PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A Cross Sectional Study of the Association between Serum Perfluorinated Alkyl Acid concentrations and Dental Caries Amongst US Adolescents (NHANES 1999-2012)
AUTHORS	Puttige Ramesh, Nithya; Arora, Manish; Braun, Joseph M

VERSION 1 – REVIEW

REVIEWER	Geary Olsen, Researcher 3M Company, Medical Department
REVIEW RETURNED	20-Jun-2018

GENERAL COMMENTS	Manuscript Title: The Association between Serum Perfluoroalkyl Substance Concentrations and Dental Caries Amongst US Children and Adolescents Aged 12-19 years. (NHANES 1999-2012) The reviewer's comments are provided such that the authors will (hopefully) consider them helpful to their manuscript. Summary of Paper. The manuscript authors conducted a cross-sectional analysis to examine the potential association between serum concentrations of per- and polyfluoroalkyl substances (PFASs) and the prevalence of dental caries among 2,869 adolescents aged 12-19 years enrolled in the National Health and Nutrition Examination Survey (NHANES), 1999-2012. Reviewer Comments: Abstract. Page 2: The authors conclude that a trend was observed "suggesting a decrease in the prevalence of caries with increasing serum PFNA concentrations.". However, the data appear not to support this conclusion. The adjusted ORs for PFNA quartiles relative to the referent group were: ORquartile 2 = 0.79 (95% CI: 0.63, 1.01), ORquartile 3 = 0.85 (95% CI: 0.67, 1.08), ORquartile 4 = 0.7 (95% CI: 0.55, 0.90). Only quartile 4 was statically significant. Furthermore, there was no evidence of a monotonic decrease in ORs (the OR for quartile 3 was higher than the OR for quartile 2) and a test for trend was not reported. As such, the appropriate study conclusion is that no associations were observed between the prevalence of dental caries and serum concentrations of PFOA, PFOS, PFHxS and PFNA. Also, the authors need to be clear in the abstract whether data are adjusted or unadjusted. P-value for trend should be reported here as well. Introduction. Pages 5-6:

	The Introduction would benefit from a more thorough description of the epidemiology of dental caries, including established risk factors for the disease (e.g., consumption of dietary sugars, oral hygiene practices, fluoride exposure etc.) and the known biological mechanism of dental caries. What are the specific bacteria most incriminated? The authors use the term PFAS to refer to "perfluoroalkyl substances". However, PFAS (plural PFASs) is the acronym used for perfluoroalkyl and polyfluoroalkyl substances. Given that the present study examined 4 perfluorinated alkyl acids (i.e. PFOA, PFOS, PFHxS and PFNA), the acronym PFAA would be more appropriate. (See Buck, RC et al., 2011 for an overview of the terminology used for perfluoroalkyl and polyfluoroalkyl substances). The authors state that PFASs have been in use for over 60 years; however, the authors fail to mention that the manufacturing practices of these chemicals in the United States changed over a decade ago and that serum levels of these chemicals have decreased substantially in the U.S. general population. For example, the percentage decline in geometric mean concentrations from 2000–2001 to 2015 in the U.S. general population were: PFHxS (61%); PFOS (88%); PFOA (77%) and PFNA (33%) (see Olsen GW et al., 2017). Similar declines have also been observed using NHANES data. The authors state that "PFAS have biological half-lives on the order of years in humans". However, this is not true for all PFASs; the half-lives vary widely depending on the chemical substance. For example, the serum elimination half-life for PFOA in humans is approximately 3.4 years, whereas the half-life for PFBS is approximately 30 days and for PFBA it is around 3 days. On top of page 6 the authors wrote, "PFASs are also potential endocrine disrupting chemicals including being associated with reduced levels of thyroid hormones . . ." This writing poorly reflects the toxicology and epidemiology literature surrounding the relationship between PFAAs and thyroid hormone measurements. Is there any consensus in the PFAA literature (per each PFAA studied) regarding TSH? T4? Free T4? Thyroid receptors? Measurement assay problems (negative bias) in measurement of T4 in toxicological studies? Epidemiological studies? Displacement of T4 from binding sites by PFAAs? The authors need to offer much more of a scholarly review then what is presented here. Given this is not the objective of the authors' study, the alternative is to refer to several different literature reviews that are now available in the literature or government-sponsored reports. The authors provide weak epidemiologic evidence to support their hypothesis that "PFASs would be associated with tooth demineralization". The authors cite a few studies reporting associations between PFAS exposure and various outcomes including, skeletal deformities, decreases in spinal bone mineral density in premenopausal women, reduced levels of thyroid hormones, and suppressed immune responses. However, the evidence as presented lacks breadth as well as depth in support of a biologically plausible hypothesis.

