the exposure period to account for pre-steady state conditions. IE, steady-state serum concentration (C_{ss}) is the administered dosage divided by the clearance, CL. If accumulation was ongoing over the exposure period, the C_{ss} value could be multiplied

by the fraction of steady state, using a rearrangement of Eq. A-7 and setting T_{ss} to the midpoint of the exposure period. To aid interspecies comparisons, and to relate the animal studies to serum concentrations reported in epidemiological studies, it would be helpful to include, along with dosage, an estimate of the average serum concentration that the animal was exposed to. The serum concentrations could also be used to create a companion set of LSE figures, as discussed below.

RESPONSE: *Tables were added to Section 2.3, which presents serum PFOA or PFOS concentrations and the associated health effects in laboratory animals.*

CHAPTER 3 – HEALTH EFFECTS

SECTION 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

Toxicity – Quality of Human Studies

QUESTION 1: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? If not, were the major limitations of the studies sufficiently described in the text without providing detailed discussions?

COMMENT: The large epidemiological literature is adequately presented. The Profile notes that most of the epidemiological studies are cross-sectional design and show associations with adverse health effects rather than causations. In addition, epidemiological studies in general cannot control co-exposure to other chemicals or environmental contaminants that might contribute to the adverse health effect, and they generally do not provide quantitative information on the intake rate of the subject compounds. These characteristics of epidemiological studies have in some instances in the Profile been invoked, which tends to undermine their import. In fact, human studies are highly relevant to the assessment of perfluoroalkyls-induced adverse effects in humans. The epidemiology database for perfluoroalkyls is much more comprehensive than for many other drinking water contaminants. It includes many studies relevant to environmental exposures (general population and communities with contaminated drinking water), as well as studies of workers with higher exposures. A number of health endpoints and diseases have been associated with exposure to perfluoroalkyls. the consistency of findings among different study populations suggests a causal relationship for some endpoints. Additionally, there is concordance between animal and human data for some effects (Post et al., 2012). This reviewer suggests that more weight be given to the human studies in the development of MRL values and that the serum concentrations produced in humans by the MRLs be considered in the context of the adverse effects associated with particular perfluoroalkyls serum concentrations in epidemiological studies.

RESPONSE: *As noted in the responses to similar comments, ATSDR does not believe that the available epidemiology data would serve as a suitable basis for an MRL. Collectively, the studies provide strong evidence for associations between serum PFOA or PFOS levels and several health effects, particularly serum cholesterol levels; however, there are a number of inconsistencies in the dose-response relationships across studies. Several studies of highly exposed individuals have not found significant associations (Emmett et al. 2006a; Olsen and Zobel 2007; Olsen et al. 2000; Wang et al. 2012); the serum levels in some of these studies were very high (e.g., 2,210 ng/mL in the Olsen and Zobel 2007 study). Other studies have found an attenuation of the effect at higher concentrations (Steenland et al. 2009b). Additionally, humans are exposed to multiple perfluoroalkyl compounds and the interaction of these compounds on the health outcome of concern is not known. Steenland et al. (2009b) found a 20–30% attenuation of the effect on serum cholesterol when PFOA and PFOS were considered together.*

Toxicity – Quality of Animal Studies

QUESTION 1: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)?

COMMENT: The quality of the animal studies appears to be high. The issue of whether the $PPAR_{\alpha}$-mediated adverse effects noted in some species are relevant to humans is well discussed. This reviewer is satisfied with the quality of the animal studies, their summary in the Profile, and the comprehensive inclusion of relevant studies in the literature.

RESPONSE: *No revision suggested.*

Levels of Significant Exposure (LSE) Tables and Figures

QUESTION 1: Are the LSE tables and figures complete and self-explanatory? Does the "Users Guide" explain clearly how to use them? Are exposure levels (units and dose) accurately presented for the route of exposure?

COMMENT: The LSE tables and figures provide an excellent summary of a large body of data. The figures in particular make dose-response relationships quickly apparent and they clearly show the adverse effects that are the most sensitive to perfluoroalkyls exposures within a species. For cross-species comparisons, however, the figures that display oral data give dosage in mg/kg/day, which does not account for large interspecies differences in pharmacokinetics. A companion set of figures that used serum concentrations rather than nominal daily dosage for the Y-axis would facilitate interspecies comparisons of the adverse effects. Human epidemiological data could be included as well which would help relate the animal study results to humans.

RESPONSE: *Due to the small number of studies in laboratory animals measuring serum PFOA or PFOS levels (10 or fewer studies for each compound), ATSDR chose to generate data tables rather than a figure. These tables, presented in Section 2.3, provide dose, serum concentration, and observed effects for several outcomes of concern in laboratory animals: hepatic, immunological, reproductive, and developmental effects. Due to concern about the epidemiology data and established a serum concern associated with a health effect, human data were not included in these tables.*

SECTION 3.4 TOXICOKINETICS

QUESTION 1: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance?

COMMENT: Absorption, distribution, metabolism and excretion of the perfluoroalkyls have been adequately presented in the Profile. As described in the Profile the mechanisms of renal excretion and the dose-dependence of the pharmacokinetics have not been fully elucidated. Methods for calculation of time to steady state, and perfluoroalkyls serum concentration during accumulation, at steady state, and during depuration are presented in the Profile. As noted above, it would be helpful to use this methodology to calculate serum levels from intake dosages as a metric of internal exposure in the oral-dose animal studies. The calculated serum concentrations would facilitate interspecies comparisons of exposure-

adverse response relationships, including comparisons with serum concentration-response associations reported in epidemiological studies. This would help unify the large body of animal and human dose-response data.

