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Title: The Association between Serum Perfluoroalkyl Substance concentrations and Dental Caries Amongst US Children and Adolescents Aged 12 to 19 years. (NHANES 1999-2012)

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Abstract:

Study Objectives: The objective of our study was to determine the relationship between PFAS exposure and dental caries prevalence in a nationally representative sample of US adolescents.

Setting/Design: We analyzed cross-sectional data from the National Health and Nutrition Examination Survey from 1999-2012 for 12-19-year-old US children.

Participants: Of the 10,856 adolescents age 12 to 19 years who had undergone a dental examination, 2,869 had dental assessments, laboratory measurements for serum PFAS concentrations, and complete covariate data and were included in our study.

Primary and secondary outcome measures: Dental caries prevalence was defined as the presence of decay or a restoration on any tooth surface, or the loss of a tooth due to tooth decay. We used multivariable logistic regression to estimate the covariate-adjusted association between serum PFAS concentrations and dental caries prevalence, and accounted for the complex survey design of the NHANES.

Results: Of 2,869 adolescents, 59% had one or more dental caries. We observed no associations between the prevalence of dental caries and serum concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), or perfluorohexane sulfonic acid (PFHxS). However, we observed a trend suggesting a decrease in the prevalence of caries with increasing serum perfluorononanoic acid (PFNA) concentrations. The odds of caries were 21% (OR: 0.79; 95% CI: 0.63, 1.01), 15% (OR:0.85; 95% CI: 0.67, 1.08), and 30% (OR:0.7; 95% CI: 0.55, 0.90) lower among children in the 2nd, 3rd, and 4th quartiles of serum PFNA concentrations compared to children in the first quartile, respectively.

Conclusion: PFOA, PFOS and PFHxS were not associated with the prevalence of dental caries. While PFNA concentrations were associated with decreased caries prevalence, the inverse

association could be confounded and additional adjustment for factors associated with higher PFAS and lower caries prevalence may attenuate it further.

Article Summary:

Strengths and Limitations of this study:

- Our study contributes to a gap in literature by examining the relationship between PFAS exposure and dental caries prevalence amongst adolescents, which to the best of our knowledge, has not been done before in published literature.
- The strengths of our study include the large sample size (2,869 participants) and the nationally representative nature of the NHANES.
- In addition, we were able to adjust for important covariates that are associated with the prevalence of dental caries and PFAS concentrations, thereby improving the strength of our inferences.
- Though our study adjusted for numerous potential confounders, misclassified or unmeasured covariates, such as variables associated with dental hygiene, could be a weakness in our methods due to the nature of data collection by NHANES in the data cycles in our study. We used serum PFAS levels to measure PFAS exposure and thus, any physiologic process that could influence the excretion of both PFAS and caries risk could have created the inverse association between PFNA and caries prevalence.
- Additionally, reverse causation is a concern in cross-sectional studies like this one, as we cannot establish temporality between PFAS exposure and caries development.

Introduction:

Poor oral health severely impacts an individual's quality of life by altering the ability to perform basic tasks such as eating or talking.[1] Of the diseases that affect the oral cavity, dental caries and periodontal infections are the most prevalent.[2] More than 91% of adults and 58% of adolescents in the US had a caries experience in 2012.[3] Children affected by dental caries have been reported to have poor growth, behavioral problems, and poor learning abilities, thus making it imperative to focus preventative efforts towards them.[4]

Although tooth decay is a biochemical process caused by the demineralization of tooth substance by bacteria, environmental factors have also been linked to dental caries.[5] Several studies have observed associations of pediatric dental caries with lead and passive tobacco smoking. [6,7] However, the role of environmental pollutants on oral health has not been adequately studied and is relatively unexplored. Children may be more sensitive to the effects of environmental toxicants on their dental health than adults due to their increased exposure to some toxicants, reduced detoxification capacity, or heightened sensitivity to environmental agents.[8]

Perfluoroalkyl substances (PFAS), are a group of compounds that have been in use for over 60 years and are predominantly used as industrial surfactants, stain repellants, and fire fighting foams.[9] Contaminated drinking water and food are the major routes of exposure, and to a lesser extent, house dust is also a minor source of PFAS exposure.[10] 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.[11,12,13,] Although there is no direct evidence available for the effect of PFAS on teeth, some indirect evidence supports the possibility of an association. Prenatal PFAS exposure has been linked to adverse skeletal deformities.[14,15] Moreover, serum

PFOA levels have been associated with a decrease in spinal bone mineral density in premenopausal women.[16,17] PFAS are also potential endocrine disrupting chemicals (EDCs), including being associated with reduced levels of thyroid hormones, which are necessary for stimulating growth plates and promoting linear growth, thereby affecting bone metabolism.[18,19] Due to the similarity in structure, chemical composition, and mineralization processes in both dentin and bone, it is plausible that PFAS could play a role in the mineralization of teeth as well.[1718] Finally, there is considerable evidence that some PFAS are immunotoxic and exposure may promote dental caries by suppressing immune responses.[20, 21, 24]

Based on this evidence we hypothesized that PFASs would be associated with tooth demineralization. Our objective was to identify the presence of any relationship between PFAS and the prevalence of dental infections in children given their potential susceptibility to environmental chemical exposures.

Methods:

Study Participants: We used a nationally representative sample of US adolescents aged 12 to 19 years. Data for this study was sourced from the National Health and Nutrition Examination Survey (NHANES) conducted from 1999 to 2012 which has a target population of non-institutionalized American civilians.[26] The 2001-2002 data was excluded since PFAS were not analyzed in individual serum samples in this cycle.

The NHANES is a cross-sectional study which combines interviews and physical examinations of children and adults living in the United States to assess their health and nutritional status. Data is collected using a complex, multi-stage probability design with over-sampling of children below the age of 5, Mexican-Americans, and non-Hispanic blacks. Information regarding interview processes, examination protocol, and sample collection can be

found elsewhere.[27] For our study, we included 10,856 adolescents age 12 to 19 years who had undergone a dental examination, amongst whom 2,869 had dental assessments, laboratory measurements for serum PFAS concentrations, and complete covariate data.

Dental Caries Assessment: A detailed report on the dental examination component of NHANES has been described in earlier studies.[28,29] Briefly, dental examinations in NHANES were performed on all participants aged 2 years or older and who did not meet the exclusion criteria such as having orofacial pain or other medical reasons, physical limitations, inability to comply, or were uncooperative.[30] Visual and tactile examination of the oral cavity were performed by trained dentists who were licensed in at least one US state. Quality control was ensured by including procedures such as having trained staff, use of standard examiners, and continuous checks on inter-examiner reliability and consistency with the standard examiner.

Our primary outcome was dental caries prevalence and it was defined as the presence of decay or a restoration on any tooth surface, or the loss of a tooth following tooth decay. All the four third molars (tooth numbers 1, 16, 17 and 32) were excluded in our analysis since caries information for these teeth were not recorded in any of the data cycles. In the data cycles 2005-2006, 2007-2008 and 2009-2010 the variables *ohxdecay* and *ohxrest* provided information about the presence of at least one decayed surface or restoration per respondent. For the remaining data cycles, a more detailed dental examination was conducted by recording the presence of caries or a restoration on each surface of the tooth. If a tooth had both decay as well a restoration, only the decay was noted. The total Decayed, Missing or Filled surfaces (DMFS) data were computed for each participant and the presence of caries was operationalized as having at least one decay or restoration per respondent to facilitate comparison with the other data

cycles. Normal eruption sequence and the age of the child were considered while evaluating DMFS for mixed dentition.

