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Subject: 3M Comments on ATSDR Draft Toxicological Profile for Perfluoroalkyls

To whom it may concern:

3M is aware that the Agency for Toxic Substances and Disease Registry (ATSDR) has issued a draft report, *Draft Toxicological Profile for Perfluoroalkyls,* and has solicited comments from the public on this document. We appreciate the opportunity to review and provide input on this profile.

Attached please find an executive summary that outlines key observations, suggestions and input that 3M has to offer in this matter. Also attached is a document containing our more detailed comments on a section-by-section basis.

The subject document represents a significant undertaking by ATSDR and needs to be based on current, relevant and well-synthesized information in order to be accurate and useful to multiple stakeholders. We trust 3M's comments will be helpful in accomplishing these objectives. Given the volume of work published since 2000, this ATSDR document should not be released until it has been updated with new information. To issue such a reference without updating it would be misleading to the public who relies on the expertise of the ATSDR.

If there are questions or comments concerning this matter, please contact either of us using the contact information provided below.

Sincerely,

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ATSDR Draft Toxicological Profile for Perfluoroalkyls

Executive Summary of 3M's Comments

The 3M Company (3M) appreciates the opportunity to review and comment on the "Draft Toxicological Profile for Perfluoroalkyls". It is evident that a great deal of effort was invested in preparation of the document and that it can be a valuable resource for many stakeholders once finalized. 3M personnel are familiar with the breadth of topics addressed by the draft profile. In fact, numerous 3M scientists are authors or contributors to many of the studies referenced in the report, especially in the areas of toxicology and epidemiology. As such, we are pleased to submit the following comments in the spirit of assisting with the development of a useful reference that is based on accurate information and sound science.

Based on 3M's review of the draft profile, we would offer the following general observations and/or suggestions:

1. A significant amount of additional scientific research has been conducted since the prior draft profile was released in 2009. In fact, many important studies have been published while this draft was in preparation or after its issuance. In our comments, we attempt to address the current state-of-the-science in various areas, especially epidemiology papers published in 2014 and 2015.
2. The ATSDR authors recognized the many inconsistent findings in the literature across a wide range of health effects. According to the draft profile, consistent findings were found for associations of serum PFOA and/or PFOS with an increase in serum lipid levels, a decrease in birth weight, an increase in uric acid levels, and alterations in biomarkers of liver damage. Furthermore, the document expresses an opinion that occupational studies support a finding of increases in deaths from several cancer types, including prostate cancer at one facility and kidney cancer at another facility. 3M respectfully disagrees with the interpretation of these associations and offers scientific evidence to refute these opinions.
3. The draft profile includes the development of Minimum Risk Levels (MRLs) for two of the thirteen perfluoroalkyls reviewed, namely PFOS and PFOA. For both of these compounds, the derived values were based on liver weight increases observed in non-human primate toxicology studies. The use of increased liver weight alone is inconsistent with current U.S. EPA guidelines and other published research. Based on the detailed information provided in the comments that follow, derivations of MRLs for PFOS and PFOA based on increased liver weight are not scientifically justified.
4. There is an unmistakable downward trend in the levels of PFOS and PFOA found in the U.S. general population in the last decade based on the Center for Disease Control's (CDC's) National Health and Nutrition Examination Survey (NHANES) data. This important observation should be documented in the final profile with the most recent data.
5. Within the last three years, through U.S. EPA's Unregulated Contaminated Monitoring Rule program (UCMR3), a considerable amount of data has been generated concerning the presence of six perfluoroalkyls in U.S. drinking water supplies. While data submissions will continue into 2016, information for PFOS, PFOA, PFNA, PFHxS, PFHpA and PFBS for 4,764 public water supplies (PWSs) is available as of October 2015. At this point, the percentage of PWSs with detectable results ranges from 0.1% (PFBS) to 2.2% (PFOA). Furthermore, seventeen (0.36%) of the PWSs have reported a PFOS level greater than U.S. EPA's Provisional Health Advisory (PHA) and none of them have detected PFOA above its PHA threshold. It is suggested that the

final profile incorporate the most recent data available from the UCMR3 program as a measure of this important potential route of exposure.

6. In its presentation, the draft profile separated the data by route of exposure. Occupational studies are categorized as 'inhalation exposures' whereas nonoccupational data are categorized as either 'oral exposures' or 'dermal exposures.' Clearly inhalation, oral, and dermal exposure would occur in an occupational setting. Although the categorization of toxicology data by exposure route is more straightforward, this artificial classification of data (especially with epidemiology data) by route of exposure prevents a robust synthesis of the available literature from which a weight-of-the-evidence evaluation becomes evident. It is important that the full breadth of relevant scientific information be considered and synthesized in developing positions and conclusions within the final document.
7. It would be helpful if the document detailed the process by which the scientific literature was searched. Specifically, information concerning the databases and years searched are recommended.
8. While this toxicology profile is intended to address perfluoroalkyls, and more specifically thirteen compounds within this broad class, for understandable reasons much of the content reflects information on PFOS and PFOA. Given the uniqueness of each compound included in this document, caution should be used in making overly broad statements concerning this class of materials.
9. Given the extensive amount of research that has been conducted and published on this class of compounds over the past 20 years, the authors face a considerable challenge in reviewing and appropriately incorporating this extent of information into the document. This is particularly evident for Section 6, Potential for Human Exposure. Much of the data cited seems almost anecdotal in nature and of questionable relevance to the broader, intended audience. A further complicating factor is temporal considerations. It is suggested that an update to the Overview for this section (Section 6.1), without all the detail that follows in the current draft, would better serve the purposes for this section and the document itself.
10. Perfluorohexanoate (PFHxA) is a key building block for fluorotelomer chemistry and is in active use in manufacturing operations worldwide. The draft document does not extensively address this compound. Given that there is relevant toxicological information available, the toxicological profile for this compound should be assessed and included.

In providing this input, it should be noted that a lack of comment on any particular detail or section within this ATSDR document does not necessarily imply agreement with that content.

ATSDR Draft Toxicological Profile for Perfluoroalkyls

Detailed Comments from 3M

The 3M Company (3M) appreciates the opportunity to review and comment on the "Draft Toxicological Profile for Perfluoroalkyls, August 2015". It is evident that a great deal of effort was invested in preparation of the document and that it can be a valuable resource for many stakeholders once finalized. 3M personnel are familiar with the breadth of topics addressed by the draft profile. In fact, numerous 3M scientists are authors or contributors to many of the studies referenced in the report, especially in the areas of toxicology and epidemiology. As such, we are pleased to submit the following comments in the spirit of assisting with the development of a useful reference that is based on accurate information and sound science.

The following comments prepared by 3M generally follow the structure of the draft profile with one notable exception as follows:

- Section 2, Relevance to Public Health, and Section 3, Health Effects. Given the clear overlap in content between these sections, and in order to provide the most coherent and helpful input, 3M has combined our comments relative to the material covered in these sections. These have been organized in the general categories of
    - Summary for Chapters 2 and 3
    - Toxicology Health Effects
    - Human Exposure Studies
- Section 4, Chemical and Physical Information
- Section 5, Production, Import/Export, Use, and Disposal
- Section 6, Potential for Human Exposure
- Section 7, Analytical Methods
- Section 8, Regulations, Advisories, and Guidelines

In providing this input, it should be noted that a lack of comment on any particular detail or section within this ATSDR document does not necessarily imply agreement with that content.

Sections 2, Relevance to Public Health, and Section 3, Health Effects

I. Summary for Chapters 2 and 3

The 3M Company (3M) appreciates the opportunity to review and comment on the "Draft Toxicological Profile for Perfluoroalkyls, August 2015". As authors or contributors of many of the human epidemiology and toxicology studies discussed in the draft documents, we offer these very detailed comments for Health Effects in Chapter 3 in the spirit of assisting with creation of an update to this section that is current, accurate and technically defensible. Given the magnitude of scientific literature that have become available in the past five years since the last Draft was released in 2009, the following high-level scientific emphases should be taken into consideration by ATSDR in Chapter 2 with the overall data integration.

(1). The document needs to detail the process by which the scientific literature was searched. The Toxicology Profile needs to state the databases and years searched. The 3M comments provided focus primarily on epidemiology papers published in 2014 and 2015 as none of these many recently published papers were cited in this draft of the ATSDR document.

(2). Vast majority of the health effect data cited by this draft document were extracted from PFOA and PFOS studies, and as such, ATSDR should be cautious and should not over-extend the conclusions (from PFOA or PFOS data) to other perfluoroalkyls. The categorical health effect on developmental and immune toxicity, for example, have not been observed with PFBuS or PFBA.

(3). The draft ATSDR Toxicology Profile for Perfluoralkyls (the document) separated the data by route of exposure. Occupational studies are categorized as 'inhalation exposures' whereas nonoccupational data are categorized as either 'oral exposures' or 'dermal exposures.' Clearly inhalation, oral, and dermal exposure would occur in an occupational setting. Although the categorization of toxicology data by exposure route is more straightforward, this somewhat artificial classification of data (especially with epidemiology data) by route of exposure prevented a robust synthesis of the available literature from which a weight of the evidence evaluation becomes evident.

(4). The purpose of establishing MRL by ATSDR is to offer guidance for the general population from exposure to certain chemical agents. Among the 13 perfluoroalkyls reviewed, minimal risk levels (MRLs) were derived for PFOA and PFOS only, which are set at 0.00002 mg/kg/day and 0.00003 mg/kg/day, respectively. The selection of these two MRLs was based on the increased liver weight observed in non-human primate toxicology studies, which, based on guidance, research and the comments provided herein, is scientifically unjustified. The use of increased liver weight alone by the ATSDR is inconsistent with current USEPA guidelines and other published peer-reviewed expert conclusions. These effects are reversible and there are distinct differences between liver hypertrophy as an adaptive (non-adverse) change vs. other biological endpoints that represent overt liver toxicity. Moreover, a significant body of mechanistic experimental

data that relates to the liver response to exposure to PFOA or PFOS strongly suggests that liver weight as an endpoint for the human-health risk assessment is inappropriate and needlessly conservative.

(5). Perfluorohexanoate (PFHxA) is a key building block for fluorotelomer chemistry and is in active use in manufacturing operations worldwide. Although ATSDR compares the toxicokinetic of PFHxA with other perfluoroalkyls, its toxicity profile was not discussed in the document. The toxicology data for PFHxA, as well as fluorotelomer-based chemistry such as 6:2 and 8:2 fluorotelomer should be assessed and included in the final toxicological profile by ATSDR because there are descriptive toxicology data available. In addition, 2-year bioassay in rats and pharmacokinetic data in several species (including humans) for PFHxA have also been published in the literature.

(6). There is an unmistakable downward trend in the levels of PFOS and PFOA found in the U.S. general population in the last decade based on CDC's National Health and Nutrition Examination Survey (NHANES) data. Mean blood levels of PFOS and PFOA in the general population have declined by approximately 80% and 60%, respectively, since 1999-2000. These reductions coincide with significant actions by industry and the U.S. EPA to decrease releases to the environment and use in manufacturing of these.

(7). This ATSDR draft profile recognized the many inconsistent findings in the literature across a wide range of health effects. According to the ATSDR document, consistent findings were found for associations of serum PFOA and/or PFOS with an increase in serum lipid levels, a decrease in birth weight, an increase in uric acid levels, and alterations in biomarkers of liver damage. Occupational exposure studies, according to the ATSDR, have found increases in deaths from several cancer types, including prostate cancer at one facility and kidney cancer at a second facility. 3M respectfully disagrees with the interpretation of several of these associations based on the following:

i. While it is recognized there have been several epidemiological studies (the majority cross-sectional) that have reported positive associations between serum PFOA or PFOS with total cholesterol, these studies, whether individually or collectively, are unable to establish methodologically a causal association. To conclude, as the ATSDR draft proposes, that there is a "strong" association is not supported by the inconsistencies of the data. There has not been evidence of increased risk for heart disease, stroke or hypertension in populations with varying levels of exposure to PFOA or PFOS. These include occupationally exposed populations and an affected community whose drinking contained PFOA. A Phase I clinical trial, where patients received high dosages of PFOA (up to 1200 mg weekly for six weeks), reported *lowered* LDL levels, a finding that is *consistent* with the hypolipidemic response observed in animal studies**.** The lipid associations with PFOA are observed generally at very low concentrations measured and not observed at more highly exposed populations. Possible non-causal biological explanations must be considered. These possibilities include a saturated response reflecting physiological conditions, and

other potential confounding factors, including binding to lipoproteins and/or a relationship with bile acids.

ii. The ATSDR document suggested PFOA and PFOS were associated with lower birth weight. It has been shown that this association is confounded by the glomerular filtration rate (GFR). Morken et al. (2014) and others have shown a significant association between glomerular filtration rate (GFR) and birth weight, thus making GFR an important confounder in the association between PFOA or PFOS and lower birth weight due to the renal elimination of the unbound perfuoroalkyl. None of these epidemiological studies that examined lower birth weight adjusted for GFR. Verner et al. (2015) subsequently developed a physiologically-based pharmacokinetic model (PBPK) that estimated 50 % of the association between PFOA and lower birth weight observed in these epidemiological studies may be attributable to confounding by GFR. This confounding may be more prevalent in studies with serum PFOA or PFOS concentrations measured later in pregnancy. Therefore, the ATSDR document should be revised to reflect the confounding attributable to GFR when studying associations between measured PFOA or PFOS and lower birth weight.

iii. In this regard, the association between uric acid and perfluoroalkyl compounds can also be confounded by GFR. Both PFOA and uric acid share the same organic anion transporter that facilitates the reabsorption of the unbound perfluoroalkyl from the renal proximal tubule. For the ATSDR to infer that PFOA is associated with increased uric acid (that can then result in hypertension) is unfounded in light of the fact that GFR was never controlled in these few epidemiologic studies related to uric acid. Another epidemiology study exquisitely showed that higher concentrations of PFOA measured in chronic kidney disease is likely the consequence of the disease, and not the result, due to the diminished GFR.

iv. The ATSDR draft document for perfluoroalkyls (primarily PFOA and PFOS) cites associations with selected hepatic clinical enzymes reported in studies of occupational populations and a community affected with PFOA from an industrial source. This ATSDR document, however, does not consider the amount of testing done (multiple comparisons), the magnitude of effects measured, the clinical relevance of the effects reported in the statistical associations, the potential for residual confounding, and the extent of medically verified diagnoses of malignant or nonmalignant liver diseases in association with exposure to PFOA or PFOS. It can be concluded that the statistical associations that were described by the ATSDR: 1) have very small absolute changes across the wide range of perfluoroalkyl exposures; 2) lack clinical relevance as the hepatic enzyme results are well-within normal reference ranges; and 3) do not correlate with malignant or nonmalignant liver disease associated with exposure to PFOA or PFOS because increased risks have not been observed in these populations.

v. An epidemiologic study of DuPont Washington Works employees exposed to PFOA as a processing aid in the polymerization of tetrafluoroethylene (PTFE) showed an association with kidney cancer mortality. TFE is a rat kidney carcinogen. PFOA is not. Because of the high correlation of exposure between TFE and PFOA in PTFE production plants, epidemiologists have been unable, in their words, to "disentangle" this association. On the other hand, workers engaged in the manufacturing of PFOA (ammonium salt) at the 3M Cottage Grove plant, in near absence of any exposure to TFE, have not shown an increased risk of kidney cancer incidence or mortality. This leads to the reasonable conclusion that the association between PFOA and kidney cancer that was reported in PTFE workers by some epidemiologists (without examining for TFE exposure) may be due to confounding from TFE or perhaps other fluoromonomer exposures known to occur at PTFE manufacturing plants – but it is unlikely due to PFOA.

vi. The ATSDR draft profile erroneously concludes that PFOA was associated with the risk of prostate cancer at the 3M Cottage Grove plant that manufactured PFOA. This association does not exist based on an extensive investigation of prostate cancer incidence and mortality data at this plant. PFOA is not associated with prostate cancer. The International Agency for Research on Cancer (IARC) reached this conclusion. The IARC considered PFOA to be a 'possible' human carcinogen (2B) based, on part, limited epidemiologic evidence on testicular and kidney cancer but IARC could not rule out chance, bias or confounding in its evaluation of this literature.

II. Toxicology Health Effects

Summary – Toxicology Health Effects

ATSDR reviewed 13 perfluoroalkyls and among them, it derived the minimal risk level (MRL) for two compounds, perfluorooctanoate (PFOA) and perfluorooctanesulfaonte (PFOS) at 0.00002 mg/kg/day and 0.00003 mg/kg/day, respectively. The selection of these two MRLs was based on the increased liver weight observed in non-human primate toxicology studies, which, in 3M's opinion, is scientifically unjustified.

Increased liver weight alone without microscopic or clinical evidence of overt liver toxicity is inappropriate as an adverse outcome for human health risk assessment and is inconsistent with current USEPA guidelines and published peer-reviewed expert conclusions. Moreover, experimental data support a reduced risk of humans to liver effects from PFOA and PFOS exposure based on: 1) investigations comparing hepatocellular responses to PFOA or PFOS exposure in laboratory animals including the use of transgenic mice that included mice humanized for PPARα; 2) the current state of our knowledge regarding the mode of action for liver effects exposed to PFOA and PFOS; 3) human relevance of these modes of action; and 4)

data to date, in particular from humans that were exposed to relatively high concentrations of PFOA or PFOS, do not demonstrate liver toxicity. All of these factors argue strongly that human health risk assessment for exposure to PFOA or PFOS should not be based on increased liver weight and/or hepatocellular hypertrophy, and that the lack of human evidence for PFOA- or PFOS-induced liver toxicity should be carefully considered by ATSDR.

In conclusion, the use of increased liver weight alone by the ATSDR as a critical effect to establish a point-of-departure for derivation of MRLs for both PFOA and PFOS is inconsistent with current USEPA guidelines and published peer-reviewed expert conclusions. There effects are reversible and there are distinct differences between liver hypertrophy as an adaptive (non-adverse) change vs. other biological endpoints that represent overt liver toxicity. Moreover, a significant body of mechanistic experimental data that relates to the liver response to exposure to PFOA or PFOS strongly suggests that liver weight as an endpoint for the human-health risk assessment is unjustified and inappropriately conservative.

The use of increased liver weight by ATSDR as a critical effect in the derivation of MRLs for both PFOA and PFOS has led to the detailed comments below addressing effects of PFOA and PFOS on liver and liver-related effects in laboratory animals. Also provided are additional comments addressing toxicology data presented by ATSDR in Chapters 2 and 3.

Comments on Increased Liver Weight As A Critical Effect for MRL Consideration

1. The Use Of Increased Liver Weight As A Critical Effect Is Inappropriate

Liver is adaptive by nature and liver growth in response to xenobiotic metabolism is a hallmark of normal adaptive change. Based on USEPA guidance document (USEPA 2002) and the general consensus of the scientific community (Hall et al. 2012), an increase in liver weight alone without other supporting evidence (microscopic or clinical data) is indicative of adaptive changes, not liver toxicity; and that liver enlargement alone should not be regarded as a sole critical endpoint for risk assessment. Therefore, ATSDR's categorization of liver enlargement as the sole endpoint for MRL derivation is not scientifically justified.

2. Increased Liver Weight Is Not Adverse

Since 2002, USEPA's Guidance Document # G2002.01 on Hepatocellular Hypertrophy (USEPA 2002) provides a framework for evaluation of hepatocellular hypertrophy and it states that liver hypertrophy does not necessarily represent liver toxicity, nor is it necessarily a precursor to a particular manifestation of toxicity. The Guidance Document # G2002.01 suggests a weight-of-evidence approach that includes evaluation of other findings, such as: 1) type and severity of observed effects; 2) onset, duration, and progression of effects; 3) study method and design; and, 4) other relevant effects and data. This guidance states that liver size or weight changes may be "indicative of adaptation which, by itself, is not necessarily adverse." In the absence of microscopic evidence of liver injury or change, at least two liver-related clinical chemistry parameters should be elevated with clinical significance ("at least 2-fold to 3-fold greater than control levels") before liver weight changes are ascribed to toxicity.

Additional guidance documents or articles similar to USEPA's Guidance Document # G2002.01 have existed for many years. More recently, in 2012, the European Society of Toxicologic Pathology published the conclusions from the 3rd International ESTP Expert Workshop, which was convened to "define more clearly when adaptive responses become adverse, and understand the long-term consequences of hepatocellular hypertrophy in order to guide scientific opinion for risk assessment in man…" (Hall et al. 2012). They provide an updated perspective on the consideration of liver hypertrophy as an adaptive versus adverse change which includes a thorough discussion of mechanistic, clinical, microscopic, and epidemiological evidence that allows for more certain interpretation of hepatic hypertrophic changes observed in experimental studies in

the context of human health risk assessment. The conclusion of the expert group with respect to liver hypertrophy as a result of enzyme induction was as follows:

> *The general opinion of the group was that liver weight increase through hepatocyte enzyme induction, in the absence of histopathologically demonstrated degenerative or necrotic changes and without significant changes in hepatic derived plasma enzymes, would not be considered adverse and would have little relevance to man in terms of risk assessment and the development of liver tumors.*

In addition, in considering the histological change caused by an increase in liver weight, the expert group suggested consideration of the following factors "in order to conclude whether the change is adverse or not":

> *1. Is there histological evidence of structural degenerative or necrotic changes such as:*
>
> - *hepatocyte necrosis, fibrosis, inflammation, and *steatotic vacuolar degeneration*
> - *biliary/oval cell proliferation, degeneration, fibrosis, and cholestasis*
> - *necrosis and degeneration of other resident cells within the liver*
>
> *(*Minimal to mild increases in steatotic macro-vesicular vacuolation without other changes indicating cellular damage should be distinguished from micro-vesicular vacuolation and considered non-adverse since this is a common change induced by feeding high-fat diets.)*
>
> *(Of note, transient increases in proliferative indices together with changes in hepatocyte ploidy, if induced through CAR/PXR/PPARα activation, is likely to be a rodent-specific phenomenon and therefore of little relevance to man even if this results in altered hepatic foci and/or primary liver tumors in chronic studies.)*
>
> *2. In the absence of histological changes, using a weight-of-evidence approach, is there clinical pathology evidence of hepatocyte damage characterized by a dose dependent and biologically significant and consistent increase in at least two liver parameters:*
>
> - *at least x2 to x3 increase in ALT (EMEA 2010, FDA 2009; HED Guidance Document 2002) or*

- *a biologically significant change in other biomarkers of hepatobiliary damage (ALP, AST, γGT, GLDH, etc.)*
- *a biologically significant change in another clinical pathology marker indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides etc.).*

The expert group also pointed out that the clinical pathology changes should "corroborate each other" and they should be:

Consistent with the expected species-specific patterns of change resulting from hepatobiliary injury, and take into account target pharmacology that may non-adversely alter one or more of these biomarkers. It should also be noted that statistical significance alone is not a reliable indicator of hepatic toxicity (HED guidance document 2002). If the above mentioned adverse criteria are not observed, then increases in liver organ weight and liver cell hypertrophy due to enzyme induction can be considered as an adaptive response to a xenobiotic and of little relevance to man.

3. Liver Weight Increase in Cynomolgus Monkeys Exposed to PFOA or PFOS Is Not Adverse

In the 6-month toxicity studies in cynomolgus monkeys with PFOA (Butenhoff et al. 2002) or PFOS (Seacat et al. 2002), albeit there were dose-dependent liver weight increases (absolute and relative) and observation of hepatic hypertrophy with PFOS (high dose group only), there were no increases in serum liver function markers such as ALT or AST, as shown in Table 1 below.

Table 1: Liver parameters in cynomolgus monkeys at the end of 6-month treatment with either PFOA or PFOS

Study	Sex	Dose Group (mg/kg/d)	Absolute liver weight (g)	Relative liver weight (%)	ALT (IU/l)	AST (IU/l)	Liver Histology
Butenhoff et al. 2002 (PFOA)	M	Control	60.2 ± 6.9	1.5 ± 0.1	68 ± 16	444 ± 191	Normal
		3	81.8 ± 2.8*	1.8 ± 0.1	43 ± 6	574 ± 290	Normal
		10	83.2 ± 9.7*	1.9 ± 0.1	53 ± 27	544 ± 181	Normal
		30/20	90.4 ± 4.2*	2.4 ± 0.5**	49 ± 8	384 ± 261	Normal
	M	Control	54.9 ± 8.1	1.6 ± 0.2	39 ± 18	--[b]	Normal

Seacat et al. 2002 (PFOS)		0.03	62.1 ± 5.3	1.7 ± 0.3	29 ± 9	--	Normal
		0.15	57.3 ± 5.5	1.8 ± 0.1	47 ± 17	--	Normal
		0.75	85.3 ±38.4	2.7 ± 0.3*	47 ± 20	--	Hypertrophy
	F	Control	51.1 ± 9.4	1.8 ± 0.2	87 ± 86	--	Normal
		0.03	56.8 ± 12.6	1.9 ± 0.0	72 ± 26	--	Normal
		0.15	57.0 ± 3.1	2.1 ± 0.2	53 ± 36*	--	Normal
		0.75	75.3 ± 13.3*	2.9 ± 0.3*	44 ± 10*	--	Hypertrophy

a Palmitoyl CoA Oxidase

b Not applicable

* Statistically significant relative to control, $p < 0.05$

** Statistically significant relative to control, $p < 0.01$

Therefore, based on the current guidance on evaluation of hepatic hypertrophy (*vide supra*) and in conjunction with the corroborating absence of evidence in the context of liver toxicity, the liver weight increase seen in cynomolgus monkeys after 6-month of oral administration with either PFOA or PFOS is not considered an adverse effect. This is further supported by the complete reversal of effects (liver weight and microscopic findings) at the end of recovery (data not shown).

4. Increased Liver Weight In Rodents Exposed To PFOA or PFOS Is An Adaptive Response Occurring Via PPAR and CAR/PXR Nuclear Receptor Activation

Majority of mammalian toxicology studies were conducted in rodents and mechanistic studies in rodents have demonstrated that adaptive hepatocellular hypertrophy can result from hepatic nuclear receptor activation in response to endogenous ligands or xenobiotics.

There are three primary nuclear receptors that can stimulate liver hypertrophy: peroxisome proliferation-activated receptor alpha (PPARα, or NR1C1), constitutive androstane receptor (CAR or NR1I3), and the pregnane X receptor (PXR or NR1I2) (Hall et al. 2012; Lake 2009; Waxman 1999). In rodents, the activations of these nuclear receptors can lead to liver enlargement as well as potential liver tumor formation with increased hyperplastic responses, however, with research tools such as humanized transgenic mouse models and availability of primary human hepatocytes, it has been well-documented that human liver lacks such hyperplastic response when exposed to PPARα and CAR/PXR activators (Corton et al. 2014; Elcombe et al. 2014; Hall et al. 2012; Klaunig et al. 2003; Lake 2009).

There is a well-established body of experimental evidence for activation of PPARα and CAR/PXR as a major factor in the rodent hepatic response to exposure to PFOA and PFOS. As Elcombe et al. (Elcombe et al. 2012a; Elcombe et al. 2012c; Elcombe et al. 2010) point out, the hypertrophic and hyperplastic response of rat liver to PFOA or PFOS exposure has clearly been demonstrated to be consistent with the criteria used to establish PPARα/CAR/PXR activation as a mode of action.

As noted above, there are fundamental difference between rodent and human liver responses to nuclear receptor activations and using cultured hepatocyte models, Bjork and Wallace (2009) demonstrated major differences between primary rat hepatocytes and human hepatocytes in response to PFOA or PFOS exposure. They concluded that:

> *While there is some similarity in the activation of metabolic pathways between rat and humans, particularly in PPARα-regulated responses; the changes in primary human cells were more subtle and possibly reflect an adaptive metabolic response rather than an overt metabolic regulation observed in rodents.*

Supporting this, the potential activation of human CAR3 isoform and human PXR has been studied *in vitro*. PFOA was not shown to activate directly either human nuclear receptor at concentrations up to 100 µM; and PFOS were not shown to activate directly either human nuclear receptor at concentrations up to 33 µM, with slight activation (much less than for positive control substances) of CAR3 and PXR occurring only at 100 µM (Ehresman et al. 2014).

5. Increased Liver Weight Exposed To PFOA or PFOS In Cynomolgus Monkeys Is An Adaptive Response Occurring Through Increased Activation of PPAR□

Due to limited number of toxicity studies available in non-human primates with PFOA and PFOS, the observation of liver weight increases in these studies can be compared to the established mode-of-action data in rodents as well as considering species difference between rodent and human, assuming that human hepatocytes is an appropriate surrogate for non-human primate hepatocytes.

The increases in liver weight seen following 6-month administration of PFOA or PFOS can be explained, in part, by peroxisome activation as demonstrated by the increased activities with liver palmitoyl CoA Oxidase (PCO), an enzyme marker for PPARα-dependent peroxisome proliferation (Table 2). Consistent with the observation reported

by Bjork and Wallace (2009) in that the extent of activation is "subtle" for primary human hepatocytes, PCO activities in monkeys were only statistically significantly higher than the respective controls for the highest dose groups (males in PFOA study and females in PFOS study). However, there were dose-dependent increasing trends for the lower dose groups in both studies.

Table 2: Liver Palmitoyl CoA Oxidase (PCO) Activity in cynomolgus monkeys at the end of 6-month treatment with either PFOA or PFOS

Study	Sex	Dose Group (mg/kg/d)	Absolute liver weight (g)	Relative liver weight (%)	Liver PCO (μmol/min/g liver)	Liver Histology
Butenhoff et al. 2002 (PFOA)	M	Control	60.2 ± 6.9	1.5 ± 0.1	0.53 ± 0.12	Normal
		3	81.8 ± 2.8*	1.8 ± 0.1	0.47 ± 0.13	Normal
		10	83.2 ± 9.7*	1.9 ± 0.1	0.90 ± 0.29	Normal
		30/20	90.4 ± 4.2*	2.4 ± 0.5**	1.36 ± 0.34*	Normal
Seacat et al. 2002 (PFOS)	M	Control	54.9 ± 8.1	1.6 ± 0.2	5.0 ± 1.5	Normal
		0.03	62.1 ± 5.3	1.7 ± 0.3	5.0 ± 1.0	Normal
		0.15	57.3 ± 5.5	1.8 ± 0.1	6.0 ± 0.6	Normal
		0.75	85.3 ±38.4	2.7 ± 0.3*	7.0 ± 1.4	Hypertrophy
	F	Control	51.1 ± 9.4	1.8 ± 0.2	4.0 ± 0.8	Normal
		0.03	56.8 ± 12.6	1.9 ± 0.0	4.0 ± 1.3	Normal
		0.15	57.0 ± 3.1	2.1 ± 0.2	6.0 ± 1.0	Normal
		0.75	75.3 ± 13.3*	2.9 ± 0.3*	7.0 ± 0.8*	Hypertrophy

* Statistically significant relative to control, $p < 0.05$

** Statistically significant relative to control, $p < 0.01$

The potential of PFOA or PFOS to activate CAR and PXR nuclear receptors in monkeys were not evaluated by Butenhoff et al. (2002) or Seacat et al. (2002). However, extrapolation from comparative metabolism and mode-of-action data in rodents (including transgenic mice) as well as *in vitro* hepatocyte work suggested that CAR and PXR might also contribute, in part, to the hepatic hypertrophic responses when exposed to either PFOA or PFOS (Bijland et al. 2011; Bjork et al. 2011; Bjork and Wallace 2009; Elcombe et al. 2012a; Elcombe et al. 2012c; Elcombe et al. 2010; Wolf et al. 2008).

It should be noted that evidence for hepatic mitochondrial biogenesis (proliferation) from exposure to PFOA has been reported in experimental studies with rodents (Cai et al. 1996; Pastoor et al. 1987; Permadi et al. 1993; Sohlenius et al. 1992a; Sohlenius et al. 1992b; Walters et al. 2009) and cynomolgus monkeys (Butenhoff et al. 2002). Thus, the increase in hepatocellular mass observed from PFOA exposure also may be, in part, attributable to mitochondrial biogenesis. Walters et al. (2009)have attributed PFOA-induced hepatic mitochondrial biogenesis in Sprague Dawley rats to activation of the

peroxisome proliferator-activated receptor γ coactivator-1 α (Pgc-1α) mitochondrial biogenesis pathway. This pathway represents a major adaptation of mitochondria and has been shown to be activated by exercise (Baar 2004; Hood 2001), cold (Puigserver et al. 1998), caloric restriction (Nisoli et al. 2005), and early stages of insulin resistance (Mitra et al. 2012). Generally recognized as an important, reversible adaptive response, mitochondrial proliferation should not be viewed as adverse.

6. Other comments on MRL Consideration

In its derivation of MRL, the ATSDR document noted an uncertainty factor of 3 for the lack of developmental data in monkeys for PFOA and PFOS. To obtain such information using monkeys is scientifically unjustifiable on the basis of animal welfare and ethical concern. A non-human primate study has not been considered as a routine toxicology test nor has it been mandated by the regulatory agencies for their risk assessment. Furthermore, the extensive (long) lag time for a monkey to reach sexual maturation and full development after birth (approximately 3 years) precludes a reasonable time frame for such a study.

7. Summary Comments on Increased Liver Weight and MRLs Consideration

For the reasons outlined above, human health risk assessment for exposure to PFOA or PFOS should not be based on increased liver weight and/or hepatocellular hypertrophy, and that the lack of human evidence for PFOA- or PFOS-induced liver toxicity should be carefully considered by ATSDR. Collectively, experimental data support a reduced risk of humans to liver effects from PFOA and PFOS exposure based on: 1) Investigations comparing hepatocellular responses to PFOA or PFOS exposure in laboratory animals including the use of transgenic mice that included mice humanized for PPARα; 2) the current state of our knowledge regarding the mode of action for liver effects exposed to PFOA and PFOS; 3) human relevance of these modes of action; and 4) data to date, in particular from humans that were exposed to relatively high concentrations of PFOS, does not demonstrate liver toxicity. As discussed in the subsequent section for Human Exposure Data, exposure to either PFOA or PFOS has not been associated with liver disease (malignant or nonmalignant) in humans.

Furthermore, the use of increased liver weight alone by the ATSDR as a critical effect to establish a point-of-departure for derivation of MRLs for both PFOA and PFOS is inconsistent with current USEPA guidelines and published peer-reviewed expert conclusions. There are distinct differences between liver hypertrophy as an adaptive

(non-adverse) change vs. other biological endpoints that represent overt liver toxicity. Moreover, a significant body of mechanistic experimental data that relates to the liver response to exposure to PFOA or PFOS strongly suggests that liver weight as an endpoint for the human-health risk assessment is unjustified and inappropriately conservative.

Liver Effects

Pages covered: 17, 157 – 162, 203, 303-304

There were scientific evidence suggesting that the increases in monkey liver weights after 6-months of PFOA or PFOS treatments can be explained, in part, by peroxisome proliferation. There are also experimental data suggesting PPARα-independent pathways such as CAR and PXR. Please refer to comments provided above for details.

Kidney Effects

Page covered: 166

ATSDR noted a renal hyperplasia finding in male rats treated with 600 mg/kg/day PFBS for 90 days. These effects likely were due to a cumulative direct irritation effect resulting from oral dosing with high concentration of PFBS (a strong surfactant). Despite this, clinical chemistry parameters related to kidney function were normal in these rats.