The Profile notes on p. 214 "As a result of these large differences in kinetics, predicting external doses that yield similar internal doses in animals and humans will require development of validated toxicokinetics models that can simulate elimination rates and internal distribution of perfluoroalkyls in animals and humans.". Considerable effort has been invested in construction of complex models to better understand some of the complexities of perfluoroalkyls pharmacokinetics. However, for the purpose of interspecies comparisons of internal exposure, a good first approximation should be possible using relatively simple pharmacokinetic equations such as those presented in Appendix A. It can be argued that more relevant than the target organ dose (AUCorgan) is the free concentration in the plasma, which is generally considered to be the best estimate of the active concentration at the site of toxicity (action). Thus, knowledge of CL, free fraction in plasma, and dose rate are all that is required to calculate the steady state total and free concentrations.

RESPONSE: *No revisions were suggested.*

SECTION 3.5 MECHANISMS OF ACTION

QUESTION 1: The purpose of this section is to provide a brief overview of known mechanisms of metabolism, absorption, distribution, and excretion, and then a discussion of any substance reactions or physiological processes that may affect these mechanisms. Have all possible mechanisms of action been discussed?

COMMENT: These are adequately presented and discussed.

RESPONSE: *No revisions were suggested.*

SECTION 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

QUESTION 1: Are the biomarkers of exposure specific for the substance or are they for a class of substances?

COMMENT: Serum concentration of perfluoroalkyls is the exposure biomarker. As the perfluoroalkyls are not metabolized the serum concentration of the perfluoroalkyls is an excellent metric to quantify the systemic exposure of an animal or human to perfluoroalkyls.

RESPONSE: *No revisions were suggested.*

QUESTION 2: Are the biomarkers of effect specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

COMMENT: Effect biomarkers are the adverse effects (disease, altered clinical chemistry, organ/tissue damage, etc.) that are observed to occur during or after exposure. The Profile well describes the several adverse effects that have been reported, and in particular describes the marker of increased liver weight that is associated with peroxisome proliferation and the $PPAR_{\alpha}$ pathway. $PPAR_{\alpha}$-associated increase in liver weight after perfluoroalkyls exposure varies among species and is not considered to be an adverse

effect per se. Increased liver weight in Cynomolgus monkeys administered PFOS or PFOA daily for six months was used in the Profile to estimate their MRLs. As suggested above, additional effect biomarkers should be considered for MRL estimation and a weight of evidence approach used to broaden the data base that supports the MRLs.

RESPONSE: *As noted in the response to General COMMENT 6, ATSDR considered several approaches to derive MRLs for PFOA and PFOS. The Agency believes that the MRLs derived from the monkey studies provide the strongest basis.*

SECTION 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

QUESTION 1: Is there a discussion of populations at higher risk because of biological differences which make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?

COMMENT: This section of the Profile is quite brief. Early life stages are not discussed here as they are covered in other sections. In addition to the effect markers described for the cardiovascular risk-factor population, increased weight gain has been associated with PFOA exposure in female offspring of CD-1 mice (Hines et al. 2009) in adulthood when exposed on GD 1-17. The LOAEL was 0.01 mg/kg/day with no NOAEL identified. No differences in food consumption were found between dose groups. Similar associations of prenatal PFOA exposure with increased body weight and changes in metabolic hormone levels in 20 year old women from the general population were reported by Halldorsson et al. (2012). In contrast, estimated life exposures to PFOA (estimated from residential history) were not associated with overweight or obesity in men and women (20-40 years of age) from the C8 Health Study population (Barry et al., 2014).

RESPONSE: *ATSDR does not believe that the available data are adequate to draw conclusions from the limited number of studies examining the possible association between early life exposure to perfluoroalkyls and adult body weight.*

SECTION 3.11 METHOD FOR REDUCING TOXIC EFFECTS

COMMENT: This section is brief; there are no known methods that effectively reduce absorption, accelerate a reduction of the body burden or interfere with mechanisms of adverse effect. This reviewer has no suggested additions to this section.

RESPONSE: *No revisions were suggested.*

SECTION 3.12 ADEQUACY OF THE DATABASE

Existing Information on Health Effects

QUESTION 1: Do you know of other studies that may fill a data gap? If so, please provide the reference.

COMMENT: The existing data base is nicely characterized in a series of tables. The Reference list appears to have captured the extensive list of publications that deal with pertinent aspects of perfluoroalkyls.

RESPONSE: *No revisions were suggested.*

CHAPTERS 4, 5, 6, 7, 8, and 9

COMMENT: The information contained in these chapters appears to serve the intent of the Profile. This reviewer has no suggestions for this part of the Profile.

RESPONSE: *No revisions were suggested.*

Comments provided by Peer Reviewer #2:

General Comments

COMMENT 1 (page 3, line 2): Add Emmett et al 2006a to references

RESPONSE 1: *ATSDR has added the suggested reference.*

COMMENT 2 (page 4, lines 12-13): Question the expression...which may be a risk factor for hypertension… Increased uric acids may be associated with hypertension but not necessarily as a risk factor, the increased uric acid could also be a consequence of hypertension.