PFAS exposure**:** Serum perfluoroalkyl substance concentrations were quantified in a random subsample of participants age 12-19 years. Serum concentrations of PFOS, PFOA, perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluoroheptanoic acid, perfluorooctane sulfonamide, 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid, 2-(Nmethyl-perfluorooctane sulfonamide) acetic acid, perfluorobutane sulfonic acid, perfluorodecanoic acid, perfluoroundecanoic acid, and perfluorododecanoic acid were quantified in 100 μL of serum using a modification of the method of Kuklenyik et al (2004)[31]. This method uses automated solid-phase extraction coupled to reversed-phase high-performance liquid chromatography–tandem mass spectrometry. Since the serum concentrations of PFOA, PFOS, PFNA and PFHxS were detectable in more than 98% of the survey participants, only these substances were included in our analysis. Other perfluoroalkyl substances were not considered due to their low detection rate and lower median concentrations relative to the other four PFAS in our study.

Covariates**:** Several covariates were considered as potential confounders based on their relationship with both PFAS exposure and dental caries. Demographic variables included the age of the participant, sex and race. Poverty to income ratio (PIR) of the child's family, which is the ratio of the family income to the poverty threshold in the year of the interview, was used to assess the socioeconomic status. A review of literature suggested that the parent or guardian's education level should be considered as a potential confounder since lower education may be associated with higher caries prevalence in the child.[32] Serum cotinine and blood lead levels were also considered as potential confounders due to studies reporting an association with

dental caries.[733] Whole blood lead concentrations were measured for all participants over the age of 1 years using a previously described laboratory procedure.[33]

Statistical Analysis: Analyses were performed using SAS survey procedures (SAS Institute Inc., version 9.3). To account for the complex NHANES survey design, we used the 2-year sampling weights (*wtmec2yr*), strata, and cluster variables to account for the complex sampling design as recommended by the National Center for Health Statistics (NCHS).[34]

We started our analyses by performing univariate analysis of serum PFAS concentrations and caries prevalence. Bivariable analysis was then conducted by examining how caries prevalence and PFOA and PFOS concentrations varied by socio-demographic, environmental, and health factors. We used logistic regression analysis with a binary outcome of dental caries to examine the association between PFAS and dental caries prevalence. Using multivariable logistic regression models, we calculated adjusted prevalence odds ratios (OR) and 95% CIs for the top three quartiles of PFAS concentrations as compared to the first. We also estimated the prevalence OR of caries with each 2-fold (i.e., $\log_2$) increase in serum PFAS concentrations.

We conducted three sets of sensitivity analyses. First, using data from 2003-2012, we adjusted for the mean total sugar intake due to its strong association with dental caries.[35] Total dietary sugar intake was assessed using 24-hour food recalls conducted on two separate days in the study years 2003 through 2012 and was considered as a confounder because dietary sugar has been identified as one of the primary risk factors for the development of caries. Second, we created a single multi-pollutant model that included $\log_2$-transformed PFOA, PFOS, PFNA and PFHxS concentrations to determine if associations of one PFAS were confounded by another. Finally, using data from the years 1999-2000, 2002-2003, 2004-2005 and 2011-2012 that had detailed DMFS scores, we calculated a count ratio of carious surfaces by PFAS concentration

using Poisson regression adjusting for race/ethnicity, age, gender, education level, family poverty to income ratio (PIR), serum cotinine and blood lead levels.

Results:

Of the 2,869 participants, 1,644 (59%) had experienced one or more dental caries (Table 1). In bivariable analyses, females had a higher prevalence of caries (63%) than males (56%). Mexican Americans had the highest prevalence of dental caries (67%) relative to other races and ethnicities. Of children with family PIR below 1.0 (living below the poverty level), 63% of those belonging to this category were found to have dental caries compared to 54% of those belonging to the highest category of family PIR (above 1.85). Dental caries prevalence was inversely related to the education level of the respondent. Higher blood lead and serum cotinine concentrations were associated with higher prevalence of dental caries.

Median (range) serum PFOA, PFOS, PFNA, and PFHxS concentrations were 3.5 ng/ml (0-22), 13 ng/mL (0-116), 0.8 ng/mL (0-6.7), and 1.8ng/mL (0-82), respectively (Table 2). 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. PFOA and PFOS concentrations were also positively associated with serum cotinine and lead concentrations.

In both unadjusted and adjusted analyses, there was no association of PFOA, PFOS, and PFHxS with dental caries prevalence (Table 3). However, in unadjusted analyses, we observed a trend suggesting an inverse association between PFNA and caries prevalence where the odds of caries were 25% (OR: 0.75; 95% CI: 0.60, 0.94), 28% (OR:0.72; 95% CI: 0.59, 0.90), and 43% (OR:0.57; 95% CI: 0.46, 0.71) lower among children in the 2nd, 3rd, and 4th quartiles of serum

PFNA concentrations compared to children in the first quartile, respectively (Table 3). After adjusting for potential confounders, the odds of caries were attenuated with increasing PFNA concentrations, where children in the 2nd, 3rd, and 4th quartiles of serum PFNA concentrations had 21% (OR: 0.79; 95% CI: 0.63, 1.01), 15% (OR:0.85; 95% CI: 0.67, 1.08), and 30% (OR:0.7; 95% CI: 0.55, 0.90) lower odds of caries compared to children in the first quartile, respectively.

In sensitivity analyses adjusting for dietary sugar intake, there was no substantive change in the association between PFAS exposure and caries prevalence in the model. (Supplemental Table 1) We observed no meaningful changes when we jointly adjusting for all four PFAS in the same model (Supplemental Table 1). Though the results were not statistically significant, PFAS concentrations were generally associated with decreased DMFS counts. (Supplemental Table 2).

Discussion:

Using data from the nationally representative NHANES, we observed no evidence that serum PFOA, PFOS and PFHxS concentrations were associated with the prevalence of dental caries in 12-19-year-old US children. However, we observed a trend suggesting a decrease in the prevalence of caries with increasing serum PFNA concentrations. Sensitivity analyses also did not elicit any meaningful changes in this association.

After adjustment for potential confounders, we observed that serum PFOA, PFOS, and PFHxS concentrations were not associated with increased odds of experiencing dental caries. This could be because of a true null association between these chemicals and caries prevalence. Another reason for the null association could be due to incorrect exposure characterization by measuring serum PFAS concentrations at the wrong time window in relation to our outcome. For example, prenatal PFAS exposures may be more important in relation to tooth development

given that teeth begin developing around 6 weeks of intrauterine life.[36] There is the potential for PFAS to have effects on other dental outcomes and these warrant additional investigation. For instance, PFAS 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.[37,39] The quantity and quality of saliva in the mouth is an important factor associated with caries incidence, and the endocrine disrupting properties of PFAS may have altered the functioning of salivary glands.[38–41] However, the NHANES does not include direct measures of salivary gland function, thus limiting our investigation into this outcome.