	The authors cite two cross-sectional NHANES studies that examined the potential association between PFAS serum concentrations and bone mineral density (Lin et al, 2014; Khalil et al., 2016) in support of their hypothesis that PFASs may be associated with tooth demineralization. However, the authors fail to acknowledge the inconsistent findings within each study and between the two studies. For example, Khalil et al (2016) found no association between PFOS and bone mineral density of the lumber spine, but did find a significant negative association between PFOS and bone mineral density of the total femur (postmenopausal women only) and femoral neck (men and postmenopausal women only). By contrast, Lin et al., (2014) reported a significant negative association between PFOS and bone mineral density of the lumbar spine (premenopausal women only). These two studies were also conflicting of the association between PFOA exposure and bone mineral density. Overall, there is inconsistent epidemiologic evidence of an association between PFAS exposure and bone mineral density and these two NHANES cross-sectional studies should be cautiously cited to support the authors' hypothesis that PFASs might be associated with tooth demineralization. The stated study objective (last paragraph in the Introduction Section) is to "identify the presence of any relationship between PFAS and the prevalence of dental infections in children…". However, the primary outcome of interest in this study is dental caries – not dental infections. Methods – Study Participants. Pages 6-7. NHANES data are released in 2 year cycles (also referred to as "waves"). A brief description of the specific NHANES cycles used in the present analysis should be included in this section along with the number of participants in each cycle. Methods – PFAS exposure. Page 8. Please describe how PFAS serum concentrations below the limit of detection (LOD) were quantified in the analysis. Methods – Covariates. Page 8-9. Several covariates were examined for their potential to confound the association between PFAS serum concentration and prevalence of dental caries. These include: age of the participant, sex, race, poverty to income ratio (PIR), education level of the respondent, serum cotinine, and blood lead levels. In a sensitivity analysis, dietary sugar intake and exposure to multiple PFASs was also examined. However, several important oral hygiene practices (e.g., teeth brushing, fluoride exposure, dental sealants and dental visits) were not considered. In addition, the authors did not adjust models by year or by NHANES survey cycle (which is standard practice in studies that analyze multiple NHANES cycles). Results Overall, the OR results (95% CIs) are adequately shown in the tables presented. However, as stated previously, there are no trend tests presented in the tables. What is the NHANES coefficient of variation of each of these 4 PFAAs? Given this coefficient of variation, is it rationale to have the quartile cutpoints as described? This question is especially directed at PFNA where quartile 2 ranges is 0.6-0.8 ng/mL and

	quartile 3 is 0.9-1.2 ng/mL. Do the authors have such a degree of confidence to detect an association with dental caries within such a narrow sub-ppb (i.e., part per trillion) range with PFNA? Perhaps the authors should collapse over quartiles 2 and 3 for PFNA. The authors did not recognize their findings that PFAA concentrations were, on average lower among Mexican-American children and higher among children of higher SES (PIR) status. This finding has been reported by NHANES 10 years ago. See Calafat et al. (Environ Sci Technol 2008;41:2237-2242). It is thus somewhat surprising that ethnicity or PIR were not found as important confounding factors in the association between PFAA and caries. Discussion. Pages 11-14. The authors correctly state that temporality between PFAS exposure and dental caries cannot be established due to the cross-sectional study design. However, the authors seem to confuse the issue of temporality (i.e., did PFAS exposure precede dental caries?) with reverse causation. For clarity, reverse causation refers to the situation in which the outcome precedes and causes the exposure instead of the other way around. The authors conclude "we observed no strong evidence suggesting an association between PFAS exposure and dental caries prevalence." As discussed previously, there was no evidence of an association. Therefore, the word "strong" should be removed from this concluding statement. On page 12 the authors write there is the potential for PFHxS, PFOS, PFOA, and/or PFNA "to have effects on other dental outcomes through interference with hormones that affect salivary gland function, which in turn alters salivary rate. Increased dryness (would) then result in poor oral clearance facilitating caries formation. The references offered (37, 39) have nothing to do with these 4 PFAAs. Thus, the authors must be very clear that this is only speculation on their part. Also, on page 12 the authors state that PFNA has been found to cause "robust" activation of PPARs. Thus, the authors speculate that PFNA is a PPAR agonist that results in anti-inflammatory properties thus being a possible inverse relationship between PFNA and dental caries prevalence. If this is the authors' speculative explanation, the authors fail to explain why such an inverse association was not seen with the equally strong PPAR agonists of PFOS, PFHxS, and PFOA. On page 13 the authors wrote that perfluorinated compounds are used in toothpastes to increase fluoride-enamel interactions. They cite references 48, 49, and 50. Reference 49 does not discuss this issue. The other two references are difficult to obtain. The authors should describe which "perfluorinated" compound. This reviewer is unaware of "perfluorinated compound" in any toothpaste. Is this a polyfluorinated compound that the authors are referring? The authors need to be specific as to the "perfluorinated" compound(s) and reference(s).