RESPONSE 2: *The text was revised to indicate that increased uric acid levels may be associated with an increased risk for high blood pressure.*

COMMENT 3 (page 5, line 20): Children aged 2 to 5 consuming drinking water contaminated with PFOA may have higher blood levels than adults consuming the same water (Emmett 2006a).

RESPONSE 3: *Although Emmett et al. (2006a) found higher serum PFOA levels in children aged 2–5 years, as compared to older children or adults <60 years of age, the study did not evaluate potential differences in water consumption. The possibility that the increased serum levels were due to increased exposure is supported by the sudden drop in serum levels in school aged children. Without information on water consumption, ATSDR believes that it is misleading to include this study in this section of the Public Health Statement. These data were added to other sections of the profile.*

COMMENT 4 (page 6, line 19): Consuming bottled water and use of activated carbon water filters have been shown to lead to lower PFOA levels in the blood over time.

RESPONSE 4: *The suggested revision was made.*

COMMENT 5 (page 6, line 30): …the median serum PFOA concentration across a range of communities was…

RESPONSE 5: *The suggested revision was made.*

COMMENT 6 (Chapter 2): There should be an introductory statement that only PFOA, PFOS and PFHxS are currently found in humans in the United States in amounts that are generally considered as likely to have potential health effects.

RESPONSE 6: *Serum levels of PFOA, PFOS, or PFHxS that are associated with potential health effects have not been established; thus, the suggested statement was not added.*

COMMENT 7 (page 11, line 26): Add " Ingestion of contaminated drinking water has been shown to be the major route of exposure for humans in communities located close to industrial facilities where PFOA is used (Emmett et al 2006a, Holzer et al 2008)."

RESPONSE 7: *The suggested revision was made.*

COMMENT 8 (page 12, line 31): Add " A study of residents in 2004-5 found a median serum PFOA concentration of 329 ng/mL in community members exposed to PFOA contaminated drinking water in 2004-5 who had no occupational exposure (Emmett 2006a)"

RESPONSE 8: *The suggested revision was made.*

COMMENT 9 (page 13, line 3): Add "Plausibility depends primarily on experimental toxicology studies that establish a plausible biological mechanism for the observed effects."

RESPONSE 9: *The suggested revision was made.*

COMMENT 10 (page 13, line 12): The term biomarker of hypertension should not be used here: at best the biomarkers are not specific for hypertension and are capable of many other explanations. Shankar et al (2011) found that the relationship to uric acid was not abolished by adjusting for hypertension.

RESPONSE 10: *The text was revised to indicate there were increases in uric acid levels.*

COMMENT 11 (page 14, line 20): There is no experimental or other evidence that give plausible support to this speculated mechanism.

RESPONSE 11: *It is an explanation suggested by Steenland et al. (2010a), which is supported by the finding of a greater change in cholesterol level per unit change in serum PFOA at the lower PFOA concentrations, as compared to the changes at higher serum PFOA levels.*

COMMENT 12 (page 16, line 34): …specific to the route of administration. Not …route specific.

RESPONSE 12: *The suggested revision was made.*

COMMENT 13 (page 16, line 29): Half-life not half-time

RESPONSE 13: *The text was revised to "elimination half-time".*

COMMENT 14 (page 22, line 15): …of liver tumors in animals.

RESPONSE 14: *The suggested revision was made.*

COMMENT 15 (page 22, line 27): This sentence is too long and convoluted.

RESPONSE 15: *The discussion of the relevance of Leydig cell tumors to humans was revised.*

COMMENT 16 (page 23, line 4): …significant pancreatic cancer hazard…

RESPONSE 16: *The suggested revision was made.*

COMMENT 17 (page 23, lines 14-15): … consistent with a PP&R mechanism.

RESPONSE17: *The suggested revision was made.*

COMMENT 18 (page 24, line 6): There is relatively little animal data and no meaningful human data on the health effects of perfluoroalkyls other than PFOA and PFOS.

RESPONSE 18: *The suggested revision was made.*

COMMENT 19 (page 25, line 25): In at least one of the community studies (Emmett et al 2006a) it was specifically shown there was no appreciable contribution from airborne exposure.

RESPONSE 19: *Data from the Emmett et al. (2006a) study were added.*

COMMENT 20 (page 24): Since the predominant exposure in highly contaminated communities is through drinking water a central question for Public Health becomes what concentration or amount in water will produce serum concentrations in exposed populations that would correspond to minimal effect levels. This question does not appear to be usefully addressed in the discussion.

RESPONSE 20: *Establishing a drinking water concentration that would correspond to a minimal effect level is site-specific and would require site-specific exposure factors.*

COMMENT 21 (pages 41-42): Serious and less serious effects. This discussion is confusing and adds little. Frequently the distinction would appear to be merely a matter of degree, a minor increase in blood lipids or uric acid would be less serious, large increases would carry significant risks of coronary disease in the case of lipids or gout in the case of uric acid and could be serious to the health of the individual.

RESPONSE 21: *As discussed in this section, serious LOAELs are effects that are manifested in severe organ impairment, severe morbidity, or mortality. Since the terms "less serious LOAEL" and "serious LOAEL" are used throughout Section 3.2, ATSDR believes that it is important to define the terms.*

COMMENT 22 (page 42, line 29): Studies of communities, not a community.

RESPONSE 22: *The suggested revision was made.*

COMMENT 23 (page 43, line 7): In highly contaminated communities exposure of residents via airborne route is theoretically possible but has been ruled out as making a meaningful contribution in at least some studies (Emmett et al 2006a). Exposure through food and water is relevant to general population exposures.