Endocrine Effects

Pages covered: 168 - 170

There has also been an increase in toxicological studies reporting on the endocrine disturbance potential with either PFOA or PFOS exposures. Most of these studies were done either under *in vitro* conditions (to which high concentrations were employed) or *in vivo* but only with a limited set of endpoints evaluated such as selected gene expressions (D'Orazio et al. 2014; Dankers et al. 2013; Dixon et al. 2012; Du et al. 2013; Du et al. 2012; Feng et al. 2015; Gao et al. 2013;

Kraugerud et al. 2011; Lopez-Doval et al. 2015; Lopez-Doval et al. 2014; Pereiro et al. 2014; Sales et al. 2013; Sonthithai et al. 2015; Wang et al. 2011; Wens et al. 2013; White et al. 2011a).

Endocrine is a very complicated system and evaluation of endocrine functions is a very highly specialized field (this is especially true in human clinical medicine). Given that both PFOA and PFOS are strong surfactants, the toxicity effects reported from the typical mono-layered *in vitro* tissue culture system offered very little insight and scientific value because the data were often compromised by the surfactant-induced toxicity. Similarly, gene expressions do not represent functionality and endocrine function is an intricate network.

Based on data from the large scale 2-generation reproductive and developmental studies (which are considered as the most comprehensive test by various agencies for evaluating endocrine functions), neither PFOA or PFOS alter the reproductive functions as the reproductive performances in both males and females were normal (*vide supra*). If they were indeed an endocrine disrupting compound, then one would expect it to directly activate endocrine receptors such as estrogen receptors or thyroid receptors. Ishibashi et al. (2007) reported that neither PFOA or PFOS can activate human estrogen receptor α or β. Yao et al. (2014) reported that PFOA did not activate mouse or human estrogen receptors and there was a lack of change in the histomorphology of uterine/cervix and vaginal tissues in female mice after receiving oral ammonium PFOA treatments. Furthermore, while triiodothyronine (T3, the active form of thyroid hormone) elicits a dose-response activation of human thyroid receptor α from 0.000001 – 0.01 uM, under the same study condition, there was no activation of human thyroid receptor α when exposed to ammonium PFOA or potassium PFOS up to 100 uM (Ehresman et al., 2014). In conclusion, 3M recommends that the entire body of research in this area be carefully reviewed.

Immunological Effects

Pages covered: 19, 177 – 179

In this section, ATSDR needs to note the strains and modes of administration cited for each of the study, as both strain and mode of administration may affect immunological response. Also, the discussion is lacking critical discussion on the potential role(s) of PPARα-mediated immune effects.

Reproductive Effects

Pages covered: 20, 191 – 194, 322

The text should be revised to reflect the results of numerous toxicological studies that have been performed which do not suggest reproductive effects of PFOA or PFOS in laboratory animals.

PFOA:

3M respectfully disagrees that PFOA is associated with reproductive toxicity. PFOA did not affect male or female reproductive functions in the laboratory animals. These included estrous cycles, sperm parameters, mating index, fertility index, and reproductive organ morphology. The potential of PFOA to influence reproductive performance has been evaluated in mice, rats, and rabbits. Gestational exposure to ammonium PFOA did not affect the number of uterine implantation sites in various strains of mice such as CD-1, Sv129, PPARα knockout, and humanized PPARα (Abbott et al. 2007; Albrecht et al. 2013; Lau et al. 2006; White et al. 2007). At inhalation dose up to 25 mg/m^3/day of ammonium PFOA or oral doses up to 100 mg/kg/day given during gestation, there was no effect on mating, pregnancy, and implantation (Staples et al. 1984). Oral administration of ammonium PFOA up to 150 mg/kg/day in rats or 50 mg/kg/day in rabbits during GD 6 – 15 (period of organogenesis) also caused reduced body-weight gain, however, they did not affect the ovaries or the reproductive contents of the dams (Gortner 1981, 1982). In a two-generation reproduction/developmental study in rats (Butenhoff et al. 2004), the reproductive outcome was not affected with oral ammonium PFOA administration up to 30 mg/kg/day (the highest dose used in the study). There were no effects on the mating or fertility indices in either male or female rats. Male rats had normal sperm parameters (count, motility, morphology) and female rats had regular estrous cycling with normal gestation lengths, and microscopic examination did not reveal any abnormalities in sex organs. Furthermore, effects of PFOA on reproductive organ morphologies in male non-human primates were evaluated from a six-month oral study and results indicated no abnormalities (Butenhoff et al. 2002).

PFOS:

3M respectfully disagrees that PFOS is associated with reproductive toxicity. PFOS did not affect male or female reproductive functions in the laboratory animals. These included estrous cycles, sperm parameters, mating index, fertility index, and reproductive organ morphology. The potential of PFOS to influence reproductive performance was evaluated in mice (Abbott et al. 2009; Thibodeaux et al. 2003), rats (Butenhoff et al. 2009; Luebker et al. 2005a), and rabbits (Case et al. 2001). Gestational exposure to PFOS did not affect the number of embryonic implantation sites in several strains of mice (CD-1, Sv129, or PPARα knockout) (Abbott et al. 2009; Thibodeaux et al. 2003, 2004). Similarly, implantations were not affected in rabbits either when exposed up to 3.75

mg/kg-d during GD 7 – 20 (period of organogenesis) albeit decreased body-weight gain and food consumption were observed (Case et al. 2001). In rats, oral administration of PFOS up to 10 mg/kg-d during GD 6 – 15 (period of organogenesis) also caused reduced body-weight gain, however, they did not affect the ovaries or the reproductive contents of the dams (Gortner 1980). In a two-generation reproduction/developmental study in rats (Luebker et al. 2005a), potassium PFOS (given as potassium salt) doses as high as 3.2 mg/kg-d were given to male and female rats for 6 weeks prior to mating, through mating and, for females, through gestation and lactation. PFOS did not adversely affect mating or fertility parameter in male or females, including fertility and pregnancy indices, estrous cycling, number of pregnancies per number of matings, number of days to inseminate, number of matings during the first week of cohabitation, epididymal sperm maturation, litter averages for corpora lutea, implantations, viable embryos, non-viable embryos, and reproductive organ histology. In particular, there were no statistically significant differences between control and potassium PFOS-treated females in the mean number of estrous cycles, rats with ≥6 consecutive days of diestrus or estrous during the 28-day evaluation period. In a developmental neurotoxicity study with PFOS, pregnant female rats received PFOS doses up to 1 mg/kg/day from gestation to lactation. No PFOS treatment-related effects were noted on maternal health or reproductive outcomes (Butenhoff et al. 2009). Furthermore, the morphologic effects of PFOS on reproductive organs in non-human primate were evaluated from a six-month oral study and results indicated no abnormalities (Seacat et al. 2002).

Developmental Effects

Pages covered: 18, 216 – 223, 310, 323 - 324

The developmental effects reported in laboratory animals for PFOA and PFOS were primarily mediated by maternal effects. In fact, experimental evidence demonstrates that developmental effects associated with PFOA or PFOS exposures in offspring are observed only where there were significant effects in the maternal animals. Evidence involving maternal effects in the outcome of the developmental toxicity, as seen in the disruption of maternal homeostasis, includes the following examples:

PFOA:

Using the mouse developmental study data reported by Lau et al. (2006), which was the critical study chosen by U.S. EPA Office of Water for the derivation of the Provisional Health Advisory for PFOA issued in 2009 (USEPA 2009), there were statistically significant ($p \leq 0.05$), dose-related increases in maternal liver weight observed at doses 1

mg/kg/day ammonium PFOA or higher (the corresponding serum PFOA concentration was 21,900 ng/mL at the end of gestaton). Various develpmental effects were reported (*e.g.*, decrased postnatal survival, decreased body weight at birth and body-weight gain thereafter, and delays in eye openings) and they were only for litters from dams receiving 3 mg/kg/day or higher. Maternal adverse responses clearly were present at doses that affected the fetus/neonate. In addition, because the influence of body weight on sexual maturation is well-described in the literature, it is not surprising that Lau et al. noted altered pubertal maturations in the offspring.

The developmental toxicity of ammonium PFOA has also been studied in rats (Butenhoff et al. 2004; Gortner 1981; Staples et al. 1984) and rabbits (Gortner 1982). In these studies, no increase in malformations relative to controls was observed at oral doses up 150 mg/kg/day in rats and 50 mg/kg/day in rabbits, as well as inhalation concentrations up to 25 mg/m^3/day (6 hours/day). In the studies by Gortner and by Staples et al., any effects on fetal or pup body weight were present at dose levels equivalent to or higher than those causing effects such as decreased body weight in the maternal animals. In a two-generation reproduction/developmental study in rats (Butenhoff et al. 2004), F1-generation pups from the highest dose group (30 mg/kg) had decreased birth weight and reduced viability that were in apparent relationship to the corresponding reduced body weight at birth and weaning. These latter effects are similar to those observed in mice by others (Abbott et al. 2007; Lau et al. 2006; Yahia et al. 2010). Even though similar to observation by Lau et al. (2006) that sexual maturation was slightly delayed (at the highest dose group only), there was no significant difference in F1 pups when days to sexual maturation was adjusted by (reduced) body weight.

PFOS:

PFOS developmental toxicity has been evaluated in several laboratory species. In rabbits, oral PFOS administration ranging from 0.1 – 3.75 mg/kg/day was given from GD 6 – 20 and decreased maternal body-weight gain was observed at 1 mg/kg dose group or higher. No abnormal fetal effects were noted except decreased fetal body weight, which was observed with 2.5 and 3.75 mg/kg/day dose groups only. Study authors concluded that "The fetal effects occurred at maternally toxic dose levels and no fetal changes were present at nontoxic maternal doses" (Case et al. 2001). In mice, there was a statistically significant ($p \leq 0.05$), dose-related increase in maternal liver weight when pregnant dams were treated during gestation at a dose as low as 1 mg/kg potassium PFOS . Various developmental effects were reported in mouse pups (e.g., decreased postnatal survival and growth deficits) but primarily for litters from dams receiving 10 mg/kg/day potassium PFOS or higher (Lau et al. 2003; Thibodeaux et al. 2003).

In addition to mice, the developmental toxicity of PFOS has also been evaluated in rats. Oral administration of PFOS during gestation to pregnant rats caused reduced maternal body-weight gain and fetal body-weight gain at 2 mg/kg/day maternal dose group or higher (Lau et al. 2003). In a two-generation reproduction/developmental study in rats by Luebker et al. (2005a), albeit reduced body weight and body weight-gain at parental generation was reported at 0.4 mg/kg or higher, developmental hallmarks similar to previously reported by others (*i.e.*, decreased fetal body weight, decreased postnatal survival, and developmental delays) were observed in pups from 1.6 mg/kg/day maternal dose groups or higher.

Mammary Gland - PFOA

Pages covered: 18, 193

There is strong scientific evidence against using the mouse mammary gland data for human risk assessment. In the recent years, there have been numerous studies that investigated the effects of PFOA on the developing mammary glands in mice as a consequence of exposure during either the *in utero* or postnatal/peripubertal window (Albrecht et al. 2013; Macon et al. 2011; Tucker et al. 2015; White et al. 2007; White et al. 2009; White et al. 2011b; Yang et al. 2009; Zhao et al. 2010). Taken together, these studies demonstrate that the effects of PFOA on mammary gland development cannot be consistently described and quantified in mouse models because these studies either found *no effect, inhibition, or stimulation* of mammary gland development (see Table 3). Furthermore, the nursing capabilities of the dams from these studies did not appear to be affected despite altered the mammary gland developments. Therefore, even though there are data available on the mammary gland development in mice, a lack of concordance among all the studies brings into question the biological significance of this phenotype and its relevance to human health.

Table 3: Summary of mouse mammary gland findings

Authors	Species (strain)	Mammary Gland Outcomes
White et al. 2007	CD-1	Stunted
White et al. 2009	CD-1	Delayed
Yang et al. 2009	C57BL6	Stimulatory (5 mg/kg) Inhibitory (10 mg/kg)
Yang et al. 2009	Balb/c	Inhibitory

	C57BL/6	Stimulated
Zhao et al. 2010	CD-1	Delayed
Macon et al. 2011	CD-1	Delayed
White et al. 2011	CD-1	Delayed
Albrecht et al. 2013	Sv/129 WT	No effect
	PPARα KO	No effect
	hPPARα	No effect
Tucker et al. 2014	CD-1	Delayed
	C57BL/6	Delayed

Neurological Effects

Pages covered: 181, 325

PFOS:

There have been a small number of studies reporting on the effects of PFOS and neurological disturbances in the laboratory animals, however, these studies were small in scale, lacked temporal trends, and did not incorporate a comprehensive battery of evaluations for all aspects of functional observations. A guideline-based developmental neurotoxicity study with PFOS was conducted by Butenhoff et al. (2009), there were no other observations among the many recorded that were suggestive of a neurotoxicological effect of PFOS on development through the PND 66 observation period. There were no abnormal histological observations in the neurological or endocrine tissues. A functional observation battery (FOB) was performed with the same sets of 20 rats per sex per group on PNDs 4, 11, 21, 35, 45, and 60 and included, stage of development permitting: ease of cage removal; ease of handling in hand; lacrimation/chromodacryorrhea; salivation; piloerection; appearance of fur; palpebral closure; respiratory rate/character; red, crusty deposits; mucous membranes/skin color; eye prominence; eye color; mobility; muscle tone; convulsions/tremors; hindlimb extension; grooming; arousal; bizarre/stereotypic behavior; urination/defecation; papillary response; backing; forelimb/hindlimb grip strength; tail pinch response; gait; and air righting. None of these FOB endpoints, including those with higher learning and memory was affected by treatment with PFOS.

ATSDR stated that "no comprehensive neurological testing has been conducted except for a study with PFHxS in rats…. and one with PFBuS". This statement is not accurate in that neurological functional observation batteries (FOBs) are integral parts of the guideline-based reproductive/developmental studies as well as long term repeated toxicity studies. In addition to PFHxS and PFBS, there are extensive guideline-based FOBs data in laboratory animals for PFOA (Butenhoff et al, 2004), PFOS (Butenhoff et al. 2009; Luebker et al. 2005b), PFBA (Butenhoff et al. 2012), and PFHxA (Chengelis et al. 2009).

Typographical Error and Data Corrections

Throughout the document:

- Be consistent with the use of "half-lives" (rather than "half-times")
- Due to the decline of PFOS and PFOA in the blood of the general population after manufacturing phase-out, to provide an accurate time-based perspective, the time period to which blood samples were collected should be specified when reporting serum concentrations.

Page 17: Correction

- It should be "expansion" of the smooth endoplasmic reticulum, not "proliferation".
- There were biochemical evidences suggesting peroxisome proliferation in both monkey studies for PFOA and PFOS, please refer to discussion above.
- Hydrophobicity may have played, in part, toward preferential enterohepatic recirculation; protein affinity may also contribute to this effect.

Page 18: Correction

- Like other peroxisome proliferator agonists, PFDeA does result in hypolipidemia in the plasma (see Van Rafelghem 1988 Lipids 23 671-678).

Pages 18 and 20: Correction

- As summarized above, mammary gland findings with PFOA in mice are subjective evaluations and all the results available to data are inconclusive, depends on the strains of mice, doses used, and research groups.

Page 19: Correction

- See comments above on neurodevelopmental effects with PFOS

- Gestation exposure to PFBuS also did not result in alterations in pup survival or pup body weight.
- It is worth noting that the immunological effects cited were high-dose effects and they were reversible upon the cessation of PFOA or PFOS exposure.

Page 20: Correction

- There was a pathology peer-review on the ovaries from the 2-year dietary study with current pathological criteria found no statistically significant increases in ovarian hyperplasia, adenoma, or adenoma and hyperplasia combined in treated groups relative to controls.

Page 27: Correction

- ALT and AST were not increased in serum from the Elcombe et al. 2012b study.

Page 243: Correction

- The time frame for the % PFBA excretion data were incorrectly reflected. Those data were for the cumulative excretion of PFBA within 24 hours after an oral does, not 96 hours.
- Sundstrom et al. (2012) did not conduct any [14C] PFHxS study in rats.

Page 275: Correction

- The estimated terminal $t_{1/2}$ values for PFBA in cynomolgus monkeys were 40 hours, not 40 days.

Page 303: Correction

- The reference for PFOS elimination should be Chang et al., 2012 (not 2001).

Page 314 (Reducing Body Burden):

- Genuis et al. (2010) presented a case history where an individual human provided stool samples before and after oral administration of bile acid sequestrant cholestyramine. There were increases in perfluoroalkyl excreted in feces after taking cholestyramine and serum levels of perfluoroalkyls were reported to have declined after regular use of cholestyramine.

Citations:

Abbott, B.D.,Wolf, C.J.,Schmid, J.E.,Das, K.P.,Zehr, R.D.,Helfant, L.,Nakayama, S.,Lindstrom, A.B.,Strynar, M.J., Lau, C.S., 2007. Perfluorooctanoic Acid (PFOA)-induced Developmental Toxicity in the Mouse is Dependent on Expression of Peroxisome Proliferator Activated Receptor-alpha (PPAR{alpha}). Toxicol Sci 98, 571-581.

Abbott, B.D.,Wolf, C.J.,Das, K.,Zehr, R.D.,Schmid, J.E.,Lindstrom, A.B.,Strynar, M.J., Lau, C., 2009. Developmental Toxicity of Perfluorooctane Sulfonate (PFOS) is not dependent on expression of Peroxisome Prooliferator activated Receptor-alpha (PPARα) in the mouse. Reproductive Toxicology 27, 258-265.

Albrecht, P.P.,Torsell, N.E.,Krishnan, P.,Ehresman, D.J.,Frame, S.R.,Chang, S.C.,Butenhoff, J.L.,Kennedy, G.L.,Gonzalez, F.J., Peters, J.M., 2013. A Species Difference in the Peroxisome Proliferator-Activated Receptor alpha-Dependent Response to the Developmental Effects of Perfluorooctanoic Acid. Toxicol Sci 131, 568-582.

Baar, K., 2004. Involvement of PPAR gamma co-activator-1, nuclear respiratory factors 1 and 2, and PPAR alpha in the adaptive response to endurance exercise. The Proceedings of the Nutrition Society 63, 269-273.

Bijland, S.,Rensen, P.C.N.,Pieterman, E.J.,Maas, A.C.E.,van der Hoorn, J.W.,Van Erk, M.J.,Havekes, L.M.,van Dijk, K.W.,Chang, S.-C.,Ehresman, D.J.,Butenhoff, J.L., Princen, H.M.G., 2011. Perfluoroalkyl Sulfonates Cause Alkyl Chain Length-Dependent Hepatic Steatosis and Hypolipidemia Mainly by Impairing Lipoprotein Production in APOE*3-Leiden CETP Mice. Toxicol Sci 123, 290-303.

Bjork, J.A., Wallace, K.B., 2009. Structure-activity relationships and human relevance for perfluoroalkyl acid-induced transcriptional activation of peroxisome proliferation in liver cell cultures. Toxicol Sci 111, 89-99.

Bjork, J.A.,Butenhoff, J.L., Wallace, K.B., 2011. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. Toxicology 288, 8-17.

Butenhoff, J.,Costa, G.,Elcombe, C.,Farrar, D.,Hansen, K.,Iwai, H.,Jung, R.,Kennedy, G., Jr.,Lieder, P.,Olsen, G., Thomford, P., 2002. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicol Sci 69, 244-257.

Butenhoff, J.L.,Kennedy, G.L., Jr.,Frame, S.R.,O'Connor, J.C., York, R.G., 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 196, 95-116.

Butenhoff, J.L.,Ehresman, D.J.,Chang, S.C.,Parker, G.A., Stump, D.G., 2009. Gestational and Lactational Exposure to Potassium Perfluoroctanesulfonate (K^+PFOS) in Rats: Developmental Neurotoxicity. Reproductive Toxicology 27, 319-330.

Butenhoff, J.L.,Bjork, J.A.,Chang, S.C.,Ehresman, D.J.,Parker, G.A.,Das, K.,Lau, C.,Lieder, P.H.,van Otterdijk, F.M., Wallace, K.B., 2012. Toxicological evaluation of ammonium perfluorobutyrate in rats: Twenty-eight-day and ninety-day oral gavage studies. Reprod Toxicol 33, 513-530.

Cai, Y.,Nelson, B.D.,Li, R.,Luciakova, K., dePierre, J.W., 1996. Thyromimetic action of the peroxisome proliferators clofibrate, perfluorooctanoic acid, and acetylsalicylic acid includes changes in mRNA levels for certain genes involved in mitochondrial biogenesis. Arch Biochem Biophys 325, 107-112.

Case, M.T.,York, R.G., Christian, M.S., 2001. Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. Int J Toxicol 20, 101-109.

Chengelis, C.P.,Kirkpatrick, J.B.,Radovsky, A., Shinohara, M., 2009. A 90-Day Repeated Dose Oral (Gavage) Toxicity Study of Perfluorohexanoic Acid (PFHxA) in Rats (with Functional Observational Battery and Motor Activity Determinations)*. Reproductive Toxicology 27, 342-351.

Corton, J.C.,Cunningham, M.L.,Hummer, B.T.,Lau, C.,Meek, B.,Peters, J.M.,Popp, J.A.,Rhomberg, L.,Seed, J., Klaunig, J.E., 2014. Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARalpha) as a case study. Crit Rev Toxicol 44, 1-49.

D'Orazio, G.,Asenio-Ramos, M.,Hernandez-Borges, J.,Fanali, S., Rodriguez-Delgado, M.A., 2014. Estrogenic compounds determination in water samples by dispersive liquid-liquid extraction and micellar electrokinetic chromatography coupled to mass spectrometry. J Chromatogr A in press.

Dankers, A.C.,Roelofs, M.J.,Piersma, A.H.,Sweep, F.C.,Russel, F.G.,van den Berg, M.,van Duursen, M.B., Masereeuw, R., 2013. Endocrine Disruptors Differentially Target ATP-Binding Cassette Transporters in the Blood-Testis Barrier and Affect Leydig Cell Testosterone Secretion In Vitro. Toxicol Sci 136, 382-391.

Dixon, D.,Reed, C.E.,Moore, A.B.,Gibbs-Flournoy, E.A.,Hines, E.P.,Wallace, E.A.,Stanko, J.P.,Lu, Y.,Jefferson, W.N.,Newbold, R.R., Fenton, S.E., 2012. Histopathologic changes in the uterus, cervix and vagina of immature CD-1 mice exposed to low doses of perfluorooctanoic acid (PFOA) in a uterotrophic assay. Reprod Toxicol 33, 506-512.

Du, G.,Hu, J.,Song, L.,Wang, X., Wu, D., 2012. Neonatal Exposure to Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) Causes Early Puberty Onset and Elevated Postpubertal Hormone Levels in Female Rats. Endocr Rev 33, SAT-587.

Du, G.,Hu, J.,Huang, H.,Qin, Y.,Han, X.,Wu, D.,Song, L.,Xia, Y., Wang, X., 2013. Perfluorooctane sulfonate (PFOS) affects hormone receptor activity, steroidogenesis, and

expression of endocrine-related genes in vitro and in vivo. Environ Toxicol Chem 32, 353-360.

Ehresman, D.J.,Webb, P.,Ayers, S.D.,Vanden Heuvel, J.,Olsen, G.W.,Chang, S.C., Butenhoff, J.L., 2014. Effects of perfluoroalkyls on the activation of human CAR3, PXR, and TR receptors in vitro (Abstract 1135 occurring in The Toxicologist, supplement to Toxicological Sciences). Toxicological sciences : an official journal of the Society of Toxicology 138, 302.

Elcombe, C.R.,Elcombe, B.M.,Foster, J.R.,Farrar, D.G.,Jung, R.,Chang, S.C.,Kennedy, G.L., Butenhoff, J.L., 2010. Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARα and CAR/PXR. Arch Toxicol 84, 787-798.

Elcombe, C.R.,Elcombe, B.M.,Foster, J.R.,Chang, S.-C.,Ehresman, D.J., Butenhoff, J.L., 2012a. Hepatocellular Hypertrophy and Cell Proliferation in Sprague-Dawley Rats from Dietary Exposure to Potassium Perfluorooctanesulfonate Results from Increased Expression of Xenosensor Nuclear Receptors PPARα and CAR/PXR. Toxicology 293, 16-29.

Elcombe, C.R.,Elcombe, B.M.,Foster, J.R.,Chang, S.-C.,Ehresman, D.J.,Noker, P.E., Butenhoff, J.L., 2012c. Evaluation of Hepatic and Thyroid Responses in Male Sprague Dawley Rats for Up to Eighty-Four Days Following Seven Days of Dietary Exposure to Potassium Perfluorooctanesulfonate. Toxicology 293, 30-40.

Elcombe, C.R.,Peffer, R.C.,Wolf, D.C.,Bailey, J.,Bars, R.,Bell, D.,Cattley, R.C.,Ferguson, S.S.,Geter, D.,Goetz, A.,Goodman, J.I.,Hester, S.,Jacobs, A.,Omiecinski, C.J.,Schoeny, R.,Xie, W., Lake, B.G., 2014. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol 44, 64-82.

Feng, X.,Wang, X.,Cao, X.,Xia, Y.,Zhou, R., Chen, L., 2015. Chronic exposure of female mice to an environmental level of perfluorooctane sulfonate suppresses estrogen synthesis through reduced histone H3K14 acetylation of the StAR promoter leading to deficits in follicular development and ovulation. Toxicol Sci in press.

Gao, Y.,Li, X., Guo, L.-H., 2013. Assessment of Estrogenic Activity of Perfluoroalkyl Acids Based on Ligand-induced Conformation State of Human Estrogen Receptor. Environ Sci Technol 47.

Genuis, S.J.,Birkholz, D.,Ralitsch, M., Thibault, N., 2010. Human detoxification of perfluorinated compounds. Public Health 124, 367-375.

Gortner, E.G., 1980. Oral teratology study of FC-95 in rats. Experiment Number 0680TR0008. Safety Evaluation Laboratory and Riker Laboratories, Inc., St. Paul, MN. USEPA Public Docket, AR-226-0016.

Gortner, E.G., 1981. Oral teratology study of T-2998CoC in rats. Experiment Number 0681TR0110. Safety Evaluation Laboratory and Riker Laboratories, Inc., St. Paul, MN. USEPA Public Docket, AR-226-0463.

Gortner, E.G., 1982. Oral teratology study of T-3141CoC in rabbits. Experiment Number 0681TB0398. Safety Evaluation Laboratory and Riker Laboratories, Inc., St. Paul, MN. USEPA Public Docket AR-226-0465.

Hall, A.P.,Elcombe, C.R.,Foster, J.R.,Harada, T.,Kaufmann, W.,Knippel, A.,Kuttler, K.,Malarkey, D.E.,Maronpot, R.R.,Nishikawa, A.,Nolte, T.,Schulte, A.,Strauss, V., York, M.J., 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes - conclusions from the 3rd International ESTP Expert Workshop. Toxicologic pathology 40, 971-994.

Hood, D.A., 2001. Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. J Appl Physiol (1985) 90, 1137-1157.

Ishibashi, H.,Ishida, H.,Matsuoka, M.,Tominaga, N., Arizono, K., 2007. Estrogenic effects of fluorotelomer alcohols for human estrogen receptor isoforms alpha and beta in vitro. Biol Pharm Bull 30, 1358-1359.

Klaunig, J.E.,Babich, M.A.,Baetcke, K.P.,Cook, J.C.,Corton, J.C.,David, R.M.,DeLuca, J.G.,Lai, D.Y.,McKee, R.H.,Peters, J.M.,Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol 33, 655-780.

Kraugerud, M.,Zimmer, K.E.,Ropstad, E., Verhaegen, S., 2011. Perfluorinated compounds differentially affect steroidogenesis and viability in the human adrenocortical carcinoma (H295R) cell assay. Toxicology Letters 205, 62-68.

Lake, B.G., 2009. Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation. Xenobiotica 39, 582-596.

Lau, C.,Thibodeaux, J.R.,Hanson, R.G.,Rogers, J.M.,Grey, B.E.,Stanton, M.E.,Butenhoff, J.L., Stevenson, L.A., 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci 74, 382-392.

Lau, C.,Thibodeaux, J.R.,Hanson, R.G.,Narotsky, M.G.,Rogers, J.M.,Lindstrom, A.B., Strynar, M.J., 2006. Effects of Perfluorooctanoic Acid Exposure during Pregnancy in the Mouse. Toxicol Sci 90, 510-518.

Lopez-Doval, S.,Salgado, R.,Pereiro, N.,Moyano, R., Lafuente, A., 2014. Perfluorooctane sulfonate effects on the reproductive axis in adult male rats. Environ Res 134C, 158-168.

Lopez-Doval, S.,Salgado, R.,Fernandez-Perez, B., Lafuente, A., 2015. Possible role of serotonin and neuropeptide Y on the disruption of the reproductive axis activity by perfluorooctane sulfonate. Toxicol Lett 233, 138-147.

Luebker, D.J.,Case, M.T.,York, R.G.,Moore, J.A.,Hansen, K.J., Butenhoff, J.L., 2005a. Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicology 215, 126-148.

Luebker, D.J.,York, R.G.,Hansen, K.J.,Moore, J.A., Butenhoff, J.L., 2005b. Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharmacokinetic parameters. Toxicology 215, 149-169.

Macon, M.B.,Villanueva, L.R.,Tatum-Gibbs, K.,Zehr, R.D.,Strynar, M.,Stanko, J.P.,White, S.W.,Helfan, L., Fenton, S.E., 2011. Prenatal perfluorooctanoic acid exposure in CD-1 mice: low dose developmental effects and internal dosimetry. Toxicol Sci 122, 134-145.

Mitra, R.,Nogee, D.P.,Zechner, J.F.,Yea, K.,Gierasch, C.M.,Kovacs, A.,Medeiros, D.M.,Kelly, D.P., Duncan, J.G., 2012. The transcriptional coactivators, PGC-1alpha and beta, cooperate to maintain cardiac mitochondrial function during the early stages of insulin resistance. Journal of molecular and cellular cardiology 52, 701-710.

Nisoli, E.,Tonello, C.,Cardile, A.,Cozzi, V.,Bracale, R.,Tedesco, L.,Falcone, S.,Valerio, A.,Cantoni, O.,Clementi, E.,Moncada, S., Carruba, M.O., 2005. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science 310, 314-317.

Pastoor, T.P.,Lee, K.P.,Perri, M.A., Gillies, P.J., 1987. Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. Exp Mol Pathol 47, 98-109.

Pereiro, N.,Moyano, R.,Blanco, A., Lafuente, A., 2014. Regulation of corticosterone secretion is modified by PFOS exposure at different levels of the hypothalamic-pituitary-adrenal axis in adult male rats. Toxicol Lett 230, 252-262.

Permadi, H.,Lundgren, B.,Andersson, K.,Sundberg, C., DePierre, J.W., 1993. Effects of perfluoro fatty acids on peroxisome proliferation and mitochondrial size in mouse liver: dose and time factors and effect of chain length. Xenobiotica 23, 761-770.

Puigserver, P.,Wu, Z.,Park, C.W.,Graves, R.,Wright, M., Spiegelman, B.M., 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92, 829-839.

Sales, L.B.,Kamstra, J.H.,Cenjin, P.H.,Van Rijt, L.S.,Hamers, T., Legler, J., 2013. Effects of endocrine disrupting chemicals on *in vitro* global DNA methylation and adipocyte differentiation. Toxicology in vitro 27, 634-643.

Seacat, A.M.,Thomford, P.J.,Hansen, K.J.,Olsen, G.W.,Case, M.T., Butenhoff, J.L., 2002. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. Toxicol Sci 68, 249-264.

Sohlenius, A.K.,Andersson, K., DePierre, J.W., 1992a. The effects of perfluoro-octanoic acid on hepatic peroxisome proliferation and related parameters show no sex-related differences in mice. Biochem J 285 (Pt 3), 779-783.

Sohlenius, A.K.,Lundgren, B., DePierre, J.W., 1992b. Perfluorooctanoic acid has persistent effects on peroxisome proliferation and related parameters in mouse liver. J Biochem Toxicol 7, 205-212.

Sonthithai, P.,Suriyo, T.,Thiantanawat, A.,Watcharasit, P.,Ruchirawat, M., Satayavivad, J., 2015. Perfluorinated chemicals, PFOS and PFOA, enhance the estrogenic effects of 17beta-estradiol in T47D human breast cancer cells. J Appl Toxicol in press.

Staples, R.E.,Burgess, B.A., Kerns, W.D., 1984. The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. Fundam Appl Toxicol 4, 429-440.

Thibodeaux, J.R.,Hanson, R.G.,Rogers, J.M.,Grey, B.E.,Barbee, B.D.,Richards, J.H.,Butenhoff, J.L.,Stevenson, L.A., Lau, C., 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. Toxicol Sci 74, 369-381.

Tucker, D.K.,Macon, M.B.,Strynar, M.J.,Dagnino, S.,Andersen, E., Fenton, S.E., 2015. The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. Reprod Toxicol 54, 26-36.

USEPA. 2002. Hepatocellular Hypertrophy. HED ToxSAC 2002 HED Guidance Doc G2002.01.

Walters, M.W.,Bjork, J.A., Wallace, K.B., 2009. Perfluorooctanoic acid stimulated mitochondrial biogenesis and gene transcription in rats. Toxicology 264, 10-15.

Wang, F.,Liu, W.,Dai, J.,Zhao, H.,Xie, Q.,Liu, X.,Yu, W., Ma, J., 2011. Interaction of PFOS and BDE-47 Co-exposure on Thyroid Hormone Levels and TH-related Gene and Protein Expression in Developing Rat Brains. Toxicol Sci 121, 279-291.

Waxman, D.J., 1999. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch Biochem Biophys 369, 11-23.

Wens, B.,De Boever, P.,Verbeke, M.,Hollanders, K., Schoeters, G., 2013. Cultured human peripheral blood mononuclear cells alter their gene expression when challenged with endocrine-disrupting chemicals. Toxicology 303, 17-24.

White, S.S.,Calafat, A.M.,Kuklenyik, Z.,Villanueva, L.,Zehr, R.D.,Helfant, L.,Strynar, M.J.,Lindstrom, A.B.,Thibodeaux, J.R.,Wood, C., Fenton, S.E., 2007. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. Toxicol Sci 96, 133-144.

White, S.S.,Kato, K.,Jia, L.T.,Basden, B.J.,Calafat, A.M.,Hines, E.P.,Stanko, J.P.,Wolf, C.J.,Abbott, B.D., Fenton, S.E., 2009. Effects of Perfluorooctanoic Acid on Mouse Mammary Gland Development and Differentiation Resulting from Cross-Foster and Restricted Gestational Exposures. Reprod Toxicol 27, 289-298.

White, S.S.,Fenton, S.E., Hines, E.P., 2011a. Endocrine Disrupting Properties of Perfluorooctanoic Acid. J Steroid Biochem Mol Biol 127, 16-26.

White, S.W.,Stanko, J.P.,Kato, K.,Calafat, A.M.,Hines, E.P., Fenton, S.E., 2011b. Gestational and Chronic Low-Dose PFOA Exposures and Mammary Gland Growth and Differentiation in Three Generations of CD-1 Mice Environ Health Perspect 119, 1070-1076.

