Reviewer’s report

Title: Immunotoxicity of perfluorinated alkylates: Calculation of benchmark doses based on serum concentrations in children

Version: 1 Date: 8 February 2013

Reviewer: Gloria Post

Reviewer’s report:

This paper presents important new information on the development of benchmark doses and approximate health-based drinking water levels for PFOA and PFOS, based on the authors’ previously reported associations with decreased vaccine antibodies with serum levels of these compounds in children. PFOA and PFOS are of interest as emerging drinking water contaminants because of their frequent occurrence in drinking water, their persistence in the environment and the human body, evidence suggesting increased risk of health effects from low exposure levels, and the greater exposures and potential susceptibilities of infants and children. Over the past few years, a large body of new data on the health effects of these compounds has become available, including epidemiology studies of the general population and communities with contaminated drinking water. However, few if any publications have focused on the development of health-based drinking water levels from these recent human data. The work presented in this paper represents a valuable initial step in this important endeavor.

A general comment is that the background information should be expanded somewhat to provide a better context for this study. For example, as currently written, a reader would not learn that associations of PFCs with many health endpoints in addition to immune effects have been evaluated in humans. Specific suggestions for relevant information that the authors should consider including are given below.

Also, since the toxicity of PFOS has some similarities and some differences with that of PFOA, it should be made clear throughout the paper whether PFOS, PFOA, or both are being discussed.

Many of the comments on PFOA are based on information from the recent review by this reviewer and colleagues (Post et al., 2012) and the citations therein, except for some additional very recent publications. Although PFOS information is not discussed in detail in Post et al. (2012), many of the studies cited in Post et al. (2012) also evaluate PFOS.

Should manuscript be reviewed by a statistician?

As noted by the authors, benchmark dose modeling based on epidemiology data is more complicated than when animal toxicology data is used. I do not have expertise in this field and suggest that the paper be reviewed by someone with
this expertise.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. Background, 2nd Paragraph. 2nd sentence. “…and, to a lesser degree, via human milk…”

Please revise to accurately reflect the relative exposure from human milk. Although the concentration of PFOA is lower in breast milk than in maternal serum, infants’ exposure through breast milk from mothers who use contaminated drinking water and/or from formula prepared with contaminated drinking water is higher than in adults exposed to the same drinking water concentration. See Post et al. (2012), Section 5.2 and Figure 3 (which was taken from Fromme et al., 2010).

2. Background, 2nd Paragraph. 3rd sentence. “…although the Faroese may be primarily exposed to PFCs through their marine diet [8].”

Relevant to this study of PFOA and PFOS, citation (8) shows association with marine diet for PFOS and some other PFCs, but not for PFOA. Also, citation (8) shows that the marine diet contributes to dietary exposure, but not necessarily that it is the primary exposure source for the Faroese. Please revise to clarify this, for example: “the marine diet may contribute to the exposure of the Faroese to some PFCs”.

3. Background, 3rd paragraph, 3rd sentence.

Please revise to include that the EPA Provisional Health Advisories are stated to rely upon subchronic data and are intended to protect for short term exposure (as opposed to chronic exposure).

4. Background, 4th paragraph. Discussion of state levels.

In addition to the Minnesota levels, please also mention the New Jersey guidance for PFOA of 0.04 ug/L which is based on endpoints identified in the EPA (2005) draft risk assessment. (For basis, see NJDEP, 2007 at http://www.nj.gov/dep/watersupply/pdf/pfoa_dwguidance.pdf; and Post, G.B., Louis, J.B., Cooper, K.R., Boros-Russo, B.J., Lippincott, R.L., 2009. Environ. Sci. Technol. 43, 4547–4554).

Please note that this NJ guidance does not consider the many recent studies showing developmental, immune, and neurobehavioral effects in animals, and associations with many health endpoints in the general population and communities with contaminated drinking water.

5. Background, 5th paragraph in general.

It should be conveyed that the primary importance of the BMDLs discussed in this paper is not that they are necessary for demonstrating that current limits are not protective, but rather that they may contribute to the future development of protective health-based limits. It is important to mention that it can readily be concluded from the human studies that the current limits for PFOA in drinking
water may not be health protective, even without consideration of the human and animal BMD modeling results discussed in this paper.

