Ecotoxicology and Environmental Safety 221 (2021) 112439

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Associations of low level of fluoride exposure with dental fluorosis among U.S. children and adolescents, NHANES 2015–2016

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ARTICLE INFO

Edited by Dr. Caterina Faggio

Keywords:
Fluoride
Dental fluorosis
Tap water
Plasma
Drinking water fluoridation

ABSTRACT

Drinking water fluoridation was a mid-twentieth century innovation based on the medical hypothesis that consuming low doses of fluoride at the teeth forming years provided protection against dental decays. Numerous studies showed that high level exposure to fluoride could cause dental and skeleton fluorosis. However, there was limited study focusing on the fluorosis effect of low levels of exposure to fluoride. Therefore, our study aimed to examine whether the low level of fluoride exposure (measured in blood plasma and household tap water) was associated with the risk of dental fluorosis based on data of the National Health and Nutrition Examination Survey (NHANES) 2015–2016. We analyzed data in 2098 children and adolescents who had Dean's Index scores, and water and plasma fluoride measures. The Dean's Index score was measured by calibrated dental examiners using the modified Dean's fluorosis classification system. Fluoride was measured in plasma and household tap water. In this study, we found that the rate of fluoride concentration in water above the recommended level of 0.7 mg/L was 25%, but the prevalence of dental fluorosis was 70%. Binary logistic regression adjusted for covariates showed that higher water fluoride concentrations (0.31–0.50, 0.51–0.70, > 0.70 compared 0.00–0.30) were associated with higher odds of dental fluorosis (OR = 1.48, 95% CI: 1.13–1.96, p = 0.005; OR = 1.92, 95% CI: 1.44–2.58, p < 0.001, and OR = 2.30, 95% CI: 1.75–3.07, p < 0.001, respectively). The pattern of regression between plasma fluoride and dental fluorosis was similar. Inclusion, our study showed that even low level of water or plasma fluoride exposure was associated with increased the risk of dental fluorosis. The safety of public health approach of drinking water fluoridation for global dental caries reduction are urgently needed further research.

1. Introduction

Fluoride is the ionic form of the naturally occurring fluorine element. People can consume adequate amounts of fluoride from fluoridated water, foods and beverages, and toothpaste and other dental products containing fluoride (Buzalaf, 2018; Levy et al., 2001). The anion increases the structural stability of teeth and bones through interactions with calcium phosphates (Bronckers et al., 2009). Oral exposure to fluoride primarily via consumption of fluoridated water has been shown to be associated with decreased prevalence of dental caries in children Featherstone, 1999). In response to these findings, community water fluoridation programs were developed to add fluoride to drinking water for preventing tooth decay. In 1962, the U.S. Public Health Service recommended fluoride concentrations in water of 0.7–1.2 mg/L to prevent dental decay (U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation, 2015).

Exposure to excessive fluoride levels can result in dental fluorosis, characterized by increased porosity of the subsurface enamel and well mineralized surface layer of the enamel. The water fluoride level of 2.0 mg/L is reported to be the threshold that can cause severe dental fluorosis in U.S. children (Selwitz et al., 1998), whereas Rango et al. (2014) found that the children barely had severe dental fluorosis with water fluoride concentrations < 4.0 mg/L in Ethiopian. Although with different thresholds of fluoride level for dental fluorosis, all these studies have confirmed high fluoride exposure can cause dental fluorosis (Ayoob and Gupta, 2006). However, the evidence on the potentially harmful effects of chronic exposure to low level of fluoride on children's dental development is relatively insufficient.

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https://doi.org/10.1016/j.ecoenv.2021.112439
Received 7 February 2021; Received in revised form 16 June 2021; Accepted 17 June 2021
Available online 22 June 2021

In many countries, small amounts of fluoride were added to drinking water, salt, or milk to reduce incidence of tooth decay. In the U.S., fluoridation of public water supplies was started in 1945 (U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation, 2015). Recent years, studies showed that the prevalence of dental fluorosis was increasing after water fluoridation programs (Neurath et al., 2019; Wiener et al., 2018). A more recent analysis of NHANES data in 200–2002 and 2011–2012 found that prevalence of dental fluorosis increased from 29.7% to 61.3% (Wiener et al., 2018). So, water fluoridation has become a controversial public health intervention these years (Peckham and Awofeso, 2014; Spencer and Limeback, 2018). In order to minimize the unwanted effect caused by water fluoridation, more research might be needed to reevaluate the current policy on water fluoridation programs.