RESPONSE 23: *The results of the Emmett et al. (2006a) study were added.*

COMMENT 24 (pages 50 and 56): Costa Study. The number of workers was relatively small but the PFOA levels were very high compared with those in most or all other studies.

RESPONSE 24: *No revisions were suggested.*

COMMENT 25 (page 56, lines 33-34): What is an observational association? Do you mean observed association?

RESPONSE 25: *The suggested revision was made.*

COMMENT 26 (page 61): Non-significant increases. Were there also any non-significant decreases?

RESPONSE 26: *Although some SMRs were less than unity, this is likely due to a "healthy worker" effect rather than indicative of a beneficial effect of perfluoroalkyl exposure.*

COMMENT 27 (page 62, line 17): Many of the studies… rather than nearly all.

RESPONSE 27: *The text changed to "most of the studies."*

COMMENT 28 (page 63, line 30): Dosage of 30/20? Please explain?

RESPONSE 28: *The following information was added: "(12 days of exposure to 30 mg/kg/day, 10 days with no exposure, 23 weeks of exposure to 20 mg/kg/day)."*

COMMENT 29 (page 65, line 17): Dong et al – what were the levels of exposure?

RESPONSE 29: *A note was added that the investigators did not report the perfluoroalkyl cut-off levels for each quartile.*

COMMENT 30 (page 66, line 18): Was the method of ascertainment of self- reported conditions in this study identical to that used in NHANES, if not why is the comparison valid?

RESPONSE 30: *Anderson-Mahoney et al. (2008) did not address whether the ascertainment of effects was similar between their study and NHANES.*

COMMENT 31 (page 71, lines 9-15): No plausible biologic rationale for an association between osteoarthritis in women was presented.

RESPONSE 31: *A statement was added that the possible mechanisms associated with these findings have not been elucidated.*

COMMENT 32 (page 80, line 15): Suggest improved wording. …perfluoroalkyls other than PFOA and PFOS

RESPONSE 32: *The suggested revision was made.*

COMMENT 33 (page 84, line 23): Incorrect reference. Should be Emmett et al 2006b NOT Emmett et al 2006a

RESPONSE 33: *The suggested revision was made.*

COMMENT 34 (page 100, line 4): In communities rather than in a community.

RESPONSE 34: *The suggested revision was made.*

COMMENT 35 (page 100, line 8): Associations not alterations. There is no proof in any study where an association was found that the relationship was causal.

RESPONSE 35: *The suggested revision was made.*

COMMENT 36 (page 113, Nolan study): High PFOA exposure was inferred from mother's residence in a community where serum PFOA levels were very high but actual PFOA levels for the mothers were not available.

RESPONSE 36: *The profile notes that the lack of biomonitoring data is a major limitation of this study.*

COMMENT 37 (page 113, line 21): A major limitation of these studies is the lack of biomonitoring data, which would allow for a more accurate examination of possible associations between maternal PFOA exposure and birth outcome. This sentence should go at the end of the paragraph at page 126 line 15. It is a limitation of each of the studies described to that point, the values were all inferred in different ways from maternal residence data $\pm$ additional estimates.

RESPONSE 37: *The suggested revision was not made because Savitz et al. (2012b) study, which also is discussed, used biomonitoring data.*

COMMENT 38 (page 154, line 14): add. … and by reductions in serum levels after exposures from water were eliminated or reduced (Emmett et al 2009, Bartell et al 2010).

RESPONSE38: *The suggested revision was made.*

COMMENT 39 (page 209, lines 19-20): It seems unlikely that any human populations have had exposures even approaching the levels reached in these studies in experimental animals.

RESPONSE 39: *A statement was made that the serum perfluoroalkyl levels were much higher in the animal studies.*

COMMENT 40 (page 219): Add to children's exposures. Children aged 2 to 5 develop higher levels of serum PFOA than older children and adults exposed to the same sources of PFOA in water. Emmett (2006a) found that children aged from 2 to 5 years in the Little Hocking Water Association District had a higher serum PFOA (median 600 ng/mL) compared with residents in all other age groups (median 321 ng/dl) except for the group aged more than 60, whose levels were similar to those in children. Factors that may have contributed to the observed high levels in children included that infants and young children proportionally drink more water per Kg of body weight than adults, and that they may tend to spend more time at home with exclusive use of residential water than people in other age groups., Olsen (2004d) also reported higher levels of serum PFOA in younger children, in a study of children aged 2 to 12.

RESPONSE 40: *As noted in the response to COMMENT 3, the Emmett et al. (2006a) study did not provide water consumption data, so it is unclear whether the increased serum levels were due to increased exposure or toxicokinetic differences.*

COMMENT 41 (page 221, line 22): The studies cited did not all use the same population.

RESPONSE 41: *The sentence was corrected to "found in highly exposed populations."*

COMMENT 42 (page 221, line 24): Nolan 2009 was a separate study and not one of the C8 Health Project studies.

RESPONSE 42: *The Nolan et al. (2009) study was removed from the string reference and a statement was added "or in another study of these communities (Nolan et al. 2009)."*

COMMENT 43 (page 223, line 15): The section should commence with a statement that the standard accepted measure of exposure in humans is the serum or whole blood concentration of the perfluoroalkyl.