Specifically, based on the 100:1 serum:drinking water ratio discussed later in your paper, chronic exposure to PFOA at the current drinking water limits cited would greatly increase body burden as measured by serum level. For example, chronic exposure to 0.3 ug/L and 0.4 ug/L would lead to serum levels of 30 ug/L to 40 ug/L (or higher), respectively. This is at least 7-10 fold above general population median of ~4 ug/L.

These elevated serum levels that would result from chronic exposure at the current guidelines cited (0.4 ug/L EPA Provisional Health Advisory; 0.3 ug/L Minnesota) must be considered in the context of the numerous human health endpoints, including the decreased vaccine antibody response discussed in this paper, that have been associated with serum levels down to and including the much lower general population range, as well as the serum levels at which effects were observed in recent animal toxicology studies. See Post et al. (2012), sections 6.1 and 10, and citations therein.

Please review and consider the discussion of these issues in pages 7-10 of Post, G. (2010). Letter to North Carolina Division of Air Quality. June 1, 2010. This is a public document which is uploaded for the authors’ consideration, since I am not sure whether it is posted online.

The following additional very recent information not included in the citations above is provided for the authors’ consideration: The C8 Science Panel recently concluded that there are probable links between exposure of the C8 Health Study population to PFOA in drinking water and the following health conditions: pregnancy induced hypertension, ulcerative colitis, thyroid disease, high cholesterol, and kidney and testicular cancer.

http://www.c8sciencepanel.org/prob_link.html

Two additional recent publications that the authors may wish to consider are:


6. Background, 6th paragraph, 4th sentence.
While the excretion of PFOA is much more rapid in female mice than in male mice, this is not true for PFOS, the compound evaluated in this study. Citation (17) states: “Differences in sensitivity between genders is most likely not related to gender differences in bioaccumulation or elimination of PFOS in these mice, as serum levels of PFOS in males and females at matching doses were not significantly different.”

7. Background, 7th paragraph. Discussion of decreased vaccine antibody
association with PFCs (Grandjean et al., 2012, citation 7).

A recent study of a different population of children found associations of maternal serum levels of four PFCs with decreased response to rubella vaccine, as well as increased episodes of common cold (PFOA and PFNA) and gastroenteritis (PFOA and PFHxS). This study should be cited. Citation: Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, van Loveren H, Løvik M, Nygaard UC. J Immunotoxicol. 2013 Jan 25. [Epub ahead of print]

8. Benchmark calculations, 3rd paragraph – choice of percentile for BMR.
Can the authors provide more justification for the choice of 5%, such as a citation for the statement that 5% is often used for human studies?

9. Discussion, 1st paragraph. Last sentence.
Was mercury exposure evaluated? Is exposure to mercury a potential confounding factor in this population for the effects that were evaluated?

10. Discussion, paragraph 4.
Please update the discussion of other relevant human studies to include the recent paper by Granum et al. (2013) discussed above.

Also, please review and consider mentioning the C8 Science Panel’s probable link evaluations for health endpoints related to immune effects. They concluded that there is a probable link between exposure to PFOA and ulcerative colitis, and no probable link with other autoimmune diseases (rheumatoid arthritis, lupus, type1 diabetes, Crohn’s disease, or multiple sclerosis)
http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Autoimmune_Disease_30Jul2012.pdf. They also concluded that there is not a probable link between exposure to C8 (also known as PFOA) and common infections, including influenza, in children or adults.
http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Infections_30Jul2012.pdf

11. Discussion, paragraph 7, 2nd sentence.
The appropriate uncertainty factors to apply to the serum level BMDL of 23 ug/L that is based on mouse mammary gland development are: 10 for intraspecies uncertainty (to protect individuals with increased vulnerability), and 3 (rather than 10 proposed by the authors) for interspecies (animal-to-human) extrapolation, for a total uncertainty factor of 30. The resulting serum level Reference Dose is 0.77 ug/L. The uncertainty factor of 10 for interspecies extrapolation typically includes two factors of 3 each, for toxicokinetic and toxicodynamic differences between humans and animals. (See: A Review of the Reference Dose and Reference Concentration Processes, http://www.epa.gov/raf/publications/pdfs/rfd-final.pdf). Since the BMDLs based on serum level rather than administered dose as is more typical, interspecies toxicokinetic differences are already accounted for, and only the factor for interspecies toxicodynamic differences should be applied.