	The authors need to elaborate/differentiate much better the concept/difference of confounding versus reverse causation. While it is true that cross-sectional studies cannot establish temporality between outcome and exposure, an association reported could be the consequence of confounding but not reverse causation. The authors therefore need to clarify their statement (page 14) “any physiologic process that could influence the excretion of both PFAAs and (dental) caries risk could have created the inverse association with PFNA.” As explicitly written, this statement defines a confounder, not reverse causation. Reverse causation occurs when the outcome (hypothesized in this paper as dental caries) results in some hypothesized (or real) time variant changes in physiology that alters concentrations of an exposure biomarker (such as PFAA). The authors have not adequately described, let alone speculated, the time variant physiologic process created by dental caries that would alter the measurement of PFAA. This reviewer is unable to think of a reasonably hypothesized reverse causation pathway for this particular outcome. On page 14 the authors state that, although preventable, the prevalence of dental caries has not declined much in the past decade in the United States (reference 51). The trend runs counter to the marked reduction of PFOA, PFOS (lesser PFHxS, and less yet with PFNA) in the children in US population (per NHANES data, see the 2018 publication by Li et al. Environ Health Perspect). This “ecologic” analysis would support the conclusion that these four PFAAs are unlikely to be related to caries in children in the United States. General Comments. The term “children” and “adolescents” is used interchangeably throughout the manuscript. Traditionally, the term “children” is used for persons aged 2-11 years and the term “adolescents” is used for persons aged 12-19 years. Since the population under study in this manuscript included only those aged 12-19 years, the term “adolescents” should be used consistently. Under the List of Abbreviations (Page 15), the list should be presented in alphabetic order. Further, PFAS refers to “per- and polyfluoroalkyl substances”, and NHANES refers to “National Health and Nutrition Examination Survey”. This reviewer cautions the authors from offering too many speculative statements about the possible reason behind null associations. Stay within the boundaries of discussing potential study biases (selection, information, confounding). Finally, the authors should acknowledge NHANES administration and staff as it is NHHANES who collected these data (not the authors)and most importantly performed the LC-MS/MS analyses. It would even seem prudent that these authors consider an NHANES investigator(s) as a co-author of this paper, given the extent of NHANES involvement, especially someone representing the NHANES laboratory analyses that were performed.

REVIEWER	Xiangqun Ju The University of Adelaide, Australia
REVIEW RETURNED	26-Jun-2018

GENERAL COMMENTS	This manuscript is of interest to readers, and presents a well written. Some minor comments: Abstract: 1) Please provide full name for PFAS (Line 6, page 2) 2) Please add a sentence to state that PFAS are a group of compounds, and includes PFOA, PFOS, PFHxS and PFNA. Otherwise, readers don't understand why serum concentrations of PFOA, PFOS …were measured. Introduction 1) Please provide full name for PFOA (Line 3, page 6) where was introduced at the first time. Methods 1) As the nationally representative data, have them been weighted? If yes, were weighted data used for data analyses? In other words, the results were weighted? 2) Some information should be introduced in the introduction section, such as socioeconomic status (Line 39-49, page 8) 3) Please add categories for each covariates in Methods section. 4) It is not clean about variable 'Education level'. Was children's 'Education level' In Table 1, and parent/guardian's 'Education level' in supplemental Table 1? Results: 1) Where can we find these results (Line 32-42, page 10)? 2) Repeating 'Supplement Table 1' (line 19 – 20, page 11)? Tables: 1) What is the unit for 'Blood lead', and PFAS? (Table 1) 2) Please make right alignment for each sub heading (Min, 25…Max) (Table 2

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Abstract. Page 2:

1. The authors conclude that a trend was observed "suggesting a decrease in the prevalence of caries with increasing serum PFNA concentrations.". However, the data appear not to support this conclusion. The adjusted ORs for PFNA quartiles relative to the referent group were: ORquartile 2= 0.79 (95% CI: 0.63, 1.01), ORquartile 3= 0.85 (95% CI: 0.67, 1.08), ORquartile 4= 0.7 (95%CI: 0.55, 0.90). Only quartile 4 was statically significant. Furthermore, there was no evidence of a monotonic decrease in ORs (the OR for quartile 3 was higher than the OR for quartile 2) and a test for trend was not reported. As such, the appropriate study conclusion is that no associations were observed between the prevalence of dental caries and serum concentrations of PFOA, PFOS, PFHxS and PFNA. Also, the authors need to be clear in the abstract whether data are adjusted or unadjusted. P-value for trend should be reported here as well.