In the U.S., fluoridation is not required by the U.S. Environmental Protection Agency (EPA), which is prohibited by the Safe Drinking Water Act from requiring the addition of any substance to drinking water for preventive health care purposes. The Centers for Disease Control and Prevention (CDC), which is one of the major operating components of HHS, provides recommendations about the optimal levels of fluoride in drinking water. A large number of studies have reported that adequate fluoride intakes can reduce the risk of dental decays (Iheozor-Ejiofor et al., 2015; Slade et al., 2018), but more and more studies showed that low level of fluoride exposure was also related with some adverse effects, such as neurotoxic to children (Agalakova and Nadei, 2020; Bai et al., 2020; Bashash et al., 2017; Malin et al., 2019a,b). They showed that low level of fluoride exposure was related with decreased IQ scores in children (Agalakova and Nadei, 2020). Another study also reported fluoride from chronic systemic exposure accumulates highly in the pineal gland, which might contribute to changes in sleep cycle regulation and sleep behaviors (Malin et al., 2019a). Dental fluorosis was the most common adverse effect caused by excessive fluoride exposure, but the dose-effect relationship between low level of fluoride and dental fluorosis were still unclear. Therefore, our study aimed to examine whether the recommend fluoride exposure (measured in blood plasma and household tap water) was still associated with dental fluorosis. This study is helpful to understanding the adverse effects of fluoride exposure and balancing the benefits with any potential risks.

2. Materials and methods

2.1. Participants

This study utilized data from the National Health and Nutrition Examination Survey (NHANES) collected from 2015 to 2016, which included both dental fluorosis clinical assessment and fluoride biomonitoring data. The NHANES is conducted biennially to collect the nationally representative sample by the Centers for Disease Control and Prevention and designed to assess health and nutrition status of people of all ages living in the U.S. Details of the NHANES research procedures are available on the NHANES website (Centers for Disease Control and Prevention, 2020). In the use of the data, we have completely followed the "data use restrictions" (Centers for Disease Control and Prevention, 2021) and ensured the data only used for statistical analysis or reporting purposes.

Dental fluorosis clinical assessment was assessed among 3478 participants aged 6–29 years. Plasma fluoride concentrations were measured among 2547 participants aged 6–19 years and tap water fluoride concentrations were measured among 4070 participants aged 0–19 years. Our analysis included children and adolescents aged 6–19 years because these participants had both fluoride measurements and dental fluorosis assessments. Our sample included participants who had fluoride measurements, dental fluorosis assessment and complete data for all covariates and outcomes. There were 2098 participants who met inclusion criteria for analyses. Of those, 1808 participants had plasma fluoride levels and 2071 participants had water fluoride levels. Participant selection was depicted in Fig. S1. Supplemental Table S1 compared demographic characteristics of the current overall study sample (n = 2098) and all participants ages 6–19 over the same years (NHANES 2015–2016).

2.2. Dental fluorosis assessment

The dental fluorosis clinical assessment was conducted at the NHANES mobile examination center (MEC) by dental examiners, who were dentists (D.D.S. or D.M.D.) licensed in at least one U.S. state. Each tooth was scored according to the Dean's Fluorosis Index (DFI) and assigned one of the DFI disease severity categories, based on the area of the tooth surface with visible fluorosis and presence of pitting (NHANES Dental Examiners Procedures Manual, 2016). Six categories were used for tooth assessment: normal (translucent, smooth, glossy, pale creamy white, DFI = 0), questionable (slight aberrations, a few white spots, DFI = 0.5), very mild fluorosis (less than 25% of tooth has small, white areas, DFI = 1), mild fluorosis (between 25% and 50% of the tooth has white areas, DFI = 2), moderate fluorosis (50% or more of the tooth with all surfaces involved, with or without brown stains, DFI = 3), or severe fluorosis (all enamel is involved and has discrete or confluent pitting, DFI = 4) (NHANES Dental Examiners Procedures Manual, 2016). Missing teeth, deciduous (primary) teeth, permanent teeth not fully erupted, and teeth in which more than one-half of the visible surface area was obscured by a restoration, caries, or orthodontic appliance were not assessed. A tooth having a non-fluoride opacity was assessed as non-fluoride opacity. The basis for classifying a person's fluorosis status was the categorization of the two most affected teeth. The lesser affected tooth was to be used to identify the person's status if the two most affected teeth were not equally affected (NHANES Dental Examiners Procedures Manual, 2016).