Wolf, D.C.,Moore, T.,Abbott, B.D.,Rosen, M.B.,Das, K.P.,Zehr, R.D.,Lindstrom, A.B.,Strynar, M.J., Lau, C., 2008. Comparative Hepatic Effects of Perfluorooctanoic Acid and WY 14,643 in PPAR-{alpha} Knockout and Wild-type Mice. Toxicol Pathol 36, 632-639.

Yahia, D.,El-Nasser, A.,Abedel-Latif, M.,Tsukuba, C.,Yoshida, M.,Sato, I., Tsuda, S., 2010. Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. The Journal of Toxicological Sciences 35, 527-533.

Yang, C.,Tan, Y.S.,Harkema, J.R., Haslam, S.Z., 2009. Differential Effects of Peripubertal Exposure to Perfluorooctanoic Acid on Mammary Gland Development in C57Bl/6, Balb/C Mouse Strains. Reprod Toxicol 27, 299-306.

Yao, P.L.,Ehresman, D.J.,Rae, J.M.,Chang, S.C.,Frame, S.R.,Butenhoff, J.L.,Kennedy, G.L., Peters, J.M., 2014. Comparative in vivo and in vitro analysis of possible estrogenic effects of perfluorooctanoic acid. Toxicology 326, 62-73.

Zhao, Y.,Tan, Y.S.,Haslam, S.Z., Yang, C., 2010. Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57Bl/6 mice. Toxicological Sciences 115, 214-224.

III. Human Exposure Studies

Specific Comments for Section 3, Health Effects

3.2 DISCUSSION of HEALTH EFECTS BY ROUTE OF EXPOSURE

3.2.1 Inhalation Exposure

3.2.1.1 Death

Page 43. *Human Exposure studies.* The ATSDR report needs to cite much more current and relevant references for the cohort mortality studies including Raleigh et al. 2014 for the series of reports on 3M Cottage Grove, Minnesota cohort, Steenland and Woskie 2012 for the DuPont Washington Works (Parkesburg, West Virginia) cohort, and include for the first time Consonni et al. 2013 for the multi-cohort, multi-company international cohort study that examined exposure to PFOA (as it is used as a processing aid) in the polymerization of tetrafluoroethylene (TFE) cohort. [Note: The DuPont Washington Works cohort is one of six cohorts included by Consonni et al.] Prior analyses of these 3M Cottage Grove (Gilliland and Mandel 1993; Lundin et al. 2008) and DuPont Washington Works (Leonard et al. 2008) cohorts should not take precedence over the most recent updates by (Raleigh et al. 2014) and (Steenland and Woskie 2012), respectively. Clearly, the most recent work should be used.

3.2.1.2 Systemic Effects

Cardiovascular Effects

Human Exposure Studies. Although Lundin et al. 2008 is discussed on page 52, the much more recent update of the epidemiologic study at the 3M Cottage Grove facility by Raleigh et al. (2014) study is not cited or discussed. This omission of Raleigh et al. is a major shortcoming throughout this ATSDR's document review of **section 3** Health Effects. Based on their extensive cumulative estimates of APFO exposure (ammonium perfluoorooctanoate that readily dissociates to PFOA in the blood) and non-APFO exposures based on the summation of daily 8-hour Time Weighted Averages (by year), Raleigh et al. provided Hazard Ratios (HR) for ischemic heart disease at the 3M Cottage Grove plant by 4 quartiles of APFO exposure in comparison to a referent plant (nearby 3M plant absent APFO exposure). Total study population was 9027 (Cottage Grove plant 4668; referent plant 4359). For ischemic heart disease, hazard ratios (HR) by increasing quartile of cumulative (mg/m^3-years) exposure were 1.00 (referent), 0.93, 0.87, 0.88, and 0.89. For cerebrovascular disease, HRs by increasing quartile of cumulative (mg/m^3-years) exposure were 1.00 (referent), 0.57, 0.70, 0.83, and 0.98. None were statistically significant.

Neither did the ATSDR draft report discuss a cohort incidence study of DuPont workers exposure to PFOA (Steenland et al. 2015). Steenland et al. interviewed 3713 Dupont Washington Works workers or their next of kin in 2008-2011and sought medical records to validate self-reported disease. These workers were the subset of workers from the community and worker cohort study (Winquist et al. 2013). In this study, Winquist et al. estimated historical

PFOA serum levels via a job-exposure matrix that was partially based on 2000 serum measurements. Non-occcupational exposure from drinking water was also estimated. There were no statistically significant trends with increasing modeled serum PFOA levels for coronary heart disease, stroke or individuals taking medications for hypertension.

There is no citation or discussion of the 3M Decatur cohort mortality study in this section. See findings from Alexander et al. 2003.

Respiratory Effects

Page 44. *Human Exposure Studies*. The Steenland et al. (2015) cohort incidence study is not cited or discussed. Steenland et al. did not observe an increased risk for the incidence of medicated asthma or COPD in these DuPont workers.

Hepatic Effects

Page 54. *Human Exposure Studies.* See first paragraph. The Grice et al. 2007 report was a study of the 3M Decatur workers – not 3M Cottage Grove workers. Steenland et al. (2015) (not cited or discussed) observed a nonsignificant trend (10 yr lag) in non-hepatitis liver disease in their cohort incidence study of 3,713 DuPont Washington Works employees. A trend was not observed among non-lagged analyses. Also not discussed in the ATSDR document in the lipid subsection regarding longitudinal studies (see page 58) is the Steenland et al. (2015) cohort incidence study of 3,713 DuPont Washington Works employees. Steenland et al. did not observe a trend between PFOA and high cholesterol in these workers (self-reported medicated hypercholesterolemia). The cutoffs for the unlagged analyses were 3030, 6160, and 11420 ng/mL-year. There were 1298 cases that self-reported taking some type of medications for high cholesterol. Relative risks by quartile were 1.00 (reference), 1.11, 1.06,and 1.05 (p value trend via categories = 0.29). No substantive differences were seen when these analyses were lagged years.

Renal Effects

Page 60. *Human Exposure Studies*. Findings from Raleigh et al. 2014 are not cited or discussed. In the most recent study (Raleigh et al. 2014) of the 3M plant, these investigators reported the following HRs based on a cohort of 9027 from APFO (ammonium perfluorooctanoate) and non-APFO production facilities. HRs for time-dependent cumulative APFO exposures (mg/m^3-years), by quartiles, using an extended Cox model for chronic renal disease were 1.00 (non-APFO referent group), 2.24, 0.94, 1.15, and 1.37. None of the HRs were statistically significant. The Steenland et al. (2015) cohort incidence study (not cited/discussed) did not observe a significant trend for chronic kidney disease among DuPont workers. For the no lagged analyses, the relative risk for chronic kidney disease by increasing quartile of exposure (cumulative serum PFOA-years), were 1.00 (reference), 0.50, 0.69, and 0.67 (p value trend – 0.22 via categories).

Endocrine effects

Pages 60 – 62. *Human exposure studies.* The 1993 analyses by Olsen et al. (1998) (see top of page 61) studied 111 workers (not 11). On page 62, Lundin et al. is discussed for the 3M Cottage Grove study but there is no citation or discussion for the updated Raleigh et al. 2014 Cottage Grove study. The HRs for diabetes were 1.00 (referent non-APFO workers and 0.27, 0.42, 0.80, and 0.72 for the four quartiles increasing cumulative APFO (PFOA) exposure (mg/m^3-years) Cottage Grove workers based on a total of 27 diabetes deaths at the 3MCottage Grove plant versus 64 deaths from diabetes at the 3M non-APFO plant (St. Paul plant). The Steenland et al. (2015) cohort incidence study is not cited or discussed that was of DuPont workers. Steenland et al. did not observe a significant trend in cumulative serum PFOA-years exposure for type 2 diabetes disease among 3713 DuPont workers based on log cumulative exposure lagged 10 years but did so when it was evaluated as quartiles of exposure lagged 10 years. Steenland et al. also reported a nonsignificant increase (10 yr lag) in male thyroid disease and an opposite nonsignificant decrease (10 yr lag) in female thyroid disease. The magnitude of these relative risk levels by quartile of cumulative serum PFOA-years exposure were rather small: 1.00 (reference), 1.06, 1.10, and 1.12. As for thyroid disease in these 3,713 workers (assumed to represent functional thyroid disorders as described by Winquist and Steenland 2014), Steenland et al. reported relative risks for cumulative serum PFOA exposures for men as 1.00 (reference), 1.64, 1.13, and 2.16. P value trend via categories = 0.10. For women, the relative risks for thyroid disease were 1.00 (reference), 1.00, 1.02, and 0.33. P value trend via categories = 0.35.

3.2.1.3 Immunological and Lymphoreticular Effects

Page 64. *Human Exposure Studies.* Not cited in this ATSDR report are the findings for an increased trend in the incidence of ulcerative colitis reported by Steenland et al. (2015) in 3,713 DuPont Washington Works employees. The 10-year lag trend by exposure quartile of serum PFOA-years (based on 28 cases) were 1.00 (referent), 3.00, 3.26, and 6.57. Each of these quartile estimates had rather wide confidence intervals. The p value was 0.05 whether the trend in incidence of ulcerative was analyzed as log cumulative exposure or via categories. A trend was also observed for the incidence of rheumatoid arthritis when analyzed by categories but it was not significant when analyzed by log cumulative exposure.

3.1.2.7 Cancer

Pages 65 – 69. *Human exposure studies.* On page 66 there is a review of 3M Cottage Grove cohort mortality study by Gilliland and Mandel (1993) that was subsequently updated by Lundin et al. (2009). However, there is no mention of the most recently updated study of the 3M Cottage Grove study by Raleigh et al. (2014). This is a serious omission especially as it relates to cancer as Raleigh et al. provided the most extensive update of not just cancer mortality but also cancer ***incidence*** data in this cohort. Although Gilliland and Mandel (1993) and Lundin et al. (2009) are often cited (as done here in this ATSDR document) as finding a nonsignificant increase in prostate cancer deaths. However, such an interpretation is extremely limited due to the low case fatality rate for prostate cancer. Therefore, prostate cancer mortality does not

present an adequate picture of the risk (diagnosis) of prostate cancer. Raleigh et al. addressed this important issue by providing prostate cancer incidence data as well as prostate cancer mortality for this cohort. The HRs for prostate cancer incidence comparing APFO cumulative exposure (mg/m3-years) quartiles to the referent (non-APFO exposed) population were 1.00 (referent), 0.80, 0.85, 0.89, and 1.11. None were statistically significant. This was based on 441 prostate cancer cases (253 referent population cases and 188 APFO-exposed population cases). Given these findings from the most recent assessment of the Cottage Grove cohort by Raleigh et al. (2018), it would be incorrect to infer that there is an increased risk of prostate cancer with APFO exposure (i.e., PFOA) in this 3M Cottage Grove cohort (see also Olsen and Ley 2015) based on the past mortality findings from Gilliland and Mandel (1993) or Lundin et al. (2009). Prostate cancer incidence was also reported by Steenland et al. (2015) (not cited or discussed). They reported a nonsignificant trend in prostate cancer incidence based on 129 cases. Relative risks by quartile of exposure among these 3713 DuPont workers were 1.00 (referent), 1.92, 1.89, and 2.15 (p value trend log cumulative exposure = 0.83; p value trend via categories = 0.11).

Raleigh presented HRs for the incidence (as well as mortality) for kidney cancer, pancreatic cancer, bladder cancer, and breast cancer. Based on 35 kidney cancer cases (19 referent cases and 16 APFO-exposed population cases), the HRs for kidney cancer comparing APFO (PFOA is the dissociated anion in the serum) cumulative (mg/m3—years) exposure quartiles to the referent population were 1.00 (referent), 1.07, 1.07, 0.98, and 0.73. Results did not change appreciably when APFO exposures were lagged 10 years. The lack of an association between kidney cancer is important because the 3M Cottage Grove cohort was exposed to APFO in near isolation to TFE (i.e., they were not engaged in TFE polymerization where PFOA is used as a processing aid). Such exposure to TFE was important in the evaluation of the DuPont Washington Works plant because TFE is a known rat kidney carcinogen.

On pages 66 – 67, the Steenland and Woskie (2012) cohort mortality study is discussed in relation to kidney cancer. Steenland and Woskie reported an increased risk of kidney cancer mortality in the DuPont Washington Works cohort by increasing cumulative exposure (ng/mL-years) to PFOA. These DuPont workers were engaged in the synthesis and polymerization of TFE (to PTFE, polytetrafluoroethylene). Steenland and Woskie stated that exposure to TFE in these DuPont workers would have been well-controlled (closed system) due to its volatile and explosive properties. Therefore, they did not construct an exposure matrix for TFE. However, this argument does not necessarily mean the DuPont workers were not exposed to TFE. As discussed by Olsen (2015), the lower explosion limit for TFE is 110,000 ppm (ACGIH 1997). The 8 hour time-weighted average (TWA) for TFE is 2 ppm. Therefore, it is likely that low level TFE exposures could have occurred through the opening of autoclaves in the polymerization area or decomposition of polytetrafluoroethylene (PTFE) as discussed by Consonni et al. (2013). Consonni et al. estimated exposures in PTFE production plants could be a few parts per million 8-hr TWA.

The relationship between TFE, PFOA, and kidney cancer was further researched by Consonni et al. (2013) who examined a combined 6 cohorts of tetrafluoroethylene synthesis and polymerization workers. The cohorts were from Gendorf, Germany; Sinetta Marengo, Italy,

Dordrecht, the Netherlands; Thronton Cleveleys, UK; Bayonne, New Jersey; and Parkersburg, West Virgina. The latter was a subset of the DuPont Washington Works cohort (Steenland and Woskie). Consonni et al. conducted exposure assessments for TFE and APFO. They found TFE and AFPO exposures were highly correlated (Spearman's = 0.72). For these 6 combined cohorts, the Standardized Mortality Ratios (SMRs) for kidney cancer were 0.98, 1.72, 2.96, and 0.00 for <5, 5-9, 10-19 and>= 20 years of exposure to TFE. When APFO was analyzed as the exposure, the findings were the same as the analyses by TFE exposure. Therefore, Consonni et al concluded that it was not possible to disentangle exposure from TFE and APFO with mortality outcomes in a PTFE plant. Of course, Steenland and Woskie did not reach this conclusion because they did not examine TFE exposure in their study. However, such a conclusion was likely because PFOA is used at the DuPont Washington Works plant as a processing aid in TFE polymerization.

In conclusion regarding kidney cancer, manufacturing workers exposed to APFO, but not to TFE, did not show an increased risk with kidney cancer mortality or incidence. DuPont Washington Works employees exposed to both TFE and APFO plants showed an association with kidney cancer mortality (Steenland and Woskie) that Consonni et al. found could not be disentangled because of the high correlation between exposures. This led Raleigh et al. to suggest that associations of PFOA with kidney cancer in PTFE workers may be due to confounding from TFE exposure. It should also be noted there was no increased incidence of kidney tumors in two two-year feeding studies of rats with APFO (Biegel 2001; Butenhoff 2002).

On pages 67-68 there is a discussion of bladder cancer mortality and incidence among 3M Decatur employees. Not cited or discussed is the cohort incidence study of DuPont Washington Works employees where Steenland et al. (2015) observed a significant decreased trend for bladder cancer with cumulative (mg/m^3-years) PFOA exposure. The relatives risks for bladder cancer incidence by quartile of exposure were 1.00 (referent), 0.32, 0.95, and 0.23.

Because the ATSDR document provides a very limited methodological critique of the perfluoroalkyl occupational (cancer-related) epidemiological studies (preferring to just provide point estimates), the ATSDR report should cite and discuss the review paper by Chang et al. (2014) in order to offer this perspective. See the following reference:

Chang ET, Adami HO, Boffetta P, Cole P, Starr TB, Mandel JS. 2014. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans. Crit Rev Toxicol 44(S1):1 – 81.

Finally, the International Agency for Research on Cancer (IARC) reached the conclusion that PFOA be considered a 'possible' human carcinogen (2B). The IARC considered PFOA to be a 'possible' human carcinogen based, in part, on limited epidemiologic evidence on testicular and kidney cancer but could not rule out chance, bias or confounding in its evaluation of this literature (Benbrahim-Talla et al. 2014). Prostate cancer as not considered as part of the epidemiologic evidence for this 2B classification as the committee felt the evidence for prostate cancer was not compelling.

3.2.2 Oral Exposure

Page 69. The ATSDR document briefly discusses the C8 Health Project (a cross-sectional study conducted in 2005-2006 of the community that involved six water districts surrounding the DuPont Washington Works plant). However, this ATSDR document needs to better define the differences between the C8 Health Project and the C8 Health Study.

The original baseline cross-sectional survey conducted in 2005-2006, referred to as the C8 Health Project, was conducted by Brookmar, Inc. (not the C8 Science Panel) which was created by physicians for the purposes of gathering health and exposure information as approved by the court system. Brookmar, Inc. took approximately one year to complete the task at a cost of about 70 million dollars (Steenland et al. 2014). Each participant was paid $400 for their participation which included a health and exposure (e.g., occupational, residential) questionnaire and the collection of a blood sample for analysis of clinical chemistries and serum PFOA and other perfluoroalkyls. A total of 69,030 individuals participated in the C8 Health Project; 54,457 were >= 20 years of age. The Department of Community Medicine at the University of West Virginia agreed to be the data hosting site that managed the C8 Health Project database. This C8 Health Project cross-sectional database was then used by the C8 Science Panel in their analysis of PFOA measurements and serum clinical chemistries, including serum cholesterol levels in children (Frisbee et al., 2010) and adults (Steenland et al. 2009).

The creation of the 3-member C8 Science Panel (Steenland et al. 2014) was also the result of a 2004 settlement of a class action lawsuit in the mid-Ohio river community between residents and DuPont through the Woods County circuit court (West Virginia). This legal agreement between the plaintiffs and defendant (DuPont) acknowledged that the C8 Science Panel had to have complete independence from both parties in order to conduct the epidemiological research that would be funded by the defendant through the state district court. Ultimately the C8 Science Panel conducted 12 C8 Health Studies over 5 years and determined "probable links" for 55 diseases evaluated, including 21 cancers and a number of conditions such as hypertension and hypercholesterolemia (Steenland et al. 2014). A "probable link" was defined as "given the available scientific evidence, it is more likely than not that among class members a connection exists between PFOA exposure and a particular human disease." See http://www.c8sciencepanel.org/prob_link.html. The C8 Science Panel was never charged by the state district court to establish causality, only whether a "*connection*" existed.

The C8 Science Panel considered there were six diseases and conditions that were "*more probably than not linked to PFOA exposure*" in this mid-Ohio river community: kidney cancer, testicular cancer, ulcerative colitis, thyroid disease, hypercholesterolemia, and pregnancy-induced hypertension (Steenland et al. 2014). However, the C8 Science Panel also stated that as more scientific evidence accumulates, "some associations may not be confirmed. Others may be identified that we (the C8 Science Panel) had missed" (Steenland et al. 2014). The C8 Science Panel *was not a public (governmental) body*. Unlike the government that solicit public comments on carcinogenic classifications, exposure guidance, and rule making, the C8 Science

Panel conducted their deliberations in private (voting of the 3 members per each disease and health condition to obtain a majority opinion). The C8 Science Panel released their consensus of the six probable link determinations to the settling parties and written conclusions on their publicly available website. While many of the individual C8 Science Panel studies have been published in the peer reviewed scientific literature, the probable link determinations were never subjected to an external scientific peer review process – as the court settlement considered the C8 Science Panel to be this review process.

3.2.2.2 Systemic Effects

Respiratory Effects

Page 72. *Human Exposure Studies.* Not cited in this ATSDR document are two studies related to PFAS (perfluoroalkyl substances) and asthma. Stein et al. (2015) examined the NHANES 2005-2006 cross-sectional survey. Stein et al. (2015) did not observe any significant positive association between serum PFOA measurements (as well as PFOS, PFHxS, and PFNA) and self-reported asthma, wheezing or allergy. A significant positive association was observed between serum PFHxS and self-reported rhinitis. This association was not observed with PFOS, PFOA, and PFNA. Granum et al. (2013) did not find an association regarding asthma in a small (n = 99) sub-cohort of the Norwegian Mother and Child Cohort Study (MoBa).

Caridovascular Effects

Page 147. *Human Exposure Studies*

The ATSDR document cited the Shankar et al. (2012) study as evidence that serum PFOA levels have been positively associated with self-reported cardiovascular disease (CVD) in an adult US population. Shankar et al. results were from NHANES (see table 1). Shankar et al. defined CVD as whether the NHANES participant was ever told by a physician that they had coronary heart disease, heart attack, or stroke. Shankar et al. combined NHANES data in the 1999-2000 and 2003-2004 time periods. The ATSDR document did not cite, however, an almost identical study by Melzer et al. (2010) that examined NHANES data in the 1999-2000, 2003-2004, and 2005-2006 time periods. Melzer et al. examined whether the NHANES participants were ever told, by a physician, that they had ischemic heart disease (coronary artery disease, angina, and/or heart attack). Substantively different results were published between Shankar et al. (2012) and Melzer et al. (2010). Whereas Shankar et al. reported a positive association between PFOA and CVD (Table 10), Melzer et al. did not (Table 2). Why the difference between the two studies of the same database (NHANES)? It appears the only difference in the data analyses was that Shankar et al. included a question about stroke in their CVD definition and Melzer included 33% more NHANES study population. Both studies adjusted for many of the same factors except Shankar et al. included hypertension (absent or present), diabetes (absent or present) and serum total cholesterol (mg/dL).

Table 1. Associations between presence of cardiovascular disease (CVD) and peripheral artery disease (PAD) and serum PFOA. NHANES, 1999-2000 and 2003-2004. See Shankar et al. Arch Intern Med (2012) 172:1397-1403.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value trend
PFOA ng/mL Women	<2.9	2.9-3.9	4.0-5.6	>5.6	
Men	<3.9	3.0-4.3	4.4-6.1	>6.1	
Cholesterol Mean (mg/dL) (SE)	206 (4.4)	212 (3.5)	212 (2.7)	218 (2.4)	0.11
Adj Odds Ratio CVD (95% CI)	1.00 (reference)	1.58 (0.80-3.12)	1.77 (1.04-3.02)	2.01 (1.12-3.60)	0.01
Adj Odds Ratio PAD (peripheral artery disease)	1.00 (reference)	0.75 (0.37-1.52)	1.18(0.47-2.96)	1.78(1.03-3.08)	0.04

*see text for adjustments.

Table 2. Association between serum PFOA concentrations (ng/mL) and self-reported prevalence odds ratio for ischemic heart disease. NHANES, 1999-2000, 2003-2004, and 2005-2006. See Melzer et al. (2010). Environ Health Perspect 118:686-692.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
PFOA ng/mL Women (mean)	1.71	3.32	4.79	9.47
Men (mean)	2.47	4.42	6.12	10.39
Adj Odds Ratio Ischemic Heart Disease (95% CI)	1.00 (reference)	0.95 (0.59-1.51)	1.02 (0.65-1.61)	1.08 (0.70-1.69)

*see text for adjustments.

The ATSDR document did not cite two C8 Health Studies related to coronary artery disease and hypertension (Winquist and Steenland 2014a) and stroke (Simpson et al. 2014). These studies were part of what the C8 Science Panel referred to as the community and worker cohort study that represented a cohort of 32,254 participants (28,541 community members and 3,713 workers). It should be noted that this cohort represented approximately 60% of the C8 Health Project adult members. See Winquist et al. (2013) for details of the construction of this community and worker cohort study population. Basically, two cohorts were combined; one from community residents and the other from DuPont plant workers. Cohort members were interviewed in 2008-2011 for their history of chronic diseases. Self-reported diseases were validated, if possible, through medical record review and disease registry matching. Historic serum PFOA exposure was modeled (i.e., estimated) by a multistage modeling procedure (Shin et al. 2011a; 2011b) that utilized an environmental fate and transport model of PFOA

concentrations in local air, surface water, and ground water. These were based on historic plant emissions and physiochemical properties of PFOA. Combined with residential information, an estimate of each person's yearly PFOA intake was calculated. A pharmacokinetic model was then used to estimate yearly serum PFOA concentrations. Ultimately, a modeled (estimated) cumulative serum PFOA-years exposure metric was calculated for the C8 Health Study community and worker cohort. Workers also had their cumulative serum PFOA estimates calculated via worker exposure models (Woskie and Steenland 2013). Table 3 and Table 4 provide findings from these two large studies. Neither study (Winquist and Steenland or Simpson et al) suggested a positive association with coronary artery disease, hypertension or stroke with modeled PFOA exposure.

Table 3. Hazard ratios (95% confidence interval) for hypertension and coronary artery disease by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Winquist and Steenland (2014) Environ Health Perspect 122:1299-1305.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Hypertension*					
	1.00	1.10 (1.02-1.19)	1.10 (1.02-1.18)	1.05 (0.97-1.12)	0.98 (0.91-1.06)
Coronary artery disease**					
	1.00	1.26 (1.10-1.45)	1.17 (1.02-1.35)	0.99 (0.86-1.14)	1.07 (0.93-1.23)

* Modeled cumulative PFOA ng/mL per year by quintile: <111; 111- <191; 191 - <471; 471 - 2763; >=2763

**Modeled cumulative PFOA ng/mL per year by quintile: <147; 147 - <248; 248 - <717; 717 - <5058; >= 5058

Table 4. Hazard ratios (95% confidence interval) for stroke by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Simpson et al. (2014) Environ Res 127:22-28.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Stroke					
Retrospective model*					
(825 cases)	1.00	1.39 (1.11-1.76)	1.36 (1.08-1.71)	1.45 (1.15-1.82)	1.13 (0.90-1.44)
Prospective model**					
(252 cases)	1.00	1.07 (0.73-1.59)	1.07 (0.72-1.58)	1.18 (0.79-1.75)	0.87 (0.58-1.30)

* Modeled cumulative PFOA ng/ml per year by quintile: <178; 178 - <319; 319 - <912; 912 - <4490; >=4490

** Modeled cumulative PFOA ng/ml per year by quintile: <244; 244 - <460; 460 - <1240; 1240 - <5500; >=5500

A study not cited in the cardiovascular effects section of this ATSDR document on pages 147 – 150 was a study by Geiger et al. (2014) that reported no association between PFOA and hypertension in 1,655 children based in an analysis of 8 years of NHANES cross-sectional

survey data. The adjusted odds ratios for PFOA for childhood hypertension by quartile of plasma concentration were 1.00 (referent), 0.89, 0.96, and 0.69. P-value trend statistic by category was 0.25. The adjusted odds ratio for PFOS for childhood hypertension by quartile plasma concentration were 1.00 (referent), 0.99, 0.73, and 0.77 (P value trend by category was 0.36).

On **pages 149 – 150** there are several citations mentioned regarding pregnancy induced hypertension. Not cited is the study by Starling et al. (2014) who conducted a case-control study (466 cases, 510 non-cases) of nulliparous pregnant women enrolled in the Norwegian Mother and Child Cohort study. Starling did not find any significant trend with PFOA or PFOS plasma concentrations and the onset of preeclampsia. The HRs for preeclampsia by quartile of exposure of PFOA were 1.00 (referent), 1.03, 0.92 and 1.01. Per ln-unit of PFOA exposure the adjusted HR was 0.89 (95 % CI 0.65 – 1.22). The adjusted HRs for preeclampsia by quartile of PFOS exposure were (1.00 (referent) 1.12, 0.88, and 1.09. Per ln-unit of PFOS exposure the adjusted HR was 1.13 (95% CI 0.84 – 1.52).

Musculoskeletal Effects

Page 152. *Human Exposure Studies* The ATSDR document should discuss Galloway et al. (2015) and their preliminary findings regarding an association between PFOA and PFOS with reduced expression of the parathyroid hormone 2 receptor gene in women and whether this may contribute to the pathogenesis of osteoarthritis.

Hepatic Effects

Pages 153 - 157. *Human Exposure Studies*. On page 153-154 the ATSDR document discusses the Gallo et al. (2012) paper which analyzed the C8 Health Project cross-sectional data by deciles of serum PFOA and PFOS measurements The ATSDR document states there was no exposure response trend when serum PFOA levels were categorized by deciles for GGT. The same conclusion can be made for ALT. See figure below from Gallo et al. For PFOA, the mean ALT value by decile increased from just under 21 IU/L to 23 IU/L as PFOA concentrations increased 60 times (5 ng/mL to >320 ng/mL). For PFOS, the ALT value went from 21.3 IU/L to 22.3 IU/L as the PFOS value increased from 5 ng/mL to approximately 60 ng/mL. It is difficult to comprehend how this figure suggests a decrease in hepatic cellular integrity as measured by ALT. In Figure 1E for the Gallo et al. paper (below), the linear association between direct bilirubin and PFOS is misleading. The serum PFOS concentration that ranges between 5 ng/mL and 60 ng/mL spans a direct bilirubin concentration of only 0.01mg/dL. The clinical relevance of a difference of 0.01 mg/dL direct bilirubin is questionable.

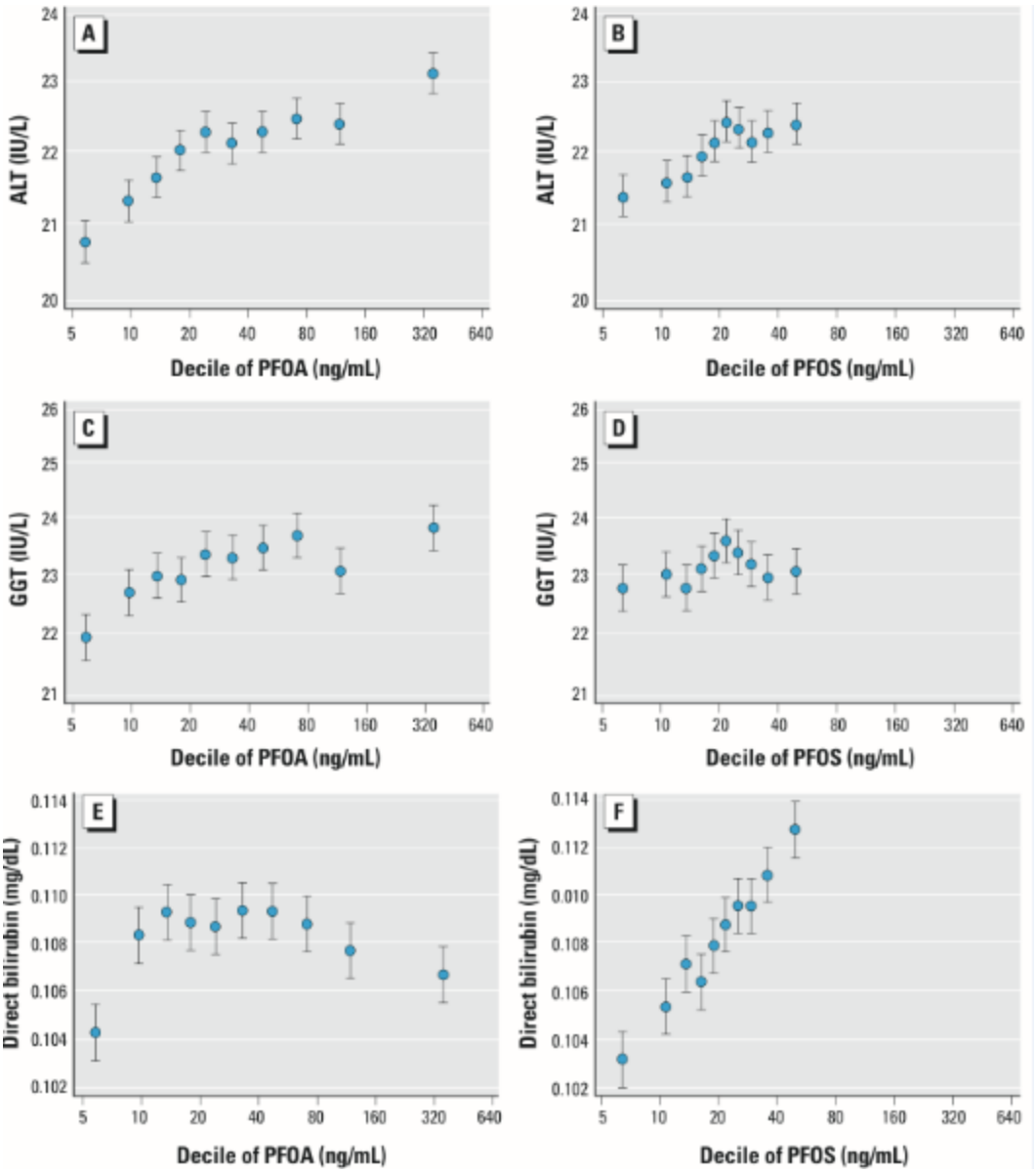


Figure 1. From Gallo et al. Environ Health Perspect 2012; 120:655-660.. Fitted values of ALT (*A*,*B*), GGT (*C*,*D*), and direct bilirubin (*E*,*F*) levels (mean and 95% CI, from fully adjusted regression model) by deciles of PFOA (*A*,*C*,*E*) and PFOS (*B*,*D*,*F*) concentrations, given the mean values of the other covariates. Graph pairs are on the same scale.

As reported in two cross-sectional studies that examined lipids (Frisbee et al. 2010; Steenland et al. 2009), a positive association between measured serum concentrations of PFOA and total cholesterol was observed. The association among adults was "steepest" among those with lower PFOA concentrations. Figure 2 is from the Steenland et al. (2009) study of 46,294 adult residents 18 years or older that showed this "steep" association with rising cholesterol levels that was observed below 50 ng/mL PFOA. Similar curves were displayed by Steenland et

al. (2009) for LDL and triglycerides but not HDL. A cross-sectional study cannot describe an effect because of its inability to separate the temporality between exposure and response. It only describes a cross-sectional study relationship between serum cholesterol and measured serum PFOA levels in these adults.

