BMJ Open

Cross-sectional study of the association between serum perfluorinated alkyl acid concentrations and dental caries among US adolescents (NHANES 1999–2012)

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To cite: Puttige Ramesh N, Arora M, Braun JM. Cross-sectional study of the association between serum perfluorinated alkyl acid concentrations and dental caries among US adolescents (NHANES 1999–2012). *BMJ Open* 2019;**9**:e024189. doi:10.1136/bmjopen-2018-024189

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-024189).

Received 14 May 2018
Revised 13 November 2018
Accepted 18 December 2018

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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: perfluorooctanoic 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 analysed cross-sectional data from the National Health and Nutrition Examination Survey from 1999 to 2012 for 12–19-year-old US adolescents.

Participants Of 10 856 adolescents aged 12 to 19 years who had a dental examination, we included 2869 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 National Health and Nutrition Examination Survey design.

Results Of 2869 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 to 1.01), 15% (OR 0.85; 95% CI 0.67 to 1.08) and 30% (OR 0.7; 95% CI 0.55 to 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.

Strengths and limitations of this study

- Our study contributes to a gap in the literature by examining the relationship between perfluoroalkyl acid exposure and dental caries prevalence among adolescents, which, to the best of our knowledge, has not been examined before.
- The strengths of our study include the large sample size (2869 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, are a weakness of our study; these data were not collected in the NHANES data cycles we used.

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 USA 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, behavioural 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 aetiological factors behind the occurrence of dental caries.[6] Although tooth decay occurs due to biochemical process caused by the demineralisation of tooth substance by these bacteria, environmental factors have also been linked to dental caries.[7] Several studies have observed associations of paediatric

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 firefighting 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 PFAAs have biological half-lives on the order of years in humans, and 95% of the US population from 1999 to 2008 had detectable serum PFAA concentrations.[14–16] Due to efforts by the US 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 with 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 perfluorooctanoic 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 mineralisation processes in both dentin and bone, it is plausible that PFAAs could play a role in the mineralisation 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 PFAAs are immunotoxic and exposure may promote dental caries by suppressing immune responses to cariogenic bacteria.[31 32]

Based on this evidence, we hypothesised that PFAA exposures would be associated with tooth demineralisation. 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-institutionalised American civilians.[33] NHANES is a cross-sectional study which combines interviews and physical examinations of children and adults living in the USA to assess their health and nutritional status. Data are collected using a complex, multistage probability design with oversampling 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 data sets are released every 2 years in cycles and we used data collected between 1999 and 2012 for our primary analysis. The 2013–2014 cycle data were used for sensitivity analyses. There were 9756–10 537 participants in each cycle. We excluded the 2001–2002 cycle because PFAAs were not analysed in individual serum samples. For our study, 10 856 adolescents aged 12 to 19 years underwent a dental examination in six cycles and we restricted our analysis to 2869 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 examinations 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 interexaminer 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 operationalised 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 perfluorooctane sulfonic acid (PFOS), PFOA, perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluoroheptanoic acid, perfluorooctane sulfonamide, 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid, 2-(N-methyl-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*.[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 PFAAs were included in our analysis. PFAAs 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 PFAAs 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 year 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, V.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 OR and 95% CIs for the top three quartiles of PFAA concentrations as compared with the first. Linear PFAA terms were used to evaluate trends and we estimated the prevalence OR of caries with each twofold (ie, $\log_2$) increase in serum PFAA concentrations.

We conducted three sets of sensitivity analyses. First, using data from 2003 to 2012, we adjusted for the mean total sugar intake (see online supplementary 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 multipollutant model that included $\log_2$-transformed PFOA, PFOS, PFNA and PFHxS concentrations to determine if associations of one PFAA was confounded by another (see online supplementary table 1. 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 PIR, serum cotinine and blood lead levels (see online supplementary table 1).

RESULTS

Of 2869 participants, 1644 (59%) experienced one or more dental caries (table 1). In bivariable analyses, women had a higher prevalence of caries (63%) than men (56%). Mexican Americans had the highest prevalence of dental caries (67%) relative to other races

Table 1 Descriptive characteristics, caries prevalence and perfluoroalkyl substance concentrations (ng/mL) by sociodemographic, environmental and health factors of 2869 aged 12–19-year-old US adolescents (National Health and Nutrition Examination Survey 1999–2012)

Covariates	N (%) with >1 caries	Perfluorooctanoic acid median (25th, 75th)	Perfluorooctane sulfonic acid median (25th, 75th)	Perfluorononanoic acid median (25th, 75th)	Perfluorohexane sulfonic acid 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 poverty to income ratio					
<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)

and ethnicities and, interestingly, the lowest median serum PFNA concentrations. Amongst adolescents with family PIR below 1.0 (ie, below the poverty threshold), 63% had one or more dental caries compared with 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.

Table 2 Univariate statistics of perfluoroalkyl acid concentrations among 2869 age 12 to 19 year-old US adolescents (National Health and Nutrition Examination Survey 1999–2012)

Variable	Min	25	Median	75	Max
Perflurooctanoic acid	<0.1	2.3	3.5	4.9	22
Perfluorooctane sulfonic acid	0.3	7.2	13	22	116
Perfluorononanoic acid	<0.1	0.5	0.8	1.2	6.7
Perfluorohexane sulfonic acid	<0.1	0.9	1.8	3.7	82

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 1.8 ng/mL (0–82), respectively (table 2). PFOA and PFOS concentrations were in general higher among men 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 (table 1).

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 to 0.94), 28% (OR 0.72; 95% CI 0.59 to 0.90) and 43% (OR 0.57; 95% CI 0.46 to 0.71) lower among adolescents in the second, third and fourth quartiles of serum PFNA concentrations compared with 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 second, third and fourth quartiles of serum PFNA concentrations had 21% (O: 0.79; 95% CI 0.63 to 1.01), 15% (OR 0.85; 95% CI 0.67 to 1.08) and 30% (OR 0.7; 95% CI 0.55 to 0.90) lower odds of caries compared with 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 (see online supplementary table 1). We observed no meaningful changes when we jointly adjusting for all four PFAA in the same model (see online supplementary table 1). Though the results were not statistically significant, PFAA concentrations were generally associated with decreased DMFS counts (see online supplementary 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.

Table 3 Unadjusted and adjusted prevalence OR of caries by perfluoroalkyl substance concentrations among aged 12–19 year-old US adolescents (National Health and Nutrition Examination Survey 1999–2012)

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

*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.
PFAA, perfluoroalkyl acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perflurooctanoic acid; PFOS, perfluorooctane sulfonic acid.

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 not have 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 antifouling 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 with 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 epidemiological study that examined the relationship between PFAA exposure and dental caries prevalence among 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 physiological 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 USA.[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.

PATIENT AND PUBLIC INVOLVEMENT

We used publicly available and de-identified NHANES data collected by the NCHS for the present study. No patients were involved in the design of our study.

Acknowledgements We would like to thank the data collection team and NHANES administration and staff for the reports made available through the NHANES website that greatly assisted us in the generation of this paper.

Contributors NPR and JMB were involved in study design, analysis and write up. NPR was responsible for literature search, preliminary analysis and initial draft.

NPR, MA and JMB were responsible for data interpretation, and have read and approved the final manuscript.

Funding A National Institutes of Environmental Health Sciences grant funded the effort of JMB (ES 024381).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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

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