2.3. Plasma fluoride measures

Plasma fluoride levels were influenced by many factors, including total fluoride intake, type of intake, renal function, rate of metabolism, etc. Fluoride concentrations were measured in blood plasma samples (Centers for Disease Control and Prevention, 2017b). Plasma samples were processed, stored, and shipped to the College of Dental Medicine, Georgia Regents University, Augusta, GA for analysis. The ion-specific electrode and hexamethyldisiloxane (HMDS) method was used to measure the plasma fluoride concentrations. Plasma fluoride was measured in duplicate using the same sample and the average of two results was employed. The lower limit of detection (LLOD) for plasma fluoride was 0.25 nmol. Approximately 68.76% (1475/2145) of detected participants in NHNES 2015–2016, had values at or above the LLOD for plasma fluoride. For analytes with analytic results below LLOD, an imputed fill value (0.18), which was the LLOD divided by the square root of 2, was assigned in the analyte results field.

2.4. Water fluoride measures

Fluoride concentrations in water samples were measured electrometrically using the ion-specific electrode (Centers for Disease Control and Prevention, 2017a). Water samples are processed, stored, and shipped to the College of Dental Medicine, Georgia Regents University, Augusta, GA for analysis. Water fluoride was measured in duplicate using the same sample and the average of two results was employed. The lower limit of detection (LLOD) for water fluoride was 0.1 mg/L. Approximately 87.66% (3495/3987) of detected participants in NHNES 2015–2016, had values at or above the LLOD for water fluoride. For analytes with analytic results below LLOD, an imputed fill value (0.07), which was the LLOD divided by the square root of 2, was assigned in the analyte results field.

2.5. Covariates

Covariates were determined according to the prior empirical evidence associated with fluoride exposure and dental fluorosis. They included: age, gender, body mass index, race/ethnicity, the ratio of family income to poverty, and season of sample collection. Questionnaires were used to collect demographic of age (yrs.), sex (male, female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, non-Hispanic Asian, other race), six-month time period when surveyed (November 1 through April 30, May 1 through October 31) and the ratio of family income to poverty. BMI and BMI categories (underweight, normal weight, overweight, and obese) were collected from body measure data.

2.6. Statistical analyses

Means and proportions were calculated for descriptive analyses of demographic variables as well as fluoride exposure and dental fluorosis measures. A Pearson correlation examined the relationship between logarithm (base 10)-transformed plasma and water fluoride concentrations. Dental fluorosis was identified according to DFI score, which was defined no fluorosis (DFI $\leq$ 0.5) and fluorosis (DFI $\geq$ 1). To examine the relationship between water fluoride exposure and dental fluorosis, water fluoride (mg/L) levels was transformed into a 4-category variable, which was: 0.00–0.30 (0 = reference level), 0.31–0.50 (1 = level 1), 0.51–0.70 (2 = level 2), and > 0.70 (3 = level 3). To examine the relationship between plasma fluoride exposure and dental fluorosis, plasma fluoride (μmol/L) levels was transformed into a 4-category variable, which was: 0.00–0.30 (0 = reference level), 0.31–0.40 (1 = level 1), 0.41–0.50 (2 = level 2), and > 0.50 (3 = level 3). Binary logistic regression analyses were used to determine the association between fluoride exposure and the occurrence of dental fluorosis, controlling for age, sex, race/ethnicity, BMI categories, the ratio of family income to poverty and six-month time period when surveyed. Data analysis was conducted with r software (R version 4.0.2). The two-sided p values < 0.05 were statistically significant.