RESPONSE 43: *The suggested revision was made.*

COMMENT 44 (page 223, line 29): References (Steenland et al. 2009a, Emmett 2006a, Holzer 2008)

RESPONSE 44: *The suggested revision was made.*

COMMENT 45 (page 224, Sections 3.9): Given doubt as to any significant role of PPARs in humans, it is not clear that the described potential interactions would be relevant to the human population.

RESPONSE 45: *A statement was added that this type of possible interaction may not be relevant to humans given that humans are less responsive to PPARα agonists.*

COMMENT 46 (page 224, line 13): Increases in serum uric acid… not biomarkers of hypertension

RESPONSE 46: *The text was changed from increases in biomarkers of hypertension to increases in serum uric acid levels.*

COMMENT 47 (page 225, line 7): Uric acid is also a risk factor for gout.

RESPONSE 47: *The text was revised to indicate that increased uric acid may be associated with an increased risk of high blood pressure. Since an association between perfluoroalkyl and gout has not been identified, the suggested revision was not made.*

COMMENT 48 (page 225, Section 3.11.2): Individuals with PFOA contamination of their drinking water sources who used bottled water have been shown to have lower levels of serum PFOA than those who drank public water. PFOA levels were also about 25% lower in those who used a carbon filter for their drinking and cooking water (Emmett 2006a). When subjects using PFOA contaminated public or well water switched to bottled water, or drank public water free of PFOA, their serum PFOA level fell around 25% over a 12 to 15 month period (Emmett et al 2009, Bartell et al 2010).

RESPONSE 48: *The second sentence of this section notes that decreasing exposure would result in decreases in body burden.*

COMMENT 49 (page 225, line 31): The low levels of PFOA found in human breast milk suggest that breast feeding may not be effective in lowering the mother's PFOA burden. Additionally, if the transfer was appreciable, the wisdom of transferring that amount of PFOA to the infant would need to be questioned.

RESPONSE 49: *The referenced statement is not advocating breastfeeding as a method to reduce body burden; rather the statement clearly states the breast milk pumping could also reduce body burden.*

COMMENT 50 (page 231, line 1): Oral studies in animals have…

RESPONSE 50: *The suggested revision was made.*

COMMENT 51 (page 235, line 29): An increase in ulcerative colitis has only been observed in a single study in which there were no reported biologic markers to indicate immunotoxicity, so the results must be interpreted with great caution.

RESPONSE 51: *A statement was added that the study did not establish whether the ulcerative colitis was due to immunotoxicity.*

COMMENT 52 (page 236, Neurotoxicity): The paragraph would benefit from a conclusion such as "Animal studies provide no basis for suspecting that perfluoroalkyls are neurotoxic"

RESPONSE 52: *The paragraph includes a statement that animal studies do not provide evidence of neurotoxicity.*

COMMENT 53 (page 237, lines 20-23): The association of PFOA and PFOS levels with certain lipid fractions across a considerable range of dose levels suggests that the cause may be physiologic rather than toxicologic, such as a result of partitioning in blood or serum consequent on protein binding. Such possibilities would need to be explored in mechanistic laboratory, rather than epidemiologic, studies.

RESPONSE 53: *A statement was added that mechanistic studies are also needed.*

COMMENT 54 (Chapter 4): No comments or suggestions

RESPONSE 54: *No revisions were suggested.*

COMMENT 55 (Chapter 5): No comments or suggestions

RESPONSE 55: *No revisions were suggested.*

COMMENT 56 (page 265, line 8): Exposure to PFOA in drinking water has been shown to be the major pathway for absorption of PFOA in individuals living in communities with high point source contamination of public or well water supplies (Emmett 2006a, Holzer 2008). Inhalation of indoor air has not been demonstrated to be an important source of exposure in these communities and that statement should be removed.

RESPONSE 56: *Inhalation of indoor air was the main source of exposure to occupationally exposed individuals in perfluorochemical plants (Vestergren and Cousins 2009). ATSDR has clarified the statement to separate this exposure group from the others.*

COMMENT 57 (page 265, line 28): suggest… is too weak. This relationship has been shown and confirmed. The data is suggestive for a minor role for ingestion of home grown fruits and vegetables (Emmett 2006a).

RESPONSE 57: *The word "suggest" was replaced by "indicate."*

COMMENT 58 (Section 6.2.2): This section would be more useful if it was introduced by a statement that ingestion of contaminated water was the major source or PFOA in communities with high blood levels of PFOA.

RESPONSE 58: *This section discusses releases to the environment; it is not intended to discuss potential exposure routes; thus, the suggested revision was not made.*

COMMENT 59 (page 311, line 1): ff text should be added. Using a stratified random sample of residents in the Little Hocking Water district in Ohio between July 2004 and February 2005 Emmett et al (2006a) found median serum PFOA levels of 329 ng/mL in residents drinking water with a mean PFOA concentration of 3.55ng/mL. Median serum PFOA was 371 ng/mL in residents for whom this was the only residential water source, and 71 ng/mL in those who used bottled, cistern or spring water. Increased serum PFOA was associated with increasing number of drinks of tap water daily and also with increasing use of water for making soups and stews and in home canning of fruits and vegetables. Use of a carbon water filter reduced PFOA levels by about 25%.