12. Discussion, paragraph 8, 1st two sentences, about 100:1 ratio between serum level and drinking water.
Please add recent citations that are more relevant to the lower drinking water levels discussed in this paper. The study cited (19) evaluated a relatively small study group (several hundred people) with extremely high PFOA levels in their drinking water. Thus, there was uncertainty about whether the 100:1 ratio observed in (19) was applicable to lower drinking water concentrations (NJDEP, 2007, http://www.nj.gov/dep/watersupply/pdf/pfoa_dwguidance.pdf).

Further work by this reviewer and colleagues showed that this approximate 100:1 ratio is applicable to the water district studied in (19) and to the 5 other water districts with lower drinking water levels in the much larger C8 Health Study (Post et al. 2009. Environ. Sci. Technol. 43, 4547–4554). More recently, the validity of this (or an even higher than 100:1 ratio) has been further confirmed by additional data from private wells, public water in other locations, and several pharmacokinetic modeling approaches (See Post et al., 2012, Section 5.1 and citations therein).

Also, relevant to the population (children) evaluated in this manuscript, it should be mentioned the serum levels in children were found to be higher than in adults using the same drinking water source both in (19) and in several more recent studies (see Post et al., section 5.2, last paragraph for citations).

13. Discussion, paragraph 8, 3rd sentence. Development of water concentration from serum-based RfD.

In developing health-based drinking water values based on a Reference Dose, a relative source contribution factor (RSC) is applied to account for non-drinking water exposures, such as diet, consumer products, air, house dust, or other sources. The default value for this factor is 20% (e.g., non-drinking water sources are assumed to provide 80% of exposure). This default value is used if the relative contributions of drinking water versus non-drinking water sources are not fully characterized, or when more than 80% of exposure comes from non-drinking water sources. This default value is thus applicable to the derivation of a health-based drinking water level for PFOA.

(http://water.epa.gov/scitech/swguidance/standards/upload/2005_05_06_criteria_humanhealth_method_complete.pdf)

Therefore, in developing a health-based drinking water level for PFOA based on the Reference Dose proposed by the authors, an RSC of 20% should be applied, resulting in a water level of 0.0002 µg/L (0.2 ng/L). Based on this 5-fold lower drinking water value, the statements about the current drinking water levels for PFOA being “100-fold” or “several hundred times” too high in this paragraph and in the Abstract also need to be revised to an even higher value.


As currently written, this statement applies only to PFOA since it cannot be assumed that the same 100:1 serum:drinking water ratio applies to PFOS. If the authors conclude that current exposure limits for PFOS are also at least 100-fold too high, a justification should be provided.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Please revise to indicate that exposures were measured as serum levels, and that BMDs were calculated in terms of serum levels.

2. Abstract, Results. 1st and last sentence. Please to indicate that the benchmark doses and doses mentioned are serum levels.

3. Background, 1st paragraph, 2nd sentence.
Although PFOA may have been produced in greater quantities than PFOS, PFOS was also produced in large amounts, as discussed in (1). Please clarify to better convey this. See also Discretionary Revisions #1, below.

4. Background, 1st paragraph, 3rd sentence.
Please clarify by adding that “…widespread PFC contamination in ground and surface waters was discovered…” As discussed in (2), PFOA was detected in drinking water sources prior to the 1990s in some locations.

5. Background, 1st paragraph, 4th sentence. The source of this information appears to be Section 2.1 of Post et al. (2012) which is citation (10) in the manuscript, rather than citation (1).
Also, “44,000 ng/L” should be corrected to “>4000 ng/L.”

6. Background, 2nd paragraph, last sentence.
Please clarify to convey the intended meaning of this sentence. Are the authors saying that not enough toxicity information has been collected for a proper risk assessment? Or that sufficient toxicity information has been collected, and therefore there is no reason that a “proper risk assessment” has not been yet developed?

7. Background, 3rd paragraph, 1st sentence.
Please revise to either state that, until recently, liver was thought to be the main target organ, or alternatively to mention other important toxicological endpoints that are now known, especially developmental effects.
Also, if the chronic rat studies are mentioned, it is relevant to mention that both PFOA and PFOS caused several types of tumors in these studies.