3. Results

3.1. Demographic characteristics

Demographic characteristics were presented in Table 1. Table S1 compared demographics between current study participants and all participants aged 6–19 years in NHANES 2015–2016. The number of overall group was 2098 with an average age of 12.19 years, including 1054 boys and 1044 girls. Among the 2098 participants, 1808 subjects had plasma fluoride concentrations and 2071 had water fluoride concentrations. The proportions of subjects in variables including age categories, sex, BMI categories, race, six-month time period when surveyed, were similar across overall group, plasma fluoride sample group, and water fluoride sample group.

3.2. Fluoride levels

Descriptive statistics for water fluoride levels and plasma fluoride levels were presented in Table 2. Geometric mean of household tap water fluoride concentration was 0.33 mg/L, which was below the U.S. Public Health Service recommended concentration of 0.7 mg/L (U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation, 2015). However, values between the 75th and 95th percentiles were above this level ranging from 0.71 to 1.02 mg/L. The water fluoride concentrations in males were comparable with those in females, but fluoride levels in plasma in males were higher than those in females (Table 2, Fig. S2). Both the water and plasma fluoride levels in children were higher than those in adolescents (Table 2, Fig. S3). Fluoride concentrations in plasma and tap water were light positively correlated (r = 0.41, p < 0.001), which presented in Fig. 1. The correlation patterns in subgroups males and females were similar (Fig. S4).

Table 1
Demographic characteristics of selected samples in NHANES 2015–2016.

Demographic characteristic	Overall sample n = 2098	Plasma fluoride sample n = 1808	Water fluoride sample n = 2071
Age (yrs.); mean (SD)	12.19 (3.77)	12.37 (3.78)	12.18 (3.77)
Age categories; N (%)			
Children (6–11 yrs.)	995 (47.43%)	819 (45.30%)	985 (47.56%)
Adolescents (12–19 yrs.)	1103 (52.57%)	989 (54.70%)	1086 (52.44%)
Sex; N (%)			
Male	1054 (50.24%)	917 (50.72%)	1038 (50.12%)
Female	1044 (49.76%)	891 (49.28%)	1033 (49.88%)
BMI; mean (SD)	21.88 (6.02)	22.12 (6.13)	21.88 (6.02)
BMI Categories; N (%):			
Underweight	57 (2.72%)	44 (2.43%)	56 (2.70%)
Normal Weight	1203 (57.34%)	1029 (56.91%)	1186 (57.27%)
Overweight	374 (17.83%)	328 (18.14%)	369 (17.82%)
Obese	464 (22.12%)	407 (22.51%)	460 (22.21%)
Race/ethnicity			
Mexican American; N (%)	456 (21.73%)	417 (23.1%)	451 (21.78%)
Other Hispanic	254 (12.11%)	232 (12.8%)	250 (12.07%)
Non-Hispanic White	612 (29.17%)	519 (28.7%)	601 (29.02%)
Non-Hispanic Black	461 (21.97%)	376 (20.8%)	457 (22.07%)
Non-Hispanic Asian	181 (8.63%)	158 (8.7%)	179 (8.64%)
Other Race-Including Multi-Racial	134 (6.39%)	106 (5.9%)	133 (6.42%)
Ratio of family income to poverty; mean (SD)	2.06 (1.49)	2.03 (1.48)	2.05 (1.49)
Six month time period when surveyed			
November 1 through April 30	984 (46.90%)	851 (47.1%)	972 (46.93%)
May 1 through October 31	1114 (53.10%)	957 (52.9%)	1099 (53.07%)

3.3. Dental fluorosis

The proportion of dental fluorosis severity by different fluoride levels in drinking water and plasma was presented in Tables 3 and 4. Generally, the proportion of participants had normal teeth was relatively low, which was just 13%. Compared with the lowest fluoride level group, severity of fluorosis increased with higher exposure to fluoride, although there were a few exceptions. For example, those exposed to > 0.70 mg/L of water fluoride had less severe fluorosis than those exposed to 0.00–0.30 mg/L (Table 4), which might just because the number of participants with severe fluorosis was too less.