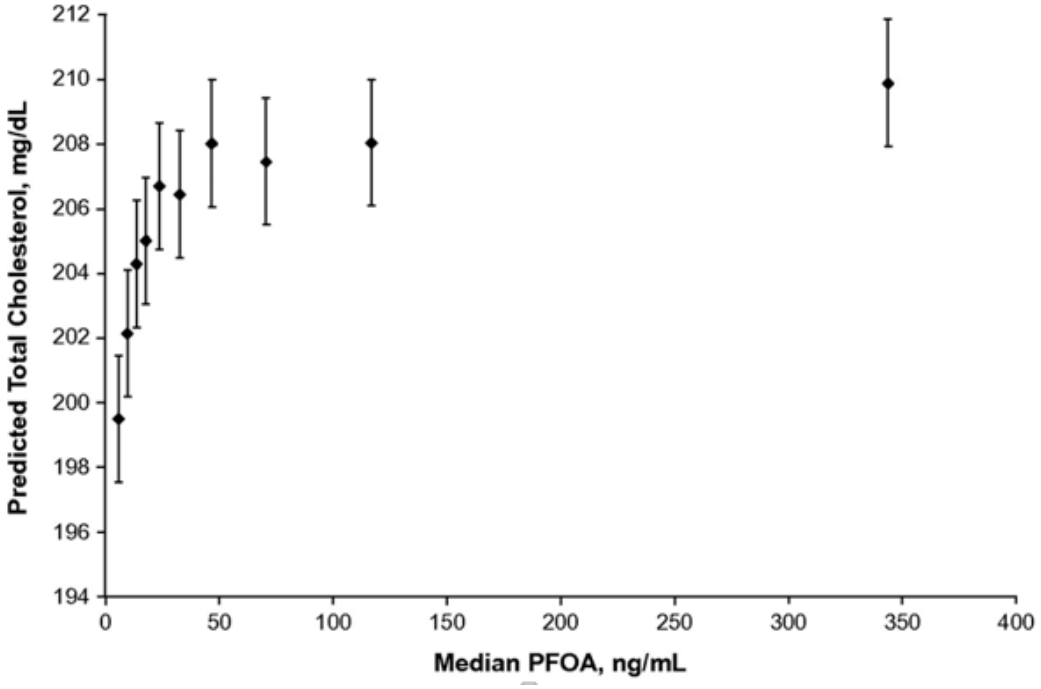


Figure 2. From Steenland et al. Am J Epidemiol 2009;170:1268-1278. Total cholesterol by decile of perfluorooctanoic acid (PFOA), with 95% confidence intervals, Ohio and West Virginia, 2005–2006. The model was adjusted for covariates. The *x*-axis uses medians of PFOA deciles. Predicted cholesterol levels are based on an "average" subject with the following characteristics: age 60–69 years, male gender, not taking cholesterol-lowering medication, never smoked, high school diploma, does not participate in regular exercise, does not drink alcohol, and body mass index between 24 and 27 kg/m^2. Confidence intervals are based on the predicted population mean given those covariate levels. Study population = C8 Health Project (cross-sectional).

Also, Steenland et al. calculated, by quartiles (not deciles as shown in Figure A), the odds ratios for high cholesterol (defined in adults as >= 240 mg/dL) and serum PFOA. These odds ratios were 1.00 (referent), 1.21, 1.31, and 1.40 that Steenland et al. suggested was an upward trend in the magnitude of the risk of high cholesterol from the 2nd through 4th quartiles of serum PFOA concentrations.

For reasons that were never discussed by the C8 Science Panel, a nearly identical "steep" curve (including confidence intervals) was published between their cross-sectional analysis of measurements of serum PFOA and uric acid in this adult C8 Health Project population (Figure 3) (Steenland et al. 2010a) as was seen with total cholesterol and PFOA (Figure 2) (Steenland et al. 2009). Similar to the hypercholesterolemia analysis provided by Steenland et al. (2009), Steenland et al. (2010a) calculated the odds ratios by quartile for hyperuricemia (> 6.0 mg/dL for women, 6.8 mg/dL for men) by quintile of PFOA: 1.00 (referent), 1.33, 1.35, 1.47, and 1.47 with the same plateauing of risks as seen with hypercholesterolemia.

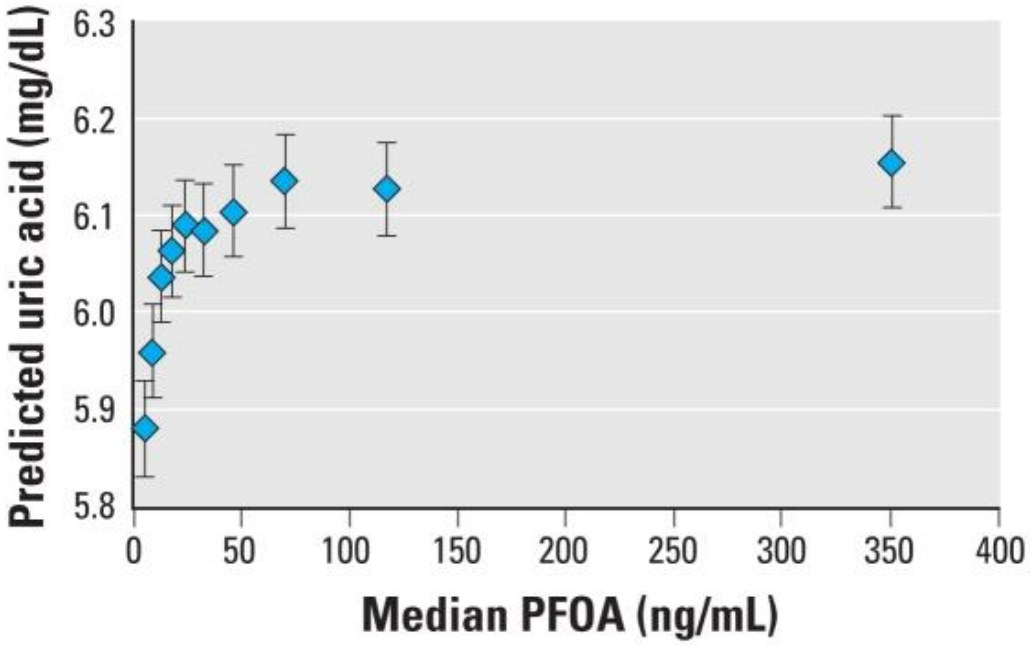


Figure 2. From Steenland et al. Environ Health Perspect 2010; 118:229-233**.** Predicted uric acid with increasing PFOA. Predicted value from regression model for an average participant: 45 years of age, 0.95 mg/dL creatinine, high school education, male, 28.55 kg/m^2 BMI, nonsmoker, nondrinker. Data are population means and 95% CIs.. Study population = C8 Health Project (cross-sectional).

While median PFOA was ≤ 50 ng/mL, both Figure 2 and Figure 3 have steep curves in the dependent variable (cholesterol or uric acid), the range of these measurements were quite narrow (199 to 207 mg/dL and 5.85 to 6.15 mg/dL for cholesterol and uric acid, respectively).

Both curves (Figures 2 and 3) could suggest similar selection bias issues that occurred while obtaining subjects with the lower concentrations of PFOA, similar confounding factors that were not considered in the analyses, or saturated responses.

If each curve is considered separately, possible explanations for the association becomes clearer, especially with uric acid. Both PFOA and uric acid bind to organic anion transporters (e.g., URAT1) in their secretion and reabsorption in the renal proximal tubules (Han et al. 2012). A positive association between PFOA and uric acid in children has also been reported in the NHANES database with lower serum PFOA levels (Geiger et al. 2013) leading Shankar et al. (2011) to suggest that PFOA was associated with chronic kidney disease in the U.S. general population. Chronic kidney disease is defined by a decline in the GFR which also results in an increase in retention of uric acid. This association, between measured PFOA and estimated GFR, was also observed in the C8 Science Panel database of healthy children (Watkins et al.

2013). However, Watkins et al. found no association with kidney function (i.e., GFR) when PFOA was estimated via their historic exposure reconstruction models for PFOA rather than using cross-sectional measurements of PFOA. This led Watkins et al. to suggest that cross-sectional associations between the estimated glomerular filtration rate and serum PFOA may be "a consequence of, rather than a cause of, decreased kidney function." Furthermore, there was not an increased risk in the diagnosis of chronic kidney disease and modeled cumulative PFOA exposure. See C8 Science Panel website http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Kidney_29Oct2012.pdf.

For the question of increased total cholesterol, similar inquiry is required before any causal inference can be considered. Could there be receptors in the gut for PFOA and cholesterol that are saturated at similar concentrations of PFOA? Frisbee et al. (2010) raised this very question of a saturated response from their cross-sectional analysis of the C8 Health Project children data (see Figure 4). (Note: The odds ratios for 'high cholesterol (defined as ≥ 170 mg/dL) showed a plateaued trend in risk with increasing quintile cutpoints of PFOA exposure: 1.00 (referent), 1.1, 1.2, 1.2, and 1.2.) Specifically, Frisbee et al. wrote "the nonlinear nature of the observed associations, particularly for PFOA, suggests a possible saturation point in an underlying physiologic mechanism." It should be noted that Steenland was a co-author of the Frisbee et al. paper. If this is a possible explanation for the children data in Figure 4 (Frisbee et al. 2010), could it not also be a viable explanation for the adult cholesterol data displayed in Figure 2 (Steenland et al. 2009)?

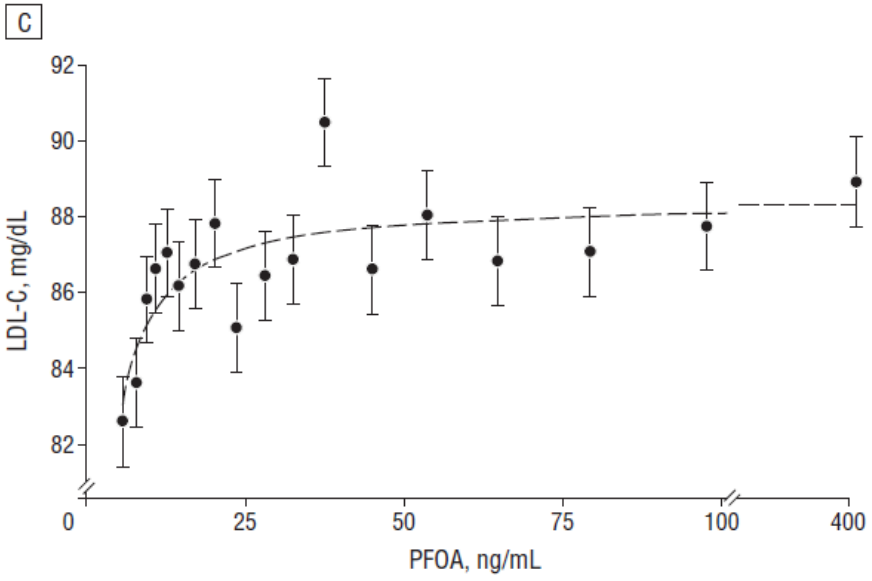


Figure 3. From Frisbee et al. Arch Pediatr Adolesc Med 2010;164:860-869. LDL cholesterol (mg/dL) with increasing PFOA (ng/mL) with 95% confidence intervals. Mid-Ohio River Ohio and West Virginia communities, children data, 2005-2006.

Other questions should be asked about the steepness of the cholesterol curve including does PFOA preferentially bind to lipoproteins? Because PFOA binds to serum proteins (*e.g.*,

albumin), Butenhoff et al. (2012) questioned whether PFOA distributes into serum lipoprotein fractions that could be evident in populations with minimum exposure to PFOA. Their analysis did not offer much support for such a hypothesis. However, it was quite limited because of its sample size. Alternative explanations might include whether high lipid diets may increase the absorption and/or retention of PFOA in the body. Other thought-provoking questions that require answers include the following: 1) Is there any evidence that this cross-sectional curve seen in Figure 2 and Figure 4, or a possible suggestion of hypercholesterolemia, results in known disease outcomes (coronary artery disease, stroke, and hypertension)? 2) Has anyone attempted to examine this association similar to Watkins et al. (2013) using modeled concentrations of PFOA rather than measured when examining lipids? and 3) At high PFOA dosages, is there any concordance with lipid findings between animal results and humans?

If a positive association exists between lower PFOA concentrations and cholesterol, that could be due to an unknown saturated response as discussed by Frisbee et al. (2010) (i.e., a non-causal association) and not observed among more highly exposed occupational populations (Olsen and Zobel 2007; Sakr et al. 2007), is there evidence that the opposite effect (hypolipidemia) actually occurs among the highest exposed humans? The answer appears to be "yes". Not cited in the ATSDR document are the findings from a Phase I dose-escalating clinical trial (conducted in Scotland) that administered PFOA (ammonium salt) to 50 cancer patients (primarily solid tumors) who had exhausted standard medical/surgical therapy and showed *reductions in LDL* at the higher administered dosages of PFOA (Macpherson et al. 2011). The weekly dose of PFOA (ammonium salt) over a six-week time period, escalated in patient groups of 3 in this trial, was from 50 mg/kg to 1200 mg/kg. The 1000 mg/kg/week dose was considered the maximum tolerated dose. Serum PFOA concentrations approached 500,000 ng/mL with these higher doses. The mode of action for lipid-lowering is probably through $PPAR_{alpha}$ activation, which is known to occur in the rodent. However, humans have less $PPAR_{alpha}$ receptors and are considered less responsive (Klauning et al. 2003).

On **page 155** there is no discussion regarding the papers that were published subsequent to Fitz-Simon et al. (2013) by Burstyn (2013) and in response to Fletcher et al. (2013) by Vanden Heuvel (2014). Fitz-Simon et al. reported that of the thousands of adults who participated in the C8 Health Project in 2005-2006, 560 subjects were selected to be measured again for serum PFOA serum concentrations as well as blood lipids in a one-time follow-up examination 4.4 years later in 2010. None of these adults stated they were on cholesterol-lowering medications during this time period. The geometric mean PFOA concentrations in these adults decreased from 74.8 ng/mL to 30.8 ng/mL primarily due to water filtration that was introduced in 2006 – 2008. Among the 560 individuals during these same two measurements (baseline and follow-up in parentheses), their geometric mean total cholesterol (192.5 vs 192.8 mg/dL), LDL cholesterol (107.7 vs. 109.2 mg/dL), HDL cholesterol (48.6 vs 47.2 mg/dL), and triglycerides (144.1 vs. 146.9 mg/dL) were unchanged from a clinical perspective despite the 58.8 percent decline in PFOA concentrations, but the variability of measurements were large among the individuals. Fitz-Simon et al. (2013) suggested a "tendency for greater decreases in LDL to occur with individuals who had greater declines of PFOA." Using a model that adjusted for age, sex, the time interval between measurements, and fasting status, Fitz-Simon et al. statistically modeled, for a person with a 50% decline in PFOA, that this individual would have had a predicted decline

in LDL of 3.6% (95% CI 1.5 to 5.7%). Therefore, Fitz-Simon et al. concluded the opposite was possible (i.e., an increase in PFOA may have been associated with an increase in LDL).

If the association between PFOA and increased cholesterol is causal and not related to chance, confounding, or bias, this would still have been unlikely to result in any clinical implications, as argued by Burstyn (2013) (not cited in this ATSDR document). Burstyn (2013) suggested that even after doubling PFOA concentrations, 97.5% of LDL values for most people, who had an initial healthy (normative range) level at baseline, would have still remained within the normal reference range for LDL. Burstyn also argued that the observed change in LDL mirrored that of NHANES data over the time period (approximately 3 percent decline of median LDL) and furthermore the average in LDL was 5 to 10 percent higher than these 560 adults. While acknowledging that the observed changes were very small, Fitz-Simon et al. (2013) counter argued that NHANES did not exclude individuals on cholesterol lowering medications and thus such a comparison was not entirely appropriate. However, Fitz-Simon et al. did not consider life-style behavioral changes that might have occurred in this mid-Ohio river valley population due to the intense scrutiny the population underwent (i.e., medical testing) in 2005-2006 that might have led to improvements in medical tests independent of their declining PFOA concentrations. Also, the 560 individuals studied by Fitz-Simons et al. were a highly self-selected population from the original C8 Health project. These 560 individuals were only one percent of the original C8 Health Project population studied. Whether such a small self-selected subsample of the original population can be used to infer causality remains untested.

Finally, it should be noted that the associations reported by Fitz-Simon et al. were observed for log transformations of PFOA and cholesterol. As Fitz-Simon stated in their supplemental material to their paper, "although the logarithmic transformation gave more credence to the assumptions of the linear regression model, this does not necessarily imply an underlying logarithmic association." When Fitz-Simons et al. fitted models for the untransformed differences, adjusted for possible time-varying confounders, they reported no associations were observed for cholesterol, LDL, HDL, triglycerides when regressed on tertiles of PFOA (see eAPPENDIX to Fitz-Simon et al. paper) as shown in Figure 5. When Fitz-Simon et al. analyzed PFOA as a continuous untransformed measurement (per 1 ng/mL), there were no statistically significant differences via regression models with lipid measurements: LDL estimate -0.10 (95% CI -1.86, 1.67); total cholesterol -0.72 (95% CI -2.34, 0.90); HDL 0.11 (95% CI -0.39, 0.61); and triglycerides -0.27 95% CI 5.97, 5.43).

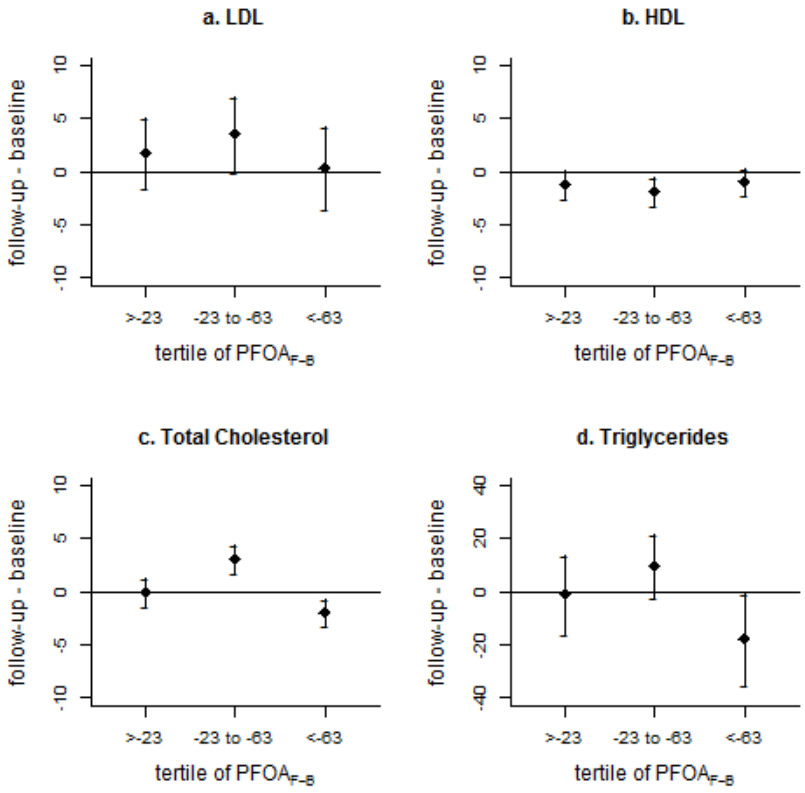


Figure 5. From Fitz-Simon et al. Epidemiology 2013;24:569-576 (eAPPENDIX). Mean follow-up – baseline (F-B) difference (95% confidence interval) in each lipid measurement (mg/dL), by tertile of decrease in PFOA (ng/mL).

In a study not cited by the ATSDR document, Fletcher et al. (2013) suggested there were gene expression changes in 290 C8 Science Panel participants that were consistent with a "hypercholesterolemic environment." Specifically, Fletcher et al. reported an inverse association between serum PFOA and whole blood expression level with NR1H2, MNPC1 and ABCG1 genes which are involved in cholesterol transport. When analyzed by sex, there was reduction in the levels of mRNAs involved in cholesterol transport in men (APC1, ABCG1, and PPARA) and women (NR1H2) gene expression. Fletcher et al. acknowledged their study limitations included their small sample size and examining whole blood rather than gene expression in target organs with more direct relevance to lipid synthesis and metabolism (not possible, of course) and that they tested only a small subset of genes that can influence cholesterol metabolism. In fact, this point was made much more clear by Vanden Heuvel (2014) (also not cited in the ATSDR document) who presented convincing evidence that the only useful conclusion from Fletcher et al. was their "thought provoking nature" but that it fell short of evidence for a "hypercholesterolemic environment" promoted by exposure to PFOA. Vanden Heuvel

summarized his findings in a figure (see Figure 1 in the Vanden Heuvel 2014 paper) that showed 67 major genes involved in the regulation of cholesterol and cholesterol ester concentrations and the transcription factors that regulate their expression. Fletcher et al. only reported on 11 of the 67 genes depicted in the Vanden Heuvel figure of which half were actually involved with reverse cholesterol transport and would therefore result in lipid lowering (not increasing) phenotype responses.

The ATSDR document also did not cite (Watkins et al. 2014 of the same C8 Short Term Follow-up study discussed above (Fletcher et al. 2013.) Watkins et al. contacted 1199 C8 Health Project participants (approximately two percent) for a short term follow-up study in 2010. A total of 973 individuals completed a telephone interview, 755 provided a blood sample, and 685 had sufficient DNA for LINE-1 methylation analysis. LINE-1 (long interspersed nuclear element 1) is a group of retrotransposon sequences that are highly methylated. Methylation of CpG regions suppresses expression of related genes (demethylation increases gene expression). According to Watkins et al. (2014), hypomethylation of LINE-1 elements is associated with ischemic heart disease, stroke, and hypertension, increased LDL and decreased HDL cholesterol. Similar to the gene expression study (see Fletcher et al. 2013), Watkins et al. regressed the 50% change in PFOA concentrations between 2005/2006 and 2010 with % LINE-1 methylation measured in 2010. There were no measurements taken in 2005-2006. LINE-1 methylation was not significantly associated with PFOA in any analysis in the Watkins et al. study.

On **page 156**, the ATSDR document discussed the findings from Nelson et al. (2010). Adding perplexity to the results from the Nelson et al. (2010) study is the analysis by Patel et al. (2013) (not cited or discussed in this ATSDR document) who conducted a systemic environmental-wide association study (EWAS) of 4 NHANES databases (1999-2006) on 188 environmental factors and serum lipids. They reported no association between the polyfluorochemicals reported in NHANES (including PFOA) and LDL HDL, and serum triglycerides in their multi-chemical evaluation for the one 2 year cycle they were able to examine (2005-2006). Why the difference between Nelson et al. and Patel et al. over analyses of the NHANES databases? Others have also expressed important general reservations about the NHANES database regarding using individual environmental chemical (biomonitoring) measurements in cross-sectional analyses (LaKind et al. 2012; Sobus et al. 2015).

How does one rectify the difference in magnitudes of effect for the difference in serum cholesterol and PFOA in the Nelson et al. analysis of NHANES data shown in Table 5 compared to those of an occupational study as shown in Table 6 (Olsen and Zobel 2007)? Clearly, the lipid association reported in Nelson et al. is not observed in the much more highly exposed occupational populations.

Table 5. Distribution of PFOA and cholesterol, persons 20-80 years of age, NHANES, 2003-2004. See Nelson et al. (2010). Environ Health Perspect 118:197-202. See supplemental material.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P trend
N	223	211	186	240	
PFOA (ng/mL)					
Median (range)	2.1 (0.1 – 2.7)	3.4 (2.8-3.9)	4.6 (4.0-5.4)	6.9 (5.5-37.3)	
Unadjusted mean values					
Total cholesterol	198.6	201.6	202.0	205.7	
HDL	56.8	54.3	52.7	54.2	
Non-HDL	141.8	147.3	149.4	151.5	
Adjusted mean differences (from reference), 20 – 80 years of age					
Total cholesterol	reference	5.40 (-2.11, 12.92)	7.50 (-3.71, 18.71)	9.76 (-0.23, 19.74)	0.07
HDL	reference	-2.01 (-3.60, -0.42)	-1.61 (-4.62, 1.40)	-1.28 (-3.45, 0.89)	0.34
Non-HDL	reference	7.41 (-0.97, 15.80)	9.11 (-0.97, 15.80)	11.03 (1.20, 20.86)	0.05

Adjusted for age, gender, race/ethnicity, socioeconomic status, saturated fat intake, exercise, time in front of a TV or computer BMI, alcohol consumption, smoking, and parity

Table 6. Adjusted mean for lipid clinical chemistry results, by PFOA decile. Cross-sectional analysis of 506 male perfluorochemical employees not taking cholesterol lowering medications. 3M Company. Antwerp, Decatur, and Cottage Grove locations. See Olsen and Zobel (2007). Int Arch Occup Ind Hyg 81:231-246.

	PFOA (ng/mL)				
Decile	Median[a]	Cholesterol[b,c]	LDL[b,c]	HDL[b,c]	Triglycerides[b,c]
1	60	214 (203-225)	137 (127-147)	50 (46-53)	145 (116-173)
2	200	211 (199-222)	135 (125-145)	51 (48-54)	124 (95-153)
3	360	209 (198-220)	128 (118-138)	51 (48-54)	153 (124-182_
4	540	210 (199-222)	133 (123-143)	50 (46-53)	145 (116-175)
5	910	217 (206-229)	140 (130-150)	48 (45-51)	162 (133-191)
6	1250	218 (206-229)	141 (130-151)	48 (45-51)	160 (131-190)
7	1630	214 (203-225)	133 (123-143)	50 (47-53)	158 (128-187)
8	2180	215 (204-226)	136 (126-146)	47 (44-50)	172 (144-201)
9	2960	221 (210-233)	140 (130-150)	48 (44-52)	165 (135-194)
10	4940	216 (204-227)	133 (123-144)	44 (41-47)	208 (179-238)

a. ng/mL
b. mg/dL
c. Adjusted for age, BMI, alcohol

Another important paper not cited or discussed by ATSDR document on pages 153 – 157 is the study by Winquist and Steenland (2014). If no associations were observed with heart disease, stroke, or hypertension with cumulative exposure to PFOA, what was the basis for the probable link of hypercholesterolemia declared by the C8 Science Panel? In large part, it appears to be from the study by Winquist and Steenland (2014) who modeled PFOA exposure and subjects' responses to a survey questionnaire that asked "did your physician ever diagnose you" as having "high cholesterol". These surveys were conducted in this mid-Ohio river community between 2008 - 2011. Age at having recalled "first physician diagnosis" was used as the age of onset for hypercholesterolemia. Participants were only included in the analysis if they reported current prescription medication use in the same survey. Winquist and Steenland wrote in their community and worker cohort study that they did not observe a continued increased risk in hypercholesterolemia with modeled cumulative concentrations of PFOA past the 2nd quintile analysis for all subjects (Table 7). The most pronounced trend for hypercholesterolemia (as defined above) was for men 40-59 years, yet, it too, had hazard ratios that essentially did not change past the second quintile 2 (Table 7 and Figure 6). Winquist and Steenland (2014) did not

report other hazard ratios but did offer a graphical display of these hazard ratios (see Figure 6). The variability of these hazard ratios by age and sex is quite apparent in Figure 6.

Table 7. Hazard ratios (95% confidence interval) for high cholesterol) by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Winquist and Steenland (2014) Environ Health Perspect 122:1299-1305.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Hypercholesterolemia*					
All subjects	1.00	1.24 (1.15-1.33)	1.17 (1.09-1.26)	1.19 (1.11-1.27)	1.19 (1.11-1.28)
Men 40-59 yrs	1.00	1.38 (1.21-1.56)	1.32 (1.17-1.50)	1.31 (1.16-1.48)	1.44 (1.28-1.62)

* Modeled cumulative PFOA ng/ml per year by quintile: <142; 142 - <234; 234 - <630; 630 - <3579; >=356

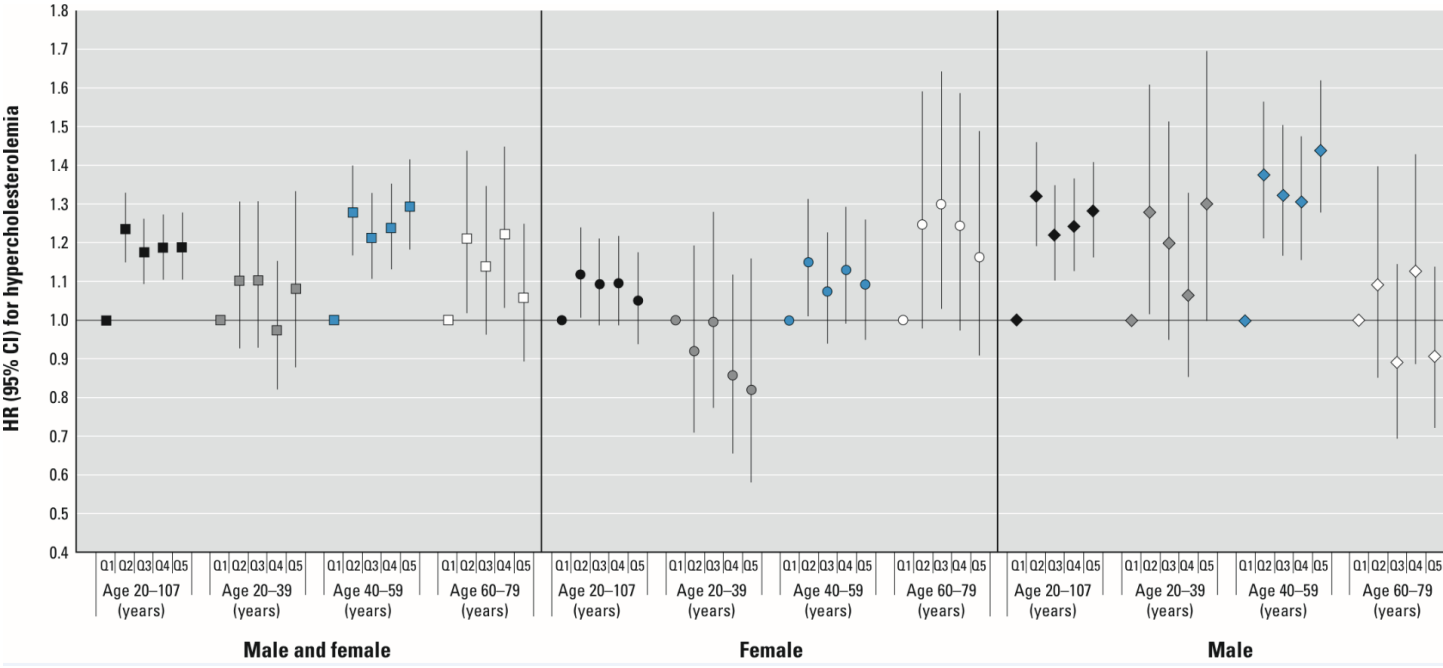


Figure 6. From Winquist and Steenland Environ Health Perspect 2014;122:1299-1305.
Hazard Ratios and 95% CIs for hypercholesterolemia in the primary retrospective analysis for the combined cohorts, cumulative exposure. Quintile (Q) cut points (μg/mL per year) were < 0.142, 0.142 to < 0.234, 0.234 to < 0.630, 0.630 to < 3.579, ≥ 3.579. The analysis included 9,653 cases of self-reported hypercholesterolemia with medication. Models were stratified by single-year birth year and were either stratified by sex or controlled for sex and the interaction between sex and age. Models also controlled for years of schooling (not time-varying; < 12 years, high school diploma/GED, some college, or ≥ bachelor's degree), race (white vs. nonwhite or missing), smoking (time-varying; current, former, none), smoking duration (time-varying), smoking pack-years (time-varying linear term created by multiplying the self-reported number of packs smoked per day by the smoking duration to that point), regular alcohol consumption (time-varying; current, former, none), BMI (at time of first study survey; underweight, normal, overweight, obese), and self-reported type 2 diabetes (time-varying according to reported age at diagnosis).

The most pronounced trend for hazard ratios for hypercholesterolemia (as defined above) in the Winquist and Steenland (2014) study appeared when the modeled PFOA exposure analyses ended in 1987 (see Winquist and Steenland, 2014 see supplement). These hazards diminished after 1987; however, it should be noted that a reduction in the modeled serum PFOA concentration estimates did not begin until 2001 (see supplement to Winquist and Steenland, 2014). Also, there were no increased trends for hypercholesterolemia in a prospective analysis through 2011 (Winquist and Steenland 2014).

There are a number of other lipid related papers regarding exposures to perfluoroalkyls that will need to be considered in finalizing the toxicological profile. Several of these are cross-sectional studies of small populations whose target populations are not well-described. These papers include Skuladottir et al. (Environ Res 2015;143:33-38); Maisonet et al. (Environ Int 2015a; 82:49-60); Zeng et al. (Sci Total Environ 2015;512-513:364-370); Fu et al. (Ecotoxicol Environ Saf 2014;106:246-252); Geiger et al. (Chemosphere 2014;98:78-83); Starling et al. (Environ Int 2014a;62:104-112); and Kerger et al. (Drug Chem Toxicol 2011;34:396-404).

In conclusion regarding this section on hepatic/lipid effects (pages 153 – 157) based on the above discussion provided, the ATSDR draft profile has incorrectly concluded on page 156 that the "available epidemiology data provide strong support for a positive association between serum PFOA and serum PFOS levels and total cholesterol and non-HDL-cholesterol . . ." The associations reported with serum lipids appears at the lower concentrations of PFOA/PFOS measured in the absence of any risk for cardiovascular disease in any population studied (occupational, exposed community, and general population). Therefore, the conclusions offered by Steenland et al. (2010) continue to reflect the weight of the evidence. Steenland et al. wrote "The strength of the association of PFOA and cholesterol varied considerably by study, making interpretation even more problematic: The lower the range of PFOA that was studied, the greater the change in cholesterol per unit change in PFOA. Thus, the studies of community populations report larger shifts in cholesterol per unit change in PFOA levels than do the occupational studies (which have higher exposures). . . . One possibility that might explain some of this discrepancy would be if the slope of an exposure–response relationship was steep at low PFOA levels and then flattened out, as might be the case, for example, if some biological pathways were saturated." This conclusion needs to be highlighted in this ATSDR document.

Renal Effects

Page 164. *Human Exposure Studies.* This section misses a very important point. What Watkins et al. exquisitely showed is that epidemiologic studies that measure perfluoroalkyl compounds need to be concerned about the potential confounding caused by the glomerular filtration rate (GFR). Perfluoroalkyls are primarily protein bound. They are not lipophilic. Renal clearance is the sum of three processes that involve GFR, renal tubular secretion, and renal tubular reabsorption. (Han et al. 2012). Specifically the renal clearance of a perfluoroalkyl or polyfluoroalkyl substances (PFAS) is a function of the unbound fraction of the PFAS and the GFR and the net result of the renal secretion and reabsorption.

Equation 1. PFAS (CL_R) = $f_u \cdot GFR + CL_S - CL_{Abs}$

Therefore, when an epidemiologic study reports an association between a measured PFAS and a health-related outcome, if the health-related outcome is associated with the GFR, then the association between the measured PFAS and the health-related outcome may be confounded by the GFR. This is a critical issue that is not understood in the Developmental Effects **section 3.2.2.6** of this ATSDR document as it relates to birth weight. It should also be understood that PFASs can also be eliminated by other routes of clearance including the gastrointestinal tract, pregnancy, menstruation, and lactation. Note: The predicted estimates for PFOA in the Watkins paper were not based on a physiologically based pharmacokinetic (PBPK) model but rather a pharmacokinetic model.

Endocrine Effects

Pages 166 – 168. *Human Exposure Studies*. This section does not contain several published studies related to the thyroid. In particular, it does not cite or discuss the Winquist and Steenland (2014b) study.

Winquist and Steenland (2014b) reported on thyroid disease based in the community and worker cohort study. As discussed before, this study consisted of 32,245 participants (28,560) from the community and subset of DuPont workers (3,713) near the DuPont Washington Works plant. The community drinking water contained PFOA. The community and worker cohort study design is detailed by Winquist et al. (2013). In brief the cohort was interviewed during 2008-2011. Reported diseases were validated through medical records review for thyroid disease. An annual model of PFOA exposure was calculated in conjunction with a pharmacokinetic model to generate estimated (not measured) PFOA serum concentrations (Shin et al. 2011a; 2011b). In the manuscript Winquist and Steenland (2014b) provided several figures regarding hazard ratios for "functional thyroid disorders" in relation to modeled cumulative serum exposure (ng/mL-years) PFOA quintiles. "Functional thyroid disorders" is defined as a report of goiter, Graves' disease, hyperthyroidism, Hashimoto's disease, hypothyroidism, thyroiditis not otherwise specified, or a thyroid function problem of unknown type. Functional thyroid disease excluded benign and malignant neoplasms, congenital disease, nodules, cysts, or a thyroidectomy without functional changes mentions; and thyroid disease of unspecified type.