We agree with the reviewer's comment and have edited our conclusion to be more nuanced. While the prevalence was reduced, there was no evidence of a monotonic dose-response relation. Our results are adjusted for covariates and we did a linear trend test with the log2-transformed PFAS concentrations. The conclusion of our abstract has now been edited to say the following:
PFOA, PFOS and PFHxS were not associated with prevalence of dental caries. The prevalence of caries was reduced with increasing serum PFNA concentrations; however, these results should be interpreted cautiously given that we were unable to adjust for several factors related to oral health.

Introduction. Pages 5-6:

2. The Introduction would benefit from a more thorough description of the epidemiology of dental caries, including established risk factors for the disease (e.g., consumption of dietary sugars, oral hygiene practices, fluoride exposure etc.) and the known biological mechanism of dental caries. What are the specific bacteria most incriminated?

We agree that this information would be helpful background information. We have now included the following text to address your suggestion:
Dental caries is known to be caused by a dynamic relationship between microbiota in dental plaque, dietary carbohydrates, the acidity and consistency of saliva, and the cariogenic potential of dental plaque. A shift in the plaque concentrations of mutans streptococci and lactobacilli is one of the primary etiologic factors behind the occurrence of dental caries. Although tooth decay occurs due to biochemical process caused by the demineralization of tooth substance by these bacteria, environmental factors have also been linked to dental caries.

3. The authors use the term PFAS to refer to “perfluoroalkyl substances”. However, PFAS (plural PFASs) is the acronym used for perfluoroalkyl and polyfluoroalkyl substances. Given that the present study examined 4 perfluorinated alkyl acids (i.e. PFOA, PFOS, PFHxS and PFNA), the acronym PFAA would be more appropriate. (See Buck, RC et al., 2011 for an overview of the terminology used for perfluoroalkyl and polyfluoroalkyl substances).

We agree and have now used the acronym PFAA in our manuscript.

4. The authors state that PFASs have been in use for over 60 years; however, the authors fail to mention that the manufacturing practices of these chemicals in the United States changed over a decade ago and that serum levels of these chemicals have decreased substantially in the U.S. general population. For example, the percentage decline in geometric mean concentrations from 2000–2001 to 2015 in the U.S. general population were: PFHxS (61%); PFOS (88%); PFOA (77%) and PFNA (33%) (see Olsen GW et al., 2017). Similar declines have also been observed using NHANES data.

We now include information in the introduction to reflect this information:
Due to efforts by the United States Environmental Protection agency (EPA) and PFAA manufacturers, a steady decline in serum PFAA concentrations has been observed in the past decade. However, those who reside near industrial sites that use PFAAs in manufacturing, or military or commercial airports that use aqueous film forming foam may have elevated PFAA exposures compared to the general population.

5. The authors state that “PFAS have biological half-lives on the order of years in humans”. However, this is not true for all PFASs; the half-lives vary widely depending on the chemical substance. For example, the serum elimination half-life for PFOA in humans is approximately 3.4 years, whereas the half-life for PFBS is approximately 30 days and for PFBA it is around 3 days.

We have now reworded our statement to the following:
Some PFAS have biological half-lives on the order of years in humans, and 95% of the US population from 1999-2008 had detectable serum PFAS concentrations.

6. On top of page 6 the authors wrote, “PFASs are also potential endocrine disrupting chemicals including being associated with reduced levels of thyroid hormones . . .” This writing poorly reflects the toxicology and epidemiology literature surrounding the relationship between PFAAs and thyroid hormone measurements. Is there any consensus in the PFAA literature (per each PFAA studied) regarding TSH? T4? Free T4? Thyroid receptors? Measurement assay problems (negative bias) in

measurement of T4 in toxicological studies? Epidemiological studies? Displacement of T4 from binding sites by PFAAs? The authors need to offer much more of a scholarly review then what is presented here. Given this is not the objective of the authors' study, the alternative is to refer to several different literature reviews that are now available in the literature or government-sponsored reports.