Interestingly, some longer chain PFAS displayed effects indicative of antibacterial action against some microorganisms[22,23,25]. We observed a decrease in the prevalence of caries with increasing serum PFNA concentrations. We speculate that the inverse association between PFNA and dental caries we observed may be due to the effect of this PFAS 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.[42] In rodent models, PFNA has been found to cause robust activation of PPARs.[43] Although the four PFAS we examined have similar chemical structures and properties, the toxicokinetics of each varies with the carbon chain length.[43,44,45] 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.[46,39,47] Long chain PFAS have displayed anti fouling properties and have shown inhibitory action on the growth of algae and certain strains of bacteria in cell cultures.[25] This could also explain why PFNA demonstrated a trend suggesting a protective association

against dental caries. However, it is also possible that the protective associations we observed for PFNA are due to confounding by factors that could not be assessed in to our study, including tooth brushing habits, use of fluoridated toothpastes, and presence of dental sealants. It is possible that the observed association between serum PFNA concentration and caries prevalence would be attenuated to a null association after adjustment for this residual confounding.

To the best of our knowledge, this is the first epidemiologic study that examined the relationship between PFAS exposure and dental caries prevalence amongst adolescents. The strengths of our study include the large sample size and nationally representative nature of the NHANES. In addition, we were able to adjust for important covariates that are associated with the prevalence of dental caries and PFAS concentrations, thereby improving the strength of our inferences. Though our study adjusted for numerous potential confounders, it is possible that our results may have been confounded by misclassified or unmeasured covariates. For instance, we were unable to adjust for the presence of dental sealants or use of fluoridated water; these may be confounders due to their protective effect on teeth and potential association with PFAS or factors associated with PFAS exposure. Patents show that some perfluorinated compounds are used in toothpastes to increase fluoride-enamel interactions.[48,49,50]. Thus, individuals who brush more could have higher PFAS exposure and lower caries, which might explain the inverse association we observed. However, we could not adjust for variables associated with dental hygiene such as tooth brushing habits or use of fluoridated toothpastes since they were not assessed by NHANES in the data cycles in our study.

We were also unable to assess earlier childhood exposure to PFAS since serum PFAS concentrations were only measured in children ages 12 years and older. Another limitation in

our study is that we could not classify specific types of caries due to lack of tooth specific data in some NHANES cycles. In addition, reverse causation is a concern in cross-sectional studies like this one, as we cannot establish temporality between PFAS exposure and caries development. We used serum PFAS levels to measure PFAS exposure and thus, any physiologic process that could influence the excretion of both PFAS and caries risk could have created the inverse association between PFNA and caries prevalence.

We observed no strong evidence suggesting an association between PFAS exposure and dental caries prevalence, despite prior studies showing that PFAS is associated with reduced bone mineral density and has actions as an endocrine disrupting compound and immunotoxicant. Future studies may try to confirm the relationship between PFNA concentrations and decreased dental caries prevalence, while adjusting for additional confounding factors that we were unable to assess in our study. Though dental caries is preventable, its prevalence has not seen much of a decline in the past decade in the United States[51]. Environmental factors are overlooked in the study of oral diseases, despite knowledge of the effects of toxicants such as tetracycline and minocycline on odontogenesis for decades.[46] Therefore, future research should consider identifying the potential effect of other environmental toxicants on oral health.

List of Abbreviations:

NHANES: National Health and Nutritional Survey
PFAS: Perfluoroalkyl Substances
PFOA: Perfluorooctanoic Acid
PFOS: Perfluorooctane Sulfonic Acid
PFHxS: Perfluorohexane Sulfonic Acid
PFNA: Perfluorononanoic Acid
EDCs: Endocrine Disrupting Chemicals
DMFS: Decayed, Missing or Filled Surfaces
PIR: Poverty to Income Ratio
NCHS: National Center for Health Statistics
OR: Odds Ratio
CI: Confidence Interval
PPARα: Peroxisome Proliferator–Activated Receptor alpha

Declarations:

Ethics approval and consent to participate:

Not applicable

Data statement:

All the datasets used are freely available from the NHANES website public archive, accessible at NHANES Questionnaires, Datasets, and Related Documentation repository, [https://wwwn.cdc.gov/nchs/nhanes/Default.aspx].

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Competing interests:

The authors declare that they have no competing interests.

Author Contributions:

NR and JB were involved in study design, analysis and write up. NR was responsible for literature search, preliminary analysis and initial draft. All authors were responsible for data interpretation, and have read and approved the final manuscript.

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Table 1: Descriptive characteristics, caries prevalence, and perfluoroalkyl substance concentrations by socio-demographic, environmental, and health factors of the 2,869 12 to 19-year-old US children and adolescents. (NHANES 1999-2012)

Covariates	N (%) with >1 caries	PFOA Median (25^{th}, 75^{th})	PFOS Median (25^{th}, 75^{th})	PFNA Median (25^{th}, 75^{th})	PFHxS Median (25^{th}, 75^{th})
Overall	1644 (59)	3.1 (2.1, 4.4)	11.0 (5.9, 17)	0.9 (0.6, 1.2)	1.7 (0.9, 3.6)
Sex					
Male	824(56)	4.0 (2.7, 5.5)	15.0 (8, 25)	1.0 (0.6, 1.3)	2.1 (1.1, 4.2)
Female	820(63)	3.1 (2.1, 4.4)	12.0 (6.7, 20)	0.7 (0.5, 1.1)	1.5 (0.8, 3)
Race					
Mexican American	591(67)	3.2 (2.2, 4.6)	12.0 (6.8, 20)	0.6 (0.4, 1)	1.4 (0.8, 2.8)
Other Hispanic	118 (60)	3.1 (2.2, 4.7)	8.0 (4.6, 16)	0.9 (0.6, 1.3)	1.1 (0.6, 2.3)
Non-Hispanic white	408 (57)	3.9 (2.7, 5.3)	15.0 (8.5, 25)	0.9 (0.6, 1.3)	2.6 (1.3, 5.1)
Non-Hispanic black	429 (53)	3.6 (2.3, 5.2)	15.0 (8.7, 25)	0.9 (0.6, 1.2)	2.0 (1.1, 3.9)
Other non-Hispanic race	98 (58)	2.7 (2, 4.1)	9.5 (4.9, 19)	0.9 (0.6, 1.2)	1.6 (0.7, 3.3)
Age					
12	164 (48)	3.7 (2.5, 5.0)	14.0 (7.1, 26)	0.8 (0.5, 1.2)	2.0 (1.1, 4.3)
13	187 (50)	3.4 (2.3, 5.0)	13.0 (5.9, 23)	0.8 (0.5, 1.2)	1.7 (0.9, 3.6)
14	200 (58)	3.2 (2.3, 4.5)	12.0 (6.8, 22)	0.9 (0.6, 1.2)	1.8 (1.0, 3.4)
15	187 (58)	3.2 (2.3, 4.7)	14.0 (7.3, 21)	0.8 (0.5, 1.1)	2.0 (0.9, 3.6)
16	207 (60)	3.6 (2.3, 5.0)	13.0 (7.4, 23)	0.7 (0.5, 1.2)	1.9 (0.9, 3.7)
17	218 (65)	3.8 (2.5, 5.3)	14.0 (8.2, 24)	0.8 (0.6, 1.3)	1.8 (1.0, 3.9)
18	255 (70)	3.4 (2.3, 5.2)	14.0 (8.1, 22)	0.8 (0.5, 1.1)	1.6 (0.8, 3.6)
19	226 (67)	3.4 (2.3, 5.1)	13.0 (7.3, 22)	0.8 (0.6, 1.2)	1.7 (0.9, 3.6)
Family PIR					
<1	668 (63)	3.2 (2.2, 4.7)	12 (6.2, 20)	0.8 (0.5, 1.1)	1.6 (0.8, 3.1)
1-1.85	388 (62)	3.4 (2.3, 4.9)	14 (7.0, 22)	0.8 (0.5, 1.2)	1.8 (0.9, 3.6)
>1.85	588 (54)	3.8 (2.6, 5.3)	15 (8.7, 25)	0.9 (0.6, 1.3)	2.1 (1.1, 4.3)
Education level of respondent					
< High school	593 (63)	3.3 (2.3, 4.7)	12.0 (6.8, 20)	0.7 (0.4, 1.1)	1.4 (0.8, 2.9)
High school	403 (61)	3.6 (2.3, 5.1)	14.0 (7.4, 24)	0.8 (0.6, 1.2)	1.9 (1.0, 3.7)
> High school	576 (55)	3.7 (2.5, 5.2)	14.0 (7.5, 24)	0.9 (0.6, 1.2)	2.2 (1.1, 4.5)
Serum cotinine (ng/ml)					
<0.05	651 (55)	3.4 (2.3, 4.9)	14.0 (7.6, 23)	0.8 (0.5, 1.2)	1.7 (0.9, 3.6)
0.05 to ≤ 3	690 (60)	3.5 (2.3, 4.9)	12.0 (6.9, 23)	0.8 (0.5, 1.2)	1.9 (1.0, 3.7)
>3	303 (70)	3.8 (2.5, 5.5)	13.0 (7.2, 21)	0.8 (0.6, 1.2)	2.0 (1.1, 4.4)
Blood Lead					
<0.69	537 (57)	2.8 (1.9, 4.2)	9.7 (5.2, 17)	0.8 (0.6, 1.2)	1.7 (0.8, 3.3)
0.7 to 1.10	544 (59)	3.7 (2.5, 5.2)	14.0 (8.4, 23)	0.9 (0.6, 1.3)	1.9 (1.0, 3.9)
>1.11	563 (62)	4.0 (2.8, 5.6)	16.0 (9.5, 26)	0.7 (0.4, 1.1)	2.0 (1.0, 4.0)