Given that most thyroid disease relate to either hyperthyroidism or hypothyroidism, the following comments and data are derived from the eTables ((http:/links.lww.com/EDE/A748) provided by Winquist and Steenland (2014b). Whereas 770 subjects self-reported hyperthyroidism and 2,395 subjects reported hypothyroidism, the number of validated cases used in the Winquist and Steenland retrospectively modeled exposure estimates of serum PFOA were 384 (49%) and 1,368 (57%), retrospectively. Among the 384 hyperthyroid cases, 304 were females (79%). Among the 1,368 hypothyroid cases, 1,123 (82%) were females. This is expected given the prevalence of these diseases in women. The number of validated cases for

the prospective analyses of modeled exposure estimates of serum PFOA were 72 hyperthyroid cases and 302 hypothyroid cases. Prospective analyses also examined newly diagnosed cases using measured PFOA obtained in the C8 Health Project (2005-2006).

Because this ATSDR document in this **section (3.2.2)** are directed at oral exposures, the study by Winquist and Steenland (2014b) regarding thyroid risk is reviewed for only their community (not worker) analyses as shown in the eTables to the supplement of Winquist and Steenland (2014). Based on 1,064 female hypothyroid cases in the community cohort, the hazard ratios for quintiles of modeled cumulative serum exposure to PFOA were 1.00 (reference) 1.31, 1.32, 1.33, 1.43, respectively (log linear (i.e., trend test) HR = 1.03, p = 0.132). (see eTable 2). The estimated modeled upper cutpoints of quintiles of cumulative exposure for PFOA were <114.7 ng/mL/yr (referent), <202.2 ng/mL/yr, < 497.3 ng/mL/yr, 2,676 ng/mL/yr, and 97,396 ng/mL/yr. When exposure was estimated as a short term effect (the serum concentration estimate for the age at diagnosis or the corresponding age for noncases), the HRs for hypothyroidism among the women community cohort were 1.00 (referent), 1.28, 1.27, 1.03, and 1.32 (log linear HR 1.03, p value = 0.161). (See eTable 2.) When Winquist and Steenland restricted their retrospective exposure assessment to a qualifying year (first age at which each person was known to have qualified for the cohort by living in the study area for at least 1 year), the HRs for cumulative exposure for hypothyroidism among women were 1.00 (referent), 0.91, 1.25, 1.19, and 1.26 (log linear HR 1.02, p value = 0.37). (See eTable 4). Using the qualifying year for the yearly exposure estimates, the HRs for hypothyroidism for women were 1.00 (reference), 1.12, 1.10, .97, and 1.14 (log linear HR 1.01, p value = 0.55). (see eTable 4). In the prospective analyses for hypothyroidism in women community members, the modeled estimate for cumulative exposure resulted in HRs of 1.00 (referent), 1.37, 0.81, 0.90, and 0.90 (log linear HR 0.95, p value = 0.315). (See eTable 6). The HRs for hypothyroidism in women cases for yearly exposure estimates were 1.00 (referent), 0.71, 0.69, 0.72, and 0.73 (log linear HR 0.93, p value = 0.225). Using the measured value at the time of the C8 Health project, the HRs for hypothyroidism among women were 1.00 (referent), 0.74, 0.72, 0.75, and 0.71 (log linear HR 0.92, p value = 0.144).

The following tables summarize the three types of modeled exposure analyses by the log linear HR and p values for each of these hypothyroid and hyperthyroid diagnoses by sex found in Winquist and Steenland (2014b).

Table 8. Retrospective survival analysis results for validated thyroid disease, community cohort only (eTable 2 from Winquist and Steenland 2014)

		Retrospective Analysis for Community Cohort (eTable 2)			
		Log linear model (Hazard Ratio (HR) and p value)			
		PFOA Modeled Cumulative		PFOA Modeled Yearly	
	N (%)	HR	p value	HR	p value
Hypothyroidism					
Women	1064 (66)	1.03	0.132	1.03	0.161
Men	189 (12)	1.10	0.075	1.05	0.381
Hyperthyroidism					
Women	291(18)	1.09	0.053	1.11	0.005
Men	64 (4)	1.00	0.984	0.97	0.710
	1608 (100)				

Table 9. Retrospective survival analysis results for validate thyroid disease starting in qualifying year, community cohort only (from eTable4, Winquist and Steenland 2014)

	Retrospective Analysis with Qualifying Year for Community Cohort (eTable 4)				
		Log linear model (Hazard Ratio (HR) and p value)			
		PFOA Modeled Cumulative		PFOA Modeled Yearly	
	N (%)	HR	p value	HR	p value
Hypothyroidism					
Women	926 (66)	1.02	0.370	1.01	0.550
Men	174 (12)	1.10	0.074	1.04	0.468
Hyperthyroidism					
Women	256 (18)	1.07	0.133	1.09	0.030
Men	57 (4)	0.98	0.812	0.94	0.482
	1413 (100)				

Table 10. Prospective survival analysis results for validated thyroid disease, community cohort only. (from eTable 6, Winquist and Steenland 2014)

		Prospective Analysis for Community Cohort (eTable 6)					
		Log linear model (Hazard Ratio (HR) and p value)					
		PFOA		PFOA		PFOA	
		Modeled cumulative		Modeled yearly		C8 Health Project	
	N (%)	HR	p value	HR	p value	HR	p value
Hypothyroidism							
Women	223 (63)	0.95	0.315	0.93	0.225	0.92	0.144
Men	58 (16)	1.28	0.012	1.08	0.499	1.11	0.330
Hyperthyroidism							
Women	57 (16)	1.11	0.279	1.18	0.100	1.19	0.075
Men	15 (6)	0.92	0.700	0.74	0.236	0.72	0.158
	353 (100)						

Although Winquist and Steenland (2014b) concluded in their abstract "higher PFOA exposure was associated with incident functional thyroid disease in this large cohort with high exposure" this appears not to be supported by a much closer inspection of their actual data as presented in the eTables for the community (non-worker) part of this cohort specific to hypothyroidism and hyperthyroidism. There is clearly no increased risk for hypothyroidism among the greatest number of subjects (women) in this community cohort whether the analyses are conducted retrospectively or prospectively. In women, hypothyroidism represents two-thirds of the cases in this community cohort study. There is a weak association for hyperthyroidism in the retrospective but not prospective analyses among the women. Among men, there is a weak association observed for hypothyroidism among men but only when PFOA is modeled as cumulative exposure, not as yearly exposure. There is no association for hyperthyroidism in men (few subjects). At the time of the measurement of actual measurement of PFOA during the C8 Health Project in 2005-2006, there was no increased increase for subsequent hypothyroidism or hyperthyroidism in women or men (Table 10). (Note: Steenland et al. (2015) reported the relative risks for thyroid disease for the 3,731 workers of this community and worker cohort. See pages 60 – 62 for the results. There were no significant risks observed.)

There are several other studies related to the thyroid that are not cited by this ATSDR document. Many of these studies are cross-sectional, some have small samples (e.g., Shrestha et al. (Environ Int 2-15;75:206-214); Webster et al. (Environ Res 2014;133:338 – 347); Pirali et al. (Thyroid 2009;19:1407-1412) and some investigators analyzed parts of the same NHANES database [Wen et al (J Clin Endocrinal Metab 2013;98:E1456-E1464); Jain (Environ Res 2013;126:51-59); Webster et al. (Environ Health Perspet 2014; doi 10.1289/ehp.1409589). Others subdivided parts of the C8 Health Project database are discussed as either related to adults [Knox et al. (J Toxicol Sci 2011;36:403-410)] or children risks for thyroid disease Lopez-Espinosa (Environ Health 2012;120:1036-1041). All of these studies reported on measured thyroid hormones but, unlike Winquist and Steenland (2014) never reported on medically validated thyroid diseases. Thyroid hormones are reported as central tendencies but discussion is lacking as to the reference range of the authors' laboratory clinical referent ranges. For example, Lopez-Espinosa et al. reported there was a significant 1.1% increase in TT4 in children 1 – 17 years of age with an interquartile shift in serum PFOS (15 to 28 ng/mL) and serum PFNA (1.2 to 2.0 ng/mL) but TSH did not significantly change. The clinical relevance of such statistical findings needs to be considered.

At least two (non-thyroid) papers are not cited in the Endocrine Effects paragraphs. They are:

Maisonet M, Calafat AM, Marcus M, et al. 2015b. Prenatal exposure to perfluoroalkyl acids and serum testosterone concentrations at 15 years of age in female ALSPAC study participants. Environ Health Perspect doi.org/10.1289/ehp.1408847

Zhang C, Sundaram R, Maisog J, et al. 2014. A prospective study of prepregnancy serum concentrations of perfluoroochemicals and the risk of gestational diabetes. Fertil Steril 103:184-189.

Body Weight Effects

Page 172. (See also **Page 71).** *Human Exposure Studies.* Contrary to the ATSDR draft document, there have been at least three human studies that examined body weight effects with inconsistent findings regarding exposure to PFOA. Halldorsson et al. (2012) reported on a prospective cohort of 665 Danish pregnant women recruited in 1988 - 1989 with follow-up of their children 20 years later. The offspring BMI, waist circumference, and biomarkers of adiposity were recorded. Halldorsson et al. observed a positive association between maternal exposure to PFOA at 30 weeks (considered to represent *in utero* exposure) and the prevalence of being overweight and high waist circumference among female offspring, but not male offspring, at 20 years of age. Likewise, maternal exposure to PFOA was associated with insulin, leptin, and adiponectin in female, but not male, offspring. Upon adjusting for PFOA concentrations, Halldorsson et al. did not observe associations with being overweight or waist circumference with other perfluoroalkyls (PFOS, PFOSA, and PFNA). In the C8 Health Study conducted by the C8 Science Panel, Barry et al. (2014) did not find PFOA exposure to be associated with being overweight or obese later in adulthood. In this study, 8764 adults aged 20 - 40 years reported height and weight based on a survey administered in 2008 - 2011. Their modeled PFOA early life (first 3 years) serum concentrations were estimated based on residential history and nearby chemical plant emissions (Shin et al. 2011). Anderson et al. (2013) reported a nonsignificant inverse association in the Danish National Birth Cohort Study between children's BMI and waist circumference and the risk of being overweight at 7 years of age based on up to 1400 maternal serum PFOA and PFOS concentrations measured early in the second trimester between 1996 - 2002.

Other Effects

Page 174. *Human Exposure Studies*. The association between measured uric acid and measured PFOA (or PFOS) is confounded by GFR. Therefore, the few cross-sectional studies that reported this association were confounded by the GFR that was not controlled in these studies. As discussed above, uric acid and PFOA both bind to URAT1, a known organic ion transporter located in the apical and basal membranes of the proximal tubule.

3.2.2.3 Immunological and Lymphoreticular Effects

Page 175. *Human Exposure Studies* Not cited or discussed in this section of the ATSDR document is the cross-sectional study by Stein et al. (2015) who examined PFAS serum concentrations in relation to measles, mumps, and rubella antibody concentrations in NHANES 1999 – 2000 and 2003-2004. Stein et al. also examined the prevalence of allergic conditions and allergic sensitization in NHANES 2005-2006. In their vaccine study, Stein et al. included 1,191 children with general population levels of PFAS values expected during the time of these NHANES surveys. Stein et al. reported no association of PFASs with measles antibody levels. A doubling of PFOS was associated with a 13.3% decrease in rubella antibodies and a 5.9%

decrease in mumps antibody concentration. Stein et al. acknowledged the uncertainty regarding the clinical relevance of this finding in a cross-sectional study. In their allergy study, children with higher PFOS serum concentrations were less likely to be sensitized to allergens. The only statistically significant finding was for increased PFOA and prevalent rhinitis (IQR shift odds ratio 1.35).

3.2.2.4 Neurological Effects

Several papers were not discussed in section 3.2.2.6 related to childhood neurodevelopment outcomes. They are the following (see also written comments on these studies are on pages 211-212):

Chen MH, Ha EH, Liao HF, et al. 2013. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology 24:800-808.

Høyer BB, Rmlau-Hansen CH, Obel C, et al. 2015. Pregnancy serum concentrations of perfluorinated substances and offspring behavior and motor development at age 5-9 years – a prospective study. Environ Health 14:2.

Lew Z, Ritz B, von Ehrenstein OS et al. 2015Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure of perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort. Environ Health Perspect 123:367-373.

Ode A, Källen K, Gustafsson P, et al. 2014. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLOS one. 9:e95891.

Stein C, Savitz DA, Belling DC. 2014. Perfluorooctanoate exposure in a highly exposed community and parent and teacher reports of behavior in 6-12 year-old children. Pediatric Perinatal Epidemiol 28:146-156.

Stein CR, Savitz DA, Bellinger DC. 2013. Perfluorooctanoate and neuropsychological outcomes in children. Epidemiol 24:590-590.

Strøm M, Hansen S, Olsen SF, et al. 2014. Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes – A prospective study with long-term follow-up, Environ Int 68:41-48.

3.2.2.5 Reproductive Effects

Pages 182 – 191. *Human Exposure Studies* Although the ATSDR document cites three general population studies that examined the possible association between serum perfluoroalkyl levels and fertility, the document does not provide any insight into this methodological controversy that has surrounded this literature. The following comments are provided to offer this insight.

The initial investigation that suggested an association between PFOA and increased infertility and decreased fecundability was a study of the Danish National Birth Cohort (DNBC) (Fei et al. 2009). The DNBC was a nationwide follow-up study of approximately 100,000 children and their mothers. Pregnant women in their first trimester were recruited through their physicians. Fei et al. randomly selected 1400 women from all participants (n = 43,045) who gave birth to a single live born child without congenital malformation and who participated in a set of 4 telephone interviews, including questions regarding the length of time required to have achieved a planned successful pregnancy. Blood samples (weeks 4 – 14 of pregnancy) were used to measure PFOA among the 1240 women who met this definition. Infertility was defined as reporting a time to pregnancy (TTP) > 12 months or infertility treatments for this current pregnancy. Fecundity odds ratios (FORs) were calculated that measured the odds of a successful conception for women who had higher levels of PFOA compared with the reference level within a given calendar month, given that pregnancy was not achieved in the prior month. FORs < 1 indicate decreased fecundity and a longer TTP.

Among the 1240 women with planned pregnancy, their mean PFOA concentration was 5.6 ng/mL. The mean PFOA concentrations by time to pregnancy (number of participants in parentheses) were 5.4 ng/mL at < 6 months (n = 861), 6.0 ng/mL at 6 – 12 months, (n = 191), and 6.3 ng/mL at >12 months (n = 188).

Provided in Table 11 are the odds ratios for infertility and fecundability from the Fei et al. study (2009). These odds ratios were adjusted for maternal age at delivery, parity, pre-pregnancy BMI, maternal SES, alcohol consumption before pregnancy, paternal age, and paternal education. There were statistically significant trends for infertility and fecundability with PFOA. Fei et al. acknowledged that the exposure time window of interest was at the start of pregnancy planning but their exposure data for PFOA were measured at 4 – 14 weeks gestation and would have been rather stable over pregnancy due to the long elimination rate for PFOA in humans. Fei et al. suggested exposure to PFOA at levels found in the general population may increase TTP and could explain some of the fertility differences among different populations developed countries.

Table 11. Association between PFOA Plasma Concentrations (ng/mL) and Subfecundity Among 910 Subjects (416 Cases, 494 Controls) Subjects from the Norwegian Mother and Child Cohort Study, Norway, 2003-2004 (Whitworth et al. 2004)

		Infertility (> 12 months TTP)			Fecundability		
		Fei et al. (2009)	Fei et al. (2012)		Fei et al. (2009)	Fei et al. (2012)	
		Adjusted OR (95% CI)			Adjusted OR (95% CI)		
Fei et al. 2009; Fei et al. 2012	PFOA (ng/mL)	All Subjects	Nulliparous	Parous	All Subjects	Nulliparous	Parous
	< LLOQ – 3.91	1.00	1.00	1.00	1.00	1.00	1.00
	3.91 – 5.20	2.06 (1.22 – 3.51)	0.79 (0.30 – 2.08)	3.39 (1.75 - 6.53)	0.72 (0.57 - 0.90)	0.98 (0.59 – 1.64)	0.61 (0.46 – 0.80)
	5.21 – 6.96	2.54 (1.47 - 4.39)	0.55 (0.21 – 1.43)	2.92 (1.44 – 5.93)	0.73 (0.58 – 0.92)	0.93 (0.56 – 1.54)	0.62 (0.46 – 0.83)
	≥ 6.97	2.54 (1.47 – 4.39)	1.30 (0.52 – 3.21)	2.99 (1.28 – 6.98)	0.60 (0.47 – 0.76)	0.63 (0.39 – 1.04)	0.63 (0.44 – 0.91)
		P = 0.006	P = 0.082	P = 0.01	P ≤ 0.001	P = 0.002	P = 0.004
Whitworth et al. 2012		Adjusted OR (95% CI)					
	PFOA (ng/mL)	All Subjects	Nulliparous	Parous			
	< 1.66	1.00	1.00	1.00			
	1.66 – 2.24	1.6 (1.1 – 2.3)	0.6 (0.3 – 1.5)	1.5 (0.9 – 2.5)			
	2.25 – 3.02	2.5 (1.5 – 3.2)	0.6 (0.3 – 1.4)	2.4 (1.4 – 4.1)			
	≥ 3.03	2.0 (1.4 – 3.0)	0.5 (0.2 – 1.2)	2.1 (1.0 – 4.4)			
	Test for trend	P ≤ 0.001	P = 0.20	P = 0.01			
Bach et al. 2015		Adjusted OR (95% CI)					
	PFOA (ng/mL)	All Subjects	Nulliparous	Parous			
	< 3.0	1.00	1.00	1.00			
	3.0 - ≤ 5.2	0.92 (0.69 – 1.22)	0.82 (0.53 – 1.26)	1.30 (0.86 – 1.98)			

	> 5.2 - ≤ 5.5	0.94 (0.71 – 1.26)	1.11 (0.73 – 1.69)	0.96 (0.66 – 1.41)	
	> 5.5	0.86 (0.63 – 1.19)	0.99 (0.64 - 1.54)	0.74 (0.48 – 1.13)	
Vestergaard et al. 2012			All Subjects		
	PFOA (ng/mL)		Adjusted OR (95% CI)	Adjusted FOR (95% CI)	
	< 5.60		1.00	1.00	
	≥ 5.60		1.21 (.67 – 2.18)	0.92 (0.65 – 1.31)	
	Log-transformed (continuous)			1.18 (0.78 – 1.78)	
Buck Louis et al. 2013				All Subjects Adjusted FOR (95% CI)	
	PFOA Log-transformed and rescaled by the SD			0.95 (0.82 – 1.11)	
Velez et al. 2014			All Subjects		
			Adjusted OR (95% CI)	Adjusted FOR (95% CI)	
	PFOA Log-transformed and rescaled by the SD		1.31 (1.11 – 1.53)	0.89 (0.83 – 0.94)	
			P = 0.001	P < 0.001	

Based on their review of the Fei et al. (2009) data, Olsen et al. (2009) discussed that parity is both an outcome of fecundity and is associated with perfluoroalkyl concentrations. Because perfluoroalkyl levels would be lower after a pregnancy (concentrations transferred in utero and through lactation), a longer interval between births would result in more time for a woman to absorb concentrations that could replace the loss incurred from the birth. In other words, there would be a longer time for re-accumulation to occur. Women who begin with comparable perfluoroalkyl concentrations and equal parity may have different perfluoroalkyl concentrations at their next birth based on the time elapsed between births (which includes the time required to become pregnant). Olsen et al. surmised if all else is equal, those women with longer TTP will have longer intervals of time between births and so may have higher perfluoroalkyl levels prior to the next pregnancy. This would result in an association between perfluoroalkyl concentrations and TTP but the direction of the causality would be backwards (*i.e.*, reverse causation).

Whitworth et al. (2012) elaborated upon this reverse causation hypothesis in a case-control study of women who originated from the Norwegian Mother and Child Cohort (MoBa) Study. Women were restricted to those who delivered a live-born child and provided a plasma

sample around 17 weeks of gestation. Subfecund cases (n = 416) were defined as TTP > 12 months. Controls (n = 494) were defined as TTP ≤ 12 months. Median PFOA concentrations were 2 ng/mL for both subfecund cases and controls. Whitworth et al. stratified their results by parity (nulliparous vs. parous). Parity was not considered a potential confounder because it is influenced by a woman's underlying fecundability. Among parous women, the interval between the 2 most recent pregnancies, the number of previous pregnancies, and the duration of breast-feeding were examined for their influence on measured levels of PFOA.

Among parous women, Whitworth et al. (2012) reported odds ratios for TTP of similar magnitude as Fei et al. for PFOA (Table 1). However, among nulliparous women, they reported odds ratios for TTP below null and the trend appeared to decrease with increasing PFOA concentrations. Whitworth et al. concluded that due to the pharmacokinetics of perfluoroalkyls during pregnancy, delivery, and lactation, associations between PFOA and subfecundity may be produced when a causal association does not exist. They recommended studying nulliparous women regarding the potential reproductive toxicity of perfluoroalkyls.

Because PFOA was not measured at the beginning of the time to pregnancy interval but after a pregnancy had been achieved, Fei et al. (2012) acknowledged in a commentary that TTP could have potentially influenced the measurement of PFOA in their original data (Fei et al. 2009). Fei et al. (2012) then reanalyzed their data by stratifying on parity and concluded there was limited evidence for reverse causation as an explanation for their results. As shown in the Table 1, upon stratification by parity, Fei et al. (2012) found the odds ratios for infertility or fecundability attenuated to the null in the nulliparous women. This suggests reverse causation.

In a third analysis of the DNBC data, Bach et al. (2015) analyzed a second (new) participant subset of the DNBC that differed somewhat in methodology, including covariates, from the original study as published by Fei et al. (2009). In this second subset, there were 65% fewer subjects (n = 440) than the original study described above. Median PFOA serum concentration was slightly less (4.0 ng/mL). For PFOA, a similar association was observed by Bach et al. among parous but not nulliparous as shown by the first subset reanalysis by Fei et al. (2012), which was similar to the reanalysis of the first DNBC subset data reported by Fei et al. (2012). See Table 1 for the Bach et al. results. This continued to suggest a "reverse causation" argument.

Other studies have been published including relatively small prospective cohort studies by Vestergaard et al. (2012) and (Buck Louis et al. 2013). Neither have shown an association between TTP and PFOA. Nor have associations been reported between TTP and PFOA by Jørgensen et al. (2014).

Vélez et al. (Vélez et al. 2015) recently reported on a data set from the Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) cohort. Information on TTP and maternal blood concentrations was collected during the first trimester of pregnancy (6 to < 14 weeks) of the current pregnancy. The median PFOA concentration was 1.7 ng/mL. A total of 1,625 subjects were included in this analysis. Concentrations were log-transformed and divided by their SDs. The adjusted odds ratio for infertility (TTP > 12 months or infertility treatment)

for PFOA was 1.31 (Table 1). Adjusted fecundability odds ratio was 0.89. However, unlike all published studies before them (except Fei et al. 2009), Vélez et al. chose not to conduct analyses stratified by parity (nulliparous vs. parous) because, in their opinion, their hypothesized causal model suggested to do so would condition on a collider (the previous time to pregnancy which they considered to be a proxy for parity). Conditioning on parity would result in collider-stratification bias according to them. However, the Velez et al. model did not acknowledge that the timing of the measurements of PFOA occurs after the conception (not before) and therefore TTP may indeed influence PFOA measurements among parous women.

In summary, women with longer TTP will have longer intervals of time between given births and therefore may re-accumulate higher PFOA levels prior to the next pregnancy compared to women with shorter TTP. This would result in longer TTP measurements associated with higher PFOA levels, but the direction of the causality would be backwards; it would be the longer time between births (including the TTP) that resulted in higher PFOA concentrations.

Other papers of interest that need to be reviewed under **3.2.2.5 Reproduction** Effects include the following:

Darrow LA, Howards PP, Winquist A, Steenland K. 2014. PFOA and PFOS serum levels and miscarriage risk. Epidemiology 25:505-514.

3.2.2.6 Developmental Effects

Pages 195 - 210. *Human Exposure Studies*

The ATSDR document discusses some epidemiologic studies that associated measured perfluoroalkyls maternal (or cord blood) concentrations with fetal growth, in particular, birth weight, in the general population. The ATSDR document, however does not provide the necessary insight to comprehend this literature related to lower birth weight and PFASs. The following comments are provided to offer this insight related to perfluoroalkyl compounds and fetal growth in the general population.

A set of 4 papers was published in *Environmental Health Perspectives* in October 2014 that provided a "comprehensive and transparent assessment on the nonhuman mammalian and human evidence of whether fetal growth, in particular birth weight at term, was inversely associated with exposure to PFOA or its salts" (Johnson et al. 2014; Koustas et al. 2014; Lam et al. 2014; Woodruff and Sutton 2014). These investigators used the Navigation Guide methodology to conduct a rigorous approach to research synthesis that has been developed to reduce bias and maximize transparency in the evaluation of environmental health information (Woodruff and Sutton 2014). The evaluation process involved three steps: 1) specify the study question; 2) select the evidence; and 3) rate the quality and strength of the evidence according to consistent criteria, and performing appropriate statistical analyses (*e.g*., meta-analyses). For each systematic review of the nonhuman mammalian and human data, the strength of evidence was defined as either sufficient, limited, inadequate, or lack of evidence of toxicity. Integration of each separate rating for nonhuman and human data resulted in an overall final strength of evidence rating.

Koustas et al. (2014) addressed the question of whether PFOA or its salts affected fetal growth in animals. They initially reviewed 21 toxicology studies relevant to the question. They determined only a subset of the data, 8 mouse gavage data sets from 7 studies, could be combined for their meta-analysis of birth weight in relation to PFOA doses administered. Only the low PFOA doses were considered in their meta-analysis in order to minimize adverse impacts from higher administered doses in these studies. The mouse species was chosen, as compared to the rat, due to its longer half-life of PFOA and pharmacokinetic differences between the sexes. The meta-analysis estimate of PFOA calculated from these 8 data sets was a change in mean pup birth weight of -0.023 g (95% CI -0.029, 0.016) per 1-unit increase in dose (mg/kg body weight per day). Koustas et al. (2014) summarized the strength of evidence across the 8 mouse gavage data sets as "sufficient evidence of toxicity" based on their a priori definition of 'one or more well-designed, well-conducted studies' and the conclusion is unlikely to be strongly affected by findings from future studies.

Similarly, Johnson et al. (2014) reported the systematic review of the human evidence by identifying 18 epidemiologic studies of which 9 data sets were considered combinable in a meta-analysis for birth weight and PFOA exposure. These 9 data sets represented 4,149 births. Reviewing each study for risk of bias (recruitment strategy. blinding, exposure assessment, confounding, incomplete outcome data, selective outcome reporting, conflict of interest, and other bias), Johnson et al. rated the quality of evidence across the studies as 'moderate.' Their meta-analysis of these 9 data sets reported an estimate of -18.9 grams (95% CI -29.8, -7.9) birth

weight per ng/mL increase in serum or plasma PFOA (see Figure 7). Johnson et al. summarized the strength of human evidence as 'sufficient evidence of toxicity' based on a reduction in birth weight associated with PFOA exposure and that chance, bias and confounding were ruled out with reasonable confidence.

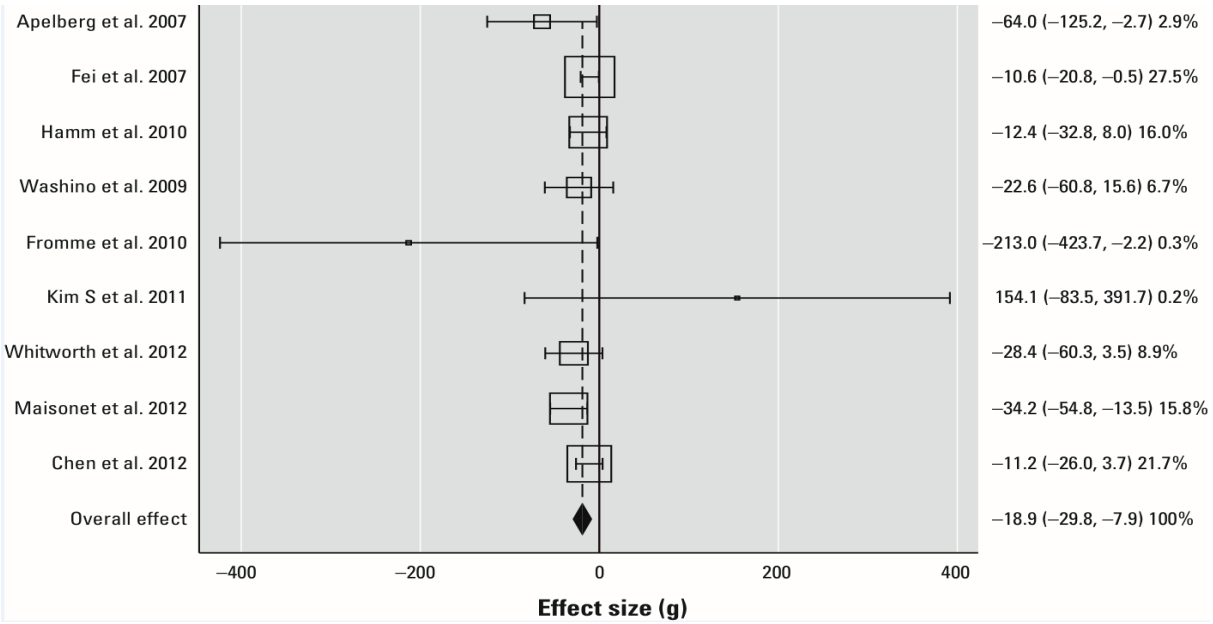


Figure 7 – from Johnson et al. 2014 Environ Health Perspect 122 1028-1039
Results of meta-analysis for birth weight (n = 9 studies, 4,149 births) shown as effect estimates [change in birth weight in grams per nanogram of PFOA per milliliter of serum or plasma (95% CIs)]. The percentages are weightings of the individual studies in the meta-analysis according to the inverse of the variance, and the sizes of the boxes are scaled accordingly. The dashed line indicates the overall effect estimate derived from the meta-analysis, and the diamond indicates the 95% CI of the overall effect estimate.

Lam et al. (2014) (same group of authors as Johnson et al. and Koustas et al.) subsequently integrated the strength of the nonhuman mammalian and human ratings and reached the conclusion that PFOA is 'known to be toxic' to human reproduction and development based on sufficient evidence of decreased fetal growth in both nonhuman mammalian and human species.

However, none of 9 epidemiologic studies included in the meta-analysis by Johnson et al. (2014) considered the potential confounding that could arise from the glomerular filtration rate (GFR). The maternal GFR increases within one month of conception (Helal et al. 2012) with maternal GFR and renal blood flow increasing by 40 – 65% and 50 - 85%, respectively, during a normal pregnancy. Whitworth et al. (2012) suggested that, because GFR is diminished in lower weight infants, this could lead to less renal elimination of PFOA; thus raising the question whether the epidemiologic studies that assessed a relationship between birth weight and PFOA were confounded by not adjusting for GFR.

While acknowledging the above hypothesis by Whitworth et al. (2012), Lam et al. (2014) believed their overall conclusion was not undermined for two reasons: 1) it was not relevant to the nonhuman mammalian data; and 2) their systematic review of the literature for this relationship between birth weight and maternal glomerular filtration rate did not suggest sufficient evidence to support this hypothesis. However, unlike their meta-analysis on PFOA and birth weight (Johnson et al. 2014), Lam et al. did not provide a systematic review in their paper as to how they reached their conclusion of a lack of an association between GFR and birth weight. Such a review by this set of authors was published a few months later by Vesterinen et al. (2015)

In their review, Vesterinen et al. (2015) presented three relationships to consider in assessing fetal growth: 1) fetal growth and GFR; 2) fetal growth and plasma volume expansion (PVE); and 3) PVE and GFR. (See Figure 1 in the Supplement to the Vesterinen et al. paper.) They examined 35 studies through the same Navigation Guide methodology. Vesterinen et al. found consistent evidence of an association among studies reporting the relationship between birth weight and PVE but they found the studies between GFR and birth weight were inconsistent and the majority had small sample sizes (range 9 to 283). They also had low confidence in the studies that examined the relationship between PVE and GFR. Vesterinen et al. concluded "the strength of the evidence of an association between fetal growth and GFR was not classifiable based on the low quality and indeterminate direction of effect of human studies and the small number and size of non-human mammalian studies which were of low quality with indeterminate direction of effect." Nevertheless, Vesterinen et al. acknowledged "A well-conducted observational human study could increase our confidence in the strength of the association" and because "the review process involved judgments, a different group of researchers *at a different time* might reach a different conclusion." (italics added).

Several months later, Verner et al. (2015), who had the distinct advantage of having one additional critically-important paper (Morken et al. 2014) not available to Lam et al. (*i.e.*, not yet published), concluded "there is reason to believe a true association exists between maternal GFR during pregnancy and birth weight." Morken et al. examined a sub-cohort of 953 women (470 women with and 483 women without preeclampsia) in the Norwegian Mother and Child Cohort (MoBa) study. The sample size represented 29 more subjects than the combined total (n = 924) from the 13 small sample studies that were available to Lam et al. at the time of their review (as discussed above). Morken et al. found a statistically significant association between maternal GFR in the second trimester and infant birth weight with using two different GFR formulas in the total cohort, but not with a third estimated GFR formula. The inclusion of women with preeclampsia in this study increased the study power because it increased the proportion of small-for-gestational age infants in the analysis. In a different analysis, Morken et al. analyzed the 953 women in a model of birth weight in relationship to the concentration of PFOA that was measured in these subjects' serum. Adjustment for GFR attenuated the PFOA coefficient by 66%.

Upon their review of the literature, and concluding there was likely a true association between maternal GFR and birth weight, Verner et al. (2015) modified an existing physiologically based pharmacokinetic model (PBPK) of pregnancy and lactation and PFOA (Loccisano et al. 2012; Loccisano et al. 2013) to address how much of the PFOA and birth

weight association might be attributable to GFR. They compared simulated estimates from their PBPK model to those from their meta-analysis of 7 epidemiologic studies (all included in the meta-analysis by Johnson et al.) See Figure 8 below.

