Author's response to reviews

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

Authors:

Kim E Innes (kinnes@hsc.wvu.edu)
Jeffrey H Wimsatt (jwimsatt@hsc.wvu.edu)
Stephanie Frisbee (sfrisbee@hsc.wvu.edu)
Alan M Ducatman (aducatman@hsc.wvu.edu)

Version: 2 Date: 28 December 2012

Author's response to reviews: see over
December 23, 2012

Re: MS: 8720488797544676. ‘Inverse Association of Colorectal Cancer to Serum Levels of Perfluorooctanoate (PFOA) and Perfluorooctane Sulfonate (PFOS) in a Large Appalachian Population’

Dear Dr. Christna,

Attached please find our draft, substantially revised to address the reviewers’ concerns. Below we detail our responses to each reviewer’s comments.

Thank you again for your consideration of our paper, and we look forward to hearing from you.

Sincerely,

Kim E. Innes, MSPH, PhD
Associate Professor, Dept Epidemiology
WVU School of Public Health

AUTHOR RESPONSES TO REVIEWER COMMENTS

Reviewer 1.

Title: it should be made clear that the association relates to the prevalence [of] colorectal cancer. We now clarify this.

Abstract: The years of the study period should be reported. We now report this.

Material and Methods: The second paragraph describes the sample rather than materials and methods, so should appear in Results. In this section a reader would expect to see only inclusion and exclusion criteria. We now include all information other than eligibility criteria in the Results section as suggested.

Page 6, paragraph 2: strata for BMI and years since diagnosis should be given, for example, below or above 30kg/cm2 for BMI. This information is now given.

All sensitivity analyses and reasons for them should be described in Methods and reported in Results. Tables 2, 3 and 4 should include the number of cases and controls for each category. We now include cases and controls in each category.
Reviewer 2.

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. Although a detailed description of the PFC assay procedures is provided elsewhere and is beyond the scope of this paper, we now provide additional information in the methods section to address the reviewer’s concerns. As is now specifically stated, internal 13C standards were used for each target PFC analyzed. Specific PFCs measured are also now specified; as is now stated, only those additional 5 PFCs detectable in at least 50% of study participants were included in any ancillary analyses. However, because this paper is focused specifically on the association of colorectal cancer (CRC) to PFOA and PFOS, findings regarding the associations of other PFCs to CRC (the topic of another paper) are not presented in detail here.

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. While toxicologists often prefer a 3 reaction product confirmation using a broad product capture range more typical of GCMS methods, the ‘selected monitoring’ approach employed in this study (and used by the U.S. Centers for Disease Control in monitoring these compounds) is appropriate and valid for the target PFAs for several reasons: 1) several of these compounds are sulfated, rendering the broader scope GCMS technique less than optimal; 2) HPLC pre-selection was used; 3) specificity was a concern; and 4) 13C internal standards for each individual target PFC were employed. This, coupled with the documented intra- and inter-assay validations and the FDA standards employed by the laboratory in testing each compound together support the use, reliability, and validity of the techniques utilized for PFC analysis in this study. Furthermore, given that detectable serum levels of PFOA and PFOS were identified in over 97% of the study population, we do not believe that assay sensitivity/detection limit was a limitation for these compounds.

4. The authors have to report the method uncertainty in order to evaluate the differences in PFCs serum concentration. As we now clarify in the limitations section, that the laboratory performing the study PFC assays followed FDA approved procedures, and the assays met the FDA standard for assay precision suggest that significant method uncertainty is unlikely. In addition, as noted above, detectable serum levels of PFOA and PFOS were identified in over 97% of the study population; as now stated in the paper, for the very small percentage of participants with levels below the detectable limit (0.5 mg) of PFOA or PFOS, a value of 0.25 mg (50% of the LOD) was substituted. Moreover, it should be noted that any uncertainty/insensitivity in the assay methods would be expected to attenuate rather than inflate risk estimates, and is thus highly unlikely to explain our consistent and robust findings.

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. As explained in the methods section of this paper, the elevation in serum PFOA in the study population is due to PFOA contamination of ground and well-water by a Dupont chemical plant. As is detailed in several previous publications, Dupont began using PFOA in the manufacturing of Teflon and other fluoropolymers at the Washington Works Plant in WV beginning in the early 1950’s. PFOA was released from the facility through both aerial emissions and liquid effluent into the Ohio river, resulting in contamination of water supplies downwind and downstream from the facility. Six water districts in the Ohio Valley were most severely affected. The C8 health project, on which this study is based, was conducted as part of the settlement of a class action law suit stemming from the spill.

Is the PFOA levels in contaminated drinking water so elevated comparing to the other PFCs? As discussed above and explained in detail in previous publications, PFOA serum levels are significantly elevated in this exposed population due to the decades long PFOA releases and emissions from a Dupont Chemical plant (the Washington Works) and ensuing contamination of well and ground water in surrounding communities. As is now clarified in the paper, serum
levels of the other PFCs detectable in at least 50% of samples, including PFOS, are similar to those of the general population in the US and other western countries.

6. The statistical data presented in Tables 2, 3 and 4 are unclear.... 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. As indicated above, we now include case and control numbers of each quartile in Tables 2-4.

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. Odds ratios are a measure often used for estimating risk/association in environmental epidemiology, so we found the first part of this statement a bit puzzling. While we do examine the association of PFOS and PFOA to CRC using serum quartiles (and ventiles), an approach that is standard in epidemiological studies of PFC and other environmental exposures (e.g. see 10-14), nowhere do we state or imply that the lowest quartile of the population is a non-exposed group; in the case of PFOA, which is significantly elevated in this population, such an assumption would be clearly erroneous. In fact, the elevated PFOA exposure in this cohort provided the rationale for conducting the sensitivity analyses restricted to participants with values comparable to the general population.

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). As discussed in out paper, while these are among the few studies to examine the association of PFC exposure to cancer in human populations, these studies differ substantially from ours in size, design, study population, and exposure determination. For example, participants were chemical plant workers and were thus not comparable to our population; as serum PFC levels were not available, likely exposure in these studies was estimated by self-reported history of work in a PFOA-exposed job.

CRC incidence rate in the Appalachian population studied should be compared with that of the general population. Unfortunately, we do not have incidence data on this population, as the C8 Health Project collected data on prevalent CRC. However, overall, incidence of CRC in West Virginia (2002-2006) is higher than that of the general US population (age-adjusted rates 69.5 vs. 59 per 100,000) and ranks among the highest in the nation.15

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. While this is a good point, again, this is a study of a specific Ohio Valley population and is the first to specifically examine the relation of PFOs to CRC levels. What the reviewer proposes, while an excellent idea, would require a separate study of a different population, as information on this association in the general population is currently lacking. With respect to the differences in strength of the dose-response association, this may, as discussed in our paper, reflect a threshold effect (for PFOA), differences in the two compounds in mechanisms of actions, or other factors.

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. We disagree, especially in light of findings from our recent experimental studies. Most drugs do, in fact have adverse health effects in addition to benefits (and most would likely be classified as toxic chemicals if broadly distributed in the environment); in many cases (e.g., most anti-cancer drugs), these adverse effects can be far more severe than those documented for PFOS. PFOS is a complex compound that may in fact, have both beneficial and adverse health effects.
References