Table 2: Univariate statistics of PFAS concentrations among 2,869 12 to 19-year-old US children and adolescents. (NHANES 1999-2012)

Variable	Min	25	Median	75	Max
PFOA	<0.1	2.3	3.5	4.9	22
PFOS	0.3	7.2	13	22	116
PFNA	<0.1	0.5	0.8	1.2	6.7
PFHxS	<0.1	0.9	1.8	3.7	82

Table 3: Unadjusted and adjusted prevalence odds ratio of caries by perfluoroalkyl substance concentrations among 12 to 19-year-old US children and adolescents (NHANES 1999-2012)

PFAS Quartile (range, ng/mL)	N caries (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[a]
PFOA			
0.0-2.3	427 (62)	Ref	Ref
2.4-3.5	400 (58)	0.85 (0.69, 1.06)	0.95 (0.74, 1.20)
3.6-4.9	410 (59)	0.87 (0.70, 1.05)	1.04 (0.82, 1.32)
5.0-22	407 (59)	0.86 (0.69, 1.06)	0.95 (0.74, 1.21)
Log2 PFOA	N/A	0.95 (0.87, 1.04)	1.00 (0.91, 1.12)
PFOS:			
0.0-7.2	421 (61)	Ref	Ref
7.3-13	399 (58)	0.91 (0.73, 1.12)	0.91 (0.72, 1.16)
14-22	421 (61)	1.01 (0.81, 1.25)	1.02 (0.81, 1.31)
23-116	403 (58)	0.87 (0.71, 1.09)	0.92 (0.72, 1.17)
Log2 PFOS	N/A	0.97 (0.91, 1.04)	0.99 (0.92, 1.07)
PFNA:			
0.0-0.5	467 (66)	Ref	Ref
0.6-0.8	422 (60)	0.75 (0.60, 0.94)	0.79 (0.63, 1.01)
0.9-1.2	407 (59)	0.72 (0.59, 0.90)	0.85 (0.67, 1.08)
1.3-6.7	348 (53)	0.57 (0.46, 0.71)	0.70 (0.55, 0.90)
Log2 PFNA	N/A	0.85 (0.78, 0.91)	0.93 (0.85, 1.01)
PFHxS:			
0.0-0.9	440 (64)	Ref	Ref
1.0-1.8	418 (59)	0.82 (0.66, 1.02)	0.87 (0.68, 1.10)
1.9-3.7	372 (54)	0.67 (0.54, 0.83)	0.78 (0.61, 0.99)
3.8-82	414 (60)	0.84 (0.68, 1.05)	1.04 (0.81, 1.33)
Log2 PFHS	N/A	0.95 (0.90, 1.00)	1.00 (0.94, 1.05)

a-Adjusted for: Child gender, race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead and serum cotinine levels.

Supplemental Tables:

Supplemental Table 1: Adjusted prevalence odds ratio of caries by perfluoroalkyl substance concentrations among 12 to 19-year-old US children and adolescents (2003-2012): Sensitivity analyses comparing adjustment for dietary sugar intake and multi-pollutant model of serum PFAS concentrations.

PFAS	Adjusted Model 1[a]	Adjusted-Model 2[b]	Adjusted-Model 3[c]
PFOA	0.95 (0.87, 1.04)	0.94 (0.84, 1.06)	0.93 (0.83, 1.05)
PFOS	0.93 (0.85, 1.01)	0.92 (0.85, 1.00)	0.92 (0.85, 1.02)
PFNA	0.95 (0.85, 1.05)	0.95 (0.85, 1.05)	0.95 (0.85, 1.05)
PFHxS	0.95 (0.90, 0.99)	0.96 (0.90, 1.02)	0.96 (0.90, 1.02)

[a] Adjusted for: Child Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead, and serum cotinine levels.
[b] Adjusted for: Child Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), log transformed PFOA concentration, log transformed PFOS concentration, log transformed PFNA concentration, log transformed PFHxS concentration, blood lead, and serum cotinine levels.
[c] Adjusted for: Dietary sugar intake, Child Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead, and serum cotinine levels

Supplemental Table 2: Sensitivity analysis demonstrating count ratios of surface level dental caries by log transformed PFAS concentrations among 12 to 19-year-old US children and adolescents (NHANES 1999-2014)

PFAS	Count Ratio[a]	95% CI[a]
PFOA	0.990	0.989-0.990
PFOS	0.948	0.947-0.948
PFNA	0.985	0.984-0.985
PFHxS	0.974	0.973-0.974

[a] Adjusted for: Child Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead, and serum cotinine levels.

