Fluoride Availability and Stability in Children's Toothpastes in Uruguay

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ABSTRACT

Purpose: The purpose of this study was to evaluate the availability and stability of fluoride in children's toothpastes in Uruguay.

Methods: Six commercial brands of children's toothpaste available in Uruguay were tested. Analyses were made when the dentifrices were purchased (fresh samples) and after one year of storage (aged samples). Total fluoride (TF) and total soluble fluoride (TSF) concentrations were determined using an ion specific electrode.

Results: Four of the children's dentifrices showed TF concentration similar to that specified on the package. Three products showed similar concentrations of TF and TSF with no variations after the one-year storage period. Two dentifrices showed an initial insoluble fluoride concentration greater than 50 percent, which increased with toothpaste aging.

Conclusion: Most tested toothpastes showed a decrease in the soluble fluoride content with aging. The high quantity of insoluble fluoride found in two tested dentifrices may compromise their anti-caries efficacy. (J Dent Child 2017;84(2):52-7)

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A significant decrease in the worldwide prevalence of dental caries has been observed over the past few decades.[1] This reduction can be attributed in large part to widespread use by the population of fluoride dentifrice,[1-3] which is considered the most reasonable method to deliver fluoride because it combines the anti-caries local effect of fluoride with the mechanical removal of dental biofilm.[4] Reduction in the prevalence and incidence of dental caries in children and adolescents in South America and Caribbean countries has been also observed, and considered to be associated with increased use of fluoride dentifrices.[1,3,5,6]

Several studies have found a contradiction between the manufacturer's information about fluoride content on the product label and the soluble and active fluoride actually present.[7-9] These investigations have reported that only a quarter of the analyzed toothpastes contained appropriate levels (89 percent or more) of the declared fluoride content compared to soluble fluoride. The fluoride concentration shown on the label of children's

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toothpaste varies between 250 and 1,500 parts per million (**ppm**) fluoride. For a fluoride dentifrice to be effective in controlling dental caries, an adequate concentration of fluoride must be soluble.[10] The soluble forms of fluoride are able to interfere with the dynamics of the caries process, thus reducing demineralization and activating remineralization of dentin and enamel.[6] Some hydrolysis of the soluble fluoride ions may occur over time, due to a combination with abrasives forming insoluble compounds.[11] Thus, the total concentration of fluoride declared by a manufacturer cannot be only the concentration of soluble and active fluoride contained in toothpaste. Furthermore, with age there is a considerable loss in the total soluble fluoride (**TSF**) concentration found in dentifrices.[12]

Uruguay has had a salt fluoridation program (250 mg/kg) since 1991. However, due to difficulties in monitoring the salt distribution and recent campaigns to avoid excess of salt consumption, fluoride dentifrice has become an important public health measure in preventing caries.

The minimum requirement for the anti-caries effect of a dentifrice is based on the available and stable fluoride in the formulation. However, Mercosur (South America's common economic market that includes Argentina, Brazil, Paraguay, and Uruguay) policies only state that toothpastes must not contain more than 1,500 ppm of total fluoride (**TF**) concentration, but there is no requirement to show the TSF.[13]

The purpose of this study was to evaluate the availability and stability of fluoride in children's toothpastes sold in Uruguay.

METHODS

Six commercial brands of fluoride dentifrice for children commercially available in Uruguay were tested in this study (Table 1). Three tubes of each brand were purchased in different supermarkets and drugstores located in Montevideo, Uruguay (N=18) to ensure they were from different lots.

Toothpastes were coded with letters (A, B, C, D, E, and F) to allow for blind analysis (Table 1). Fluoride concentration analysis was done by researchers in the Laboratory of Oral Biochemistry, Faculty of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. All samples were analyzed before their expiration dates, and fluoride determination was made in duplicate. The analysis was carried out immediately upon acquisition of the dentifrices (fresh samples) and after 12 months of storage at room temperature (aged samples).

The concentrations of three forms of fluoride (**F**) available in the toothpastes were determined: fluoride ion (**FI**), TSF, which includes FI and fluoride as monofluorophosphate (**MFP**), and TF. TF is the sum of TSF plus insoluble fluoride (**InsF**), representing the F bound to the abrasive. From these analyses, the concentrations of F as MFP and the percentage of FI were calculated. Analyses were carried out in duplicates, according to the modified procedure described by Cury et al.[14] Ninety to 110 mg of toothpaste were weighted (±0.01 mg) and homogeinized in 10 mL of deionized water; duplicates of 0.25 mL of the suspension were transferred to test tubes for TF analysis.

The remaining suspension was centrifuged (3,000 x *g* for 10 minutes) to remove InsF bound to the abrasive. Duplicates of 0.25 mL of the supernatant were transferred to assay tubes to determine TSF and FI concentrations. For the TF and TSF tubes, 0.25 mL of 2.0 molar (M) hydrochloric acid (HCl) was added; after one hour at 45 degrees Celsius, the samples were neutralized with 0.5 mL 1.0 M sodium hydroxide

Table 1. TOOTHPASTES TESTED

Commercial brand (manufacturer city, state, country)	Code	Type of fluoride	Lot number	Expiration date	Abrasive system
Picojener Kids (Laboratórios Abarly, Montevideo, Uruguay)	A	NaF	87381 94104 96303	04/2013 02/2014 05/2014	Not declared
Picojener Junior (Laboratórios Abarly, Montevideo, Uruguay)	B	NaF	90441 94141 97522	09/2013 02/2014 07/2014	$CaCO_3$*
Colgate Smiles 0-2 years (Colgate Palmolive, Ciudad de Mexico, CDMX, Mexico)	C	NaF	0306MX1161 0335MX1162 11132MX1161	11/2012 12/2012 05/2013	SiO_2†
Colgate Smiles >5 years (Colgate Palmolive,Ciudad de Mexico, CDMX, Mexico)	D	NaF	1027MX1162 1131 MX1161 1164MX1163	01/2013 05/2013 06/2013	SiO_2
Ultra Action Kids (Laboratorio Boniquet, São Bernardo do Campo, SP, Brazil)	E	NaF	H0024 I0015 I0021	11/2012 04/2013 05/2013	SiO_2
Bitufo (Bitufo Indústria de Produtos de Higiene/ Cosméticos Ltda, Itupeva, SP, Brazil)	F	MFP	5939711020302 5939711020302 6015211030225	02/2014 02/2014 02/2014	$CaCO_3$ SiO_2

** $CaCO_3$= calcium carbonate. † SiO_2= silica.*

(NaOH) 1.0 M and buffered with 1.0 mL of total ionic strength adjustor buffer II (TISAB II, 1.0 M acetate buffer, pH 5.0, containing 1.0 M sodium chloride (NaCl) and 0.4 percent cyclohexylenedinitrilotetraacetic acid). In the FI tubes, 0.50 mL of 1.0 M NaOH was added and buffered with 1.0 mL of TISAB II and 0.25 mL of 2.0 M HCl. The analyses were carried out using a fluoride ion specific electrode (Orion model 96-09, Orion Research, Cambridge, Mass., USA) connected to an ion analyzer (Procyon Instrumentos Científicos, São Paulo, São Paulo, Brazil). Calibration of the electrode was done by researchers in triplicate with fluoride standard solutions containing 0.8, 1.6, 3.2, and 6.4 µg F per ml, prepared with the same reagents as the samples. The analyses were validated using internal standards, and a coefficient variation lower than three percent was considered acceptable. The readings were expressed in millivolt and transformed to ppm F by linear regression curve.

Descriptive analysis was performed, and means and standard deviations of the concentrations of TF, TSF, and InsF percentage of fresh and aged samples of the three samples for each toothpaste were calculated using Excel software, version 2007 (Microsoft Corp., Redmond, Wash., USA).

RESULTS

Table 1 shows the characteristics (manufacturer; type, and concentration of F; expiration date and abrasive system) of the six dentifrices analyzed in this study. Three products had a fluoride concentration labeled by the manufacturer as 1,000 ppm F or more, and three other toothpastes were labeled as less than 1,000 ppm or low fluoride concentration. Five samples contained sodium fluoride (**NaF**) as the fluoride compound and only one contained MFP.

There was no significant difference between the concentrations of TF in fresh and aged samples (Tables 2). TF concentrations found in the toothpastes ranged from 475.6 to 991.3 ppm F for fresh samples and from 437.6 to 906.5 ppm F for aged samples. Fresh samples of all dentifrices had TF content similar to that declared by the manufacturer, except for one (Picojener Kids, Laboratorios Abarly, Montevideo, Uruguay) that had a lower TF concentration than adverstised (Table 2). To consider toothpaste containing less than what was advertised, a disagreement of greater than 10 percent had to be found between the concentration labeled in the tube and the measured values.

The concentrations of TSF ranged from 219.2 to 993.9 ppm F in fresh samples and from 168.4 to 943.9 ppm F in aged samples. Fresh samples of four toothpastes showed similar concentrations of TF and TSF. However, Picojener Kids and Picojener Junior (Laboratorios Abarly, Montevideo, Uruguay) toothpastes presented TSF content lower than 50 percent of TF, indicating that most F content was insoluble in the product. The aged samples of all tested toothpastes showed a decrease of TSF concentration compared to fresh samples (Table 2).

The Figure shows the percentage of InsF contained in the fresh and aged samples in each toothpaste. In the fresh samples, the percentage of InsF varied from 0 to 55.8 percent and from 0 to 64.9 percent in aged samples. Picojener Kids and Picojener Junior toothpastes showed 55.7 percent and 55.4 percent of InsF in fresh samples, increasing respectively to 60.1 percent and 64.9 percent in aged samples.

Table 2. TOTAL FLUORIDE (TF) AND TOTAL SOLUBLE FLUORIDE (TSF) CONCENTRATIONS IN FRESH AND AGED SAMPLES (MEAN±SD)

Dentifrice	Fluoride concentration shown on label	Fresh samples		Aged samples	
		TF	TSF	TF	TSF
A	1,000	838.2±48.6	380.8±234.5	776.6±6.3	309.8±184.4
B	500	475.6±70.1	219.2±89.3	469.1±68.8	168.4±64.5
C	500	505.4±8.2	512.7±10.4	437.6±18.8	431.7±26.5
D	1,100	988.0±22.5	993.9±12.3	906.5±6.7	943.9±69.2
E	1,100	991.3±35.6	959.8±13.8	903.9±6.7	904.9±30.3
F	750	684.2±8.1	505.0±116.1	667.9±0.9	470.2±157.5

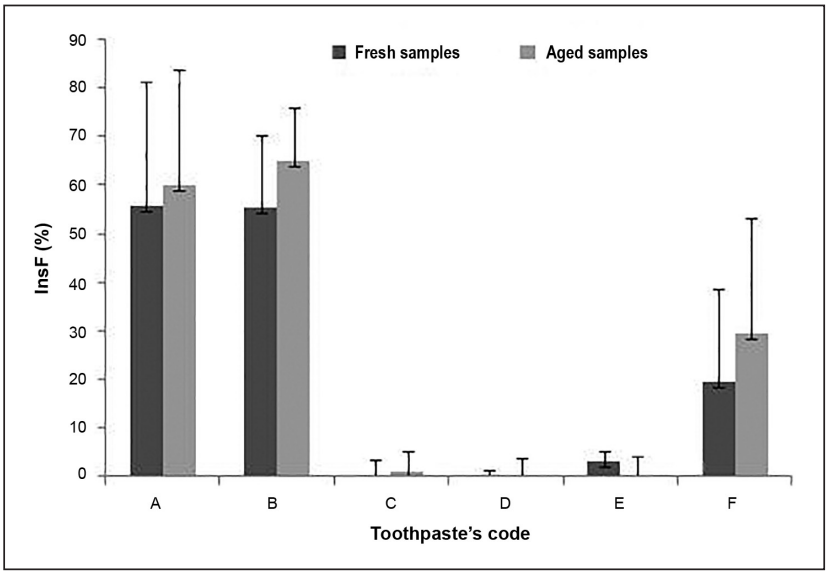

Figure. Percentages of insoluble fluoride (InsF) in fresh and aged samples. Bars correspond to mean values±SD.

DISCUSSION

Some studies have shown that many fluoride toothpastes from different countries report in their packages only the information of TF concentration. However, TF concentration may also contain insoluble and other ineffective fractions of fluoride.[12,15,16] Therefore, lack of consistency between labeled and actual F content in toothpastes can compromise its effectiveness. In the present study, the TF concentration of five tested toothpastes showed a similar concentration of TF as that specified on the package, and in accordance with the Mercosur legislation. Only one dentifrice showed TF concentration lower than that reported on the label.

Fluoride in dentifrices must be present in a soluble form to guarantee activity against caries. Depending on the formulation, part of the fluoride may be inactive; this occurs mainly in the presence of calcium (**Ca^{++}**) and fluoride ions (**F^{-}**). MFP is more stable in the presence of Ca^{++}; however, because fluoride is linked covalently to phosphate, it undergoes hydrolysis over time and releases F^{-}, which reacts with Ca^{++} to form InsF that is inactive against caries.[6,17,18] Recently, the American Dental Association (**ADA**) published acceptance program requirements for fluoride-containing dentifrices to address requirements necessary to determine their anti-caries efficacy. They recommend that at least 90 percent of the labeled amount of fluoride must be available in both fresh and aged samples.[10] Only three of the six dentifrices analyzed in the present study presented the amount of available F in fresh and aged samples, as recommended by the ADA. The other three dentifrices presented values below the recommended one, which could compromise their efficacy against caries in children.

TSF concentration was lower than that for TF content in most of the tested toothpastes in this study. This lower value of active fluoride is due to the incompatibility between the abrasive agent and fluoride. Dentifrices containing both free ionic fluoride and Ca++ lose their effectiveness against dental caries as a consequence of the formation of low solubility products that inactivate fluoride.[14,15] This reaction is fast, and within a week of toothpaste production more than 60 percent of the fluoride will be insoluble. For this reason, dentifrice formulations containing both NaF and calcium carbonate (**$CaCO_3$**) are considered inadequate.[19] Toothpastes containing MFP and $CaCO_3$ are considered more appropriate and are a more economical option for developing countries.[21] However, it has been observed that, if MFP hydrolyzes in the product, it can react partially with Ca++, reducing the amount of soluble fluoride.[12,15,19,20]

In the present study, five dentifrices used NaF and only one used MFP. One toothpaste did not inform the abrasive system, and another (containing NaF) had $CaCO_3$ in its composition. One can speculate that the dentifrice that did not advertise also used $CaCO_3$ as abrasive because the manufacturer used it on another toothpaste. These characteristics could explain, in part, the higher concentration of InsF in this dentifrice (more than 50 percent of TF content). Dentifrice also showed a decrease in the TSF concentration with age, even after having MFP associated with two abrasives [$CaCO_3$ and silica (SiO_2)]. In three dentifrices that used NaF and SiO_2, the concentration of TSF was almost the same as that of TF, and the concentration remained stable after 12 months.

Stability was evaluated in this study over a period of one year; however, it should be emphasized that the estimated time that dentifrices stay on the store shelves is less than this evaluated period. This shelf time of the dentifrices would be sufficient to maintain soluble fluoride and indicates that dentifrices can be effective in preventing dental caries.

All dentifrices showed a tendency toward decreases in the concentration of TSF and an increase in the percentage of InsF after one year of storage at room temperature. This may be explained by the hydrolysis of fluoride compounds, which releases free ionic fluoride and links with the Ca++ of the abrasive resulting in InsF.

This study showed that more than 50 percent of fluoride content in the Picojener dentifrices was insoluble, which can be explained by their composition with NaF and $CaCO_3$ as abrasive. These commercial dentifrices are made by the same Uruguayan manufacturer, and are very popular.

Compared with water fluoridation, community salt fluoridation demonstrated a similar beneficial reduction in the prevalence of caries and provided a choice to consumers. Individual choice is an interesting option, but the consumption of fluoridated salt among individuals is not similar.[21] Because of difficulties in monitoring the distribution of fluoridated salt and frequent campaigns to avoid overconsumption of salt, fluoride dentifrice has become an important public health measure for preventing caries in Uruguay.

Almost all commercial toothpastes in Uruguay are fluoridated, so it is necessary to control the quality of commercial toothpastes and establish the minimum concentration of soluble fluoride to have anti-caries efficacy. Unfortunately, Mercosur does not establish how much TF content should be maintained as soluble in toothpaste formulation. A review the guidelines on fluoride dentifrices for children in Uruguay and Mercosur is necessary to ensure optimum benefit.

Our results showed that, except for Picojener Kids, all the toothpastes tested corresponded with the TF concentrations declared and the content found in the analyses. However, only two dentifrices (Colgate Smiles >5 years, Colgate Palmolive, Ciudad de Mexico, CDMX, Mexico, and Ultra Action Kids, Laboratório Boniquet, São Bernardo do Campo, São Paulo, Brazil) had the minimum concentration of TSF necessary to have an anticaries effect.[22-25]

It was expected that the TF concentration would remain stable after 12 months of storage, but there may be chemical changes from one form of fluoride to another as a result of product stability.[15,21] In the present study, all the toothpastes showed a small but insignificant variation between the values for TF concentration in fresh and aged samples. These small variations are expected and usually occur due to issues related to the methodology of biochemical analysis itself.

CONCLUSIONS

Based on the results of this study, the following conclusions can be made:

1. Two of the five toothpastes for children commercialized in Uruguay showed low content of TSF and more than 50 percent of insoluble and inactive fluoride content in their composition, which may influence their anti-caries effect.
2. Although the tested toothpastes showed stability in TF concentration, there is a reduction in the TSF content with aging in the most of them.

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