We have now summarized findings from a recent systematic review to support our statement.

In a recent systematic review by Ballesteros et al, the authors reported consistent positive associations of maternal and adolescent serum PFAA concentrations with circulating TSH concentrations in several studies.

7. The authors provide weak epidemiologic evidence to support their hypothesis that "PFASs would be associated with tooth demineralization". The authors cite a few studies reporting associations between PFAS exposure and various outcomes including, skeletal deformities, decreases in spinal bone mineral density in premenopausal women, reduced levels of thyroid hormones, and suppressed immune responses. However, the evidence as presented lacks breadth as well as depth in support of a biologically plausible hypothesis.

Additional information from prior studies has been added to support our hypothesis.
Prior studies show that thyroid hormones influence the maturation of teeth and cause early life changes in periodontal tissues. Moreover, children and adolescents with reduced thyroid hormone levels exhibit enamel hypoplasia, causing the enamel layer of teeth to be thin and deficient, thereby making them more susceptible to caries.

8. The authors cite two cross-sectional NHANES studies that examined the potential association between PFAS serum concentrations and bone mineral density (Lin et al, 2014; Khalil et al., 2016) in support of their hypothesis that PFASs may be associated with tooth demineralization. However, the authors fail to acknowledge the inconsistent findings within each study and between the two studies. For example, Khalil et al (2016) found no association between PFOS and bone mineral density of the lumber spine, but did find a significant negative association between PFOS and bone mineral density of the total femur (postmenopausal women only) and femoral neck (men and postmenopausal women only). By contrast, Lin et al., (2014) reported a significant negative association between PFOS and bone mineral density of the lumbar spine (premenopausal women only). These two studies were also conflicting of the association between PFOA exposure and bone mineral density. Overall, there is inconsistent epidemiologic evidence of an association between PFAS exposure and bone mineral density and these two NHANES cross-sectional studies should be cautiously cited to support the authors' hypothesis that PFASs might be associated with tooth demineralization.

We have edited our statement to reflect this nuance.
In rodent studies, prenatal PFAA exposure has been linked to adverse skeletal deformities. Moreover, serum perflurooctanoic acid (PFOA) levels have been associated with a decrease in spinal bone mineral density in premenopausal women. However, inconsistencies in results were observed when different bone sites (such as lumbar spine) were assessed and by menopausal status in women.

9. The stated study objective (last paragraph in the Introduction Section) is to "identify the presence of any relationship between PFAS and the prevalence of dental infections in children...". However, the primary outcome of interest in this study is dental caries – not dental infections.

This has now been corrected to say dental caries instead of infections.

Methods – Study Participants. Pages 6-7.

10. NHANES data are released in 2 year cycles (also referred to as “waves”). A brief description of the specific NHANES cycles used in the present analysis should be included in this section along with the number of participants in each cycle.

We have now included the following information in the methods section.
NHANES datasets are released every two years in cycles and we used data collected between 1999-2012 for our primary analysis. The 2013-2014 cycle data was used for sensitivity analyses. There were 9,756-10,537 participants in each cycle. We excluded the 2001-2002 cycle because PFAA were not analyzed in individual serum samples. For our study, 10,856 adolescents age 12 to 19 years underwent a dental examination in six cycles and we restricted our analysis to 2,869 who had laboratory measurements for serum PFAA concentrations and complete covariate data. Approximately equal proportions of adolescents from each cycle contributed to our analysis.

Methods – PFAS exposure. Page 8.
11. Please describe how PFAS serum concentrations below the limit of detection (LOD) were quantified in the analysis.

We have now added information regarding this under the PFAS exposure subsection in our methods.
PFAA below the limit of detection (LOD) were quantified by dividing the LOD by the $\sqrt{2}$.

Methods – Covariates. Page 8-9.
12. Several covariates were examined for their potential to confound the association between PFAS serum concentration and prevalence of dental caries. These include: age of the participant, sex, race, poverty to income ratio (PIR), education level of the respondent, serum cotinine, and blood lead levels. In a sensitivity analysis, dietary sugar intake and exposure to multiple PFASs was also examined. However, several important oral hygiene practices (e.g., teeth brushing, fluoride exposure, dental sealants and dental visits) were not considered. In addition, the authors did not adjust models by year or by NHANES survey cycle (which is standard practice in studies that analyze multiple NHANES cycles).