3.4. Regression analysis between fluoride levels and dental fluorosis

Regression results for fluoride levels and fluorosis were presented in Table 5 and adjusted variables in the regression were presented in Tables S4 and S5. Binary logistic regression adjusted for covariates showed that higher water fluoride concentrations (0.31–0.50, 0.51–0.70, > 0.70 compared 0.00–0.30) were associated with higher odds of dental fluorosis (OR = 1.48, 95% CI: 1.13–1.96, p = 0.005; OR = 1.92, 95% CI: 1.44–2.58, p < 0.001, and OR = 2.30, 95% CI: 1.75–3.07, p < 0.001, respectively). The pattern of regression between plasma fluoride and dental fluorosis was similar, which showed the higher plasma fluoride concentrations (0.31–0.40, 0.41–0.50, > 0.50 compared 0.00–0.30) were associated with higher odds of dental fluorosis (OR = 1.49, 95% CI: 1.14–1.96, p = 0.004; OR = 1.61, 95% CI: 1.15–2.29, p = 0.007, and OR = 1.64, 95% CI: 1.18–2.28, p = 0.003, respectively). We also further explored regression analysis for fluoride levels and

Table 2
Descriptive statistics of fluoride exposure levels.

Measure	Number	Arithmetic mean (standard deviation)	Geometric mean	Median	5th percentile	25th percentile	75th percentile	95th percentile
Water fluoride (mg/L)								
All	2071	0.46 (0.40)	0.33	0.44	0.07	0.16	0.70	1.02
Male	1038	0.48 (0.41)	0.33	0.44	0.07	0.16	0.70	1.04
Female	1033	0.47 (0.38)	0.33	0.44	0.07	0.17	0.69	1.00
Children	985	0.52(0.44)	0.36	0.47	0.07	0.18	0.72	1.12
Adolescents	1086	0.43(0.35)	0.31	0.37	0.07	0.15	0.68	0.86
Plasma fluoride (μmol/L)								
All	1808	0.35 (0.22)	0.31	0.30	0.18	0.18	0.41	0.71
Male	917	0.36 (0.19)	0.32	0.32	0.18	0.18	0.43	0.70
Female	891	0.34 (0.25)	0.29	0.29	0.18	0.18	0.39	0.71
Children	819	0.38 (0.24)	0.33	0.33	0.18	0.25	0.45	0.73
Adolescents	989	0.32 (0.20)	0.29	0.28	0.18	0.28	0.38	0.66

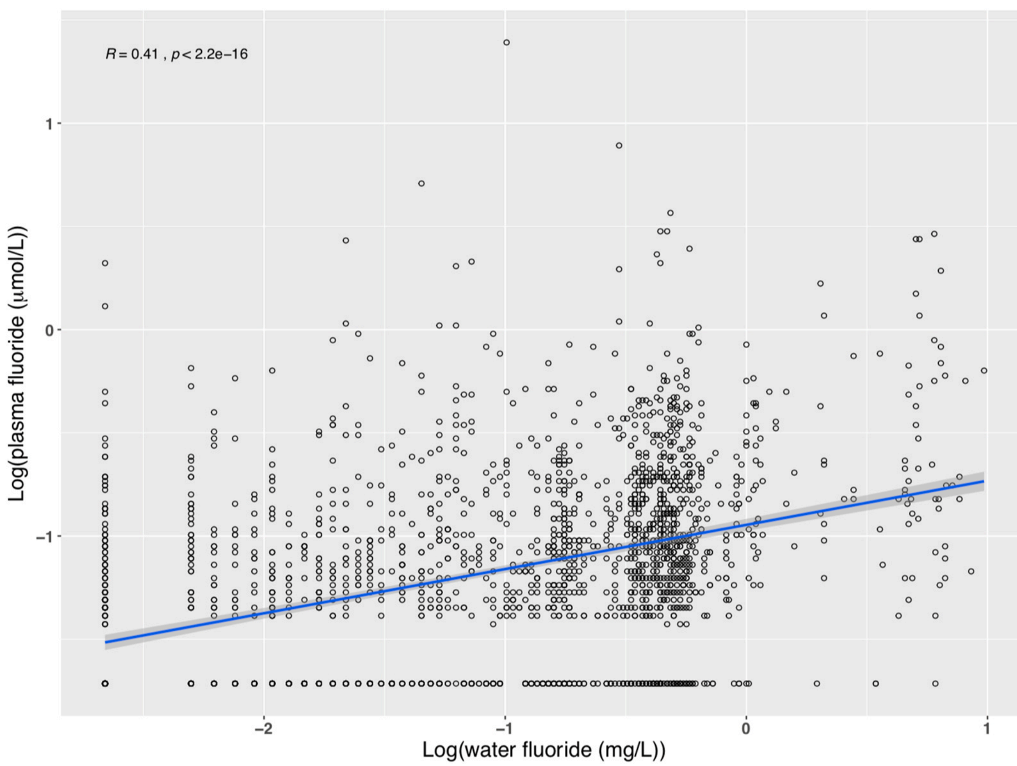

Fig. 1. Pearson's correlations between log 10-transformed water fluoride and plasma fluoride (n = 2107).

