Reviewer's report

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

Version: 5 Date: 19 March 2013

Reviewer: Gloria Post

Reviewer's report:

The authors have addressed many of the comments on the earlier version of the paper, and the revised version is greatly improved. However, there are some remaining comments which need to be addressed before the paper is published.

1. Background, paragraph 2, sentence 3. “Due to global dissemination of PFCs, ….” Please clarify whether “global dissemination” refers to global environmental transport, or to global exposure to PFCs from use of consumer products, or both.

2. Background, paragraph 3, sentence 1 (1st part of sentence). The liver was identified as the main target organ for PFOA, but I am not sure if this statement applies equally to PFOS, since thyroid was also identified as an important target in early studies. The authors should review Lau et al. 2007. Tox. Sci. 99: 366–394, the draft ATSDR Toxicological Profile for Perfluoroalkyls, and/or other reviews of PFOS toxicity and decide whether to revise the sentence.

3. Background, paragraph 3, sentence 1 (2nd part of sentence). Citation (10) which discusses the fact that chronic toxicity data have only been published for the rat refers only to PFOA, not PFOS. Citation (1) or Lau et al. (2007) can be cited for a discussion of the chronic data for PFOS.

4. Background, paragraph 3, sentence 2. This sentence is out of context as currently placed, and the reader might misinterpret it to mean that the Benchmark Doses discussed are based on liver data and/or chronic rat data (from the previous sentence). Since these Benchmark Doses are the basis for the Minnesota health-based water levels discussed later in the same paragraph, and they are based on the health endpoints mentioned there, this sentence should be moved to the later section of the paragraph where the basis for the MN values are discussed.

5. Background, paragraph 3, sentence 2 (end of sentence). Citation (10) does not discuss the derivation of health-based drinking water levels for PFOA or PFOS and should be removed from this sentence.

6. Background, paragraph 3, sentences 4 and 6. When discussing health-based limits for drinking water contaminants, it is very important to specify the exposure duration for which they are intended, since drinking water limits that are intended to be protective for short term exposures are not necessarily protective for chronic exposures. Also, the EPA Provisional Health Advisories were developed
in 2009; they have not been in force since 2005 as stated in the authors’ response to my earlier comments. (See citation (14) at http://water.epa.gov/action/advisories/drinking/upload/2009_01_15_criteria_drinking_pha-PFOA.pdf) Sentence 4 should be reworded to convey this information. For example, “EPA has issued provisional health advisories of 0.4 ug/L for PFOA and 0.2 ug/L for PFOS for short-term exposure to drinking water, but has not yet issued chronic drinking water guidance.” Sentence 6 could then be revised to say that, “State authorities have issued CHRONIC limits for PFC concentrations in drinking water.”

7. Background, paragraph 4, sentence 1. The recent evidence discussed is for PFOA and may not necessarily apply to other PFCs. This should be clarified.

8. Background, paragraph 4, and/or Discussion, paragraph 4. A recent study (Granum et al., 2013) of a different population of children than the one studied by the authors 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). In the response to the earlier reviewer comments, the authors state that this study differs in design from their study on vaccine antibody response and perfluorinated compounds in children, and that it does not provide data relating to benchmark dose calculations. However, this study will be of interest to readers interested in the effects of PFCs on vaccine antibody response, and it is relevant to the general information on immunotoxicity of PFCs that is presented in the Background and Discussion sections. It should be mentioned in one of these places. 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]

9. Benchmark Calculations, paragraph 3, line 1. Typo: “t” should be changed to “to”.

10. Discussion, paragraph 7, sentence 1. It should be mentioned that this BMDL is for PFOA.

11. Discussion, paragraph 8, sentence 3. The cited studies (10, 16, 35) evaluated populations in 6 water districts located in both Ohio and West Virginia. “West Virginia” should be added to the sentence.

12. Discussion, paragraph 8, sentence 2. The drinking water concentration was about 3500 ng/L (3.5 ug/L), not 3500 ng/ml.

13. Discussion, paragraph 8, sentence 2. It is stated that an approximate 100:1 serum:drinking water ratio for PFOA was observed with water containing 3500 ng/L (3.5 ug/L). This is a very high drinking water concentration, and there was initially uncertainty about whether or not it applied to lower drinking water levels that are more commonly found (See http://www.nj.gov/dep/watersupply/pdf/pfoa Dwguidance.pdf, p. 9). Since this ratio is the basis for the suggested PFOA drinking water concentration based on
the Benchmark Dose that is presented in this paper, it is important to state that this 100:1 ratio was later shown to apply to lower drinking water concentrations that are more relevant to the water concentrations being discussed in this paper (as discussed in citations (10) and (16)).

14. Discussion, paragraph 8, sentence 4. The current EPA limit for PFOA (400 ng/L) is 400-fold higher than the water concentration of 1 ng/L suggested by the authors, not 300-fold higher.

15. Tables 1-3. In the earlier comments, it was requested by two reviewers that the units for serum PFOA concentrations in these tables be changed from ng/ml to the equivalent units ug/L for consistency with the rest of the paper. The authors’ response indicates that this change was made, but the tables have not yet been changed. Please make this change.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.