We used appropriate survey weighted procedures in our analysis to account for the design of the NHANES data, which do indirectly adjust for survey cycle. The masked variance units (which are akin to the PSUs) are calculated for each year and are thus a form of adjustment for year. It is described in our statistical analysis section as follows:
To account for the complex NHANES survey design, we used the 2-year sampling weights, strata, and cluster variables to account for the complex sampling design as recommended by the National Center for Health Statistics (NCHS).

Unfortunately, we could not assess oral hygiene practices in our study since they were not measured by NHANES and we have mentioned this as a limitation in our study. We now also add a note about this in our methods section:
Though significant contributors to dental caries risk, factors such as oral hygiene practices could not be accounted for since they were not measured in these NHANES cycles.

Results
13. Overall, the OR results (95% CIs) are adequately shown in the tables presented. However, as stated previously, there are no trend tests presented in the tables.

We evaluated linear trends using the log2-transformed PFAAs concentrations as a predictor in separate multivariable linear regression models (Table 2).

14. What is the NHANES coefficient of variation of each of these 4 PFAAs? Given this coefficient of variation, is it rationale to have the quartile cutpoints as described? This question is especially directed at PFNA where quartile 2 ranges is 0.6-0.8 ng/mL and quartile 3 is 0.9-1.2 ng/mL. Do the authors have such a degree of confidence to detect an association with dental caries within such a narrow sub-ppb (i.e., part per trillion) range with PFNA? Perhaps the authors should collapse over quartiles 2 and 3 for PFNA.

The reviewer raises a valid point about the degree of variation at low levels due to lab measurement error. While this would certainly have implications at the individual level with regard to assigning someone into one category vs. another, it is a manifestation of exposure misclassification at the population level. We would expect this to be non-differential with respect to our outcomes and on average, this misclassification would be likely to attenuate our estimates towards the null. In addition, collapsing these two categories is unlikely to yield any new information that could not be gained from looking at the table results. The adjusted ORs for the 2nd and 3rd quartiles of PFNA are 0.85 and 0.79. Thus, the pooled OR would be ~ 0.83.

15. The authors did not recognize their findings that PFAA concentrations were, on average lower among Mexican-American children and higher among children of higher SES (PIR) status. This finding has been reported by NHANES 10 years ago. See Calafat et al. (Environ Sci Technol 2008;41:2237-2242). It is thus somewhat surprising that ethnicity or PIR were not found as important confounding factors in the association between PFAA and caries.

We have added information to reflect this in our introduction as well as results sections:
Mexican Americans had the highest prevalence of dental caries (67%) relative to other races and ethnicities and interestingly, the lowest median serum PFNA concentrations. Adolescents with family PIR below 1.0 (i.e., below the poverty threshold), 63% had one or more dental caries compared to those belonging to the highest category of family PIR (above 1.85, 54%). Dental caries prevalence was inversely related to the education level of the respondent.

Discussion. Pages 11-14.
16. The authors correctly state that temporality between PFAS exposure and dental caries cannot be established due to the cross-sectional study design. However, the authors seem to confuse the issue of temporality (i.e., did PFAS exposure precede dental caries?) with reverse causation. For clarity, reverse causation refers to the situation in which the outcome precedes and causes the exposure instead of the other way around.

We agree with the reviewer and have revised this to drop the mention of reverse causation. We have edited this section as follows:
Critically, establishing temporality is a concern in cross-sectional studies like this one, as we cannot determine the sequence of occurrence of PFAA exposure and caries development. Moreover, because we used serum PFAA concentrations to assess PFAA exposure, any physiologic process that influences the excretion of PFAA and caries risk could have confounded the association between PFAA and caries prevalence.

17. The authors conclude “we observed no strong evidence suggesting an association between PFAS exposure and dental caries prevalence.” As discussed previously, there was no evidence of an association. Therefore, the word “strong” should be removed from this concluding statement.

The word “strong” has now been removed.

18. On page 12 the authors write there is the potential for PFHxS, PFOS, PFOA, and/or PFNA "to have effects on other dental outcomes through interference with hormones that affect salivary gland function, which in turn alters salivary rate. Increased dryness (would) then result in poor oral clearance facilitating caries formation. The references offered (37, 39) have nothing to do with these 4 PFAAs. Thus, the authors must be very clear that this is only speculation on their part.

We now clarify that it is speculative on our part.

For instance, we speculate that PFAA may interfere with hormones that affect salivary gland function, which in turn alters salivary rate in the oral cavity. Decreased salivation leads to dryness in the mouth and poor oral clearance, thereby facilitating caries formation.