8. Background, 3rd paragraph, 2nd sentence.
The USEPA (2005) draft PFOA risk assessment concluded that PFOA is a suggestive human carcinogen. In its review of the draft risk assessment, the USEPA Science Advisory Board (2006) disagreed and concluded that PFOA is a likely human carcinogen. If the draft EPA risk assessment is to be mentioned, the SAB’s differing conclusion should also be mentioned for completeness.
9. Background, 4th paragraph, 1st sentence.
Please revise to convey that a few U.S. states have issued limits that are intended to be protective (rather than “thought to pose little or no appreciable risk”) for chronic (or lifetime) exposure.

10. Background, 4th paragraph. Last sentence.
The cited reference (10) (Post et al., 2012) does not discuss these BMD calculations. The citation for the BMD for PFOA is: Butenhoff, J. L., Gaylor, D. W., Moore, J. A., Olsen, G. W., Rodricks, J., Mandel, J. H., & Zobel, L. R. (2004). Regul. Tox. Pharmacol. 39, 363-380. The PFOS BMD was done by the same research group, but I do not have the citation.

Also, the values cited are BMDL10 (lower confidence limit on BMD for 10% change), not BMDs.

11. Background, 5th paragraph, 1st sentence.
This sentence appears to refer to the BMDLs for liver toxicity in the preceding paragraph. These BMDLs are based on studies in primates, not rodents.

12. Background, 5th paragraph, 1st sentence.
Delete “modent”.

13. Background, 5th paragraph, 2nd sentence.
Please clarify that this refers to PFOA. To my knowledge, mammary gland development has not been evaluated for PFOS.

Also, it would be more informative to expand this sentence to say that a number of developmental effects have recently been found to be more sensitive endpoints than liver toxicity for PFOA, and, of these, mammary gland development is the most sensitive animal endpoint yet identified. (See Post et al., 2012, and citations therein).

14. Background, 5th paragraph. 3rd sentence.
Sentence should be revised to clarify that BMDLs rather than BMDs are being discussed, and that these BMDLs are for mammary gland development effects. Also, please add the citation for the BMDLs presented (Post et al., 2012).

15. Background, 5th paragraph, 4th sentence.
Please clarify the intended meaning of “If this is true,...” Does this mean, “If these BMDs are based on sound modeling,” or “If the mouse mammary gland development effects are relevant to humans,” or something else?

16. Background, 6th paragraph, last sentence.
Please remove citation (19) from this sentence. It is a study of a community exposed to drinking water contaminated with PFOA, not PFOS, and is thus not relevant to the PFOS serum levels being discussed.
17. Background, paragraph 7, 1st sentence.
I assume that the authors are referring to concern about potential immunotoxicity and developmental toxicity from exposure to environmental contaminants. Please revise to make this clear.

18. Methods, 3rd sentence.
Suggest adding that three other PFCs were measured as well and/or naming the three others, especially since the others are referred to a few sentence later as: “Of the PFCs, PFOS and PFOA showed the highest concentration....”

19. Methods. 6th sentence.
The word “concentrations” is misspelled.

20. Results, 1st paragraph. “Children, who participated in one clinical examination, but not the other,...” and “those cohort subjects, who...”
Remove commas after “children” and “those”.

21. Results, final paragraph.
This sentence is confusing as written. The BMDLs in Table 3 are being compared both to BMDLs from the same model in Table 2 and to BMDLs from other low-dose models in Table 3 in different parts of the same sentence. It would be clearer to divide into two sentences, for example as: “Using the low-dose threshold models with a flat dose-response below the lowest observed exposure levels, the BMDL results were about 5-fold higher than those from the original dose-response model for the linear curve. The results of the low-dose model for both the piecewise and the logarithmic curves approximated those obtained for the low-dose model using a linear slope (Table 3).”

22. Discussion, paragraph 5, first 2 sentences.
Please clarify that toxic mechanisms for immune effects are being discussed here. PPAR#-dependent and -independent pathways for liver, developmental, and other effects have also been studied, so the sentence is confusing as written.

Also, clarify as to whether these statements apply to PFOA, PFOS, or both.

23. Discussion, paragraph 5, 2nd to last sentence.
It appears that the study reported in (32) involved in vitro PFOS exposure of white blood cells from human volunteers. Please clarify the sentence, since, as written, it could be interpreted to mean that humans volunteered to be dosed with PFOS and that 0.1 ug/L refers to the serum level in these dosed volunteers.