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	3
Study design	#4	Present key elements of study design early in the paper	2, 6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	9
Study size	#10	Explain how the study size was arrived at	7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	8
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	9
	#12b	Describe any methods used to examine subgroups and interactions	9
	#12c	Explain how missing data were addressed	9
	#12d	If applicable, describe analytical methods taking account of sampling strategy	9
	#12e	Describe any sensitivity analyses	9
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	10
	#13b	Give reasons for non-participation at each stage	6
	#13c	Consider use of a flow diagram	6
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	10

	#14b	Indicate number of participants with missing data for each variable of interest	10
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	10
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
	#16b	Report category boundaries when continuous variables were categorized	10
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
Key results	#18	Summarise key results with reference to study objectives	11
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	14
Generalisability	#21	Discuss the generalisability (external validity) of the study results	14
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

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A Cross Sectional Study of the Association between Serum Perfluorinated Alkyl Acid concentrations and Dental Caries Amongst US Adolescents (NHANES 1999-2012)

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Title: A Cross Sectional Study of the Association between Serum Perfluorinated Alkyl Acid concentrations and Dental Caries Amongst US Adolescents (NHANES 1999-2012)

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Abstract:

Study Objectives: 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.

Setting/Design: We analyzed cross-sectional data from the National Health and Nutrition Examination Survey from 1999-2012 for 12-19-year-old US adolescents.

Participants: Of 10,856 adolescents age 12 to 19 years who had a dental examination, we included 2,869 with laboratory measurements for serum PFAA concentrations and complete covariate data in our study.

Primary and secondary outcome measures: Dental caries prevalence was defined as the presence of decay or a restoration on any tooth surface, or the loss of a tooth due to tooth decay. We used multivariable logistic regression to estimate the covariate-adjusted association between serum PFAA concentrations and dental caries prevalence, accounting for the complex NHANES survey design.

Results: Of 2,869 adolescents, 59% had one or more dental caries. We observed no associations between the prevalence of dental caries and serum concentrations of PFOA, PFOS, or PFHxS. The adjusted odds of caries were 21% (OR: 0.79; 95% CI:0.63, 1.01), 15% (OR:0.85; 95% CI:0.67, 1.08), and 30% (OR:0.7; 95% CI:0.55, 0.90) lower among adolescents in the 2nd, 3rd, and 4th serum PFNA concentration quartiles compared to adolescents in the first quartile, respectively. The linear trend for this association was not statistically significant.

Conclusion: 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.

Article Summary:

Strengths and limitations of this study:

- Our study contributes to a gap in literature by examining the relationship between PFAA exposure and dental caries prevalence amongst adolescents, which to the best of our knowledge, has not been examined before.
- The strengths of our study include the large sample size (2,869 participants) and the nationally representative nature of the National Health and Nutrition Examination Survey (NHANES).
- Although we adjusted for potential confounders, misclassified or unmeasured covariates, such dental hygiene, is a weakness of our study; these data were not collected in the NHANES data cycles we used.

Patient and public involvement: We used publicly available and de-identified National Health and Nutrition Examination Survey data collected by the National Center for Health Statistics for the present study. No patients were involved in the design of our study.

Introduction:

Poor oral health severely impacts an individual's quality of life by altering the ability to perform basic tasks such as eating or talking.[1] Of the diseases that affect the oral cavity, dental caries and periodontal infections are the most prevalent.[2] More than 91% of adults and 58% of adolescents in the US had a caries experience in 2012.[3] Dental caries also disproportionately affects adolescents from low socioeconomic backgrounds.[4] Children affected by dental caries have poor growth, behavioral problems, and poor learning abilities, thus making it imperative to focus preventative efforts towards reducing the risk of dental caries.[5]

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.[6] 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.[7] Several studies have observed associations of pediatric dental caries with lead and passive tobacco smoking.[8,9] However, the role of other environmental pollutants on oral health has not been adequately studied and is relatively unexplored. Children and adolescents may be more sensitive to the effects of environmental toxicants on their dental health than adults due to their increased exposure to some toxicants, reduced detoxification capacity, or heightened susceptibility to environmental agents.[10]

Perfluoroalkyl acids (PFAAs), are a group of compounds that have been in use for over 60 years and are predominantly used as industrial surfactants, stain repellants, and fire fighting foams.[11] Contaminated drinking water and food are the major routes of exposure, and to a lesser extent, house dust is also a minor source of PFAA exposure.[12,13] Some PFAA have

biological half-lives on the order of years in humans, and 95% of the US population from 1999-2008 had detectable serum PFAA concentrations.[14–16,] 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.[17] 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.[17–20] Prior studies also report that PFAA levels are higher in men than women and those of higher socioeconomic status.[21]

Although there is no direct evidence available for the effect of PFAA on dental caries, some indirect evidence supports the possibility of an association. In rodent studies, prenatal PFAA exposure has been linked to adverse skeletal deformities.[22] Moreover, serum perflurooctanoic acid (PFOA) levels have been associated with a decrease in spinal bone mineral density in premenopausal women.[23] However, inconsistencies in results were observed when different bone sites (such as lumbar spine) were assessed and by menopausal status in women.[24] PFAA are also potential endocrine disrupting chemicals (EDCs), and have been associated with reduced levels of thyroid hormones, which are necessary for stimulating growth plates and promoting linear growth, thereby affecting bone metabolism.[24,25] Due to the similarity in structure, chemical composition, and mineralization processes in both dentin and bone, it is plausible that PFAAs could play a role in the mineralization of teeth as well.[26,27] 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.[28] Prior studies show that thyroid hormones influence the maturation of teeth and cause early life changes in periodontal tissues.[29] 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.[30] Finally, there is considerable evidence that some PFAA are immunotoxic and exposure may promote dental caries by suppressing immune responses to cariogenic bacteria.[31,32]

Based on this evidence we hypothesized that PFAA exposures would be associated with tooth demineralization. Our objective was to identify the presence of any relationship between PFAA exposure and the prevalence of dental caries in adolescents given their potential heightened susceptibility to environmental chemical exposures.

Methods:

Study Participants: We used a nationally representative sample of US adolescents aged 12 to 19 years. Data for this study came from the National Health and Nutrition Examination Survey (NHANES), which recruits non-institutionalized American civilians.[33] The NHANES is a cross-sectional study which combines interviews and physical examinations of children and adults living in the United States to assess their health and nutritional status. Data is collected using a complex, multi-stage probability design with over-sampling of children below the age of 5, Mexican-Americans, and non-Hispanic blacks. Information regarding interview processes, examination protocols, and sample collection can be found elsewhere.[34,35]

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.

Dental Caries Assessment: A detailed report on the dental examination component of NHANES has been described in earlier studies.[36,37] Briefly, dental examinations in NHANES were performed on all participants aged 2 years or older and who did not meet the exclusion criteria including orofacial pain or specific medical conditions, physical limitations, inability to comply, or being uncooperative.[38] Visual and tactile examination of the oral cavity were performed by trained dentists who were licensed in at least one US state. Quality control was ensured by including procedures such as having trained staff, use of standard examiners, and continuous checks on inter-examiner reliability and consistency with the standard examiner.

Our primary outcome was dental caries prevalence and it was defined as the presence of decay or a restoration on any tooth surface, or the loss of a tooth following tooth decay. All the four third molars (tooth numbers 1, 16, 17 and 32) were excluded in our analysis since caries information for these teeth were not recorded in any of the data cycles. In the data cycles 2005-2006, 2007-2008 and 2009-2010 the variables *ohxdecay* and *ohxrest* provided information about the presence of at least one decayed surface or restoration per respondent. For the remaining data cycles, a more detailed dental examination was conducted by recording the presence of caries or a restoration on each surface of the tooth. If a tooth had both decay as well a restoration, only the decay was noted. The total Decayed, Missing or Filled surfaces (DMFS) data were computed for each participant and the presence of caries was operationalized as having at least one decay or restoration per respondent to facilitate comparison with the other data cycles. Normal eruption sequence and the age of the child were considered when evaluating DMFS for mixed dentition.