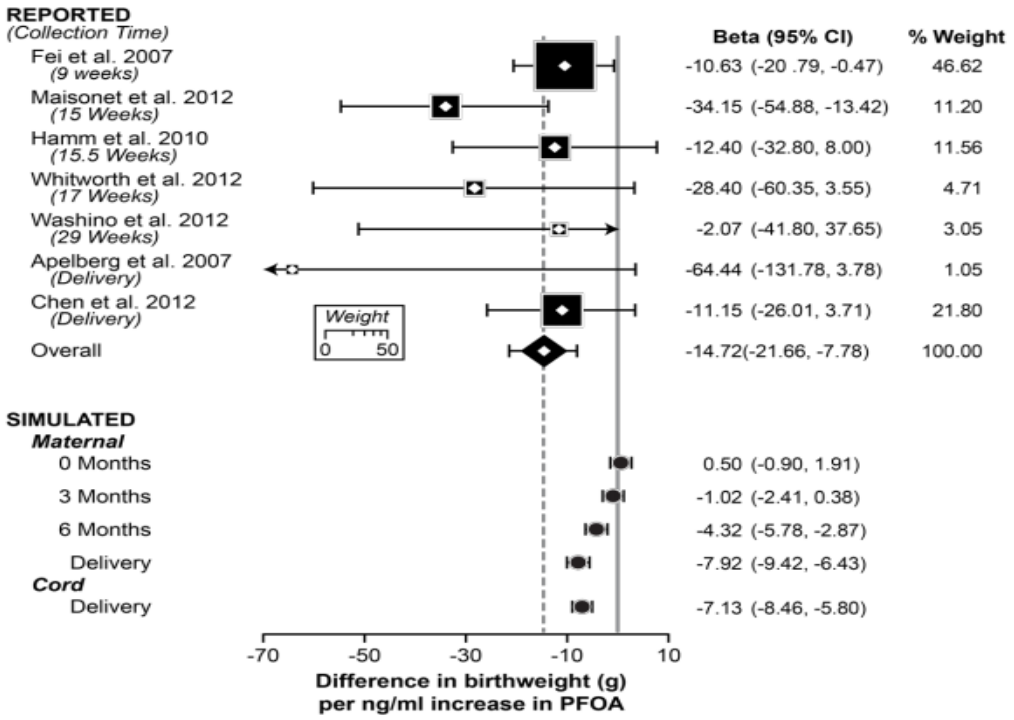


Figure 8 – from Verner et al. 2015, Environ Health Perspect DOI: 10.1289/ehp.1408837.
Difference in birth weight (g) per 1 ng/mL increase in Reported (meta-analysis) and Simulated Model PFOA levels. The Simulated Model showed a -7.92 g birth weight per ng/mL maternal PFOA and -7.13 g birth weight per ng/mL cord plasma PFOA. The size of the square represents the weight of each study in the calculation of the overall meta-analytic association.

Using Monte Carlo and sensitivity analyses, Verner et al. reported the association between maternal plasma levels (per 1 ng/mL increase) and birth weight only appeared after the first trimester (see Figure 8, Simulated Model). The association was strongest at birth. The association between simulated PFOA levels (per 1 ng/mL increase) and birth weight was comparable for maternal plasma at term (-7.9 g birth weight (95% CI -9.4, -6.4)) and cord plasma (-7.1 g birth weight (95% CI -8.5, -5.8)). Their meta-analysis of the 7 epidemiologic studies (see Figure 8, Reported Model) found a summary meta-analysis coefficient of -14.7 g (95% CI -21.7, -7.8) birth weight for each 1 ng/mL increase of PFOA (as compared to the -18.8 g for 1 ng/mL increase in PFOA as reported by Johnson et al. as illustrated in Figure 7). Verner et al. concluded a substantial proportion of the association between maternal PFOA and birth weight may be attributable to confounding by GFR. Also, Verner et al. concluded epidemiologic studies that measured PFOA early in pregnancy may have been less confounded by GFR than those who measured PFOA late in pregnancy.

Shifting emphasis to PFOS (not PFOA), based on a qualitative literature review of PFOS and birth weight, Bach et al. (2015) identified 8 epidemiologic studies (Apelberg et al. 2007;

Chen et al. 2012; Darrow et al. 2013; Fei et al. 2007; Hamm et al. 2010; Inoue et al. 2004; Maisonet et al. 2012; Washino et al. 2009) that examined PFOS as a continuous variable. All eight studies were from general populations. Six of these studies reported an association between PFOS and lower birth weight (Apelberg et al. 2007, Chen et al. 20120, Darrow et al. 2013, Fei et al. 2007, Maisonet et al. 2012, Washino et al 2009) but only three (Washino et al. 2009, Chen et al 2012, Maisonet et al. 2012) were statistically significant. Bach et al. concluded PFOS exposure was associated with decreased average birth weight but the impact on public health was not clear. Bach et al. did not conduct a meta-analysis.

A meta-analysis on prenatal PFOS and birth weight was subsequently performed by Verner et al. (2015) (see Figure 9, Reported Model). They included the same above mentioned studies, except Darrow et al. (2013) and Inoue et al. (2004) but included Whitworth et al. (2012) which Bach et al. (2015) did not. Verner et al. reported a reduced association with birth weight in six of these seven studies. The summary meta-analysis estimate for the seven studies was -5.0 g (95% CI -8.9, -1.1) birth weight per ng/mL increase in PFOS

However, none of these epidemiologic studies cited above considered the potential confounding that could arise from the glomerular filtration rate (GFR). The maternal GFR increases within one month of conception (Helal et al. 2012) with maternal GFR and renal blood flow increasing by 40 – 65% and 50 - 85%, respectively, during a normal pregnancy. Whitworth et al. (2012) suggested that, because GFR is diminished in lower weight infants, this could lead to less renal elimination of PFOS; thus raising the question whether the epidemiologic studies that assessed a relationship between birth weight and PFOS were confounded by not considering for GFR.

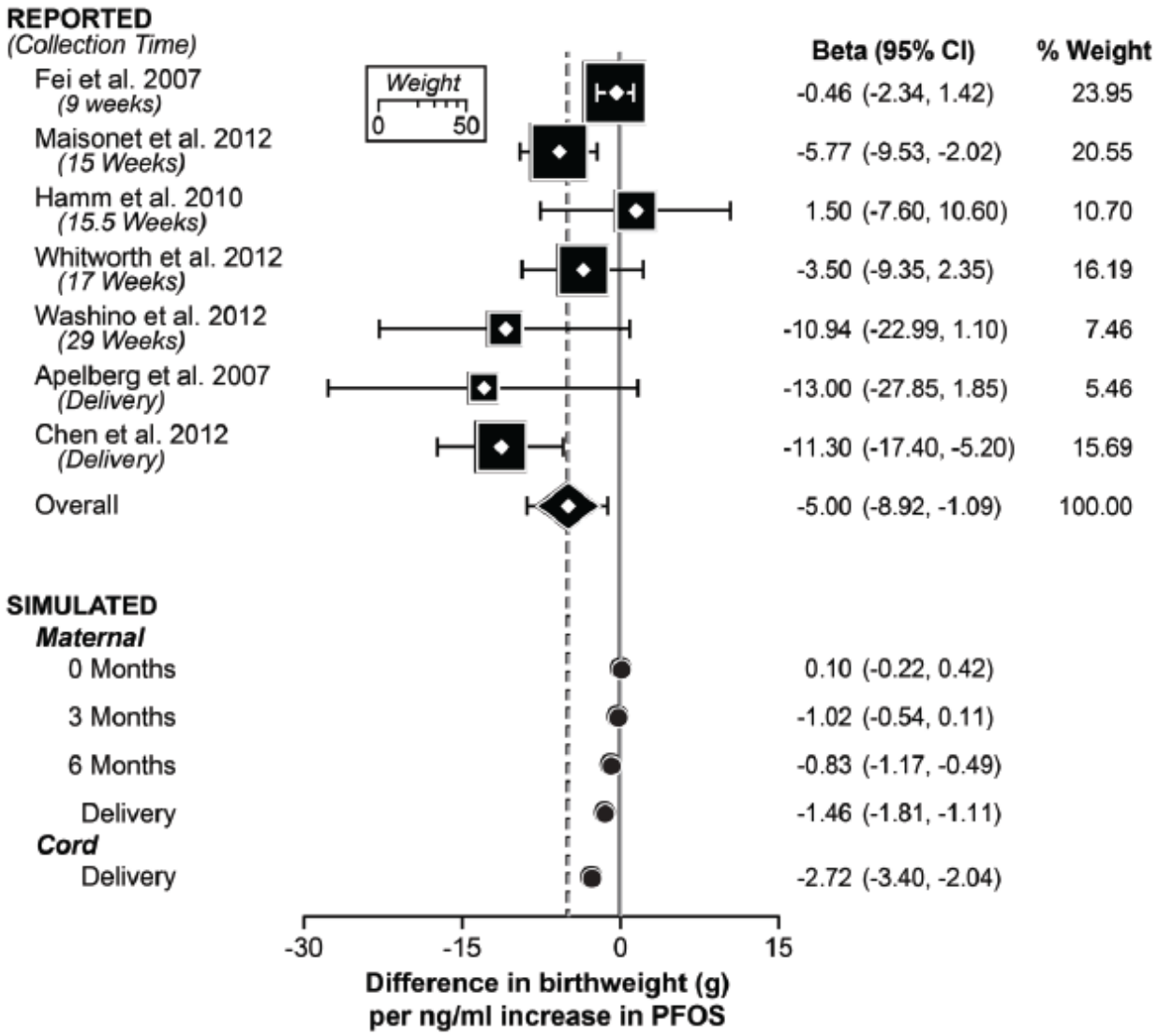


Figure 9 - from Verner et al. 2015, Environ Health Perspect DOI: 10.1289/ehp.1408837.
Difference in birth weight (g) per 1 ng/mL increase in reported and simulated PFOS levels. Simulated model provided the overall maternal (-1.46 g) and cord (-2.72 g) per ng/mL PFOS. The size of the square represents the weight of each study in the calculation of the overall meta-analytic association.

Upon their review of the literature and concluding there was likely a true association between maternal GFR and birth weight (see above discussion), Verner et al. (2015) then modified an existing physiologically based pharmacokinetic model (PBPK) of pregnancy and lactation and PFOS (Loccisano et al. 2012; Loccisano et al. 2013) to address how much of the PFOS-birth weight association might be attributable to GFR. They compared simulated estimates from their PBPK model to those from their meta-analysis of 7 epidemiologic studies (see Figure 9, Reported Model). Using Monte Carlo and sensitivity analyses, Verner et al. reported the association between maternal plasma levels (per 1 ng/mL increase) and birth weight was strongest at birth as the association between simulated cord plasma levels and birth weight was -2.7 g (95% CI -3.4, -2.0) per ng/mL increase PFOS compared to 1.5 g (95% CI -1.8, -1.1) based on maternal plasma levels (see Figure 9, Simulated Model). Verner et al. concluded a substantial proportion of the association between prenatal PFOS and birth weight may be attributable to confounding by GFR. Also, Verner et al. concluded epidemiologic studies that measured PFOS early in pregnancy may have been less confounded by GFR than those who measured PFOS late in pregnancy.

In summary, epidemiological associations between maternal/cord blood measurements of PFOA or PFOS and birth weight are confounded by GFR.

Additional papers not cited by ATSDR regarding fetal growth include:

Bach CC, Bech BH, Nor EA, et al. 2015. Perfluoroalkyl acids in maternal serum and incides of fetal growth: The Aarhus birth cohort. Environ Health Perspect doi:10.1289/EHP.1510046.

Lee ES, Han S, Oh JE. 2015. Association between perfluoroinated compound concentrations in cord serum and birth weight using multiple regression models. Reprod Toxicol 2015;doi:10.1016/j.reportox.2015.10.020.

Lenters V, Portengen L, Rignell-Hydobom A, et al. 2015. Pernatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environ Health Perspect doi:10.1289/ehp.1408933.

Kishi R, Hakajima T, Goudarzi H, et al. 2015 The association of prenatal exposure to perfluoroinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: The Hokkaio Study. Environ Health Perspect 123:1038-1045.

Page 209. Paragraph related to birth outcome (birth defects).

Using the C8 Health Project as the measurement for serum PFOA (2005-2006), Stein et al. examined the measured serum PFOA concentrations for related major birth defects (n = 325) among 10,262 live singleton or multiple births prior to this time period (1990 – 2006). They reported no associations with the possible exception of brain defects but the number of cases were small (n =13) which yielded an odds ratio adjusted for year of conception of 2.6 (95% CI 1.3 – 5.1) for an increase in the interquartile range. Liew et al. (2014) conducted a case-cohort study of congenital cerebral palsy in children identified form the Danish National Birth Cohort. Six PFAS compounds were measured during the early part of the maternal second trimester, including PFOA and PFOS. They reported a dose response association for PFOA and PFOS with cerebral palsy but in male children only. The risk ratio was 1.7 (95% CI 1.0 – 28) for PFOS and 2.1 (95% CI 1.2-36) for PFOA per a 1-unit (natural-log ng/ml) increase.

Pages 211 – 212. Paragraph related to the discussion on neurodevelopment

The Gump et al. study should not be presented (see top of page 212) between the findings from the Stein and Savitz (2011) and Stein et al. (2013) studies. The latter two studies are part of the C8 Health Studies. The Gump et al. study should be presented separately so that this distinction is understood by the reader.

There are at least 7 papers not cited or discussed in this paragraph on childhood neurodevelopment. Stein et al. (2013a) recruited 326 children, aged 6 – 12 from the C8 Health Project for a survey, to review mother and teacher reports of the child's behavior in school. Aggregate findings did not suggest adverse effects of PFOA on behavior. Findings were not consistent between mothers' and teachers' reports or by sex of the child. Stein et al. (2013b) also examined the childrens' Intelligence Quotient (IQ), reading and math skills, language, memory and learning, visual-spatial processing, and attention. Children in the highest vs. lowest quartile of estimated *in utero* PFOA exposure had an increase in IQ and decrease in characteristics of attention deficit/hyperactivity disorder. There were negligible associations between estimated in utero PFOA exposure and reading and math skills or neuropsychological function. Høyer et al. examined child motor development and behavior 8 to 10 years after follow-up in a maternal cohort of 1,106 mother-child pairs from Greenland, Ukraine or Poland. They reported a significant increase in hyperactivity (odds ratio 3.1) for children prenatally exposed to the highest PFOA tertile compared to the lowest tertile. A nonsignificant association was observed with PFOS. Ode et al. (2014) conducted a case-control study of 206 Swedish children with/without ADHD. PFOA and PFOS concentrations were at general population levels. There was no support for an association between fetal exposure to PFOS, PFOA, or PFNA (perfluorononanoate) and ADHD. Liew et al. (2015) randomly selected 220 cases of ADHD and childhood autism from the Danish National Birth Cohort study and compared them to 550

controls frequency matched by the child's sex. The study found no consistent evidence to suggest that prenatal PFAS exposure increased the risk of ADHD or childhood autism in children. Strøm et al. (2014) reported on the offspring diagnoses of various neurodevelopmental outcomes from a cohort of 965 women who had their maternal serum samples measured for PFOA and PFOS at approximately 30 weeks of gestation. Behaviors measured included ADHD, depression, and scholastic achievement. They found no association for maternal levels of PFOA or PFOS with offspring behavioural disorders or scholastic achievement. Chen et al. examined 239 mother-infant pairs from the Taiwan Birth Panel study. Serum concentrations of PFOA and PFOS were measured at mean 2.5 and 7.0 ng/mL, respectively (i.e., general population levels). Comprehensive Developmental Inventory for Infants and Toddlers was used by specially trained physical therapists to assess children's neurodevelopment at 2 years of age. There were five domains to this test:cognitive, language, motor, social, and self-help. An adverse dose response association was observed in the gross-motor subdomain for PFOS but not PFOA.

Page 212. Paragraph related to the discussion on the development of the reproductive system

Based on the cross-sectional C8 Health Project data obtained in 2005-2006, Lopez-Espinosa et al. (2011) categorized 3,067 boys and 2,931 girls aged 8 – 18 years as whether they had reached puberty based on sex steroid hormone levels or onset of menarche. Using total testosterone (> 50 ng/dL) or free testosterone (> 5 ng/dL) in boys and self-reported menarche and estradiol >20 pg/mL in girls as markers of puberty, PFOA (girls only) was associated with median delays of three to six months based on quartile analyses. The authors acknowledged that clearance may have an explanatory role, as an earlier menarche would result in behavioral and physiologic changes, including menstrual blood loss, which may result in lower perfluoroalkyl levels. The delayed menarche association reported by Lopez-Espinosa et al. was inconsistently reported in two longitudinal studies (Christensen et al. 2011; Kristensen et al. 2013). Christensen et al. (2011) conducted a nested case-control study within a cohort of approximately 14,000 pregnant women in 1991-1992. Cases were defined as female offspring who self-reported early menarche before 11.5 years (n = 218) with a median PFOA concentration of 3.9 ng/mL compared to 3.6 ng/mL amongst the controls (menarche after 11.5 years (n = 230)). The adjusted odds ratios for a natural log transformed unit of PFOA was 1.01 (95% CI 0.61 – 1.68) for an age at menarche having occurred at less than 11.5 years of age. Kristensen et al. (2013) examined the recalled age of menarche among 343 daughters aged 20 years whose mothers had an archived blood sample measured while at pregnancy week 30. Mean age at menarche was 13.2 years, median maternal PFOA was 3.6 ng/mL. Daughters exposed to PFOA *in utero* had a 5.3 months (95% CI 1.3 – 9.3) later age of self-reported menarche among the highest exposed group (maternal PFOA level 4.4 – 19.8 ng/mL) compared to the referent group (0.1 – 3.0 ng/mL PFOA maternal level).

A Monte Carlo PBPK simulation model was developed that incorporated significant points of pubertal development that included growth spurts and menarche (Wu et al. 2015). The model included compartments for plasma, gut, liver, fat, rest of body, kidney, filtrate, and storage. Tissue volumes and tissue blood flow rates were estimated based on body weight, body

height, body surface area, and body mass index. Daily exposure to PFOA in plasma was from several sources but it was only drinking water and absorbed into gut for PFOA (per the mid-Ohio river population studied by Lopes-Espinosa et al. 2011). PFOA concentrations were simulated for a distribution of individuals 2 to 20 years of age with similar physiologic characteristics as those reported by Lopez-Espinosa et al. Models of growth were based on simulated population matches of the 5^{th}, 50^{th}, and 95^{th} percentiles of the NHANES 2003-2004 data. Monte Carlo simulations showed the distribution of serum PFOA concentrations to be very similar between the PBPK model and the Lopez-Espinosa et al. study population. The delay in menarche in days per natural log of PFOA was approximately one-third that reported in the Lopez-Espinosa et al. paper (Table 12).

Table 12. Comparison of association between plasma PFOA concentrations and age at menarche in simulated (Wu et al. 2014) and observed (Lopez-Espinosa et al. 2011) girls.

Exposure	Simulated Wu et al. study			Lopez-Espinosa study		
	OR	95% CI	Delay (days)	OR	95% CI	Delay (days)
PFOA-Q2	0.95	0.88 – 1.02	12	0.54	0.35 – 0.84	142
PFOA-Q3	0.91	0.84 – 0.98	18	0.5	0l32 – 0.77	163
PFOA-Q4	0.82	0.76 – 0.88	48	0.57	0.38 – 0.89	130
LnPFOA	0.94	0.92 – 0.96	15	0.83	0.83 – 0.95	42

In summary, the association between serum PFOA concentrations and delayed age at menarche may be due, in part, to dilution (through growth of adolescents) and excretion (via menstruation).

3.2.2.7 Cancer

The ATSDR draft profile did not cite or discuss the study by Ducatman et al. (2015). Using C8 Health Project data, Ducatman et al. examined prostate-specific antigen (PFA) and PFAS concentrations. No PFAS concentrations measured in this study provided consistent evidence of an association with clinically significant increases in PSA across age groups. The ratio of geometric means of PFOA for men age 20 – 49 (n = 9169 with PSA values >= 4.0 versus < 4.0 was1.15 (95CI 0.67 – 1.98). This ratio in men 50 – 69 years of age (n = 3819) was 0.96 (95 CI 0.77 – 1.20). Ducatman concluded this evidence did not suggest PFAS exposure (including PFOA) is associated with findings from PSA tests.

3.4.2 Distribution

Page 253. Maternal-fetal transfer. Additional studies with small sample sizes that should be included in Table 3-11 include the following:

Zhang T, Sun H, Lin Y, et al. 2013. Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environ Sci Technol 47:7974-7981.

Yang L, Wang Z, Shi Y et al. 2015 Human placental transfer of perfluoroalkyl acid precursors: Levels and profiles in paired maternal and cord serum. Chemosphere 144:1631-1638.

3.4.4 Elimination and Excretion

Page 269. Elimination of perfluoroalkyls in humans.

There are four important papers that are not cited in this section. All four papers should be part of this discussion. Bartell (2012) and Russell (2015) essentially discuss the same topic - bias in calculation of the serum elimination rate in humans in the presence of background exposures. Zhang et al. (2013) estimated the biological half-life of perfluoroalkyl acids by using urine concentrations and calculated it by specific isomers. Beesoon and Martin (2015) discussed why greater binding affinity of the linear isomer resulted in its slower clearance for both PFOS and PFOA. Also, this isomer-specific binding affinity may explain why PFOS has a longer biological half-life than PFOA. References for these four papers are the following:

Bartell SM. Bias in half-life estimates using log concentration regression in the presence of background exposures, and potential solutions. J Expos Sci Environ Epidemiol 2012;22:299-303.

Russell MH, Waterland RL, Wong F. 2015. Calculation of chemical elimination half-life from blood with an ongoing exposure source: The example of perfluorooctanoic acid. Chemosphere 129:210-216.

Zhang Y, Beesoon S, Zhu L, Martin JW. 2013. Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environ Sci Technol 47:10619-10627.

Beesoon S, Martin J. 2015.. Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. Environ Sci Technol 49:5722-5731.

Page 274. Last paragraph. The Olsen et al. (2009) reference is about PFBuS. As this paragraph is currently written, it appears this sentence concerns PFBA. This sentence should read, "Olsen et al. (2009) estimated serum $t_{1/2}$ for PFBuS in six fluorochemical workers."

3.4.5 Physiologically Based Pharmacokinetic (PBPK/Pharmacodynamic (PD) Models

There are two important PBPK models that are not cited or discussed in this section. They were briefly reviewed in the comments provided for the **3.2.2.6 section** of this ATSDR document. These two models need to be thoroughly discussed in this **3.4.5 section**. Both are adapted models of the Loccisano et al. (2011, 2013) human models discussed in the **3.4.5.2 section**. These citations are:

Verner MA, Loccisano AE, Morken NH, et al. 2015. Associations of perfluoroalkyl substances (PFASs) with lower birth weight: An evaluation of potential confounding by glomerular filtration
rate using a physiologically based pharmacokinetic model (PBPK). Environ Health Perspect doi: 10.1289/ehp.1408837

Wu H,Yoon M,Verner MA, et al. 2015. Can the observed association between serum perfluoroalkyl
subtances and delayedmenarche be explained on the basis of puberty-related changes in physiology
and pharmacokinetics. Environ Int 82, 61-68.

3.6 Toxicities Mediated Through the Neuroendocrine Axis. This section is quite superficial, and has been covered in much more detail, as written, in Section 3.2. Unless the ATSDR report authors decide to greatly expand this section to cover parts of Section 3.2, the recommendation is that **section 3.6** be deleted.

3.7 Children's Susceptibilities

Page 309. Last sentence of last paragraph. As commented above, the PBPK model by Wu et al. (2015) showed, via simulations, that growth dilution and menstruation may result in approximately 1/3 of the delayed menarche association reported in the Lopez-Epinosa et al. (2011) study. Please read the written comments on **section 3.2.2.6.**

Page 310. Second paragraph. Comments are provided concerning this ATSDR draft document which have discussed that the probability the lower birth weight association with perfluoroalklys reported in multiple epidemiologic studies is due, in part, to confounding by the glomerular filtration rate. Please read the written comments on **section 3.2.2.6.**

3.8 Biomarkers of Exposure and Effect

This section should refer the reader to **section 7.** Analytical Methods.

3.10 Populations That Are Unusually Susceptible

Extensive comments are provided concerning this ATSDR draft profile regarding cholesterol and cardiovascular effects in **section 3.2.** These comments need to be reemphasized for **section 3.10** given the perfunctory comments provided in this section of the draft profile. Bottom line: there is not an apparent cardiovascular risk based on a weight of evidence review for cardiovascular disease in either occupational populations, an affected community population, or general populations. The ATSDR draft report has separated the occupational data (inhalation exposure) from the community data (oral exposure). This lack of synthesis of both sets of data is a shortcoming to this review.

The ATSDR document suggests the cholesterol association, as viewed by the report's authors, may result in an increased risk to the population susceptible to cardiovascular disease. First, the preceding comments that were provided regarding an association between perfluoroalkyls and serum cholesterol do not suggest a strong association. They do suggest the possibility of a saturated transporter in common between perfluoroalkyls and cholesterol and that much more research is necessary in this area as it has been done for renal transporters (organic anion transports).

The only strong evidence of an effects with serum lipids and PFOA comes from a phase 1 clinical trial that orally administered APFO (ammonium perfluorooctanoate) to the subjects and resulted in their lowered serum cholesterol, as it does in experimental studies in animals, at sufficiently high concentrations. Second, is there any increased risk in cardiovascular disease observed in the epidemiology studies among the higher PFOA exposed populations? The answer appears to be "No". This need to be acknowledged in this section if the ATSDR profile is going to conjecture a susceptible population is at increased for cardiovascular risk (coronary artery disease, stroke, hypertension) due to their perfluoroalkyl exposure (increased lipids) Whether it was an occupational cohort mortality study of the DuPont Washington Works plant that used PFOA as a processing aid in the polymerization of tetrafluoroethylene (Table 13), a disease incidence cohort study of the same DuPont population (Table 14), or a cohort study of the mid-Ohio river valley community whose water contained PFOA from the environmental emissions of the DuPont plant (Tables 15 and 16), increased risks have not been reported for coronary artery disease, stroke, and hypertension. Furthermore, Raleigh et al. (2014) examined the 3M Cottage Grove cohort that actually manufactured PFOA (ammonium salt) and did not observe increased risks for heart disease or stroke (Table 17). All of the occupational studies used internal referent groups to avoid the healthy worker effect in their analyses.

Table 13. Standardized Mortality Ratios (95% confidence intervals) for ischemic heart disease and stroke. DuPont Washington Works plant. Reference = other DuPont workers in the Appalachian region. See Steenland and Woskie. (2012). Am J Epidemiol 176:909-917.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Ischemic Heart Disease				
No lag*	1.07 (0.85-1.32)	1.02 (0.80-1.28)	0.87 (0.67-1.11)	0.93 (0.86-1.09)
(N = 287 deaths)				
10 year lag**	0.95 (0.76-1.18)	1.01 (0.79-1.27)	0.93 (0.71-1.20)	0.59 (0.93-1.20)
(N = 273 deaths)				
20 year lag***	1.00 (0.79-1.24)	0.92 (0.70-1.18)	1.05 (0.79-1.37)	0.89 (0.65-1.18)
(N=243 deaths)				
Stroke				
No lag*	0.63 (0.85 (1.32)	0.78 (0.39-1.39)	1.34 (0.82-2.07)	0.69 (0.32-1.31)
(N = 50 deaths)				

10 and 20 year lags not provided

*Modeled cumulative PFOA by quartile: 0 - <ppm-years; 904 – 1520 ppm-years; 1,520 - <2700 ppm-years; >-2700 ppm-years where ppm = 1000 ng/mL

**Modeled cumulative PFOA by quartile: 0 - <798 ppm-years; 798 - < 1379 ppm-years; 1379 - <2384 ppm-years; >=2384 ppm-years

***Modeled cumulative PFOA by quartile: 0 –<515 ppm-years; 515 – 1057 ppm-years; 1057 - <1819 ppm-years; >=1819 ppm-years

Table 14. Disease-specific relative risks (95% confidence interval) from Cox regression models of a cohort incidence study of the DuPont Washington Works plant and modeled PFOA exposure. See Steenland et al. J Occup Environ Med 72:373-380

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Hypertension medications (N = 1430 cases)				
No lag	1.00	0.95 (0.81-1.11)	0.97 (0.82-1.15)	1.04 (0.87-1.25)
P value trend = 0.99				
10 year lag	1.00	0.95 (0.81-0.98)	0.91 (0.75-1.09)	0.95 (0.77-1.16)
P value trend = 0.12				
Coronary artery disease (N = 380 cases)				
No lag	1.00	1.03 (0.71-1.49)	1.23 (0.84-1.78)	1.03 (0.70-1.52)
P value trend = 0.39				
10 year lag	1.00	1.20 (0.82-1.75)	1.06 (0.71-1.58)	0.93 (0.61-1.41)
P value trend = 0.99				
Stroke (N = 108 cases)				
No lag	1.00	2.63 (1.06-6.59)	2.13 (0.83-6.44)	2.07 (0.81-5.29)
P value trend = 0.17				
10 year lag	1.00	1.48 (0.56-3.69)	1.53 (0.60-3.89)	1.33 (0.51-3.43)
P value trend = 0.22				

*Quartile cut-offs for the no lag analysis were (ng/mL-year): 3030; 6160; 11420

*Quartile cut-offs for the 10-year lag analysis were (ng/mL-year): 800; 3440; 7040

Table 15. Hazard ratios (95% confidence interval) for hypertension and coronary artery disease by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Winquist and Steenland (2014) Environ Health Perspect 122:1299-1305.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Hypertension*					
	1.00	1.10 (1.02-1.19)	1.10 (1.02-1.18)	1.05 (0.97-1.12)	0.98 (0.91-1.06)
Coronary artery disease**					
	1.00	1.26 (1.10-1.45)	1.17 (1.02-1.35)	0.99 (0.86-1.14)	1.07 (0.93-1.23)

* Modeled cumulative PFOA ng/mL per year by quintile: <111; 111- <191; 191 - <471; 471 - 2763; >=2763

**Modeled cumulative PFOA ng/mL per year by quintile: <147; 147 - <248; 248 - <717; 717 - <5058; >= 5058

Table 16. Hazard ratios (95% confidence interval) for stroke by quintiles of modeled PFOA cumulative serum exposure. Community/worker cohort of the mid-Ohio river area. See Simpson et al. (2014) Environ Res 127:22-28.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Stroke					
Retrospective model*					
(825 cases)	1.00	1.39 (1.11-1.76)	1.36 (1.08-1.71)	1.45 (1.15-1.82)	1.13 (0.90-1.44)
Prospective model**					
(252 cases)	1.00	1.07 (0.73-1.59)	1.07 (0.72-1.58)	1.18 (0.79-1.75)	0.87 (0.58-1.30)

* Modeled cumulative PFOA ng/ml per year by quintile: <178; 178 - <319; 319 - <912; 912 - <4490; >=4490

** Modeled cumulative PFOA ng/ml per year by quintile: <244; 244 - <460; 460 - <1240; 1240 - <5500; >=5500

Table 17. Hazard ratios (95% confidence intervals) for ischemic heart disease and cerebrovascular disease (stroke) by PFOA (ammonium salt) quartiles of exposures to a non-exposed referent plant population. 3M Cottage Grove manufacturing plant. See Raleigh et al. (2014). Occup Environ Med 71:500-506.

	Non PFOA Production Plant	PFOA Manufacturing Plant			
Disease	Reference*	Quartile 1 (95%CI)**	Quartile 2	Quartile 3	Quartile 4
Ischemic Heart N = 444 deaths (reference)	1.00	0.93 (0.73-1.18)	0.87 (0.66-1.13)	0.88 (0.57-1.53)	0.89 (0.66-1.21)
N = 248 deaths (PFOA plant)					
Stroke N = 112 deaths (reference)	1.00	0.57 (0.32-1.02)	0.70 (0.39-1.24)	0.93 (0.57-1.53)	0.98 (0.53-1.81)
N = 57 deaths (PFOA plant)					

*Reference: Non PFOA 3M manufacturing plant located in same Minneapolis-St. Paul metropolitan area

**Quartile cutpoints were ($\mu g/m^3$ years): 2.9×10^{-5}; 1.5×10^{-4}; 7.9×10^{-4}

In summary, it is difficult for the ATSDR report authors to suggest there is a susceptible population for cardiovascular effects when there is no increased risk observed among the highest exposed (to PFOA) populations which would contain a subset of higher risk individuals.

3.12 Adequacy of the Database

Page 320. Chronic-Duration Exposure and Cancer.

See prior comments regarding serum lipid levels and small decreases in birth weight.

There is no increased risk for prostate cancer as a consequence of PFOA exposure in the 3M Cottage Grove employee population. The results from Raleigh et al. (2014) clearly do not show a risk for prostate cancer mortality or incidence. Gilllliland and Mandel (1993) and Lundin et al. (2009) were earlier analyses of this cohort that used duration of employment at Cottage Grove or a qualitative exposure ranking as their exposures for PFOA. The much more comprehensive study done by Raleigh et al. should be the primary citation for the 3M Cottage Grove study.

The Raleigh et al. (2014) study is not cited or discussed. Raleigh et al. did not observe an an association between PFOA and kidney cancer incidence or mortality. Raleigh et al. examined the 3M Cottage Grove cohort which manufactured PFOA in near absence of exposure to tetrafluoroethylene See prior comments provided in **section 3.1.2.7**.

Page 322. Reproductive Toxicity

The ATSDR document cites the Knox et al. (2011) paper that suggested there is an association between serum PFOA and PFOS levels and earlier onset of menopause. Not cited in the ATSDR draft document is the paper by Taylor et al. that examined NHANES data and showed that early onset of menopause would likely result in higher concentrations of PFOA due to the cessation of this clearance pathway for perfluoroalkyl compounds. Thus, the association that Knox et al. reported could be due to reverse causality. In addition, Taylor et al. showed the act of artificial menopause by a hysterectomy subsequently resulted in higher concentrations of perfluoroalkyl compounds. In addition, Verner and Longecker (2015) have shown that menstrual fluid consists of blood and endometrial transudate. Both of these matrices are high in albumin (binding site for perfluoroalkyls) which would facilitate the clearance of perfluoroalkyl compounds. Development of the final ATSDR profile needs to include a review of the following two papers:

Taylor KW, Hoffman K, Thayer KA, Daniels JL.2014. Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). Environ Health Perspect 122:145-150.

Verner MA, Longnecker MP. 2015. Comment on "enhanced elimination of perfluorooctanesulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. Environ Sci Technol 49:5836-6837.

Page 323. Developmental Toxicity.

The epidemiologic association between perfluoroalkyls and lower birth weight is confounded by the glomerular filtration rate. Please see the extensive comments provided concerning the ATSDR draft profile in **section 3.2.2.6**.

Page 327. Biomarkers of Exposure and Effect

The references provided are quite outdated. Recommend ATSDR authors use the most recent CDC NHANES data. There is a clear and unmistakable downward trend for PFOA and PFOS concentrations in the general population. Serum PFOS concentration has declined 80 % in NHANES between the 1999-2000 and 2011-2012 biomonitoring surveys. Serum PFOA concentrations have declined 60 % between 1999-2000 and 2011-2012 biomonitoring surveys. See graphs provided in **section 6.5.**

3.12.3 Ongoing Studies

Given the many health effects studies that have been published in 2014 and 2015, the National Institutes of Health (NIH) RePORTER (2014) is an unreliable database to be used to reflect the extent of ongoing perfluoroalkyl epidemiology and toxicology studies.