Table 3
Number and frequency (percent) of Dean's Index score for children aged 6–19 years in the 3 sample groups.

	Fluorosis severity level					
	Normal (DFI = 0)	Questionable (DFI = 0.5)	Very mild (DFI = 1)	Mild (DFI = 2)	Moderate (DFI = 3)	Severe (DFI = 4)
Overall sample n = 2098	288 (13.73)	348 (16.59)	1223 (58.29)	202 (9.63)	34 (1.62)	3 (0.14)
Water fluoride sample n = 2071	285 (13.76)	346 (16.71)	1206 (58.23)	197 (9.51)	34 (1.64)	3 (0.14)
Plasma fluoride sample n = 1808	243 (13.44)	297 (16.43)	1054 (58.30)	181 (10.01)	30 (1.66)	3 (0.17)

fluorosis by age (Table S2) and gender (Table S3). The patterns of regression results in children (aged 6–11 years) and adolescents (aged 12–19 years) were similar, but the patterns in different gender were changed. Higher plasma fluoride concentrations were associated with higher odds of dental fluorosis in females, but the associations in male groups were almost disappeared (Table S3).

4. Discussion

No fluoride deficiency disease had ever been documented for humans. However, municipal fluoridation was a mid-twentieth century innovation based on the medical hypothesis that consuming low doses of fluoride at the teeth forming years provided protection against dental decays. In this study, we found that the rate of fluoride concentration in water above the recommended level of 0.7 mg/L was 25%, but the prevalence of dental fluorosis was 70% in the NHANES 2015–2016 survey, which was higher than that in the previous 2010–2012 survey of 65% (Neurath et al., 2019). The rate of combined moderate and severe degrees was relatively low with 1.8%. To accurately assess the impact of low levels of fluoride exposure on children and adolescents, we selected

Table 4
Number and distribution (percent) of fluorosis severity level by different fluoride levels in drinking water and plasma.

	Fluorosis severity level						
	Normal (DFI = 0)	Questionable (DFI = 0.5)	Very Mild (DFI = 1)	Mild (DFI = 2)	Moderate (DFI = 3)	Severe (DFI = 4)	Total
Water fluoride (mg/L)							
0.00–0.30	134 (15.46)	198 (22.84)	483 (55.71)	39 (4.50)	11 (1.27)	2 (0.23)	867 (41.86)
0.31–0.50	68 (19.21)	42 (11.86)	186 (52.54)	51 (14.41)	7 (1.98)	0 (0.00)	354 (17.09)
0.51–0.70	29 (7.83)	61 (16.49)	241 (65.14)	31(8.38)	7 (1.89)	1 (0.27)	370 (17.87)
> 0.70	54 (11.25)	45 (9.38)	296 (61.67)	76 (15.83)	9 (1.88)	0 (0.00)	480 (23.18)
Total	285 (13.76)	346 (16.71)	1206 (58.23)	197 (9.51)	34 (1.64)	3 (0.14)	
Plasma fluoride (μmol/L)							
0.00–0.30	136 (14.66)	179 (19.29)	533 (57.44)	65 (7.00)	14 (1.51)	1 (0.11)	928 (51.33)
0.31–0.40	50 (12.38)	57 (14.11)	247 (61.14)	45 (11.14)	4 (1.00)	1 (0.25)	404 (22.35)
0.41–0.50	28 (12.73)	28 (12.73)	131 (59.55)	27 (12.27)	5 (2.27)	1 (0.45)	220 (12.17)
> 0.50	29 (11.33)	33 (12.89)	143 (55.86)	44 (17.19)	7 (2.73)	0 (0.00)	256 (14.16)
Total	243 (13.44)	297 (16.43)	1054 (58.30)	181 (10.01)	30 (1.66)	3 (0.17)	