PFAA exposure: Serum PFAA concentrations were quantified in a random subsample of approximately one-third of participants age 12-19 years.[39] Serum concentrations of PFOS, PFOA, perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluoroheptanoic acid,

perfluorooctane sulfonamide, 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid, 2-(Nmethyl-perfluorooctane sulfonamide) acetic acid, perfluorobutane sulfonic acid, perfluorodecanoic acid, perfluoroundecanoic acid, and perfluorododecanoic acid were quantified in 100 μL of serum using a modification of the method of Kuklenyik et al (2005).[40] This method uses automated solid-phase extraction coupled to reversed-phase high-performance liquid chromatography–tandem mass spectrometry. Since the serum concentrations of PFOA, PFOS, PFNA and PFHxS were detectable in more than 98% of the survey participants, only these PFAA were included in our analysis. PFAA below the limit of detection (LOD) were quantified by dividing the LOD by the √2. Other perfluoroalkyl substances were not considered due to their low detection rate and lower median concentrations relative to the other four PFAA in our study.

Covariates**:** Several covariates were considered as potential confounders based on their relationship with both PFAA exposure and dental caries. Demographic variables included the age of the participant (continuous in years), sex (male vs. female) and race/ethnicity (Mexican American, Other Hispanic, Non-Hispanic white, Non-Hispanic black, vs Other non-Hispanic race). We included two measures of family socioeconomic status. First, poverty to income ratio (PIR) of the child's family, which is the ratio of the family income to the poverty threshold in the year of the interview, was used to assess household income. Second, we adjusted for the parent or guardian's education level (less than, equal to and greater than high school level of education) since lower education may be associated with higher caries prevalence in the child.[41] Serum cotinine and blood lead levels were also considered as potential confounders due to studies reporting an association between these exposures and dental caries.[8,42] Whole blood lead and serum cotinine concentrations were measured for all participants over the age of 1 years using a previously described laboratory procedure.[43] 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.

Statistical Analysis: Analyses were performed using SAS survey procedures (SAS Institute Inc., version 9.3). 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).[44]

We started our analyses by performing univariate analysis of serum PFAA concentrations and caries prevalence. Bivariable analysis was then conducted by examining how caries prevalence and PFAA concentrations varied by covariates. We used logistic regression with a binary outcome of dental caries to examine the association between PFAA and dental caries prevalence. Using multivariable logistic regression models, we calculated adjusted prevalence odds ratios (OR) and 95% CIs for the top three quartiles of PFAA concentrations as compared to the first. Linear PFAA terms were used to evaluate trends and we estimated the prevalence OR of caries with each 2-fold (i.e., $\log_2$) increase in serum PFAA concentrations.

We conducted three sets of sensitivity analyses. First, using data from 2003-2012, we adjusted for the mean total sugar intake (Supplemental table 1).[45] Total dietary sugar intake was assessed using 24-hour food recalls conducted on two separate days in the study years 2003 through 2012 and was considered as a confounder because dietary sugar has been identified as one of the primary risk factors for the development of caries. Second, we created a single multi-pollutant model that included $\log_2$-transformed PFOA, PFOS, PFNA and PFHxS concentrations to determine if associations of one PFAA was confounded by another (supplemental table 2). Finally, using data from the years 1999-2000, 2002-2003, 2004-2005, 2011-2012 and 2013-2014 that had detailed DMFS scores, we calculated a count ratio of carious surfaces by PFAA concentration using Poisson regression adjusting for race/ethnicity, age, gender,

parent/guardian education level, family poverty to income ratio (PIR), serum cotinine and blood lead levels (supplemental table 2).

Results:

Of 2,869 participants, 1,644 (59%) experienced one or more dental caries (Table 1). In bivariable analyses, females had a higher prevalence of caries (63%) than males (56%). 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. Higher blood lead and serum cotinine concentrations were associated with higher prevalence of dental caries.

Median (range) serum PFOA, PFOS, PFNA, and PFHxS concentrations were 3.5 ng/ml (0-22), 13 ng/mL (0-116), 0.8 ng/mL (0-6.7), and 1.8ng/mL (0-82), respectively (Table 2). PFOA and PFOS concentrations were in general higher among males and non-Hispanic whites. They were also higher among adolescents from wealthier families and respondents with more education. PFOA and PFOS concentrations were also positively associated with serum cotinine and lead concentrations.

In both unadjusted and adjusted analyses, there was no association of PFOA, PFOS, and PFHxS with dental caries prevalence (Table 3). However, in unadjusted analyses, we observed a trend suggesting an inverse association between PFNA and caries prevalence where the odds of caries were 25% (OR: 0.75; 95% CI: 0.60, 0.94), 28% (OR: 0.72; 95% CI: 0.59, 0.90), and 43% (OR: 0.57; 95% CI: 0.46, 0.71) lower among adolescents in the 2nd, 3rd, and 4th quartiles of serum PFNA concentrations compared to adolescents in the first quartile, respectively (Table 3). After

adjusting for potential confounders, the odds of caries were attenuated with increasing PFNA concentrations, where adolescents in the 2nd, 3rd, and 4th quartiles of serum PFNA concentrations had 21% (OR: 0.79; 95% CI: 0.63, 1.01), 15% (OR: 0.85; 95% CI: 0.67, 1.08), and 30% (OR: 0.7; 95% CI: 0.55, 0.90) lower odds of caries compared to adolescents in the first quartile, respectively.

In sensitivity analyses adjusting for dietary sugar intake, there was no substantive change in the association between PFAA concentrations and caries prevalence (Supplemental Table 1). We observed no meaningful changes when we jointly adjusting for all four PFAA in the same model (Supplemental Table 1). Though the results were not statistically significant, PFAA concentrations were generally associated with decreased DMFS counts. (Supplemental Table 2).

Discussion:

Using data from the nationally representative NHANES, we observed no evidence that serum PFOA, PFOS and PFHxS concentrations were associated with the prevalence of dental caries in 12-19-year-old US adolescents. However, we observed a trend suggesting a decrease in the prevalence of caries with increasing serum PFNA concentrations. Our sensitivity analyses did not elicit any meaningful changes in this association.

The null association that we observed of serum PFOA, PFOS, and PFHxS concentrations with dental caries prevalence could be because of a true null association. However, there are several other potential explanations. First, we may have not observed an association because we did not assess PFAA exposure during a susceptible time period of development in relation to our outcome. For example, prenatal PFAA exposures may be more important in relation to tooth development given that teeth begin developing around 6 weeks of intrauterine life.[46] Second, there is the potential for PFAA to have effects on other dental outcomes and these warrant additional investigation. 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.[47,48] The quantity and quality of saliva in the mouth is an important factor associated with caries incidence, and the endocrine disrupting properties of PFAA may have altered the functioning of salivary glands.[25,49] However, the NHANES does not include direct measures of salivary gland function, thus limiting our investigation into this outcome.