RESPONSE 59: *The suggested revision was made.*

COMMENT 60 (page 311, lines 1-5): insert text. In a follow-up study, 231 study participants in the Little Hocking Water District were evaluated 15 months later. 88% were now using bottled water exclusively, 8% had made other changes to their ingestion of residential water including use of activated carbon water filters. PFOA levels had decreased an average of 26% from the initial levels (Emmett et al 2009). Missing Reference and Citation Emmett 2009. Emmett EA, Zhang H, Shofer FS, Rodway N, Desai C, Freeman D, Hufford M. Development and successful application of a "community-first" communication model for community based environmental health research. J Occup. Environ. Med. 51: 146-156, 2009.

RESPONSE 60: *ATSDR has added this citation to the profile.*

COMMENT 61 (page 311, line 19): The analysis of pathways of exposure to PFOA and PFOS by Trudel et al (2008) was limited in that it did not reference or incorporate information demonstrating the importance of ingested water as a source of PFOA in communities near industrial facilities using PFOA.

RESPONSE 61: *No change was made since the profile indicates that the analysis of Trudel et al. (2008) is for the general population and not specifically for populations residing near sites with heavy PFOA or PFOS contamination.*

COMMENT 62 (page 316): Add text to section on children. Emmett (2006a) found that children aged from 2 to 5 years had a higher serum PFOA (median 600 ng/mL) in the Little Hocking Water Association district compared with residents in all other age groups (median 321 ng/dl) except for the group aged more than 60, whose levels were similar to those in young children. Several factors may have contributed to the observed high levels in children: infants and young children proportionally drink more water per Kg of body weight than adults, children and also the elderly tend to spend more time at home with exclusive use of residential water than other age groups, trans-placental and breast milk exposures could also contribute to levels in children. Olsen (2004d) also reported higher levels of serum PFOA in younger children.

RESPONSE 62: *The suggested revision was made.*

COMMENT 63 (page 323, line 6): PFOA levels are not similar for adults and children.

RESPONSE 63: *The text was revised to clarify that the serum levels in older children (12–19 years of age) were similar to adults.*

COMMENT 64 (page 330, lines 17-22): Trudel did not appear to incorporate findings that water is the major source of exposure for highly exposed communities into his analyses. Children aged 2 to 5 living in highly exposed communities appear to have higher PFOA levels than older children and adults given the same residential drinking water (Emmett 2006a)

RESPONSE 64: *No change was made since the profile indicates that the analysis of Trudel et al. (2008) is for the general population. It is stated numerous times within the profile that ingestion of contaminated drinking water is the major exposure pathway for special populations residing near sites where there are PFOA- and PFOS-contaminated public and private water supplies.*

COMMENT 65 (Chapter 7): No comments or suggestions

RESPONSE 65: *No revisions were suggested.*

COMMENT 66 (Chapter 8): Regulations from the other countries and particularly the EU are not included. This may reflect current ATSDR policy but does appear shortsighted since many countries have issues with PFOA and PFOS, and pollution of seawater with these compounds is global.

RESPONSE 66: *The intent of this chapter is to provided U.S. regulations and guidelines; it is beyond the intended scope of this profile to include regulations from other countries.*

Comments provided by Peer Reviewer #3:

General Comments

COMMENT 1: Overall, the readability of the chapters is good, the text is generally clear (although see some specific comments below). Recognizing that these profiles have had a specific format historically, it would be far more useful to interpretation of the health effects if the chapters on chemical and physical information, production/use, potential for human exposure, and analytical methods and toxicokinetics preceded the chapter on health effects as it would provide the context necessary to evaluate these findings. Currently, there is a brief mention of background and exposures prior to the summary of health effects.

RESPONSE 1: *ATSDR will consider the Reviewer's suggested revisions in future versions of the profile format.*

COMMENT 2: The lack of any evaluation of study quality is troubling in the health effects chapter. Overall, the human studies are discussed as if they were all equivalent, with a stronger emphasis consequently on the number of positive vs. negative findings. Clearly, some studies are of better quality than others and thus would be more heavily weighted. Not even sample size is considered with respect to power to detect effects across the studies; studies are presented with samples sizes of 20-40 as if they were equivalent to those with sample sizes of thousands. Thus, it does not read as if a thorough 'review' of the literature was done, rather just a summation or a tabulation of positive vs. negative findings. For example, p. 62 lines 5-7 simply states that there were inconsistent results across studies may be due to differences in exposures or to exposures to other compounds, i.e., does not differentiate quality of studies.

RESPONSE 2: *Study quality was taken into consideration when evaluating the weight of evidence for effects. Although the statistical power is higher in studies with thousands of subjects, these studies are not necessarily of higher quality than studies with fewer subjects. A statement was added to the introduction of the weight-of-evidence approach used to evaluate whether the available data support a link between exposure and a health effects (Section 2.2) that ATSDR considered study quality in this assessment.*

COMMENT 3: Overall, similar comments apply to the animal studies, where strengths and limitations of studies are generally not provided. Furthermore, the review of animal studies does not generally appear to consider differences in potency of the different perfluoroalkyls and how this influences comparative effects.

RESPONSE: *As with the human studies, ATSDR considered the quality of the individual studies. ATSDR did not compare the toxicity of the different perfluoroalkyl compounds.*

COMMENT 4: The Tables in Chapter 3 could be expanded to include strengths and limitations of cited studies to provide this assessment of quality and indicate which studies should be given greater weight. In addition, readability of the tables would be significantly improved by the addition of horizontal lines to separate the studies that are being cited.