24. Discussion, paragraph 5, last sentence.
As above, citation (19) is a study of PFOA, not PFOS, and is thus not relevant to the PFOS study (32).
25. Discussion, paragraph 6, 1st sentence.
It is not clear if the authors mean that the mouse model and the in vitro studies provide qualitative support that these types of effects are caused by PFCs, or support for the numeric values of the BMDLs of about 1 µg/L. Please revise to clarify.

Also, please change units (1 ng/ml to 1 µg/L) to be consistent with units used in the rest of the paper.

26. Discussion, paragraph 8, 1st sentence.
The community studied in (19) was Little Hocking, Ohio (not West Virginia).

27. Tables 1 - 3. Please change units for PFOA and PFOS serum concentrations from ng/ml to µg/L for consistency within the paper.

28. Table 3. The word “showing” is misspelled in the title of the figure.

29. Figure 1. State in the legend that the raw data are displayed on bottom of graph.

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

1. Abstract, and throughout paper.
Suggest using a term such as “serum level benchmark dose levels” instead of simply “benchmark dose levels” for clarity, since “benchmark dose levels” usually refers to administered dose (e.g. mg/kg/day) rather than serum level (internal dose).

2. Background, Paragraph 1, 2nd sentence.
Consider adding that phaseout of PFOS manufacture in the US was completed in 2002 as discussed in (1), and that PFOA manufacture and use is currently being phased out in U.S. by major manufacturers. However, environmental occurrence is expected to be ongoing due to persistence, formation from precursors, and continued production overseas and in the U.S. (see Section 1. of Post et al. (2012) and citations therein).

Especially since this is a study of a non-US population, it is suggested that the non-US production of PFOA and PFOS also be mentioned, especially since it may increase as US production is being phased out. See: USEPA. 2009. Long-Chain Perfluorinated Chemicals (PFCs) Action Plan. http://www.epa.gov/opptintr/existingchemicals/pubs/pfcs_action_plan1230_09.pdf

3. Background, 2nd Paragraph. 1st and 3rd sentence.
Since this is a study of a non-US (Faroese) population, consider citing general information on serum levels worldwide, in addition to the US information. Several review articles on this topic are cited in Section 4.1 of Post et al. (2012).
4. Background, paragraph 4, 3rd sentence. Discussion of BMD for PFOA (23 mg/L).

This reviewer has evaluated both the 6-month cynomolgus monkey PFOA study that was modeled and the basis for the BMD itself. It was concluded that the study is problematic for use in risk assessment, and also that the data from the study do not support BMD modeling. The authors should review this evaluation and consider whether to discuss the basis for this BMD that is cited in their paper.

See discussion on pages 2-4 of Post, G. 2010. Letter to North Carolina Division of Air Quality. June 1, 2010. This is a public document, but I am not sure whether or not it is posted online. It will be uploaded with this review.

5. Background, 8th paragraph, last sentence.

Please note that unintended PFOA exposure in the control groups is also an issue for toxicological studies of PFOA in experimental animals, including the study which is the basis for the BMD based on mammary gland development. See Post et al. (2012), section 6.2.3. As discussed in Section 7 of Post et al. (2012), the serum PFOA level in the control group was accounted for in the BMD modeling, and the serum level BMD and BMDL likely would have been lower if the baseline serum level had been lower.

6. Benchmark calculations, 1st paragraph, 2nd sentence.

It would be helpful to provide the clinical cut-offs for antibody levels that represent long-term protection with a citation for the interested reader.

7. Benchmark calculations, 3rd paragraph.

The EPA BMDS package (http://www.epa.gov/ncea/bmds/) provides models in addition to those used in this study for BMD modeling of continuous response. If appropriate for modeling of these human data, the authors could consider investigating the results of BMD modeling using other models, such as those available in the BMDS package.

8. Benchmark calculations, 3rd paragraph – choice of percentile for BMR.

It might be relevant to mention here that a relatively small change in this or other health-related parameters caused by exposures to environmental contaminants can potentially shift individuals on the lower end of the “clinically normal” range into the “clinically abnormal” range.


Consider adding that future research could evaluate the feasibility of developing serum-level BMDLs, Reference Doses, and health-based water levels from the data for other health endpoints associated with PFOA and PFOS in the general population and in communities with contaminated drinking water. This research would complement and extend the research presented in this paper.
Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.