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.[52,55,56] However, it is also possible that the protective associations we observed for PFNA are due to confounding by factors that could not be assessed in to our study, including tooth brushing habits, use of fluoridated toothpastes, and presence of dental sealants. Indeed, our adjusted results were attenuated towards the null compare to unadjusted results and further adjustments for residual

confounding could completely attenuate the observed association between serum PFNA concentration and caries prevalence. Although they did not reach significance, our sensitivity analyses also showed a trend towards an inverse association between serum PFNA concentrations and caries prevalence.

To the best of our knowledge, this is the first epidemiologic study that examined the relationship between PFAA exposure and dental caries prevalence amongst adolescents. The strengths of our study include the large sample size and nationally representative nature of the NHANES. In addition, we were able to adjust for several important covariates that are associated with the prevalence of dental caries and PFAA concentrations, thereby improving the strength of our inferences. Though our study adjusted for numerous potential confounders, it is possible that our results may have been confounded by misclassified or unmeasured covariates. For instance, we were unable to adjust for the presence of dental sealants or use of fluoridated water; these may be confounders due to their protective effect on teeth and potential association with PFAA or factors associated with PFAA exposure. Patents show that some perfluorinated compounds containing 7-8 carbon atoms are used in toothpastes to increase fluoride-enamel interactions.[57] Thus, individuals who brush more could have higher PFAA exposure and lower caries, which might explain the inverse association we observed. However, we could not adjust for variables associated with dental hygiene such as tooth brushing habits or use of fluoridated toothpastes since they were not assessed in the data cycles we examined.

We were also unable to assess earlier childhood exposure to PFAA since serum PFAA concentrations were only measured in children ages 12 years and older. Another limitation in our study is that we could not classify specific types of caries due to lack of tooth specific data in some NHANES cycles. 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.

We observed no evidence suggesting an association between PFAA exposure and dental caries prevalence, despite prior studies showing that PFAA is associated with reduced bone mineral density and has actions as an endocrine disrupting compound and immunotoxicant. Future studies may try to confirm the relationship between PFNA concentrations and decreased dental caries prevalence, while adjusting for additional confounding factors that we were unable to assess in our study. Though dental caries is preventable, its prevalence has remained relatively stable for the past decade in the United States.[58] Environmental factors are overlooked risk factors in the study of oral diseases, despite knowledge of the effects of toxicants such as tetracycline and minocycline on odontogenesis for decades.[59] Therefore, future research should consider identifying the potential effect of other environmental toxicants on oral health.

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List of Abbreviations:

CI: Confidence Interval
DMFS: Decayed, Missing or Filled Surfaces
EDCs: Endocrine Disrupting Chemicals
NCHS: National Center for Health Statistics
NHANES: National Health and Nutrition Examination Survey
OR: Odds Ratio
PFAA: Perfluroinated Alkyl Acids
PFAS: Per- and polyfluoroalkyl Substances
PFHxS: Perfluorohexane Sulfonic Acid
PFNA: Perfluorononanoic Acid
PFOA: Perfluorooctanoic Acid
PFOS: Perfluorooctane Sulfonic Acid
PIR: Poverty to Income Ratio
PPARα: Peroxisome Proliferator–Activated Receptor alpha

Declarations:

Ethics approval and consent to participate:

Not applicable

Data statement:

All the datasets used are freely available from the NHANES website public archive, accessible at NHANES Questionnaires, Datasets, and Related Documentation repository, [https://wwwn.cdc.gov/nchs/nhanes/Default.aspx].

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Competing interests:

The authors declare that they have no competing interests.

Author Contributions:

NR and JB were involved in study design, analysis and write up. NR was responsible for literature search, preliminary analysis and initial draft. NR, MA and JB were responsible for data interpretation, and have read and approved the final manuscript.

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Table 1: Descriptive characteristics, caries prevalence, and perfluoroalkyl substance concentrations (ng/mL) by sociodemographic, environmental, and health factors of 2,869 age 12 to 19-year-old US adolescents. (NHANES 1999-2012)

Covariates	N (%) with >1 caries	PFOA Median (25th, 75th)	PFOS Median (25th, 75th)	PFNA Median (25th, 75th)	PFHxS Median (25th, 75th)
Overall	1644 (59)	3.1 (2.1, 4.4)	11.0 (5.9, 17)	0.9 (0.6, 1.2)	1.7 (0.9, 3.6)
Sex					
Male	824(56)	4.0 (2.7, 5.5)	15.0 (8, 25)	1.0 (0.6, 1.3)	2.1 (1.1, 4.2)
Female	820(63)	3.1 (2.1, 4.4)	12.0 (6.7, 20)	0.7 (0.5, 1.1)	1.5 (0.8, 3)
Race					
Mexican American	591(67)	3.2 (2.2, 4.6)	12.0 (6.8, 20)	0.6 (0.4, 1)	1.4 (0.8, 2.8)
Other Hispanic	118 (60)	3.1 (2.2, 4.7)	8.0 (4.6, 16)	0.9 (0.6, 1.3)	1.1 (0.6, 2.3)
Non-Hispanic white	408 (57)	3.9 (2.7, 5.3)	15.0 (8.5, 25)	0.9 (0.6, 1.3)	2.6 (1.3, 5.1)
Non-Hispanic black	429 (53)	3.6 (2.3, 5.2)	15.0 (8.7, 25)	0.9 (0.6, 1.2)	2.0 (1.1, 3.9)
Other non-Hispanic race	98 (58)	2.7 (2, 4.1)	9.5 (4.9, 19)	0.9 (0.6, 1.2)	1.6 (0.7, 3.3)
Age					
12	164 (48)	3.7 (2.5, 5.0)	14.0 (7.1, 26)	0.8 (0.5, 1.2)	2.0 (1.1, 4.3)
13	187 (50)	3.4 (2.3, 5.0)	13.0 (5.9, 23)	0.8 (0.5, 1.2)	1.7 (0.9, 3.6)
14	200 (58)	3.2 (2.3, 4.5)	12.0 (6.8, 22)	0.9 (0.6, 1.2)	1.8 (1.0, 3.4)
15	187 (58)	3.2 (2.3, 4.7)	14.0 (7.3, 21)	0.8 (0.5, 1.1)	2.0 (0.9, 3.6)
16	207 (60)	3.6 (2.3, 5.0)	13.0 (7.4, 23)	0.7 (0.5, 1.2)	1.9 (0.9, 3.7)
17	218 (65)	3.8 (2.5, 5.3)	14.0 (8.2, 24)	0.8 (0.6, 1.3)	1.8 (1.0, 3.9)
18	255 (70)	3.4 (2.3, 5.2)	14.0 (8.1, 22)	0.8 (0.5, 1.1)	1.6 (0.8, 3.6)
19	226 (67)	3.4 (2.3, 5.1)	13.0 (7.3, 22)	0.8 (0.6, 1.2)	1.7 (0.9, 3.6)
Family PIR					
<1	668 (63)	3.2 (2.2, 4.7)	12 (6.2, 20)	0.8 (0.5, 1.1)	1.6 (0.8, 3.1)
1-1.85	388 (62)	3.4 (2.3, 4.9)	14 (7.0, 22)	0.8 (0.5, 1.2)	1.8 (0.9, 3.6)
>1.85	588 (54)	3.8 (2.6, 5.3)	15 (8.7, 25)	0.9 (0.6, 1.3)	2.1 (1.1, 4.3)
Education level of respondent					
< High school	593 (63)	3.3 (2.3, 4.7)	12.0 (6.8, 20)	0.7 (0.4, 1.1)	1.4 (0.8, 2.9)
High school	403 (61)	3.6 (2.3, 5.1)	14.0 (7.4, 24)	0.8 (0.6, 1.2)	1.9 (1.0, 3.7)
> High school	576 (55)	3.7 (2.5, 5.2)	14.0 (7.5, 24)	0.9 (0.6, 1.2)	2.2 (1.1, 4.5)
Serum cotinine (ng/ml)					
<0.05	651 (55)	3.4 (2.3, 4.9)	14.0 (7.6, 23)	0.8 (0.5, 1.2)	1.7 (0.9, 3.6)
0.05 to ≤ 3	690 (60)	3.5 (2.3, 4.9)	12.0 (6.9, 23)	0.8 (0.5, 1.2)	1.9 (1.0, 3.7)
>3	303 (70)	3.8 (2.5, 5.5)	13.0 (7.2, 21)	0.8 (0.6, 1.2)	2.0 (1.1, 4.4)
Blood Lead (μg/dL)					
<0.69	537 (57)	2.8 (1.9, 4.2)	9.7 (5.2, 17)	0.8 (0.6, 1.2)	1.7 (0.8, 3.3)
0.7 to 1.10	544 (59)	3.7 (2.5, 5.2)	14.0 (8.4, 23)	0.9 (0.6, 1.3)	1.9 (1.0, 3.9)
>1.11	563 (62)	4.0 (2.8, 5.6)	16.0 (9.5, 26)	0.7 (0.4, 1.1)	2.0 (1.0, 4.0)