RESPONSE 4: *Major limitations of some of the epidemiology studies (lower quality studies) are noted in the text of Section 3.2. It is beyond the intended scope of the profile to discuss all of the strengths and*

limitations of each study discussed in Section 3.2. ATSDR will take into consideration the Reviewer's suggested revision to the table format in future revisions to the profile format.

COMMENT 5: Page 25, lines 13-34 describe the basis for the decision to not derive an MRL for PFOA or PFOS. The rationales described are not compelling, for several reasons, including some of those cited above with respect to the failure to actually evaluate data in the context of study strengths and weaknesses. Given that there are serum levels available, what difference does the route of exposure make? Further, if there are effects in population studies, where exposures are the lowest, why would those be discounted if equivalent effects are not necessarily seen in very different populations, i.e., occupationally exposed populations. Furthermore, how discrepant are results when studies are actually critically evaluated, i.e., when the strongest studies are considered? What difference does it make that mechanisms have not been fully established given that there are human exposure levels? Mechanisms of action are not fully established for many chemicals for which MRLs or other exposure guidelines are set.

RESPONSE 5: *Although one single issue does not provide strong support for not using the epidemiology data as the basis of the MRL, ATSDR believes that these issues collectively greatly decrease the confidence in the MRL.*

COMMENT 6: Even with the issues raised above, Chapter 3 provides little in the way of any types of conclusions. It is not clear from the text which studies were used by the document to arrive at its conclusions, which studies were eliminated, and why these decisions were made. It would be very useful to the reader to include conclusions for each of the health effects that are discussed in any detail. Perhaps it would make more sense, albeit changing the traditional document structure, to abbreviate the summary of health effects and incorporate that into the health effects chapter in appropriate places, which would end with a discussion and section on how the advisories were decided upon based on the health effects.

RESPONSE 6: *ATSDR has provided summary discussions of the data in the more extensive subsections. However, the type of discussion suggested by the Reviewer would involve collapsing the data across routes of exposure. The current format of the profile does not allow for this type of analysis in Section 3.2. Because of the importance of examining the epidemiology data across routes of exposure and the need to use a weight-of-evidence approach for interpreting the epidemiology data, ATSDR has included the type of discussion suggested by the Reviewer in Section 2.2 of the profile.*

COMMENT 7: The document in many places dismisses effects, some statistically significant, as not being 'biologically significant'. What exactly does that mean? Is a value that moves outside the range of what is defined as clinically normal (ranges which are often extremely broad) required for biological significance? Does not the fact that a change is occurring in the wrong direction, i.e., increased cholesterol levels but not yet above the clinical danger mark, count as biological significance? In many places the document implies that in the absence of clinical illness, the effect is without importance.

RESPONSE 7: *Although there is more than one definition of "biologically relevant," these effects are typically within the established normal ranges and are not likely to result in an impairment of health. ATSDR considered all increases in serum cholesterol levels to be biologically relevant.*

COMMENT 8: Similar questions apply to the discussion of the level of the seriousness of effects (p. 42), which seems highly subjective; it is stated that ATSDR has established guidelines for this and the corresponding URL should be cited.

RESPONSE 8: *The suggested revision was made.*

COMMENT 9: The document is highly repetitive. It might be more readable if health effects were summarized by target organ and this included each route of exposure, rather than formulating the chapter on different exposure route. This would also make more sense given uncertainties within the human data as to actual routes of exposure in many cases.

RESPONSE 9: *ATSDR will consider the Reviewer's suggested revisions in future versions of the profile format.*

COMMENT 10: Furthermore, within each target organ section, it would be extremely helpful to include subtitles in chapter 3 that separate the text for each different perfluoroalkyl, as was done in Chapter 2, as well as for human vs. animal studies; right now this information simply all merges together and it is difficult to obtain any sense of differences among the compounds being evaluated. Tables should be inserted in the text in the section in which they are referred to.

RESPONSE 10: *Within a given effect section, the following subtitles were added: Human Exposure Studies, Laboratory Animal Exposure Studies—PFOA; Laboratory Animal Exposure Studies—PFOS; Laboratory Animal Exposure Studies—Other Perfluoroalkyls. It would be very difficult to separate the discussion of the human studies by perfluoroalkyl compound, since many of the studies examined multiple compounds, particularly PFOA and PFOS; separating the compounds would greatly increase the redundancy of the profile.*

COMMENT 11: A further improvement in readability would be the addition of summary tables that compare the effects across perfluoroalkyls to the extent that data make that possible.

RESPONSE 11: *ATSDR disagrees with the Reviewer that a table comparing the toxicity of the different perfluoroalkyl compounds would improve the readability. Given the differences in the type of available toxicity data and differences in the toxicokinetic properties of the different compounds, ATSDR did not include a comparison of the toxicity of the different compounds.*

COMMENT 12: The word 'however' is used frequently in Chapter 3 (Health Effects), but often does not appear to be used appropriately, i.e., to signify information that would be in contrast to what was stated prior to that point. This makes for some confusion in the reading.

RESPONSE 12: *The text in Chapter 3 has been edited to decrease the use of "however" and to assure the proper usage of "however".*

COMMENT 13: P. 73 describes the findings of U-shaped curves for these compounds, findings that could be of direct relevance to the human studies in which apparent discrepancies occur in cases e.g., where effects are seen at quintile exposure levels 2 and 4, but not 3, e.g., p. 107 lines 23-33. The possibility of U-shaped dose effect curves is never again mentioned.