References (Human Health)

Andersen CS, Fei C, Gamborg M, et al. 2013. Prenatal exosures to perfluorinated chemicals and anthropometry at 7 years of age. Am J Epidemiol 178:921-927.

Apelberg BJ, Goldman LR, Calafat AM, et al. 2007. Determinants of fetal exposure to polyfluoroalkyl compound in Baltimore, Maryland. Environ Sci Technol 41:3891-3897.

Bach CC, Bech BH, Brix N, et al. 2015a. Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: A systematic review. Crit Rev Toxicol 45:53-67.

Bach CC, Liew Z, Bech BH, et al. 2015b. Perfluoroalkyl acids and time to pregnancy revisited: an update from the Danish National Birth Cohort. Environ Health doi10.1186/s12940-015-0040-9.

Bach CC, Bech BH, Nor EA, et al. 2015c. Perfluoroalkyl acids in maternal serum and incides of fetal growth: The Aarhus birth cohort. Environ Health Perspect doi:10.1289/ehp.1510046.

Barry V, Darrow LA, Klein M, et al. 2014. Early life perfluorooctanoic acid (PFOA) exposure and overweight and obesity risk in adulthood in a community with elevated exposure. Environ Res 132:62-69.

Bartell SM, Calafat AM, Lyu C, et al. 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ Helath Perspect 118:222-228.

Bartell SM. 2012. Bias in half-life estimates using log concentration regression in the presence of background exposures, and potential solutions. J Expos Sci Environ Epidemiol 22:299-303.

Beesoon S, Martin J. Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. Environ Sci Technol 2015;49:5722-5731.

Benbrahim-Tallaa L, Lauby-Scretan B, Loomis D, et al. 2014. Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone. Lance Oncol 15:924-925.

Burstyn I. 2013. Country doctor versus epidemiologist. How uncertainty analysis can help see what is in plain view. Epidemiology 24:577-580.

Buck Louis GM, Sundaram R, Shisterman EF, et al. 2013. Persistent environmental pollutants and couple fecundity: the LIFE study. Environ Health Perspect 121:231-236.

Butenhoff JL, Pieterman E, Ehresman DJ, et al. 2012. Distribution of perfluorooctanesulfonate and perfluorooctanoate into human plasma lipoprotein fractions. Toxicol Lett 5:360-365.

Chang ET, Adami HO, Boffetta P, et al. 2014. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans. Crit Rev Toxicol 44: 1-81.

Chen MH, Ha EH, Wen TW, et al. 2012. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. Plos One 7:e42474.

Chen MH, Ha EH, Liao HF, et al. 2013. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology 24:800-808.

Consonni D, Straif K, Symons JM, et al. 2013. Cancer risk among tetrafluoroethylene synthesis and polymerization workers. Am J Epidemiol 178:350-358.

Costa G, Sartori S, Consonni D. 2009. Thirty years of medical surveillance in perfluorooctanoic acid production workers. J Occup Environ Med 51:364-372.

Darrow LA, Howards PP, Winquist A, Steenland K. 2014. PFOA and PFOS serum levels and miscarriage risk. Epidemiology 25:505-514.

Ducatman A, Zhang J, Fan H. 2015. Prostate-specific antigen and perfluoroalkyl acids in the C8 Health Study population. J Occup Environ Med 57:11-114.

Emmet EA, Zhang H, Shofer FS, et al. 2006. Community exposure to perfluorooctanoate:relationships between serum levels and certain health parameters. J Occup Environ Med 48:771-779.

Ericksen KT, Raaschou-Nielsen O, McLaughlin JK, et al. 2013. Association between plasma PFOA and levels and total cholesterol. In a middle-aged Danish population. Plos One. 8:e56969

Fei C, McLaughlin JK, Tarone RE, et al. 2007. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environn Health Perspect 115:1677-1682.

Fei C, Weinberg CR, Olsen J. 2012. Perfluorinated chemicals and time to pregnancy. A link based on reverse causation? Epidemiology 23:264-266.

Fisher M, Arbuckle TE, Wade M, et al. 2013. Do perfluoroalkyl substances effect metabolic function and plasma lipids? – Analysis of the 2007-2009 Canadian Health Measures Survey (CHMS) Cycle 1. Environ Res 121:95-103.

Fitz-Simon N, Fletcher T, Luster MI, et al. 2013a. Reductions in serum lipids with a 4-year decline in perfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology 24:569-576.

Fitz-Simon N, Fletcher T, Armstrong B. 2013b. Understanding uncertainties in a change verus change study. Epidemiology 24:580-581.

Fletcher T, Galloway TS, Melzer D, et al. 2013. Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. Environ Int 57-58:2-10.

Frisbee SJ, Brooks AP, Maher A, et al. 2009. The C8 Health Project: design, methods, and participants. Environ Health Perspect 117:1873-1882.

Frisbee SJ, Shankar A, Knox SS, et al. 2010. Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. Arch Pediatr Adolesc Med 164:860-869.

Fromme H, Mosch C, Morovitz M, et al. 2010. Pre- and postnatal exposure to perfluorinated compounds (PFCS). Environ Sci Technol 44:7123-7129.

Fu Y, Wang T, Fu Q, et al. 2014. Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Eco Environ Safety 106:246-252.

Gallo V, Leonardi G, Genser B, et al. 2012. Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS_ concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environ Health Perspect 120:655-660.

Galloway TS, Fletcher T, Thomas OJ, et al. 2015. PFOA and PFOS are association with reduced expresson of the parathyroid hormone 2 receptor (PTH2R) gene in women. Chemosphere 120:555-562,

Geiger SD, Xiao J, Ducatman A, et al. 2014. The association between PFOA, PFOS, and serum lipid levels in adolescents. Chemosphere 98:78-83.

Geiger SD, Xiao J, Shankar A. 2014. No association between perfluoroalkyl chemicals and hypertension in children. Int Blood Pres Control. 7:1-7.

Geiger SD, Xiao J, Shankar A. 2013. Positive association between perfluoroalkyl chemicals and hyperuricemia in children. Am J Epidemiol 177:1255-1262.

Granum B, Haug LS, Namork E, et al. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol 10:373-379.

Grice MM, Alexander BH, Hoffbeck R, et al. 2007 Self-reoprted medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. J Occup Environ Med 49:722-729.

Gump BB, Wu Q, Dumas AK, et al. 2011. Perfluorochemcial (PFC) exposure in children: associations with impaired response inhibition. Environ Sci Technol 45:8151-8159.

Halldorsson T, Rytte D, Haug LS, et al. 2012. Prenatal exposure to perflurooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ Health Perspect 120:668-673.

Hamm MP, Cherry NM, Martin JW, et al. 2010. Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol 20:589-597. Environ Health Perspect 117:660-687.

Han X, Nabb DL, Russell MH, et al. 2012. Renal elimination of perfluorocarboxylates (PFCAs). Chem Res Toxicol 25:35-46.

Høyer BB, Rmlau-Hansen CH, Obel C, et al. 2015. Pregnancy serum concentrations of perfluoinated substances and offspring behavior and motor development at age 5-9 years – a prospective study. Environ Health 14:2.

Jain RB. 2013. Association between thyroid profile and perfluoroalkl acids:data from NHANES 2007-2008. Environ Res 126:51-59.

Johnson PI, Sutton P, Atchley DS et al. 2014. The Navigation Guide – Evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environ Health Perspect 122:1028-1039.

Kerger BD, Copeland TL, DePario AP. 2011. Tenuous dose-response correlations for common disease states: case study of cholesterol and perfluorooctanoate/sulfonate (PFOA/PFOS) in the C8 Health Project. Drug Chem Toxicol 34:396-404.

Kim S, Choi K, Ji K, et al. 2011. Transplacental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol 45:7465-7472.

Kishi R, Hakajima T, Goudarzi H, et al. 2015. The association of prenatal exposure to perfluoroinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: The Hokkaio Study. Environ Health Perspect 123:1038-1045.

Klaunig JE, Babich MA, Baetcke KP, et al. 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol 33:655-780.

Knopp RH, Bergelin RO, Wahl PW, et al. 1985. Relationships of infant birth size to maternal lipoproteins, apoproteins, fuels, hormones, clinical chemistries, and body weight at 36 weeks gestation. Diabetes 34 Supp l2:71-77.

Knox SS, Jakson T, Frisbee SJ, et al. 2011. Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project. J Toxicol Sci 36:403-410.

Koustas E, Lam J, Sutton P et al. 2014. The Navigation Guide – Evidence-based medicine meets environmental health: systematic review of nonhuman evidence for PFOA effects on fetal growth.

LaKind JS, Goodman M, Naiman DQ. 2012. Use of NHANES data to link chemical exposures to chronic diseases: A cautionary tale. PLoS One 7:e51086.

Lam J, Koustas E, Sutton P et al. 2014. The Navigation Guide – Evidence-based medicine meets environmental health:integration of animal and human evidence for PFOA effects on fetal growth. Environ Health Perspect 122:1040-1051.

Lee ES, Han S, Oh JE. Association between perfluoroinated compound concentrations in cord serum and birth weight using multiple regression models. Reprod Toxicol 2015;doi:10.1016/j.reportox.2015.10.020.

Lenters V, Portengen L, Rignell-Hydobom A, et al. 2015 Pernatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environ Health Perspect doi:10.1289/ehp.1408933.

Leonard RC, Kreck mann KH, Sakr CJ, et al. 2008. Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. Ann Epidemiol 18:15-22.

Liew Z, Ritz B, Bonefield-Jørgnensen EC, et al. 2014. Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children. Am J Epidemiol 180:574-581.

Lew Z, Ritz B, von Ehrenstein OS et al. 2015. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure of perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort. Environ Health Perspect 123:367-373.

Loccisano AE, Longnecker MP, Campbell JL, et al. 2013. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. J Toxicol Environ Health A 76:25-57.

Lopez-Espinosa MJ, Mondal D, Armstrong B, et al. 2012. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ Health Perspect 120:1036-1041.

Lundin JI, Alexander BH, Olsen GW, Church TR. 2009. Ammonium perfluoroocatnoate production and occupational mortality. Epidemiology 20:921-928.

MacPherson IR, Bissett D, Petty RD et al. 2011. A first-in-human phase 1 clinical trial of CXR1002 in patients with advanced cancer. J Clin Oncol 29: (suppl; abstr 3063).

Maisonet M, Terrell ML, McGeehin MA, et al. 2012. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environ Health Perspect 120:1432-1437.

Maisonet M, Näyhä S, Lawlor DA, et al. 2015a. Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females. Environ Int 82:49-60.

Maisonet M, Calafat AM, Marcus M, et al. 2015b. Prenatal exposure to perfluoroalkyl acids and serum testosterone concentrations at 15 years of age in female ALSPAC study participants. Environ Health Perspect 2015 doi.org/10.1289/ehp.1408847.

Melzer D, Rice N, Depledge MH, et al. 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. Environ Health Perspect 118:686-692.

Morken NH, Travlos GS, Wilson RE et al. 2014 Maternal glomerular filtration rate in pregnancy and fetal size. PLoS One 9(7):e101897.

Nelson JW, Hatch EE, Webster TF. 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ Health Perspect 118:197-202.

Ode A, Källen K, Gustafsson P, et al. 2014. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLOS One. 9:e95891.

Olsen GW, Gilliland FD, Burlew MM, et al. 1998. An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. J Occup Environ Med 40:614-622.

Olsen GW, Zobel LR. 2007. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentration in fluorochemical production workers. Int Arch Occup Environ Health 81:231-246.

Olsen GW, Butenhoff JL, Zobel LR. 2009. Perfluoroalkyl chemicals and human fetal development: an epidemiologic review with clinical and toxicological perspectives. Reprod Toxicol 27:212-230.

Olsen GW, Burris JM, Burlew MM, et al. 2010. Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. Drug Chem Toxicol 23:603-620.

Olsen GW, Ehresman DJ, Buehrer BD, et al. 2012. Longitudinal assessment of lipid and hepatic clinical parameters in workers involved with the demolition of perfluoroalkyl manufacturing facilities. J Occup Environ Med 54:974-983.

Olsen GW, Ley CA. 2015. Prostate cancer and PFOA. 57:e60.

Patel CJ, Cullen MR, Ioannidis JPA, et al. 2013. Systematic evaluation of environmental factors: persistent pollutants and nutrients correlated with serum lipid levels. Int J Epidemiol 41:828-843.

Pirali B, Negri S, Chytiris S, et al. 2009. Perfluorootane sulfonate and perfluorooctanoic acid in surgical thyroid specimens of patients with thyroid diseases. Thyroid 19:1407-1412.

Raleigh KR, Alexander BH, Olsen GW, et al. 2014 Mortality and cancer incidence in ammonium perfluorooctanoate production workers. Occup Environ Med 71:500-506.

Russell MH, Waterland RL, Wong F. 2015. Calculation of chemical elimination half-life from blood with an ongoing exposure source: The example of perfluorooctanoic acid. Chemosphere 129:210-216.

Sakr CJ, Krecmann KH, Green JW, et al. 2007a. Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as a part of a general health survey in a cohort of occupationally exposed workers. J Occup Environ Med 49:1088-1096.

Sakr CJ, Leonard RC, Krecmann KH et al. 2007b. Longitudinal study of serum lipids and liver enzymes in workers with occupational exposures to ammonium perfluorooctanoate. J Occup Environ Med 49:872-879.

Savitz DA. 2007. Biomarkers of perfluorinated chemicals and birth weight. [Editorial] Environ Health Perspect 115:A528-A529.

Savitz DA, Stein CR, Bartell SM, et al. 2012a. Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposd community. Epidemiology 23:386-392.

Savitz DA, Stein CR, Elston B, et al. 2012b. Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio valley. Environ Health Perspect 120:1201-1207.

Shankar A, Xiao J, Ducatman A. 2010. Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am J Epidemiol

Shankar A, Xiao J, Ducatman A. 2011. Perfluoroalkyl chemicals and elevated serum uric acid US adults. Clin Epidemiol 3:251-258.

Shankar A, A, Xiao J, Ducatman A. 2012. Perfluoroctanoic acid and cardiovascular disease in US adults. Arch Intern Med 172:1397-1403.

Shin HM, Vieira VM, Ryan PB, et al. 2011a. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works facility in West Virginia. Environ Sci Technol 45:1435-1442.

Shin HM, Vieira VM, Ryan PB, et al. 2011b. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. Environ Health Perspect 119:1760-1765.

Shrestha S, Bloom MS, Yucel R, et al. 2015. Perfluoroalkyl substances and thyroid function in older adults. Environ Int 75:206-214.

Simpson C, Winquist A, Lally C, Steenland K. 2013. Relation between perfluorooctanoic acid exposure and strokes in a large cohort living near a chemical plant. Environ Res 127:22-28.

Skuladottir M, Ramel A, Rytter D, et al. 2015. Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environ Res doi:10.1016/j.envres.2015.09.001.

Sobus KR. DeWoskin RS, Tan YM et al. 2015. Use of NHANES biomarker data for chemical risk assessment: trends, challenges, and opportunities. Environ Health Perspect doi.10.1289/ehp.1409177,

Starling AP, Engel SM, Whitworth KW, et al. 2014a. Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child cohort study. Environ Int 62:104-112.

Starling AP, Engel SM, Richardson DB, et al. 2014b. Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child cohort study. Am J Epidemio 179:824-833.

Steenland K, Tinker S, Frisbee S, et al. 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am J Epidemiol 170:1268-1278.

Steenland K, Tinker S, Shankar A, et al. 2010. Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with uric acid among adults with elevated community exposure to PFOA. Environ Health Perspect 118:229-223.

Steenland K, Fletcher T, Savitz D. 2010. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). Environ Health Perspect 118:1100-1108.

Steenland K, Woskie S. 2012. Cohort mortality study of workers exposed to perfluorooctanoic acid. Am J Epidemiol 176:909-917.

Steenland K, Savitz DA, Fletcher T. 2014. Class action lawsuits. Can they advance epidemiologic research? Epidemiology 25:167-169.

Steenland K, Zhao L, Winquist A. 2015. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). J Occup Environ Med doi:10.1136/oemed-2014-102364.

Stein C, Savitz DA, Belling DC. 2013a. Perfluorooctanoate exposure in a highly exposed community and parent and teacher reports of behavior in 6-12 year-old children. Pediatric Perinatal Epidemiol 28:146-156.

Stein CR, Savitz DA, Bellinger DC. 2013b Perfluorooctanoate and neuropsychological outcomes in children. Epidemiol 24:590-590.

Stein CR, Savitz DA, Elston B, et al. 2014. Perfluorooatanaoate exposure and major birth defects. Reprod Toxicol 47:15-20.

Stein CR, McGovvern KJ, Pajak AM, et al. 2015. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12 – 19 years: National Health and Nutrition Examination Survey. Doi:10.1038/pr.2015.213.

Strøm M, Hansen S, Olsen SF, et al. 2014. Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes – A prospective study with long-term follow-up, Environ Int 68:41-48.

Taylor KW, Hoffman K, Thayer KA, Daniels JL. 2014. Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES). Environ Health Perspect 122:145-150.

Timmermann CA, Rossing LI, Grøntved A, et al. 2014. Adiposity and glycemic control in children exposed to perfluorinated compounds. J Clin Endocrinol Metab 99:E608-614.

Vanden Heuvel JP. 2014. Comment on "Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism." Environ Int 61:150-153.

Velez MP, Arbuckle TE, Fraser WD. 2015. Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. Hum Reprod 30:701-709.

Verner MA, Loccisano AE, Morken NH, et al. 2015a. Associations of perfluoroalkyl substances (PFASs) with lower birth weight: an evaluation of potential confounding by glomerular filtration rate using a physiologically based pharmacokinetic model (PBPK). Environ Health Perspect doi.org/10.1289/ehp.1408837.

Verner MA, Longnecker MP. Comment on "enhanced elimination of perfluorooctanesulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. Environ Sci Technol 2015b;49:5836-6837.

Vestergaard S, Nielsen F, Andersson AM, et al. 2012. Assoications between perfluorinated compouds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. Hum Reprod 27:873-880.

Vesterinen HM, Johnson PI, Atchley D et al. 2015. Fetal growth and maternal glomerular filtration rate: a systematic review. J Matern Fetal Neonatal Med 28, 2176-2181.

Wang JS, Zhang YT, Zhang W, et al. 2012. Association of perfluorooctanoic acid with HDL cholesterol and circulating miR-26b and miR-199-3p in workers of a fluorochemical plant and nearby residents. Environ Sci Technol 46:9274-9278.

Washino N, Saijo Y, Sasaki S, et al. 2009. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth.

Watkins DJ, Josson J, Elston B, et al. 2013. Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environ Health Perspect 121:625-630.

Watkins DJ, Wellenius GA, Butler RA, et al. 2014. Associations between serum perfluoroalkyl acids and LINE-1 DNA methylation. Environ Int 63:71-76.

Weber KE, Fischl AFR, Murray PJ, et al. 2014. Effect of BMI on cardiovascular and metabolic syndrome risk factors in an Appalachian pediatric population. Diabetes, Metabolic Syndrome and Obesity:Target and Therapy 47:445-453.

Webster GM, Venners SA, Mattman A, et al. 2014. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environ Res 133:338-348.

Webster GM, Rauch SA, Ste Marie N, et al. 2015. Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. adults: variation according to TPOAb and iodine status (NHANES 2007-2008). Environ Health Perspect doi:10.1289/ehp.1409589.

Wen LL, Lin LY, Su TC, et al. 2013. Association between serum perfluorinated chemicals and thyroid function in U.S. adults: the National Health and Nutrition Examination Survey 2007-2010, J Clin Endocrinol Metab 98:E1456-E1464 doi:10.1210/jc.2013-1282.

Whitworth KW, Haug LS, Baird DD, et al. 2012. Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort study. Am J Epidemiol 175:1209-1216.

Winquist A, Lally C, Shin HM, et al. 2013. Design, methods, and population for a study of PFOA health effects among highly exposed Mid-Ohio Valley community residents and workers. Environ Health Perspect 121:893-899.

Winquist A, Steenland K. 2014a. Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. Environ Health Perspect 122:1299-1305.

Winquist A, Steenland K. 2014b. Perfluorooctanoic acid exposure and thyroid disease in community and worker cohorts. Epidemiology 25:255-264.

Woodruff TJ, Sutton P. 2014. The Navigaton Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. Environ Health Perspect 122:1007-1014.

Woskie SR, Gore R, Steenland K. 2012. Retrospective exposure assessment of perfluorooctanoic acid serum concentrations at a fluoropolymer manufacturing plant. Ann Occup Hyg 56:1025-1037.

Wu H.,Yoon M,Verne, MA, et al 2015. Can the observed association between serum perfluoroalkyl subtances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics. Environ Int 82, 61-68.

Yang L, Wang Z, Shi Y et al. Human placental transfer of perfluoroalkyl acid precursors: Levels and profiles in paired maternal and cord serum. Chemosphere 2015;144:1631 -1638,

Zeng XW, Qian Z, Emo B, et al. 2015. Association of polyfluoroalkyl chemical exposure with serum lipids in children. Sci Tot Environ 512-513:364-370.

Zhang C, Sundaram R, Maisog J, et al. 2014. A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational Diabetes. Fertil Steril 103:184 – 189.

Zhang T, Sun H,Lin Y, et al. 2013. Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environ Sci Technol 27:7974 – 7981.

Zhang Y, Beeson S, Zhu L, et al. 2013. Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environ Sci Technol 47:10619 – 10627.

Section 4, Chemical and Physical Information

ATSDR (August 2015) - Summary of Chemical and Physical Information Section 4 Comments on Draft Toxicological Profile for Perfluoroalkyls

Section 4. Page 333

Comment:
The last sentence of the 2nd paragraph under Section 4.2 references thermal decomposition. This sentence may be miss-leading to a lay reader. Beyond the basic decomposition of the parent compound, what it converts to, rate and conditions are all important features to understand in order to make proper use of this information. If the authors' intent is to address the chemical stability of the material, they should consider a separate paragraph to accomplish this. One alternative would be to separate environmentally relevant conversion from laboratory or extreme condition conversion.

Comment:
In the first sentence of the 3rd paragraph under Section 4.2, We suggest removing "long" as some PFCAs and PFSAs are not long according to the generally accepted definitions (greater than or equal to 7 perfluorinated carbons for PFCAs and 6 perfluorinated carbons for PFSAs; see Buck et al. 2011).

Comment:
The last sentence of the 3rd paragraph under Section 4.2 might be expanded as follows - Neutral or uncharged perfluoroalkyls or very long chain constituents are expected to form separate layers when mixed with hydrocarbons and water. Conversely, charged species, salts, and ionized species at relevant pH (i.e. PFOS, PFOA, PFHpA, PFNA) and short chain species (i.e. PFBA, PFBuS) have relatively good solubility in water and alcohol. Both the potential to form separate layers when mixed with hydrocarbons and water and the propensity for charged or ionized perfluoroalkyls to concentrate at interfaces makes the measurement of the n-octanol water partition coefficient impractical (3M 1999, EPA 2005a).

Comment:
It is suggested that the 2nd sentence in the 4th paragraph under Section 4.2 be modified as follows – "…when in contact with water at environmental and physiologically relevant pHs."

Section 4. Table 4-1. Page 334

Comment:

The systematic name for PFBA could be added - 2,2,3,3,4,4,4-heptafluorobutanoic acid. Consistency in naming convention throughout the table and document is recommended.

Section 4. Table 4-1. Page 335

Comment:
PFUA is missing its systematic name.

Section 4. Table 4-2. Page 339

Comment:
3M records indicate that PFBA is “miscible” in water. In other words, >100 g PFBA dissolves into 100 g water.

Section 4. Table 4-2. Page 339

Comment:
Many of the references listed for Table 4-2 are difficult to locate. It is recommended that the authors provide full citations.

Section 5, Production, Import/Export, Use, and Disposal

ATSDR (August 2015) - Summary of Production, Import/Export, Use, and Disposal Section 5 Comments on Draft Toxicological Profile for Perfluoroalkyls

Section 5. Page 345

Comment:
The 1st paragraph suggests that "perfluoroalkyls" are a "chemical" that is not subject to the referenced regulations. Of course, perfluoroalkyl substances (PFASs) are a class of chemicals, each of which has distinctive properties and potential uses, and none of which are governed by the referenced U.S. regulations. Rewording of this paragraph to more accurately describe this situation is suggested.

Comment:
In the 2nd paragraph, the statement that PFOS and PFOA have been the "most important" PFASs in terms of production is a very subjective position that cannot be supported and probably does not belong in such a technical document. Furthermore, it is misguided in that perfluorooctane sulfonyl fluoride (POSF), not PFOS, was the dominant chemical in this portion of 3M's manufacturing portfolio. Furthermore, the tense of the last part of this sentence should really be shifted from "present" to "past". It is suggested that the following might better convey the intent of this sentence – "Two specific chemicals that have resulted from manufacturing involving PFASs, namely PFOS and PFOA, are of worldwide interest given their detection in multiple media in the environment. However, these substances and related long-chain perfluoroalkyl compounds have been essentially phased out as a joint effort by EPA and industry. Given their unique properties, certain narrow exceptions exist for specific applications."

Comment:
In the 1st sentence of the 3rd paragraph, the following modification is suggested – "…substantially completed the phase-out of its PFOS production…" An additional comment concerning this sentence applies to the 3M reference provided. It seems a bit problematic to point to a 7+ year old URL as a reference in a report like this; not surprisingly, this link no longer exists.

Comment:
The 3rd paragraph discusses SNURs issued by EPA. According to EPA's website, see link below, two SNURs were issued in 2002. In addition, a final SNUR was issued on September 30, 2013 relative to carpets and carpet treatment. Slight modifications/updates to this paragraph are suggested.

http://www2.epa.gov/sites/production/files/2014-04/documents/factsheet_contaminant_pfos_pfoa_march2014.pdf

Lastly, while 3M no longer manufactures PFOS anywhere in the world and does not have specific information regarding other manufacturers, we note that current production may not be limited to China.

Comment:
The 4th paragraph discusses EPA's PFOA Stewardship Program. It is suggested that this paragraph be modified to reflect EPA's view that "Results [based on 2014 annual reports] show that the companies are on track to reach the program's goal of phasing out these chemicals by the end of 2015." (http://www2.epa.gov/assessing-and-managing-chemicals-under-tsca/20102015-pfoa-stewardship-program) In addition, Table 5-1 should be updated to reflect the most recent reported information.

Section 5. Page 346

Comment:
The following replacement for the last sentence of the 1st full paragraph is suggested – "The 3M Company has developed a new emulsifier to replace PFOA in its fluoropolymer manufacturing operations (Gordon, S.C., 2011. Toxicological evaluation of ammonium 4,8-dioxa-3H-perfluorononanoate, a new emulsifier to replace ammonium perfluorooctanoate in fluoropolymer manufacturing. Regulatory toxicology and pharmacology : RTP 59, 64-80). In addition, perfluorobutane sulfonyl fluoride, PBSF, has become a building block for fluorochemical modified polymers that have been used to replace certain POSF-based products affected by the company's announced phaseout of perfluorooctanyl chemistries. Other POSF-based products were simply discontinued."

Comment:
The 2nd to last sentence of the 2nd full paragraph indicates that PFBA was last produced in 1998. 3M records indicate that a small amount of production continued until 2001.

Comment:
The last sentence of the 2nd full paragraph should be modified to reflect the success of EPA's PFOA Stewardship Program (see comment above).

Section 5. Page 350

Comment:
The first sentence of the 1st paragraph is probably no longer true based on the success of EPA's PFOA Stewardship Program.

Comment:
There is a typo in the sentence under Section 5.2; "is" should be deleted.

Comment:
Section 5.4 (Disposal) appears to be quite fragmented, limited in extent and out-of-date. For example, the work on incineration attributed to reference "EPA 2008f" is no longer active. (It should also be noted that the URL listed for this reference is also no longer active.) Given that "perfluoroalkyls" represents such and broad and diverse set of compounds, it follows that treatment/disposal alternatives will be equally diverse. One option would be to simply make such a statement as a summary for this section. Alternatively, or in addition, it may be worth noting that incineration at conventional temperatures is a proven technology for treating wastes containing PFASs. The last two sentences of this section appear to adequately support this position.

Section 6, Potential for Human Exposure

ATSDR (August 2015) - Summary of Potential for Human Exposure Section 6 Comments on Draft Toxicological Profile for Perfluoroalkyls

Section 6. Page 353

Comment:
Authors should consider the following relative to the 2nd paragraph:

- In the first sentence, because some company actions were taken independently, the better wording for the sentence might be "…have been working in concert with EPA…"
- Concerning the second sentence, 3M suggests the following as an accurate and succinct summary of the company's actions – "As a result of its phase-out decision in May 2000, 3M no longer manufactures perfluorooctanyl compounds. The company ceased manufacturing and using the vast majority of these compounds within approximately two years of the phase-out announcement, and ceased all manufacturing and the last significant use of this chemistry by the end of 2008."
- Concerning the 3rd and 4th sentences, the following is suggested as an updated and more accurate summary – "PFOA, PFOA precursors, and higher homologues have essentially been phased out by the eight major fluoropolymer and telomere manufacturers that accepted U.S. EPA's invitation to participate in its global PFOA Stewardship Program (insert updated EPA reference). Industrial releases of these perfluoroalkyls in the United States have essentially been eliminated based on company reports submitted to the EPA (insert updated EPA reference)."
- Concerning the last sentence, to avoid subjectivity and potential inaccuracy, the following is suggested – "In the past, perfluoroalkyls were released to the air, water, and/or soil in and around fluorochemical facilities (list references)."

Comment:
In the third paragraph, perfluoroalkyl phosphonates should be included with perfluorocarboxylates and sulfonates in the 2nd sentence and supported with a reference. The sentence addressing carpets and textiles might be better worded "…for carpets and textiles may be expected to result…"

Section 6. Page 355

Comment:
The carry-over sentence from page 354 states that background concentrations have not been located for perfluoroalkyls in drinking water. Given the considerable amount of drinking water data that has been generated under U.S. EPA's Third Unregulated Contaminant Monitoring Rule (UCMR3) program, this point should be modified. For example, it could be stated (based on

data reported through October 2015), that the percentage of results above the minimum reporting levels for each of the 6 PFASs in the program is 1% or less. Furthermore, the percentage of results that are above reference concentrations are 0.1% and 0% for PFOS and PFOA, respectively.

Comment:
The last sentence in the carry-over paragraph is subjective and overly broad. The following modification is suggested – "Certain perfluoroalkyls have been measured in air, water, soil, sediment and/or biota at or near fluorochemical industrial facilities (references)."

Comment:
In the second full paragraph, serum concentrations should be updated based on more recent studies.

Section 6. Page 356

Comment:
In the 2nd full paragraph, the statement is made that workers at fluorochemical facilities "may have higher exposure" compared to the general population. The sentence also indicates that this is because of "…elevated concentrations of these substances measured in air, soil, sediment, surface water, groundwater, and vegetation surrounding these facilities." The following modification is suggested as more accurate – "Individuals who work at fluorochemical facilities generally have had higher perfluoroalkyl serum levels than the general population based on exposures in the work environment."

Comment:
The last sentence of the 2nd full paragraph falls short of accurately representing the nature of the work cited. The data that was represented was potential estimated doses from cumulative exposure from multiple pathways. The potential cumulative exposure scenarios for off-site receptors were developed on the basis of plausible combinations of individual exposure pathways.

Section 6. Page 357

Comment:
Concerning the 3rd paragraph, the following changes are suggested:

- Change 2nd sentence to read "…work toward elimination of these substances in products by 2015 (reference)."
- Change 3rd sentence to read "Progress reports have been submitted annually beginning in 2007."
- Deletion of the last sentence.
- Addition of a statement, as supported by EPA's website, that all the companies are on track to meet the goals by the end of 2015.

Comment:
The work by Prevedouros et al. (2006), like a similar and more recent publication by Wang et al. (2013), contains results that are unreliable. The data accuracy and overall data integrity relating to the reported levels of Global Emission Inventories for C4–C14 Perfluoroalkyl Carboxylic Acid (PFCA) Homologues are unverifiable. Although generally the authors have cited numerous references for sources of data applied to the inventories, they have not adequately documented explicitly the source data used in the compilation of the inventories, the data treatment procedures and assumptions applied to the key source inventory data, or provided a reconstructible quantitative uncertainty analysis of the source data or of the emission inventories. Established procedures are not adequately documented for defining and determining analytical data acceptance criteria for reporting, analytical data accuracy, analytical data precision, and overall analytical data uncertainty. Without reconstructible data and data treatment procedures, the reported data for these inventories could result in unverifiable biased results or highly inaccurate results. In summary, the reported results for these inventories are unreliable.

Section 6. Page 358

Comment:
Table 6-1 should be updated based on the most recent (2014) reports submitted to U.S. EPA.

Section 6. Page 359

Comment:
If Table 6-2 remains (see previous comment), the title should clearly reflect that the data represents an estimated historical summary.

Section 6. Page 360

Comment:
Modifications to the first two sentences of the 1st paragraph are suggested as follows – "3M announced its decision to phase-out perfluorooctanyl compounds in May 2000 and had essentially ceased manufacturing and using the vast majority of these compounds within approximately two years of the phase-out announcement (references). EPA has since established three significant new use rules (SNURs) to limit…"

Comment:
Concerning the 2nd paragraph under Section 6.2.1, it should be noted that air emissions of PFOA from the facility were not significant. Furthermore, the second sentence would be more accurate as "This company states that there are currently no process-related air emissions at this facility (3M 2008b)."

Comment:
Concerning the 3rd paragraph under Section 6.2.1, it is suggested that the first sentence be reworded to indicate that "…consumer products such as treated carpets and textiles may be sources of releases…"

Section 6. Page 362

Comment:
In the 3rd sentence of the 3rd paragraph, use of the term "higher concentrations" is vague and subjective. The following is suggested as an alternative – "…found to have detectable concentrations of perfluoroalkyl compounds."

Comment:
Relative to the 3rd paragraph discussion on wastewater treatment plants (WWTPs) as potential sources of PFASs, other references may warrant inclusion. For example:

- Schultz, Melissa et al.; Fluorochemical Mass Flows in a Municipal Wastewater Treatment Facility; ES&T 2006, 40 (23) pp 7350-7357.
- Schultz, Melissa et al.; Chromatography Tandem Mass Spectrometry Characterization of Municipal Wastewaters; ES&T 2006, 40 (1) pp 289-295.

Section 6. Page 363

Comment:
Section 6.2.3 is intended to be a section to review environmental releases to soil. Given the very limited and essentially anecdotal information presented, it is suggested that this section be reduced to only the 1st paragraph. More specifically, the 2nd paragraph discusses waste management practices at two 3M manufacturing facilities without making a connection to how these impact releases to soils in the general environment. Furthermore there are errors in the statements about the 3M facilities: 1) the sludge at Decatur was not "disposed of" but rather used as agricultural amendment under the state NPDES program; 2) the referenced document concerning Cottage Grove did not state that perfluoroalkyl-containing wastes were disposed of at the listed landfills.

Comment:
In the last sentence of the 2nd paragraph, it is not clear what "EPA regulations" are being referred to.

Comment:
In the last paragraph, the references to biodegradation studies does not seem particularly germane or well-developed relative to this being a section on "Releases to the Environment".