Table 2: Univariate statistics of PFAA concentrations among 2,869 age 12 to 19-year-old US adolescents. (NHANES 1999-2012)

Variable	Min	25	Median	75	Max
PFOA	<0.1	2.3	3.5	4.9	22
PFOS	0.3	7.2	13	22	116
PFNA	<0.1	0.5	0.8	1.2	6.7
PFHxS	<0.1	0.9	1.8	3.7	82

Table 3: Unadjusted and adjusted prevalence odds ratio of caries by perfluoroalkyl substance concentrations among age 12 to 19-year-old US adolescents (NHANES 1999-2012)

PFAA Quartile (range, ng/mL)	N caries (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[a]
PFOA			
0.0-2.3	427 (62)	Ref	Ref
2.4-3.5	400 (58)	0.85 (0.69, 1.06)	0.95 (0.74, 1.20)
3.6-4.9	410 (59)	0.87 (0.70, 1.05)	1.04 (0.82, 1.32)
5.0-22	407 (59)	0.86 (0.69, 1.06)	0.95 (0.74, 1.21)
Log_2 PFOA	N/A	0.95 (0.87, 1.04)	1.00 (0.91, 1.12)
PFOS			
0.0-7.2	421 (61)	Ref	Ref
7.3-13	399 (58)	0.91 (0.73, 1.12)	0.91 (0.72, 1.16)
14-22	421 (61)	1.01 (0.81, 1.25)	1.02 (0.81, 1.31)
23-116	403 (58)	0.87 (0.71, 1.09)	0.92 (0.72, 1.17)
Log_2 PFOS	N/A	0.97 (0.91, 1.04)	0.99 (0.92, 1.07)
PFNA			
0.0-0.5	467 (66)	Ref	Ref
0.6-0.8	422 (60)	0.75 (0.60, 0.94)	0.79 (0.63, 1.01)
0.9-1.2	407 (59)	0.72 (0.59, 0.90)	0.85 (0.67, 1.08)
1.3-6.7	348 (53)	0.57 (0.46, 0.71)	0.70 (0.55, 0.90)
Log_2 PFNA	N/A	0.85 (0.78, 0.91)	0.93 (0.85, 1.01)
PFHxS			
0.0-0.9	440 (64)	Ref	Ref
1.0-1.8	418 (59)	0.82 (0.66, 1.02)	0.87 (0.68, 1.10)
1.9-3.7	372 (54)	0.67 (0.54, 0.83)	0.78 (0.61, 0.99)
3.8-82	414 (60)	0.84 (0.68, 1.05)	1.04 (0.81, 1.33)
Log_2 PFHxS	N/A	0.95 (0.90, 1.00)	1.00 (0.94, 1.05)

a-Adjusted for: Adolescents' gender, race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead, and serum cotinine levels.

Supplemental Tables:

Supplemental Table 1: Adjusted prevalence odds ratio of caries by perfluoroalkyl substance concentrations among age 12 to 19-year-old US adolescents (2003-2012): Sensitivity analyses adjusting for dietary sugar intake and multi-pollutant model of serum PFAA concentrations.

PFAA	Adjusted Model 1[a]	Adjusted-Model 2[b]	Adjusted-Model 3[c]
PFOA	0.95 (0.87, 1.04)	0.94 (0.84, 1.06)	0.93 (0.83, 1.05)
PFOS	0.93 (0.85, 1.01)	0.92 (0.85, 1.00)	0.92 (0.85, 1.02)
PFNA	0.95 (0.85, 1.05)	0.95 (0.85, 1.05)	0.95 (0.85, 1.05)
PFHxS	0.95 (0.90, 0.99)	0.96 (0.90, 1.02)	0.96 (0.90, 1.02)

[a] Adjusted for: Adolescent's Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead, and serum cotinine levels.

[b] Adjusted for: Adolescent's Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), log transformed PFOA concentration, log transformed PFOS concentration, log transformed PFNA concentration, log transformed PFHxS concentration, blood lead, and serum cotinine levels.

[c] Adjusted for: Dietary sugar intake, Adolescent's Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead, and serum cotinine levels

Supplemental Table 2: Sensitivity analysis of count ratios of surface level dental caries by log_2 transformed PFAA concentrations among age 12 to 19-year-old US adolescents (NHANES 1999-2014)

PFAA	Count Ratio[a]	95% CI[a]
PFOA	0.990	0.989-0.990
PFOS	0.948	0.947-0.948
PFNA	0.985	0.984-0.985
PFHxS	0.974	0.973-0.974

[a] Adjusted for: Adolescent's Age, Gender, Race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, other non-Hispanic race), Education level of the parent/guardian (<12; 12; some college education), Family PIR (<1, 1-1.85, >1.85), blood lead, and serum cotinine levels.

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	3
Study design	#4	Present key elements of study design early in the paper	2, 6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	7

	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	9
Study size	#10	Explain how the study size was arrived at	7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	8
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	9
	#12b	Describe any methods used to examine subgroups and interactions	9
	#12c	Explain how missing data were addressed	9
	#12d	If applicable, describe analytical methods taking account of sampling strategy	9
	#12e	Describe any sensitivity analyses	9
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	10
	#13b	Give reasons for non-participation at each stage	6
	#13c	Consider use of a flow diagram	6
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	10

	#14b	Indicate number of participants with missing data for each variable of interest	10
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	10
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
	#16b	Report category boundaries when continuous variables were categorized	10
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
Key results	#18	Summarise key results with reference to study objectives	11
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	14
Generalisability	#21	Discuss the generalisability (external validity) of the study results	14
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16