19. Also, on page 12 the authors state that PFNA has been found to cause "robust" activation of PPARs. Thus, the authors speculate that PFNA is a PPAR agonist that results in anti-inflammatory properties thus being a possible inverse relationship between PFNA and dental caries prevalence. If this is the authors' speculative explanation, the authors fail to explain why such an inverse association was not seen with the equally strong PPAR agonists of PFOS, PFHxS, and PFOA.

We speculate that this could be due to the carbon chain length of the compounds and have edited the discussion as follows:
Interestingly, some longer chain PFAA display effects indicative of antibacterial action against some microorganisms.32,50 Long chain PFAA have displayed anti fouling properties and have shown inhibitory action on the growth of algae and certain strains of bacteria in cell cultures.50 This could also explain why PFNA, the longest chain length PFAA we examined, demonstrated a trend suggesting a protective association against dental caries. We also speculate that the inverse association between PFNA and dental caries we observed may be due to the effect of this PFAA on the peroxisome proliferator–activated receptor alpha (PPARα). PPARα is a transcription factor that regulates the gene expression of enzymes and it has been shown to have anti-inflammatory properties.51 In rodent models, PFNA has been found to cause robust activation of PPARs.52 Although the four PFAA we examined have similar chemical structures and properties, the toxicokinetics of each varies with the carbon chain length.53,54 We speculate that PFNA, and not PFOA, PFOS, or PFHxS was inversely associated with decreased dental caries prevalence by causing reduced inflammation as its longer chain length is associated with more PPARα agonism compared to PFOA, PFOS, and PFHxS.

20. On page 13 the authors wrote that perfluorinated compounds are used in toothpastes to increase fluoride-enamel interactions. They cite references 48, 49, and 50. Reference 49 does not discuss this issue. The other two references are difficult to obtain. The authors should describe which "perfluorinated" compound. This reviewer is unaware of "perfluorinated compound" in any toothpaste. Is this a polyfluorinated compound that the authors are referring? The authors need to be specific as to the "perfluorinated" compound(s) and reference(s).

We have edited the references to match our statement. The evidence we gained was from a patent that stated the use of perfluorinated compounds having 7-8 carbon atoms in their dentrifice. We have edited our text to reflect this change.
Patents show that some perfluorinated compounds containing 7-8 carbon atoms are used in toothpastes to increase fluoride-enamel interactions.

21. The authors need to elaborate/differentiate much better the concept/difference of confounding versus reverse causation. While it is true that cross-sectional studies cannot

establish temporality between outcome and exposure, an association reported could be the consequence of confounding but not reverse causation. The authors therefore need to clarify their statement (page 14) “any physiologic process that could influence the excretion of both PFAAs and (dental) caries risk could have created the inverse association with PFNA.” As explicitly written, this statement defines a confounder, not reverse causation. Reverse causation occurs when the outcome (hypothesized in this paper as dental caries) results in some hypothesized (or real) time variant changes in physiology that alters concentrations of an exposure biomarker (such as PFAA). The authors have not adequately described, let alone speculated, the time variant physiologic process created by dental caries that would alter the measurement of PFAA. This reviewer is unable to think of a reasonably hypothesized reverse causation pathway for this particular outcome.

We agree with the reviewer and have revised this to drop the mention of reverse causation. We have edited this section as follows:
Critically, establishing temporality is a concern in cross-sectional studies like this one, as we cannot determine the sequence of occurrence of PFAA exposure and caries development. Moreover, because we used serum PFAA concentrations to assess PFAA exposure, any physiologic process that influences the excretion of PFAA and caries risk could have confounded the association between PFAA and caries prevalence.

22. On page 14 the authors state that, although preventable, the prevalence of dental caries has not declined much in the past decade in the United States (reference 51). The trend runs counter to the marked reduction of PFOA, PFOS (lesser PFHxS, and less yet with PFNA) in the children in US population (per NHANES data, see the 2018 publication by Li et al. Environ Health Perspect). This “ecologic” analysis would support the conclusion that these four PFAAs are unlikely to be related to caries in children in the United States.

The reviewer is correct that there is not an ecological association between PFAA levels and dental caries prevalence over time. However, this does not preclude there being a relation between PFAAs and dental caries at the individual level (i.e., ecological fallacy; see Idrovo, EHP, 2011). For example, motorcycle helmets may reduce the risk of head injuries at the individual level, but statewide motorcycle helmet laws do not since people chose not to ride their motorcycles in those states.

23. General Comments. The term “children” and “adolescents” is used interchangeably throughout the manuscript. Traditionally, the term “children” is used for persons aged 2-11 years and the term “adolescents” is used for persons aged 12-19 years. Since the population under study in this manuscript included only those aged 12-19 years, the term “adolescents” should be used consistently.