Section 6. Page 364

Comment:
The first complete sentence indicates an "extrapolated vapor pressure" for PFOA. Given the unique physico-chemical properties of PFASs, the use of modeling and extrapolation techniques are not generally viewed as reliable in projecting physical parameters. Furthermore, given its pKa, PFOA will not be volatile under most environmental conditions. The statement is misleading and should be deleted.

Section 6. Page 380

Comment:
In the 1st full paragraph, reference is made to Table 6-9, a summary of select surface water and groundwater data from three distinct manufacturing facilities. Besides being considerably out-of-date, the relevance of this information to the general environment and the general population is highly doubtful (i.e., these are very localized results that are presented without sufficient context to yield meaningful interpretation). It is suggested that EPA's UCMR3 database be referenced as a far more robust, current and relevant set of data to characterize both surface water and groundwater supplies across the U.S.

Comment:
In the 1st full paragraph, data is cited from a study by Hanson performed in November 2000. Such data is obviously very much out-of-date, especially considering 3M's perfluorooctanyl phaseout that was executed, in part, at the 3M Decatur, AL facility. Analytical data has also improved dramatically in quality and reliability in recent years. Furthermore, use of maximum detected values to characterize the entire dataset from the study is questionable. As noted above, EPA's UCMR3 database is far more relevant and contains recent data for the same portion of the Tennessee River evaluated by Hanson et al. Use of more recent data is recommended for the final ATSDR profile.

Section 6. Page 388

Comment:
In the 1st full paragraph, the data referenced is for samples collected in 2005 and early 2006. As such, it is badly out-of-date. More current, comprehensive (analytes beyond just PFOA) and relevant data can be found in EPA's UCMR3 database. This statement is true generally and also for the specific stretch of the Tennessee River referenced in the paragraph.

Comment:
In the 3rd full paragraph, the sentence referencing the study by Chang is misleading. A better statement might be – "According to Chang et al. (2008a), concentrations of PFBA in precipitation, surface waters and water treatment facility effluents have been measured in the low

ng/L range while similar measurements in public and private wells have ranged up to low ug/L concentrations."

Section 6. Page 389

Comment:
The data presented in Section 6.4.3, Sediment and Soil, was obtained from specific site assessments conducted in a 2006-2007 timeframe at two manufacturing facilities that formerly produced perfluorooctanyl products. As such, the relevance of the information to levels found in the general environment is highly questionable. For example, recitation of percent detection and maximums from site investigation work, where the objective is typically to characterize areas with known or suspected highest concentrations, does not seem in any way comparable to levels in the environment at-large.

Section 6. Pages 390-391

Comment:
The relevance of the data presented in Table 6-11 is highly questionable since it is obtained from site investigations at perfluorochemical manufacturing locations. In addition, the information is now arguably out-of-date.

Section 6. Pages 392

Comment:
There appears to be an incorrect reference in the 1st paragraph under Section 6.4.4. The paragraph discusses a food study while reference "3M 2001" is listed as a 28-day oral (gavage) toxicity study.

6.5 General Population and Occupational Exposure.

Section 6.5 is currently missing key information on exposure to perfluorooctanyl chemistry:

1. The history of the phasing out of perfluorooctanyl chemistry and significant efforts that restrict manufacturing, import and use of this chemistry in the United States; and

2. The full extent to which residual levels of perfluorooctanyl chemistry (e.g., PFOS and PFOA) have declined since 1999, as rigorously measured by the Center for Disease Control's (CDC's) National Health and Nutrition Examination Survey (NHANES).

It is recommended that additional information be included on these items such that the general population and occupational exposure are characterized more fully. Suggested below is additional text on these topics to support a more accurate representation of available information.

1. Information on the history of the perfluorooctanyl chemistry phaseout and significant efforts that restrict manufacturing, import and use of perfluorooctanyl chemistry in the United States should be added to Section 6.5.

PFOA

In May 2000, 3M announced that it was voluntarily phasing out the production of perfluorooctanyl chemistry, including PFOA. This goal was reached by 2008. In 2006, the US Environmental Protection Agency (EPA) invited eight major fluoropolymer and telomer manufacturers (Arkema, Asahi, BASF (successor to Ciba), Clariant, Daikin, 3M/Dyneon, DuPont, and Solvay Solexis) to join in a global stewardship program with two goals: 1) To commit to achieve, no later than 2010, a 95 percent reduction, measured from a year 2000 baseline, in both facility emissions to all media of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, and product content levels of these chemicals; and 2) To commit to working toward the elimination of these chemicals from emissions and products by 2015. In January 2015, EPA released the most recent reports that showed these companies were on track to reach the program's goal of phasing out these chemicals by the end of 2015. Annual progress reports can be found at http://www.epa.gov/oppt/pfoa/pubs/stewardship/preports8.html (accessed October 7, 2015).

EPA has and continues to promulgate Federal Regulations that restricts the manufacture and use of PFOA and PFOA-precursors.

PFOS

3M was the only known manufacturer of PFOS and PFOS-precursor products in the United States. From the early 1960s until 2000, these materials were used in an increasingly wide variety of consumer and industrial products. In 1998, 3M scientists reported to the US Environmental Protection Agency (EPA) that they had identified PFOS in the blood of the general population (at levels measured in parts per billion, ng/mL). In May 2000, 3M announced that it was voluntarily phasing out of production of PFOS and products that could degrade or metabolize to PFOS.

After 3M ceased the manufacture of PFOS, the US EPA promulgated federal regulations that require notification by other manufactures (as well as 3M) for manufacturing or importing PFOS

or PFOS precursors, subject to a handful of very narrow critical use exceptions with limited exposure potential approved by EPA. See 40 Code of Federal Regulations §721.9582, listing several hundred PFOS precursors that cannot be manufactured or imported without EPA permission, and the permissible uses approved by EPA via its Significant New User Rule (SNUR). EPA's rules allowed the continuation, without being subject to notification requirements, of a few specifically limited, highly technical uses of these chemicals for which no alternatives were available, and which were characterized by very low volume, low exposure and low releases. Any other uses of these chemicals would require prior notice to and review by the Agency.

2. Additional information on the full extent to which residual levels of perfluorooctanyl chemistry (e.g., PFOA and PFOS) have declined since 1999, as rigourously measured by the CDC's NHANES should be added.

The CDC's National Health and Nutrition Examination Survey (NHANES), a nationally representative sample of the U.S. population (noninstitutionalized), has conducted biomonitoring of selected environmentally-present chemicals every 2 years since 1999-2000. This includes PFOA and PFOS. The geometric mean concentration of PFOA in the serum (blood) of the general population has *declined by approximately sixty percent* since 1999-2000 (Figure 1A). The geometric mean concentration went from 5.41 ng/mL (1999-2000) to 2.08 ng/mL (2011-2012). The 95th percentile has declined from 11.9 ng/mL (1999-2000) to 5.68 ng/mL (2011-2012) (Figure 1B). This decline in PFOA was observed across both sexes (see Figures 1A and 1B), as well as for all age groups (see Figures 2A and 2B), and ethnicity/race groups (see Figures 3A and 3B). Data obtained for these figures are found in the Fourth National Report on Human Exposure to Environmental Chemicals (http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf, accessed October 7, 2015).

Similar to PFOA, the concentration of PFOS in the serum (blood) of the general population has declined by approximately eighty (80) percent since 2000 as reported for the geometric mean and the 95th percentile according to the NHANES. Like PFOA, this decline in PFOS is observed across males and females (see Figures 1A and 1B), age (see Figures 2A and 2B), and ethnicity/race (see Figures 3A and 3B). Data obtained for these figures can be found in the Fourth National Report on Human Exposure to Environmental Chemicals (See http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf, accessed October 9, 2015). The 80% decline in the geometric mean in the general population between 2000 and 2012, given the absence of production activities in the United States, is reasonably consistent with a published geometric mean serum elimination "half-life" of 4.8 years in retired occupationally exposed 3M production workers (Olsen et al. 2007).

Figure 1A. Trends in NHANES by Sex:
Geometric Mean Serum PFOA Concenrations (ng/mL)

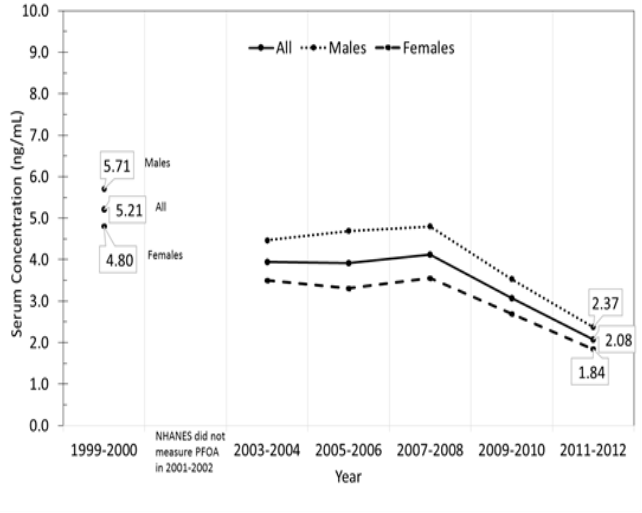


Figure 1B. Trends in NHANES by Sex:
95th Percentile Serum PFOA Concentrations (ng/mL)

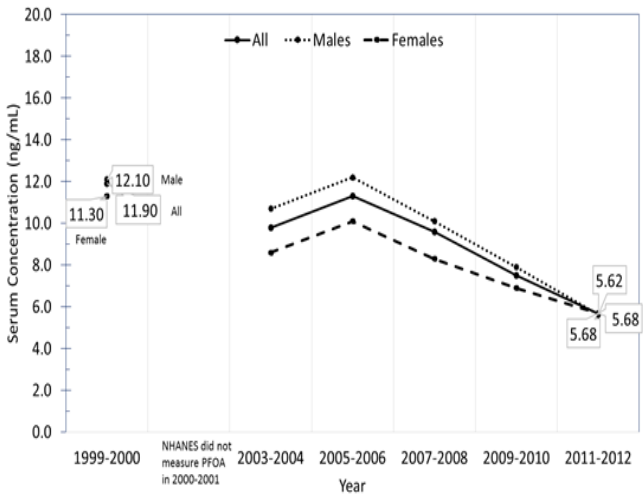


Figure 2A. Trends in NHANES by Age:
Geometric Mean Serum PFOA Concentrations (ng/mL)

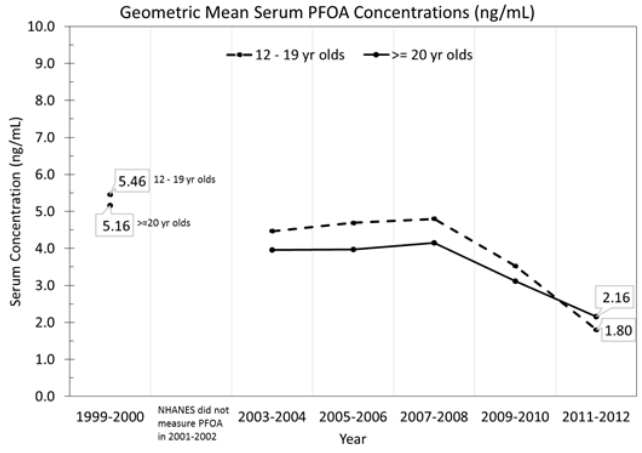


Figure 2B. Trends in NHANES by Age:
95th Percentile Serum PFOA Concentrations (ng/mL)

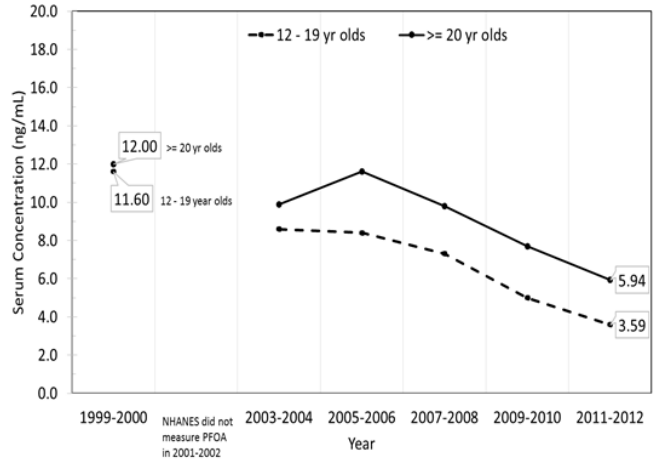


Figure 3A. Trends in NHANES by Ethnicity/Race:
Geometric Mean by Serum PFOA Concentration (ng/mL)

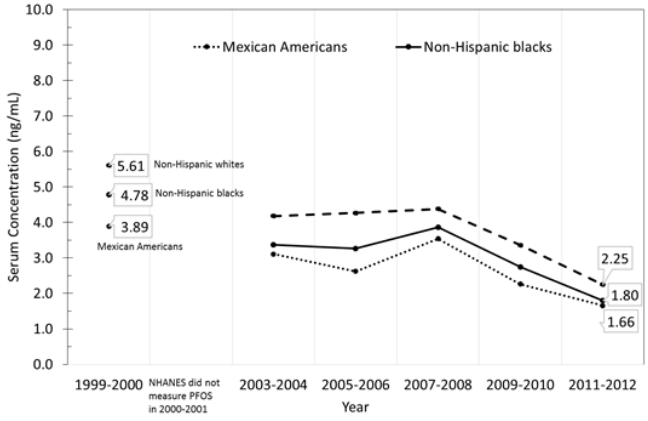
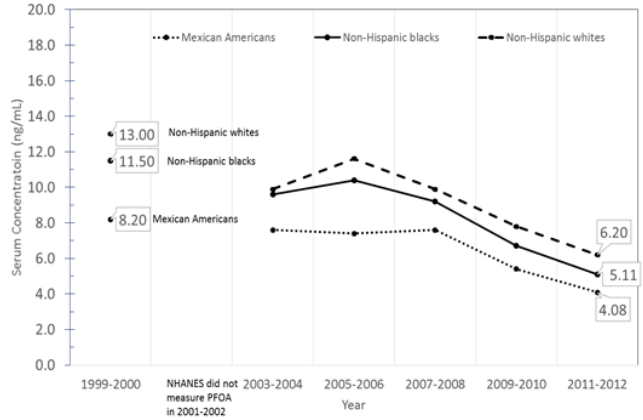


Figure 3B. Trends in NHANES by Ethnicity/Race:
95th Percentile Serum PFOA Concentrations (ng/mL)



Other cross-sectional biomonitoring studies, including analyses from six American Red Cross blood donation centers, between 1999-2000 and 2010, have shown similar declining trends in PFOA and PFOS serum concentrations (Olsen et al. 2003; Olsen et al. 2008; Olsen et al. 2012).

As a result of the EPA PFOA product stewardship program, it is anticipated that PFOA serum concentrations will continue to decline. Furthermore, given the absence of PFOS production in the United States since 2002, and subsequent US EPA regulatory decisions, it is anticipated that PFOS serum concentrations will also continue to decline.

Biomonitoring data for perfluoroalkyls for the 2013-2014 period is anticipated to be released by NHANES within two years. The American Red Cross blood donor study is currently analyzing blood samples collected in July 2015 from the same six donation centers in their previous studies conducted in 2000, 2006, and 2010 (Olsen et al. 2003; Olsen et al. 2012; Olsen et al. 2008). Assuming it is available, inclusion of this additional and most recent data in the final toxicological profile would be informative.

Figure 1A. Trends in NHANES by Sex:
Geometric Mean Serum PFOS Concentrations (ng/mL)

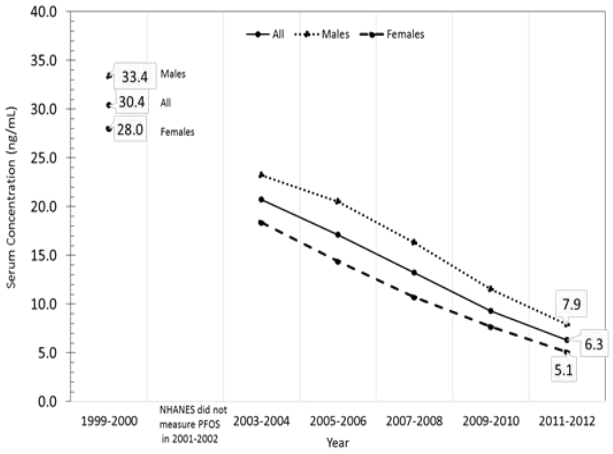


Figure 1B. Trends in NHANES by Sex:
95th Percentile of Serum PFOS Concentrations (ng/mL)

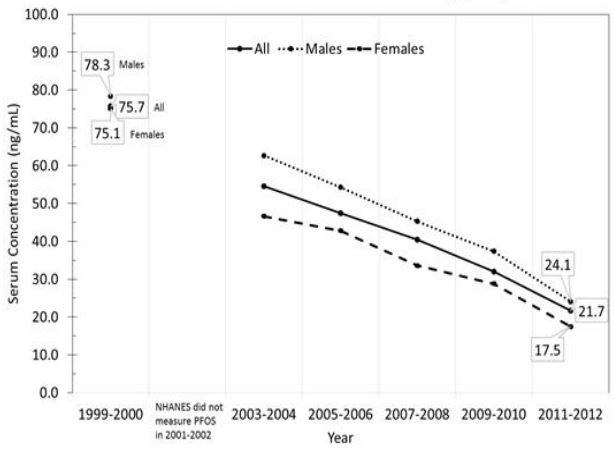


Figure 2A. Trends in NHANES by Age:
Geometric Mean Serum PFOS Concentrations (ng/mL)

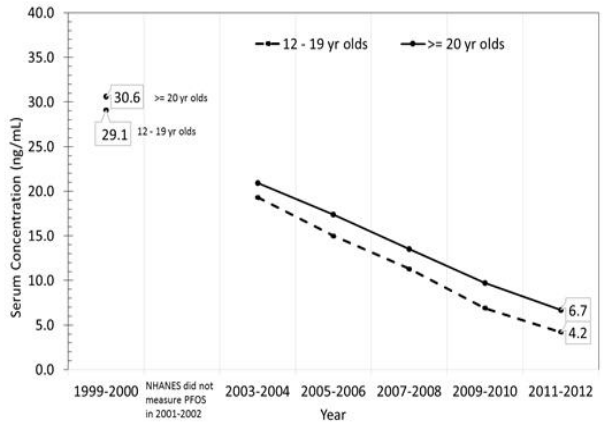


Figure 2B. Trends in NHANES by Age:
95th Percentile of Serum PFOS Concentration (ng/mL)

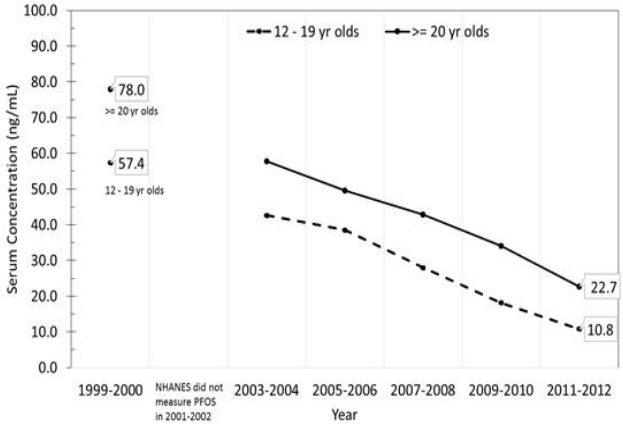


Figure 3A. Trends in NHANES by Ethnicity/Race:
Geometric Mean Serum PFOS Concentrations (ng/mL)

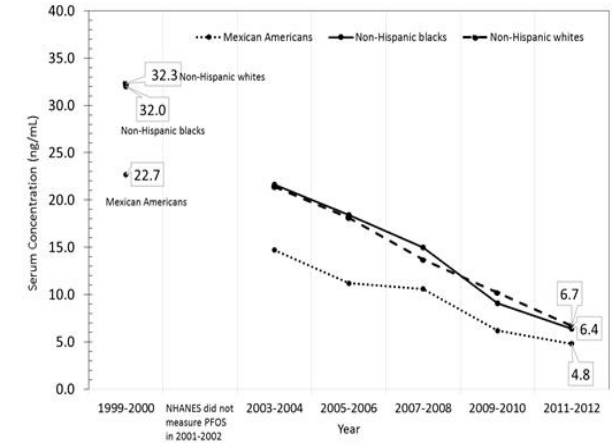
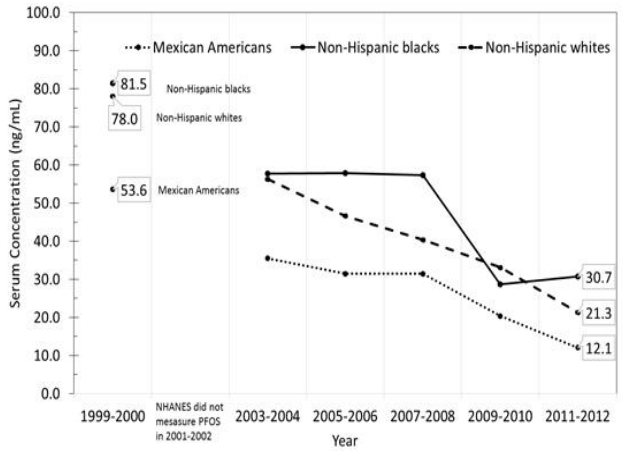


Figure 3B. Trends in NHANES by Ethnicity/Race:
95th Percentile of Serum PFOS Concentration (ngmL)



6.7 Populations with Potentially Higher Exposures

This section does not include the findings from much of the published literature on this topic. For a review of this literature regarding populations with potentially higher exposure, please see the following reference:

Olsen GW, 2015. Chapter 4. PFAS Biomonitoring in Higher Exposed Populations. (In) Toxicological Effects on Perfluoroalkyl and Polyfouroalkyl Substances. (ed) Dewitt JC. London: Humana Press. pages 77 – 125.

6.8 Adequacy of the Database

6.8.2 Ongoing studies. The US Environmental Protection Agency (EPA) PFOA Stewardship Program consists of eight major fluoropolymer and telomer manufacturers: Arkema, Asahi, BASF (successor to Ciba), Clariant, Daikin, 3M/Dyneon, DuPont, and Solvay Solexis.

Section 7, Analytical Methods

ATSDR (August 2015) - Summary of Analytical Section 7 Comments on Draft Toxicological Profile for Perfluoroalkyls

Section 7. Page 425

Comment:
Authors should clarify that the two standard method citations 2009 EPA and 2009 ISO are applicable to PFCs in water and that standard PFC methods in other environmental and biological matrices are not yet available. LC/MS/MS method validation guidance and recent validated test method publications in other environmental and biological matrices should be included.

See, for example:

- Malinsky M.D., Jacoby C.B., Reagen W.K.; "Determination of Perfluorinated Compounds (PFCs) in Fish Fillet Homogenates: Method Validation and Application to Fillet Homogenates from the Mississippi River", Analytica Chimica Acta, 683, 2011, 248-257.
- S. T. Wolf, W.K. Reagen, "Method for the Determination of Perfluorinated Compounds (PFCs) in Water by Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)" Anal Methods; 3 (7), 1485-1493, 2011.
- G. W. Olsen, W.K. Reagen, et.al, "Temporal Trends of Perfluoroalkyl Concentrations in American Red Cross Adult Blood Donors, 2000−2010" Environ. Sci. Technol.; 46, 6330-6338, 2012.
- S. T. Wolf, W.K. Reagen, "Method and Validation for the Analysis of Perfluorinated Compounds in Water by Pre-Sampling Isotope Dilution-Direct Injection- LC/MS/MS" Anal Methods; 5 (10), 2429-2636, 2013.
- J. A. Shoemaker, Development and multi-laboratory verification of U.S. EPA method 538 for the analysis of drinking water contaminants by direct aqueous injection LC/MS/MS, Anal. Methods, 2011, 3, 1628-1636.
- Guidance for Industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001.
- Viswanathan C.T., et. al.; Workshop/Conference Report - Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays, The AAPS Journal 2007; 9(1) article 4, E30-E42.

Comment:
An interlaboratory study concluded that PFC standard reference materials (SRMs) should be incorporated into analytical method standard operating procedures (SOPs) to provide comparable precision and accuracy metrics across different laboratories and methods. Several SRMs in a

variety of environmental test matrices have recently become available as performance quality control standards for testing laboratories. Add recent citations from the National Institute of Standards and Technology (NIST) and the Institute for Reference Materials and Measurements (IRMM).

See, for example:

- Keller J.M., Reagen W.K., et al; "Determination of perfluorinated alkylacid concentrations in human serum and milk standard reference materials", Anal Bioanal Chem, 397, 2010, 439-451.
- J. L. Reiner, W.K. Reagen, et. al.; "Determination of Perfluorinated Alkyl Acid Concentrations in Biological Standard Reference Materials"; Anal Bional Chem; 404, 2683-2692, 2012.
- J. L. Reiner, W.K. Reagen, et. al.; "Perfluorinated Alkyl Acids in Abiotic Standard Reference Materials"; Anal Bioanal Chem; 407, 2975-2983, 2015.
- Ramos M. D., et.al.; "Certification Of The Mass Fraction Of Perfluoroalkyl Substances (PFASs) In Fish Tissue (Pike-Perch): IRMM-427"; JRC Reference Materials Report 2015; European Commisssion, Joint Research Center, Institute for Reference Materials and Measurements (IRMM).
- Ramos M. D., et.al.; "The Certification Of The Mass Concentration Of Perfluoroalkyl Substances (PFASs) In Water: IRMM-428"; JRC Reference Materials Report 2015; European Commisssion, Joint Research Center, Institute for Reference Materials and Measurements (IRMM).

Section 7. Page 426

Comment:

Concerning the 1st carry-over sentence at the top of this page, it would be helpful to add the Riddell et.al. citation that demonstrates significant variation in data accuracy with PFOS isomer quantification methods.

- Riddell N., et.al; "Branched Perfluorooctane Sulfonate Isomer Quantification and Characterization in Blood Serum Samples by HPLC/ESI-MS(/MS). Environ. Sci. Technol., 2009, 43, 7902-7908.

Section 7.1 Page 426

Comment:

Add more recent and validated methodology citations for fish fillet and blood matrices to Table 7-1.

- Malinsky M.D., Jacoby C.B., Reagen W.K.; "Determination of Perfluorinated Compounds (PFCs) in Fish Fillet Homogenates: Method Validation and Application to Fillet Homogenates from the Mississippi River", Analytica Chimica Acta, 683, 2011, 248-257.

- G. W. Olsen, W.K. Reagen, et.al, “Temporal Trends of Perfluoroalkyl Concentrations in American Red Cross Adult Blood Donors, 2000−2010” Environ. Sci. Technol.; 46, 6330-6338, 2012.

Section 7.2 Page 429

Comment:
Add more recent and validated LC/MS/MS methodology citations in water matrices to Table 7-2.

- S. T. Wolf, W.K. Reagen, “Method for the Determination of Perfluorinated Compounds (PFCs) in Water by Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)” Anal Methods; 3 (7), 1485-1493, 2011.
- S. T. Wolf, W.K. Reagen, “Method and Validation for the Analysis of Perfluorinated Compounds in Water by Pre-Sampling Isotope Dilution-Direct Injection- LC/MS/MS” Anal Methods; 5 (10), 2429-2636, 2013.
- J. A. Shoemaker, Development and multi-laboratory verification of U.S. EPA method 538 for the analysis of drinking water contaminants by direct aqueous injection LC/MS/MS, Anal. Methods, 2011, 3, 1628-1636.

Section 7.3.1 Page 432

Comment:
Reference the Riddell et.al. citation that demonstrates significant variation in data accuracy with PFOS isomer quantification methods.

- Riddell N., et.al; "Branched Perfluorooctane Sulfonate Isomer Quantification and Characterization in Blood Serum Samples by HPLC/ESI-MS(/MS). Environ. Sci. Technol., 2009, 43, 7902-7908.

Comment:
Reference the additional interlaboratory study conclusion was the need for standard PFC reference materials (SRMs) to be incorporated into analytical method standard operating procedures (SOPs) to provide comparable precision and accuracy metrics across different laboratories and methods. Several SRMs in a variety of environmental test matrices have recently become available as performance quality control standards for testing laboratories. Add recent citations from the National Institute of Standards and Technology (NIST) and the Institute for Reference Materials and Measurements (IRMM).

See, for example:
- Keller J.M., Reagen W.K., et al; “Determination of perfluorinated alkylacid concentrations in human serum and milk standard reference materials”, Anal Bioanal Chem, 397, 2010, 439-451.
- J. L. Reiner, W.K. Reagen, et. al.; “Determination of Perfluorinated Alkyl Acid Concentrations in Biological Standard Reference Materials”; Anal Bional Chem; 404, 2683-2692, 2012.

- J. L. Reiner, W.K. Reagen, et. al.; “Perfluorinated Alkyl Acids in Abiotic Standard Reference Materials”; Anal Bioanal Chem; 407, 2975-2983, 2015.
- Ramos M. D., et.al.; “Certification Of The Mass Fraction Of Perfluoroalkyl Substances (PFASs) In Fish Tissue (Pike-Perch): IRMM-427”; JRC Reference Materials Report 2015; European Commisssion, Joint Research Center, Institute for Reference Materials and Measurements (IRMM).
- Ramos M. D., et.al.; “The Certification Of The Mass Concentration Of Perfluoroalkyl Substances (PFASs) In Water: IRMM-428”; JRC Reference Materials Report 2015; European Commisssion, Joint Research Center, Institute for Reference Materials and Measurements (IRMM).

Section 8, Regulations, Advisories, and Guidelines

ATSDR (August 2015) - Summary of Regulations, Advisories, and Guidelines Section 8 Comments on Draft Toxicological Profile for Perfluoroalkyls

Section 8. Page 435

Comment:
Authors should consider starting section with an introductory paragraph indicating that few environmental regulations have been adopted for PFASs, especially beyond PFOS and PFOA. Rather, various public health and environmental agencies have tended to establish advisories or guidance to help inform situations involving these constituents. This tendency reflects the ongoing research and development of information in this area.

Comment:
In the second and third paragraphs, the authors summarize ATSDRs development of intermediate-duration oral MRLs for PFOA and PFOS, respectively. References should be made to Appendix A of the report where additional information and worksheets are provided for each substance.

Comment:
Fourth paragraph; suggest wording change to "…values for any perfluoroalkyl compounds (IRIS2014)."

Comment:
Fifth paragraph; it is suggested that the sentence (and section) be expanded to include actions taken by various states within the United States. See details in comments provided on Table 1.

Section 8. Table 8-1

Comment:
Under "International", it should be noted that based in a meeting held 3-10 June 2014, IARC made a determination to classify PFOA as *possibly carcinogenic to humans (Group 2B).* Volume 110, in preparation, will summarize this action.

Comment:
Under "International", it should be noted that in 2007, the United Kingdom Health Protection Agency adopted Drinking Water Quality Guidelines of 0.3 ug/L for PFOS and 10 ug/L for PFOA.

https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/317725/PFOS_and_PFOA_properties_incident_management_toxicology.pdf

Comment:
Under "International", it should be noted that Germany has implemented a graduated scale of guidance values for composite (summed) PFOA and PFOS concentrations in drinking water. The health based precautionary value (HPV_1) is 0.1 ug/L.

This is Germany's long term minimum quality goal for non-genotoxic substances. The strictly health based guidance value (GV) for safe lifelong exposure of all population groups is 0.3 ug/L. The precautionary action values for infants and adults are 0.5 ug/L and 5 ug/L, respectively.

http://www.umweltbundesamt.de/sites/default/files/medien/pdfs/pft-in-drinking-water.pdf

Comment:
Under "International", it should be noted that Health Canada has developed Health-based Drinking Water Guidance Values for the following PFASs: PFOS – 0.3 ug/L, PFOA – 0.7 ug/L, PFBS – 15 ug/L, PFBA – 30 ug/L. In addion, it has indicated that the PFOS value (0.3 ug/L) can be used as a screening value for PFHxS and that the PFOA value (0.7 ug/L) can be used as a screening value for PFPeA, PFHxA, PFHpA and PFNA.

Drinking Water Guidance Value for Various perfluorinated alkyl compounds; Health Canada; January 26, 2011.

Comment:
Under "National", it should be noted that in 2009, U.S. EPA Region 4 adopted residential soil screening levels of 6 mg/kg for PFOS and 16 mg/kg for PFOA.

http://www2.epa.gov/sites/production/files/2014-04/documents/factsheet_contaminant_pfos_pfoa_march2014.pdf

Comment:
Under "National", it should be noted that a screening level drinking water threshold of 400 ug/L for PFBS can be derived based on the reference dose established by the U.S. EPA National Center for Environmental Assessment in their Provisional Peer-Reviewed Toxicity Value report on this substance. EPA's Regional Screening Level (RSL) calculator can be used establish this value.

http://hhpprtv.ornl.gov/issue_papers/PerfluorobutaneSulfonate.pdf

Comment:
It is suggested that a section be added for "States within U.S." Entries might include:

Agency	Description	Information	Reference
MN Dept of Health	Health Risk Limits for groundwater	PFOS – 0.3 ug/L PFOA – 0.3 ug/L PFBA – 7 ug/L PFBS – 7 ug/L	http://www.health.state.mn.us/divs/eh/risk/guidance/gw/table.html
MN Pollution Control Agency	Soil Reference Values	Industrial PFBA – 500 mg/kg PFOA – 13 mg/kg PFOS – 14 mg/kg Residential PFBA – 77 mg/kg PFOA – 2.1 mg/kg PFOA – 2.1 mg/kg Recreational PFBA – 94 mg/kg PFOA – 2.5 mg/kg	www.pca.state.mn.us/index.php/view-document.html?gid=3154

		PFOS – 2.6 mg/kg	
NJ	Preliminary Drinking Water Guidance Value	PFOA – 0.04 ug/L	http://www.nj.gov/dep/watersupply/dwc_quality_pfoa.html
NC	Interim Maximum Allowable Concentration for groundwater	PFOA – 1 ug/L	http://daq.state.nc.us/toxics/risk/sab/ra/PFOA_Pending.pdf
MI	Surface Water Quality Values	Drinking water PFOA – 0.42 ug/L Non-drinking water PFOA – 12 ug/L	http://www.michigan.gov/documents/deq/wrd-swas-rule57_372470_7.pdf
TX	Protective Concentration Levels	Soil and groundwater values for 16 PFASs for several different exposure scenarios	http://www.tceq.texas.gove/assets/public/remediation/trrp/pcls2014.xlsx
IL	Provisional Groundwater Remediation Objectives	Class I PFOA – 0.4 ug/L PFOS – 0.2 ug/L Class II PFOA – 0.2 ug/L PFOS – 0.2 ug/L	
ME	Remedial Action Guidelines; Groundwater Residential	PFOA – 0.06 ug/L PFOS – 0.1 ug/L	http://www.maine.gov/dep/spills/publications/guidance/rags/final_5-8-2013/2%20ME-RAGS_Final_5-8-2013%20Corrected%20Copy.pdf
WV	U.S. EPA/Dupont Consent Agreement	PFOA action level – 0.5 ug/L	
OH	U.S. EPA/Dupont Consent Agreement	PFOA action level – 0.5 ug/L	