Table 5
Associations between water fluoride, plasma fluoride and occurrence of dental fluorosis.[ab]

Fluoride levels	n	Fluorosis[a]	
		Odds ratio (95%CI)	*p*-value
Water fluoride (mg/L)			
0.00–0.30	867	Reference	
0.31–0.50	354	1.48 (1.13–1.96)	0.005**
0.51–0.70	370	1.92 (1.44–2.58)	< 0.001**
> 0.70	480	2.30 (1.75–3.07)	< 0.001**
Plasma fluoride (μmol/L)			
0.00–0.30	928	Reference	
0.31–0.40	404	1.49 (1.14–1.96)	0.004**
0.41–0.50	220	1.61 (1.15–2.29)	0.007**
> 0.50	256	1.64 (1.18–2.28)	0.003**

** $p < 0.01$.
[a] Fluorosis: 0 = No fluorosis (DFI ≤ 0.5); 1 = Fluorosis (DFI ≥ 1).
[b] Regression analyses were adjusted for age, sex, race/ethnicity, body mass index categories, ratio of family income to poverty, and six month time period when surveyed. The regression analysis was carried out separately for water fluoride and plasma fluoride.

both water fluoride and plasma fluoride as external and internal exposure indicators, respectively, and observed that the levels of both were positively associated with the increased risk of dental fluorosis.

People of different ages have different excretion rates of fluoride. For adults, about 50% of absorbed fluoride is retained, and stored in bones and teeth. The other 50% is excreted in urine (VidaZohoori and MarslandDuckworth, 2017). However, in young children, up to 80% of absorbed fluoride is retained because of the more need for the development of the body (Whitford, 1999). In our study, we found that the concentration of plasma fluoride in children was higher than that in adolescents, which could be contributed by the less excretion fluoride in children. But there was a strange result that the water fluoride concentration in children was also higher than that in adolescents, which also contributed the higher level of plasma fluoride in children. As more and more researches had indicated that even low-to-moderate exposure to fluoride was related to a number of adverse health effects in children, such as neurotoxicity (Agalakova and Nadei, 2020; Green et al., 2019; Spencer and Limeback, 2018), changes in sleep cycle (Malin et al., 2019a), alteration of kidney and liver function (Malin et al., 2019b), et al. All these studies implicated that younger children were the suspected population to fluoride. However, all people with different ages were exposed to the same level of fluoride (0.7 mg/L) in drinking water with the water fluoridation system. So, in order to against the adverse effect by fluoride exposure in youngers, children should be provided with alternative sources of drinking water.

In our study, the level of plasma fluoride in males was higher than that in females, when the level of water fluoride was similar with each other. The reasons for this were complex. One possible reason for this might be that males might intake more fluoride from drinking water than females, because males had more weight than females (p = 0.009, showed in Fig. S5) and needed more water. Once absorbed, a portion of fluoride was deposited in the skeleton and most of the remainder was excreted in urine, and to a smaller degree in feces and sweat. Another reason might be a differential excretion rate of fluoride between genders, which might cause different effects. In Green et al. study, they reported that maternal exposure to higher levels of fluoride was associated with lower IQ scores in boys but not significant in girls (Green et al., 2019). Zhou et al. (2019) also reported that gender potentially modified the associations of dental prevalence with relative mitochondrial DNA levels, which showed a stronger inverse relationship between dental fluorosis prevalence and relative mitochondrial DNA levels in boys than in girls.

The main type of drinking water sources in U.S. was being mainly from tap water. Previous analysis of NHANES 2005–2014 showed that 85% of the U.S. children and adolescents on average drunk tap water (Sanders and Slade, 2018). In order to reduce the risk and severity of dental caries of children, the U.S. Public Health Service had recommended the addition of fluoride to drinking tap water since 1945, and 63.4% of the U.S. population had accessed to a fluoridated community water system in 2018 (Centers for Disease Control and Prevention, 2018). So, there was easy to understand that fluoride concentrations in plasma was correlated with that in tap water. But the correlation coefficient was not high. One reason for this might be that only about 60% of fluoride intake was from fluoridated drinking water (U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation, 2015).