We have now changed the term “children” to “adolescent” to be consistent.

24. Under the List of Abbreviations (Page 15), the list should be presented in alphabetic order. Further, PFAS refers to “per- and polyfluoroalkyl substances”, and NHANES refers to “National Health and Nutrition Examination Survey”.

We have done this.

25. This reviewer cautions the authors from offering too many speculative statements about the possible reason behind null associations. Stay within the boundaries of discussing potential study biases (selection, information, confounding).

We have removed our discussion on reverse causation.

26. Finally, the authors should acknowledge NHANES administration and staff as it is NHHANES who collected these data (not the authors) and most importantly performed the LC-MS/MS analyses. It would even seem prudent that these authors consider an NHANES investigator(s) as a co-author of this paper, given the extent of NHANES involvement, especially someone representing the NHANES laboratory analyses that were performed.

We have now acknowledged the NHANES administrative staff as per the standard practice.
Reviewer: 2Abstract:1) Please provide full name for PFAS (Line 6, page 2)

This has been implemented. The line now reads as:
Perfluoroalkyl acids (PFAAs) are a class of anthropogenic and persistent compounds that may impact some biological pathways related to oral health. The objective of our study was to estimate the relationship between dental caries prevalence and exposure to four PFAA: perflurooctanoic acid(PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS) in a nationally representative sample of US adolescents.
2) Please add a sentence to state that PFAS are a group of compounds, and includes PFOA, PFOS, PFHxS and PFNA. Otherwise, readers don't understand why serum concentrations of PFOA, PFOS …were measured.

We have edited it as mentioned above.
Introduction1) Please provide full name for PFOA (Line 3, page 6) where was introduced at the first time.
We have done this.

Methods1) As the nationally representative data, have them been weighted? If yes, were weighted data used for data analyses? In other words, the results were weighted?

Yes, we weighted the data and this is mentioned in the statistical analysis section of our results as follows:
To account for the complex NHANES survey design, we used the 2-year sampling weights, strata, and cluster variables to account for the complex sampling design as recommended by the National Center for Health Statistics (NCHS).
2) Some information should be introduced in the introduction section, such as socioeconomic status (Line 39-49, page 8)

We agree and have now added text in two different paragraphs to include this information for both our exposure and outcome as follows:
Dental caries also disproportionately affects adolescents from low socioeconomic backgrounds.
Prior studies also report that PFAA levels are higher in men than women and those of higher socioeconomic status.
Results: Mexican Americans had the highest prevalence of dental caries (67%) relative to other races and ethnicities and interestingly, the lowest level of PFNA serum concentrations.
PFOA and PFOS concentrations were in general higher amongst males and non-Hispanic whites. They were also higher among children from wealthier families and respondents with more education.
3) Please add categories for each covariates in Methods section.

This has now been done.
4) It is not clean about variable 'Education level'. Was children's 'Education level' In Table 1, and parent/guardian's 'Education level' in supplemental Table 1?

They were both referring to the parent/guardian's education level and we have now clarified this in the footnotes for the tables.

Results: 1) Where can we find these results (Line 32-42, page 10)?

The manuscript has now been edited to direct readers to Supplemental table 1.

2) Repeating 'Supplement Table 1' (line 19 – 20, page 11)?

We have now corrected it to Supplemental table 2.

Tables:

1) What is the unit for 'Blood lead', and PFAS? (Table 1)

It is microgram/dL and the table has now been updated to state this.

2) Please make right alignment for each sub heading (Min, 25…Max) (Table 2)

This has been done.

VERSION 2 – REVIEW

REVIEWER	Geary Olsen 3M Company, Medical Department
REVIEW RETURNED	20-Nov-2018

GENERAL COMMENTS	The authors' responses are quite responsive to this reviewer's comments and questions. Congratulations to the authors' on their well-written re-submitted manuscript.

REVIEWER	Xiangqun Ju Adelaide Dental School, The University of Adelaide, Australia
REVIEW RETURNED	28-Nov-2018

GENERAL COMMENTS	General comments This revised manuscript presents a well written, and addressed my main concerns. Minor points 1) In the Method section, the second sensitivity analysis was in the 'Supplemental table 1', not in 'Supplemental table 2' (see line 47, page 10). Please change it. 2) In the Results section, these results were from Table 1 ('PFOA and PFOS concentrations were….lead concentration.' See line 32-41, page 11). Please label them.