RESPONSE 13: *Although not referred to as a "U-shaped curve," the finding of greater changes in serum lipid levels at lower serum perfluoroalkyl levels is discussed in numerous places in the profile,*

particularly in the comparisons of the results of occupational exposure studies to the general population studies.

COMMENT 14 (page 14, lines 24-26): Statements are often made about the fact that animal studies find decreases in cholesterol, whereas human studies find the opposite effects. Is this really a discrepancy? It seems clear that in both humans and animals, the pathways related to cholesterol metabolism are influenced by these compounds. While that possibility is raised on lines 27-28, it is not consistently included in chapter 3.

RESPONSE 14: *ATSDR disagrees with the Reviewer that the association between serum perfluoroalkyls and serum lipid levels in humans and animals is not discussed in Chapter 3. The Hepatic Effects discussions in Sections 3.2.1.2 and 3.2.2.2 include extensive discussions of the increases in cholesterol levels observed in humans and decreases in cholesterol levels observed in animals. Additionally, this is identified as a sensitive effect (referred to generically as increases in serum lipids) in humans in several other places in Chapter 3, including Sections 3.8.2, 3.10, and 3.12.*

COMMENT 15: There are numerous references in the document to unpublished information summarized by the Organization for Economic Co-Operation and Development. Unfortunately, these references make for lack of transparency of what studies are being cited, their quality, etc. Can further information about these studies be provided? Or, should unpublished data be included. What criterion is being used?

RESPONSE 15: *ATSDR was unable to obtain copies of these studies. Given the limited number of studies examining the toxicity of airborne perfluoroalkyls in laboratory animals, ATSDR believed that it was important to briefly mention that these studies exist and the results that were found.*

COMMENT 16 (page 61, lines 11-12): Do you actually mean that it cannot be ascertained given the confounding?

RESPONSE 16: *The study did not find a significant increase in the risk of bladder cancers.*

COMMENT 17 (page 69, line 6): But weren't the controls gavaged as well? How would the irritation only occur in the treated group?

RESPONSE 17: *It was noted that the lesions were likely due to irritation from repeated gavage administration with PFBuS.*

COMMENT 18 (page 86, lines 19-20): Seem disconnected from the rest of the text, were these supposed to be bolded?

RESPONSE 18: *The text should not be bolded; this was correct.*

COMMENT 19 (page 88, line 14-16): What does "clinical relevance' mean here? Does it mean that it requires a clinical diagnosis to be an adverse effect?

RESPONSE 19: *Since there were no other indications of a hypothyroid response or alterations in thyroid histology, the decrease in T3 levels was not likely an adverse effect.*

COMMENT 20 (page 97, line 17): What is meant by a 'reliable' NOAEL or LOAEL?

RESPONSE 20: *A "reliable" NOAEL or LOAEL is a value identified in higher quality studies.*

COMMENT 21 (page 138, lines 31-34): States that changes in locomotor activity were seen on PND 17 but not other days, which is apparently intended to indicate a lack of reliability of the findings. That conclusion (albeit not directly stated) ignores the fact that there is an ontogenic trajectory of locomotor activity levels across this stage of life in rodents, and thus having changes at one day but not another is not necessarily indicative of no effect.

RESPONSE 21: *This statement is not intended to indicate a lack of reliability; rather, it is suggestive of a transient effect.*

COMMENT 22 (page 143, lines 12-17): Appears to rely totally on the interpretation of the published paper that there were no carcinogenic changes when relying on comparisons to untreated aging rats, even though there was a significant difference from concurrent controls. Again, which conclusion is the document agreeing with?

RESPONSE 22: *The text was revised and the investigators' conclusion that PFOA was not carcinogenic was deleted. The statement that the incidences were similar to historical controls was retained.*

COMMENT 23 (page 154, line 27): Appears to be missing the word "higher" prior to 'when administered'.

RESPONSE 23: *The suggested revision was made.*

COMMENT 24 (page 221, lines 20-21): While decreases were small, leading to the conclusion that they were 'not likely biologically relevant', how do they compare to a very large human literature on low for birth weight?

RESPONSE 24: *As discussed in Section 3.2.2.6, the decreases in birth weight were not high enough for the infants to be considered low birth weight (<2,500 g).*

COMMENT 25 (page 236, line 27): What is meant by 'demeanor'?

RESPONSE 25: *The text was changed to clinical observations.*

COMMENT 26 (page 236, line 31): States that changes in tail flick latency are of unknown toxicological significance. In fact, this endpoint is used extensively in the pharmacology and behavioral pharmacology literature and the draft document needs to examine the changes cited in the context of that literature; this dismissal of significance appears to be done totally arbitrarily.

RESPONSE 26: *The phrase "of unknown toxicological significance" was deleted.*

COMMENT 27 (pages 315-316): Tables 6-14 and 6-15 presents concentrations in ppm whereas Tables 6-12 and 6-13 use ppb. Why can't the same metric be used to facilitate comparison across the Tables? Tables 6-15 forward then goes back to ppb.

RESPONSE 27: *No changes were made to the profile. Table 6-15 uses units of ng/mL (ppb) as do Tables 6-12 and 6-13. These tables largely represent general population exposures, while Table 6-14 provides serum levels of occupationally exposed individuals, which are much higher than the general population and thus, the ppm unit is more convenient. In some cases, levels on the order of $1x10^6$ ppb would be required in Table 6-14. Moreover, ppm was the unit used by all of the authors cited in Table 6-14, with the exception of Emmett et al. (2006a).*