Reviewer's report

Title: Inverse Association of Colorectal Cancer to Serum Levels of Perfluorooctanoate (PFOA) and Perfluorooctane Sulfonate (PFOS) in a Large Appalachian Population

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Reviewer: Leondios Leondiadis

Reviewer's report:

Major Compulsory Revisions

1. In this work, the authors are trying to prove an inverse association between the diagnosis of CRC and PFOS and PFOA, a group of compounds that have already been characterized as persistent, bioaccumulative and toxic by EFSA, Stockholm Convention and EPA, and their use has been restricted in several countries. In my opinion the paper needs major compulsory revisions

2. The analytical method applied for the determination of the perfluorinated compounds in serum is not described adequately. The reader has to follow several threads of references in order to understand that it has been developed and validated only for PFOA measurement and that only PFOA 13C-labeled standard was used. However, in the present study, it is applied for the ascertainment of PFOA, PFOS and eight other perfluorocarbon compounds (the authors do not specify exactly which other PFCs are "ascertained", or what results they obtained) without the use of the relevant PFCs 13C-labeled standard.

3. In the Materials and Methods section, the authors mention that “Detection was performed using a triple quadrupole mass spectrometer in selected monitoring mode”. In fact, selected monitoring mode is not considered an adequate approach for the quantification of PFCs, where multiple reaction monitoring (MS/MS) is necessary for adequate detection limit.

4. The authors have to report the method uncertainty in order to evaluate the differences in PFCs serum concentration.

5. PFOA levels were found to be elevated 5 times in this population compared to general US population while PFOS levels were similar to those in the general population. The authors should try to explain the unusual fact. Is the PFOA levels in contaminated drinking water so elevated comparing to the other PFCs?

6. The statistical data presented in Tables 2, 3 and 4 are unclear. To begin with, no data about the number of individuals allotted to each quartile of PFOS and PFOA concentration and the number of respective CRC diagnosed cases is included. This can possibly mislead the reader.
7. The only parameter that is presented in the Tables is the odds ratio, which however is calculated (although this merely implied and not explicitly stated) based on the erroneous assumption that the population of the lowest concentration quartile is a “non-exposed” group. The authors could refer to similar papers of this kind where statistical data are presented more clearly (Grice M.M. et al., J. Occup. Environ. Med. 2007, 49(7) 722-729 and Olsen J.W. et al., J. Occup. Environ. Med. 2004, 46(8) 837-847).

8. CRC incidence rate in the Appalachian population studied should be compared with that of the general population.

9. Although PFOA was elevated 5 times in this population compared to general US population, the inverse correlation with CRC was found modest. On the other hand PFOS was found in similar levels to those in the general population but the inverse correlation with CRC was found strong. The correlation of PFOS and CRC in the general population has to be considered as well and if it differs, it has to be commented.

10. I think that the suggestion in the conclusion that PFOA and PFOS, which are classified toxic chemicals, can potentially be used for prevention and therapy to colorectal cancer, is excessive.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests