

Is the fluoride intake by diet and toothpaste in children living in tropical semi-arid city safe?

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Abstract: Data about total fluoride intake in children living in a tropical semi-arid climate city is scarce, thus we conducted this study. Fifty-eight children aged two to five years, living in a Brazilian tropical city with optimally fluoridated water were selected. Dietary samples were collected using the duplicate diet method on two non-consecutive days in the children's home. Water was collected separately from the other drinks. The intake from toothpaste was determined by subtracting the amount of fluoride recovered after brushing from the amount placed on the toothbrush. The mean total dose (SD) of fluoride intake was 0.043(0.016) mg F·kg⁻¹·d⁻¹, with the major (60.6%) contribution from water. The factors associated with the ingestion of fluoride from toothpaste were fluoride concentration of the toothpaste ($p = 0.03$) and the use of kids toothpaste ($p = 0.02$). The findings suggest that children have a low fluoride intake, measured by at-home meals and use of fluoride toothpaste; drinking water is the main source of fluoride ingestion.

Keywords: Fluorides; Fluorosis, Dental; Toothpastes; Diet.

Introduction

In recent decades, there has been a decline in the worldwide prevalence of dental caries in the population, which is associated with the use of fluorides.¹ Fluoridation of the public water supply and the use of fluoride toothpastes are the most important methods of fluoride delivery.^{2,3} However, an increase in the prevalence of dental fluorosis due to the intake of multiple sources of fluoride during tooth formation has been observed.⁴ Burt⁵ empirically determined a fluoride intake dose considered safe: from 0.05 to 0.07 mg F/kg body weight/day.

Some studies showed that children's fluoride intake from diet (solid and liquid) in a subtropical region of Brazil with fluoridated water was not relevant, with doses of approximately 0.04 mg F/kg body weight/day.^{6,7} However, Lima and Cury⁷ reported that the amount of fluoride ingested during the hot season was 19% higher than during the cold season. Such data can be troubling for people living in tropical climate regions, because they tend to consume more water than people from cooler regions. In addition, children are at risk of ingesting fluoride from other sources, such as toothpaste.

Previous studies have evaluated the dose of fluoride intake by children from tropical semi-arid climates.^{8,9,10} Omena et al.⁸ evaluated fluoride intake from drinking water and from fluoride toothpaste, without considering

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others sources of fluoride intake (others liquids and food). Buzalaf et. al.⁹ reported fluoride intake from the entire diet (water, other beverages and solid foods) and fluoride toothpaste in a city with naturally fluoridated water. In addition, Lima et al.¹⁰ evaluated fluoride intake from diet (liquid and solid foods) and fluoride toothpaste in a city with artificially fluoridated water; however, the authors reported a low intake of water maybe because children spent most of their time in the classroom. Furthermore, another study developed in a city with tropical semi-arid climate found an increase in cases of dental fluorosis with esthetically undesirable appearance.¹¹

Aiming to expand the existing knowledge, this study was developed in a city with tropical semi-arid climate to determine the total daily dose of fluoride intake from diet (water, other beverages and solid diet, collected at children's home) and from use of fluoride toothpaste in children living in a city with optimally fluoridated water and with high temperatures throughout the year.

Methodology

This study was approved by the Ethics Committee of the Federal University of Piauí (Opinion 667,541), according to the guidelines of Declaration of Helsinki.

Teresina, Piauí, is a Northeastern Brazilian city with a tropical climate and average annual maximum temperatures above 33.5°C observed over the last 10 years. The public water supply has been fluoridated since 1978 in accordance with the Brazilian legislation (optimal recommended concentration of 0.7 mg/L, ranging from 0.6 to 0.8 mg/L for locations with average maximum temperatures of 26.7 to 32.5°C).¹²

Children were selected from a private school (convenience sample), living in Teresina, aged between two and five years, which is considered the critical period for the development of fluorosis in aesthetically important teeth.^{13,14,15}

For sample size calculation, we used the formula $n = 2s^2 (t_{\alpha/2} + t_{\beta})^2 / d^2$, where "s" is the estimated variation of fluoride intake ($s = 0.2$); " $t_{\alpha/2}$ " is the value of the t distribution to 95%; " t_{β} " is the power of the test ($t_{\beta} = 20\%$) and "d" is the difference to be detected in the fluoride intake ($d = 0.1$).¹⁶ Thus, the minimum

sample size required was 62 children. Considering the possibility of losses during the study, 30% was added to the minimum value, to give a total of 81 children.

Children were included in the study if they regularly consumed water from the fluoridated public water supply, used fluoride toothpaste, and whose caregivers signed the consent form. Children were ineligible for the study if they had any acute or chronic infection or were taking any medicine or fluoride supplement.

Details of the research were explained to the children's caregivers. A questionnaire about demographic data (age, gender, and mother education level), eating habits (food diary of the collection day), and oral hygiene (brushing frequency, who performs the brushing, type of toothpaste, and amount of toothpaste used) was sent to the caregivers. Samples were collected from May to August 2014. All children were weighed on each day of diet collection (twice), on a digital scale (Dayhome Comercial LTDA, São Paulo, Brazil) to determine the fluoride dosage (mg F/kg body weight).

Fluoride intake was determined by the duplicate diet method, as described by Guha-Chowdhury et al.¹⁷ but with a modification, because the collection was made at home and held over two days, separated by an interval of two months. Solid food, water, and other beverages were collected in plastic bottles and packed separately. Solid foods included rice, bread, beans, soup, meat, biscuits, fruit, vegetables, and pasta, among others. Other beverages included juice, yogurt, milk, coffee, and soda. The food was collected and placed in a refrigerator by the parents, on a day when both the child and the parent were home.

The samples were homogenized in laboratory. The weight of solids and the volume of liquids were recorded for the analysis of fluoride. Deionized water was added to the solid foods and homogenized with a blender. The other beverages were also homogenized using a blender. Aliquots of 15 mL of solid food, water, and other beverages were stored in a plastic container at -4°C for further analysis.

The fluoride obtained from diet samples was extracted using the microdiffusion technique facilitated by hexamethyldisiloxane (HMDS).¹⁸ The fluoride analyses were performed using a fluoride ion-specific electrode (Orion Model 96-09, Orion

Research Incorporated, Cambridge, MA, USA) and an ion analyzer (Orion Star A211), previously calibrated with standard solutions (0.0156 ± 2.0 mg F/mL) under the same conditions as the samples. All diet samples were analyzed in triplicate. To calculate the daily dose, an average of the two sampling days was calculated.

The fluoride intake from toothpaste was determined by subtracting the amount of fluoride recovered after brushing from the amount placed on the toothbrush,¹⁷ as collected during a brushing session on the second day of diet collection. It was recommended for children to perform their habitual brushing routine, using their own toothpaste and toothbrush. The researcher supervised and observed how the brushing was performed. Deionized water was provided to assist in brushing and in washing the brush. Saliva and deionized water were used to rinse the mouth and toothbrush after brushing, and the liquid was collected in a plastic cup. These brushing residues were homogenized, the volume recorded, and a 15 mL aliquot stored at -4°C for further analysis. The toothpastes of all children were collected for analysis of total fluoride and total soluble fluoride (TSF).

Samples were centrifuged (3,000 g, 10 min) and the TSF in the supernatant was determined, after performing 2 M HCl hydrolysis, neutralization with 1 M NaOH and buffering with TISAB II. Fluoride analyses were performed using a specific fluoride ion electrode (Orion Model 96-09, Orion Research Incorporated, Cambridge, USA) and an ion analyzer (Orion Star A211), previously calibrated with standard solutions (0.125 to 32 μg F/ml) under the same conditions as the samples. All samples were analyzed in duplicate. The TSF present in the toothpastes was determined; values used in the calculations accounted only for the bioavailable fluoride.^{6,19,20} If the total fluoride present in toothpastes was considered, the ingested dose could be overestimated.²¹ The daily brushing frequency and weight of the child were used to determine fluoride intake.

The maximum daily average temperatures ($^{\circ}\text{C}$) from May to August were provided by the National Institute of Meteorology - INMET (Source: INMET Network Data). Tap water samples were collected from the residence of each child on the days of diet collection for fluoride analysis.

A descriptive analysis was conducted using one-dimensional contingency tables. The chi-square test for equality of proportions and basic statistics for the analysis of numerical variables were used. In order to evaluate the associations between measures, Pearson correlation coefficients were calculated, two-dimensional contingency tables were constructed and the V Cramer coefficient was adopted to quantify the association. The chi-square test was used to determine the p-value for the likelihood ratio (G2). Repeated measures analysis of variance based on generalized linear mixed models was used to compare the means of fluoride intake from different sources. The calculations were performed using the SAS system and all statistical tests adopted a significance level of 5% ($\alpha = 0.05$).

Results

Of the 81 children whose parents agreed to participate in the study, 13 were not included in the sample because diet collection was not performed. After analysis of the water used at home, 10 children were excluded as they drank mineral bottled water without fluoride. Thus, the final sample consisted of 58 children (36 girls and 22 boys) with a mean age of $49.8 (\pm 11.9)$ months and an average weight of $17.9 (\pm 3.6)$ kg.

The average fluoride concentration in tap water was $0.6 (\pm 0.1)$ mg F/l, which is the lower limit according to Brazilian regulations. The average maximum temperature during the days of sample collection was $33.5 \pm 1.1^{\circ}\text{C}$.

Fluoride intake by children is described in Table 1. The largest contribution was from water (60.6% of the total diet).

The total average fluoride intake from diet and toothpaste was low. However, 12.1% of study participants exceeded the upper threshold of 0.07 mg $\text{F}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ for being at risk of esthetically undesirable fluorosis, and 29.3% of children reached the lower threshold of 0.05 mg $\text{F}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$.

Most children used flavored kids toothpastes that contain NaF and silica for abrasiveness (72.41%). Toothpastes containing 1,100 ppm F were the most frequent (Table 2).

Table 1. Contribution of each source of fluoride to total fluoride intake ($\text{mg F} \times \text{kg}^{-1} \times \text{d}^{-1}$) for children from diet and fluoride toothpaste.

Measure	Mean	Standard deviation	Minimum	Maximum
Intake from diet				
Water	0.021	0.009	0.007	0.043
Other drinks	0.009	0.008	0.001	0.040
Solid diet	0.005	0.004	0.001	0.022
Total Dose from diet	0.033	0.013	0.011	0.073
Variables related to the use of fluoridated toothpaste				
TSF* ($\mu\text{g F/mg}$)	1065.684	171.520	518.400	1440.200
Amount of toothpaste (g)	0.305	0.272	0.010	1.040
Brushing time (s)	95.862	40.266	21.000	255.000
Fluoride intake from brushing (%)	27.032	19.159	0.000	74.300
Fluoride from toothpaste ingestion	0.010	0.010	0	0.043
Total fluoride intake				
Diet + Toothpaste	0.043	0.016	0.011	0.083

*TSF: Total soluble fluoride.

Table 2. Toothpastes characteristics used in the sample and chi-square test for equal proportions between the variables.

Variables	Characteristics		Chi-square test	
	Frequency	Percentage	Statistic χ^2	p-value
Type of toothpaste				
Adult	13	22.41	17.65	0.0001
Child	45	77.59		
Type of fluoride				
MFP*	16	27.59	11.65	0.0006
NaF**	42	72.41		
Abrasive				
Calcium carbonate	12	20.69	19.93	0.0001
Silica	46	79.31		
Fluoride concentration ($\mu\text{g F/mg}$)				
500	3	5.17	115.79	0.0001
750	2	3.45		
1,100	39	67.24		
1,350	1	1.72		
1,450	12	20.69		
1,500	1	1.72		

*MFP: Sodium monofluorophosphate; **NaF: Sodium fluoride.

Table 3 describes the brushing habits of children. Most children had their teeth brushed by their mother, 2 to 3 times per day and with $\frac{1}{4}$ of the brush covered with toothpaste.

The fluoride intake from toothpaste was divided into three categories: < 0.0001 , from 0.0001 to 0.0025 and $> 0.0025 \text{ mg F} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, allowing association analyses between this source of fluoride intake and the variables related to brushing (Table 4). Higher amounts of fluoride were ingested with the use of 1,100 ppm F toothpastes ($p=0.0384$) and kids toothpastes ($p = 0.0209$).

Fluoride intake from water was significantly higher than from all other sources (Figure). Additionally, the intake of fluoride from toothpaste was significantly higher than from solid diet.

Discussion

According to the average fluoride intake, children in this study were exposed to safe levels concerning the risk of dental fluorosis. Fluoride exposure obtained in this study was lower than the that obtained in other studies conducted in Northeastern Brazil,^{8,9,10,22} which can be explained by the small amount of toothpaste used for brushing and consequent reduction of fluoride intake from toothpaste. In addition, Teresina has the minimum recommended concentration of fluoride in the public water supply.

Comparing dietary fluoride ingestion in this study with those of others conducted in tropical

Table 3. Brushing characteristics of the sample.

Variables	Characteristics		Chi-square test	
	Frequency	Percentage	Statistic χ^2	p-value
Brushing frequency (daily)				
1	1	1.72	50.41	0.0001
2	29	50.00		
3	27	46.55		
4	1	1.72		
Amount of toothpaste				
¼ brush	30	51.72	8.93	0.0115
Brush Half	15	25.86		
The entire brush	13	22.41		
Act of wetting the brush				
Yes	35	60.34	2.48	0.1151
No	23	39.66		
Brushing technique				
Horizontal	44	75.86	47.62	0.0001
Vertical	5	8.62		
Fones	9	15.52		
Position brushing				
Facing the child	34	68.00	32.92	0.0001
Child on her lap	1	2.00		
Starkey (adult behind the child)	15	30.00		
Who was brushing				
Mother	34	58.62	62.17	0.0001
Father	1	1.72		
Nanny	13	22.41		
Other caregiver	2	3.45		
Child	8	13.79		

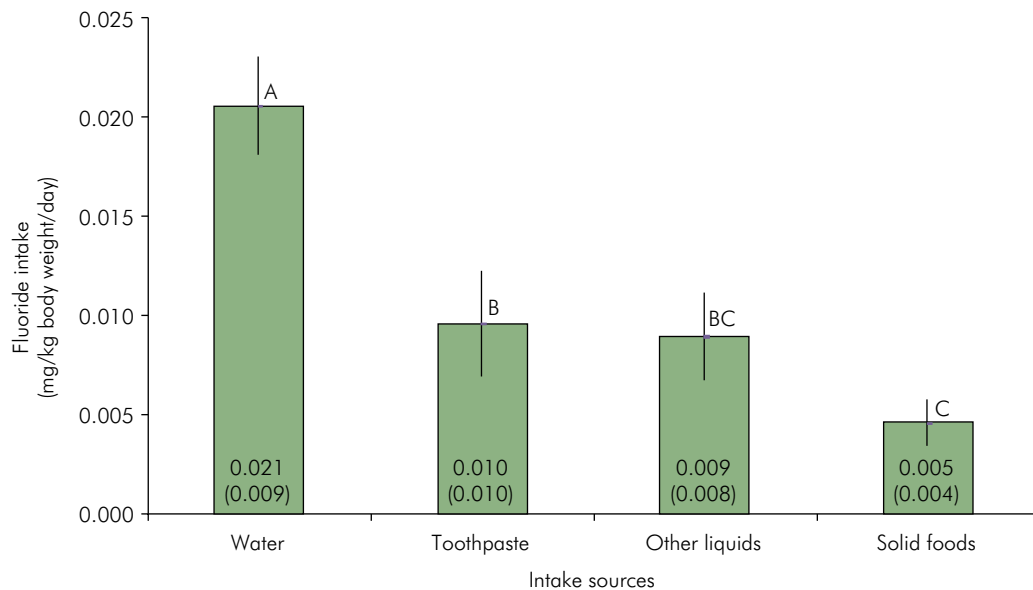


Figure. Mean (SD), confidence intervals (95%), and Tukey’s post test to compare the fluoride intake. Bars with the same letter are not significant ($p > 0.05$).

Table 4. Frequency (percentage), statistical V Cramer and p-value of the chi-square likelihood ratio (G2) to assess the associations between the amount of ingested fluoride and the variables related to brushing.

Brushing-related variables	Ingested fluoride from toothpaste (mg.kg ⁻¹ .day ⁻¹)		
	(< 0.0001)	(0.0001–0.0025)	(> 0.0025)
Fluoride concentration in toothpaste (mg F/mg F) (V: 0.2908 – p-value: 0.0384)			
500–750	2 (40.00)	3 (60.00)	0 (0.00)
1,100	4 (10.53)	30 (7.95)	4 (10.53)
1,350–1,500	6 (46.15)	7 (53.85)	0 (0.00)
Type of toothpaste (V: 0.3754 - p-value: 0.0209)			
Adult	6 (50.00)	6 (50.00)	0 (0.00)
Child	6 (13.64)	34 (77.27)	4 (9.09)
Type of fluoride (V: 0.1782 – p-value: 0.2569)			
MFP*	4 (28.57)	10 (71.43)	0 (0.00)
NaF**	8 (19.05)	30 (71.43)	4 (9.52)
Abrasive (V: 0.2121 – p-value: 0,2110)			
Calcium carbonate	4 (36.36)	7 (63.64)	0 (0.00)
Silica	8 (17.78)	33 (7.33)	4 (8.89)
Brushing frequency (V: 0.0515 – p-value: 0.9282)			
1 a 2	7 (23.33)	21 (70.00)	2 (6.67)
3 a 4	5 (19.23)	19 (73.08)	2 (7.69)
Wetting the brush (V: 0.2824 – p-value: 0.0546)			
Sim	5 (14.71)	25 (73.53)	4 (11.76)
Não	7 (31.82)	15 (68.18)	0 (0.00)
Person performing (V: 0.3513 – p-value: 0.0672)			
Mother	9 (27.27)	24 (72.73)	0 (0.00)
Father	1 (100.00)	0 (0.00)	0 (0.00)
Nanny	1 (8.33)	9 (7.00)	2 (16.67)
Other caregiver	0 (0.00)	2 (100.00)	0 (0.00)
Child	1 (1.50)	5 (62.50)	2 (25.00)

*MFP: Sodium monofluorophosphate; **NaF: Sodium fluoride.

cities, such as in Rodrigues et al.,²² we found lower values. Fluoride intake from drinks in this study was 0.03 mg F.kg⁻¹.d⁻¹, which is higher than the dose observed by Lima et al.¹⁰ (0.025 mg F.kg⁻¹.d⁻¹). This is probably due to a higher consumption of water.

Teresina is a city that experiences high temperatures throughout the year and provides fluoridated water supply for collective use; thus, the greatest contribution to fluoride intake is from water, which is confirmed

by Rodrigues et al.²² The observed value was similar to the findings of Omena et al.,⁸ despite differences between the ambient temperatures in the three cities. Teresina is much warmer (33.5°C to 24°C) and the fluoride concentration of the public water supply is lower (0.6 vs. 0.94 mg/ml). These factors may explain the similarities among studies.

The fluoride intake from toothpaste was 0.010 mg F.kg⁻¹.d⁻¹. This result is in disagreement with other studies conducted in Northeastern Brazil^{8,10} as well as in other Brazilian regions,^{6,18,19,23,24} where higher values were observed. In the present study, flavored toothpaste was the most frequently used and almost all children brushed at least twice a day, factors that have been cited in the literature as associated with the risk of fluorosis.²³ However, tooth brushing was performed in the majority of cases by a caregiver, which explains the smaller amount of toothpaste used in comparison to other studies.^{6,17,19} In our study, fluoride dosage was calculated by the TSF method rather than considering the concentration indicated on the toothpaste label. Moreover, our participants had in general a high socioeconomic status and were consequently more informed about oral health care and the risk of using excess toothpaste. This sample was chosen for having a diversified diet, free access to water, and an easy diet collection at home.

The largest amounts of fluoride ingested by children during tooth brushing were observed in those who used flavored toothpaste, in agreement with the observations of Kobayashi et al.²⁵ and Oliveira et al.²⁰. The average concentration of fluoride of these toothpastes was 1,100 ppm.

In this study, water was the greater contributor to total fluoride intake, followed by toothpaste, other beverages, and solid foods, resulting in significant differences between sources. In other studies,^{6,8,18,23,26,27,28} toothpaste was the main source of fluoride intake.

Teresina has no fixed system for water fluoridation. A research conducted in 2001 showed concentrations ranging from 0.8 to 0.9 ppm F¹² in different months. However, a recent control (2012) showed that the averages were closer to 0.6 ppm F,²⁹ as was observed in this study. This value proved to be safe with regard to ingestion by children and may have contributed to caries control. According to the latest national

epidemiological survey, the mean DMFT (number of decayed, missing, and filled teeth) in children up to 12 years of age in Teresina was 1.5, which is lower than the national average (2.1) and other cities in Northeastern Brazil that do not have fluoridated water.³⁰ Although the average fluoride intake was low, probably due to the low ingestion of toothpaste, some children exceeded the safety threshold. One way to reduce the intake of fluoride from toothpaste is through the orientation on the needed amount of toothpaste to be placed on the brush and parent supervision when brushing,^{20,26} to prevent children from swallowing. Moreover, it is important to periodically control the fluoridation of the public water supply.

In this study, diet was the greatest source of fluoride (more precisely from water), differing from Lima et al.¹⁰, who found that dentifrice was the largest source in a sample of the same city. A study is therefore needed to assess the total fluoride intake in children of low socioeconomic status, as they may be exposed to higher levels of fluoride.

One of the limitations of this study was that it was performed on two non-consecutive days, with an interval of two months between each collection, unlike other studies that collected data on two consecutive days^{6,10,18,22} to improve the accuracy of

the information. An attempt to reduce this limitation was made by collecting food samples on days that parents were at home, as was done in the study by Oganessian et al.³¹ Another advantage is diet stability, since on consecutive days the type of food eaten can be similar. An additional limitation is that the duplicate diet was collected by the caregiver. To minimize this limitation, verbal and written instructions were given on how the collection should be performed. In addition, the high education level of the parents contributed to the methodology be followed more precisely.

In conclusion, most of the evaluated children from a tropical semi-arid municipality of high temperatures and a fluoridated public water supply had low fluoride intake from diet and toothpaste; water had the greatest contribution in fluoride intake.

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