In order to minimize the unwanted effect caused by water fluoridation, we might need to reevaluate the current policy on national water fluoridation program, which is overseed by the Department of Health and Human Services (HHS). Water fluoridation had become a controversial public health intervention these years (Peckham and Awofeso, 2014; Spencer and Limeback, 2018). Fluoridation was not required by EPA, which was prohibited by the Safe Drinking Water Act from requiring the addition of any substance to drinking water for preventive health care purposes. As some areas of the country had high levels of naturally occurring fluoride which could dissolve easily into ground water as it moved through bedrock, EPA had a non-enforceable standard for fluoride of 2.0 mg/L in drinking water to protect children against dental fluorosis (https://www.epa.gov/sdwa/drinking-water-regulations-and-contaminants). As there were numerous studies supported that low level of fluoride consumption had been shown to be associated with decreased prevalence of dental caries (Featherstone, 1999; Iheozor-Ejiofor et al., 2015). In many countries, including the U.S., small amounts of fluoride were added to drinking water, salt, or milk to reduce the incidence of tooth decay. In the U.S., fluoridation of public water supplies was started in 1945. The Centers for Disease Control and Prevention (CDC), which is one of the major operating components of

HHS, provides recommendations about the optimal levels of fluoride in drinking water. However, a large increase in prevalence of dental fluorosis occurred among recent 30 years, which might relate with the widespread use of fluoride toothpastes and dental treatments (Neurath et al., 2019).

In our study, we observed that even low level of water or plasma fluoride exposure was associated with increased the risk of dental fluorosis. This result was consistent with a European review, which concluded that water fluoridation was a crude and rather ineffective policy to prevent dental caries without a detectable threshold for dental damage (European Commission, 2011). Previous studies reported there was a linear dose-response relationship between the serious of dental fluorosis and fluoride intake, and indicated that dental fluorosis could occur even at very low fluoride intake from water (Butler et al., 1985; Fejerskov et al., 1996). In Peckham's review, the authors concluded that available evidences suggested that fluoride had a potential to cause major adverse human health problems, while having only a modest dental caries prevention effect (Peckham and Awofeso, 2014). Therefore, the intervention of drinking water fluoridation is really needed further research.

Our study also had some limitations. Due to the cross-sectional design, this study had less power in terms of the causal inference of the associations between fluoride exposure and dental fluorosis. Secondly, the assessment of drinking water fluoride and plasma fluoride might not be satisfactory in reflecting exposure level in the years when the permanent teeth of the participants forming (birth to 8 years). As fluoridated water policy have implemented since 1960s, to a certain extent, we hypothesized that a single measurement of blood fluoride and water fluoride reflected the level of long-term exposure. However, since participants were enrolled during or after 2015, the year that the HHS recommended lowering water fluoride concentrations from 0.7 to 1.2 mg/L to 0.7 mg/L to minimize the risk of dental fluorosis (Fluoridation 2015), the water fluoride concentrations during the years when the permanent teeth of the participants forming might be higher than those observed in this study. We also collected the water fluoride data in the year of 2013–2014 from NHANES (Centers for Disease Control and Prevention, 2020), and found that water fluoride concentrations were reduced significantly after lowering the recommended water fluoride concentration (Fig. S6). Thirdly, NHANES did not provide data on participants' length of time at their current residence, thus we could not get their duration of exposure to the water fluoride concentrations measured in this study.

5. Conclusions

Low level of water or plasma fluoride exposure was associated with increased risk of dental fluorosis. The safety of public health approach of drinking water fluoridation for global dental caries reduction are urgently needed further research.

CRediT authorship contribution statement

Haitao Dong: Conceptualization, Writing - original draft. **Xin Yang**: Investigation, Software. **Shixuan Zhang**: Data curation, Resources. **Xueting Wang**: Investigation. **Chunlan Guo**: Software. **Xinyuan Zhang**: Methodology. **Junxiang Ma**: Data curation. **Piye Niu**: Project administration. **Tian Chen**: Software, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The study was supported by the Fundamental Research Funds for the Central Universities (No. 3332019030), and Youth Program of Peking Union Medical College Hospital Foundation (No. PUMCH 201910847), the National Natural Science Foundation of China (81703198).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112439.

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