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ADEMIR MELO LEITE FILHO

CONCENTRAÇÃO DE FLUORETO EM DENTIFRÍCIOS COMERCIALIZADOS PARA CRIANÇAS NO BRASIL E MÉXICO, E DISCUSSÃO SOBRE AS REGULAMENTAÇÕES VIGENTES

FLUORIDE CONCENTRATION IN DENTIFRICES MARKETED TO CHILDREN IN BRAZIL AND MEXICO, AND DISCUSSION ON CURRENT REGULATIONS

Piracicaba
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Orientador: Prof. Dr. Antônio Pedro Ricomini Filho

Coorientador: Prof. Dr. Jaime Aparecido Cury

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RESUMO

De acordo com a melhor evidência científica disponível, os dentifrícios fluoretados devem conter concentração mínima de 1.000 ppm de fluoreto (F), o qual deve estar quimicamente solúvel para assegurar o efeito anticárie, sendo que esta premissa é também válida para os dentifrícios fluoretados comercializados para crianças. Neste estudo foram avaliadas as concentrações de fluoreto total (FT = Fluoreto Solúvel Total + Fluoreto insolúvel) e fluoreto solúvel total (FST = íon Flúor + íon FPO_3^{2-}) em dentifrícios comercializados para crianças adquiridos no Brasil e no México. Em acréscimo, foram analisadas as regulamentações vigentes em ambos os países sobre dentifrícios fluoretados. Dentifrícios comercializados para crianças foram adquiridos no Brasil, um total de 24 marcas (n=2-3/marca), e no México, um total de 6 marcas (n=2-3/marca), todos com lotes diferentes. Os dentifrícios foram adquiridos em setembro/outubro de 2019 e analisados ainda frescos (outubro de 2019), e os formulados com Na_2FPO_3/ $CaCO_3$ foram analisados novamente em abril de 2021 para verificar a estabilidade das concentrações de FST ao longo do tempo de armazenamento. Para a análise dos dentifrícios, uma quantidade de 100 mg (± 10) de dentifrício foi ressuspendida em 10 mL de água, homogeneizada, e duplicatas foram utilizadas para análise de FT. A suspensão restante foi centrifugada e duplicatas do sobrenadante foram utilizadas para análise de FST. Para dentifrícios que contêm monofluorfosfato de sódio (Na_2FPO_3), é necessário realizar hidrólise ácida do íon FPO_3^{2-}, portanto, nos tubos para análise do FT e FST foi adicionado HCl 2 M, e após 1 hora a 45°C, as soluções foram neutralizadas com NaOH 1 M e tamponadas com TISAB II. Para análise do fluoreto iônico (FI) em dentifrícios à base de Na_2FPO_3, duplicatas do sobrenadante foram transferidas para tubos de ensaio, e foram adicionados NaOH 1 M, TISAB II e HCl 2 M, nessa ordem. Para os dentifrícios que contêm fluoreto de sódio (NaF), utilizou-se a técnica simplificada para determinação de FT e FST, na qual não é requer hidrólise ácida, portanto, aos tubos foi adicionado apenas alíquotas da suspensão, as quais foi adicionado TISAB II. As concentrações de fluoreto foram analisadas com eletrodo íon-específico, calibrado com padrões de fluoreto, preparados nas mesmas condições das amostras. Os resultados foram expressos em ppm (μg F/g). No Brasil, 20,8% (5 entre 24) dos dentifrícios continham Na_2FPO_3 em sua formulação, sendo que 2 destes apresentavam sílica (SiO_2) como abrasivo, e 3 continham carbonato de cálcio ($CaCO_3$); e 79,8% formulados com NaF e SiO_2 como abrasivo. Por outro lado, no México, todos os dentifrícios adquiridos eram formulados com NaF/SiO_2. Os dentifrícios adquiridos em ambos os países apresentaram concentração de

FT inferior a 1.500 ppm F, com valores de 476,0 ± 10,0 a 1.385,3 ± 3,2. O FST encontrado foi muito próximo ao FT encontrado nos dentifrícios formulados com SiO_2 como abrasivo, comercializados no Brasil ou México, e os dentifrícios formulados com $CaCO_3$ como abrasivo (adquiridos no Brasil) tinham concentrações de F insolúvel variando entre 17,0 e 27,7%. Verificou-se que 70,9% dos dentifrícios brasileiros e 83,4% mexicanos apresentaram concentração superior a 1.000 ppm F, variando de 1,005,1 ± 16,0 a 1.110,5 ± 57,8 nos brasileiros e de 1.066,9 ± 6,3 a 1.398,8 ± 11,0 nos mexicanos. Ao realizar a análise das formulações à base de $Na_2FPO_3/CaCO_3$ em 2021, verificou-se que todas as formulações apresentaram aumento significativo na concentração de fluoreto insolúvel, com valores variando de 43,3% a 63,8%. Com relação às resoluções vigentes, ambos os países apenas estabelecem a concentração de 1.500 ppm de fluoreto total como limite máximo permitido, sem fazer menção ao conteúdo de fluoreto solúvel, o qual é necessário para efeito anticárie. Em conclusão, a maioria dos dentifrícios comercializados para crianças no Brasil e no México têm concentração de fluoreto suficiente disponível para promover o controle de cárie. As concentrações de fluoreto total presentes nos dentifrícios estão de acordo com as resoluções de ambos os países, porém, não estão de acordo com as melhores evidências científicas disponíveis, pois não consideram a necessidade de fluoreto solúvel na formulação do dentifrício. Todos os dentifrícios contendo $Na_2FPO_3/CaCO_3$ não foram capazes de manter uma concentração mínima de fluoreto solúvel dentro do prazo de validade.

Palavras-chave: fluoreto, dentifrício, creme dental, cárie.

ABSTRACT

According to the best scientific evidence available, fluoride toothpastes must contain a minimum concentration of 1000 ppm of fluoride (F), which must be chemically soluble to ensure the anti-caries effect, and this parameter is also valid for fluoride toothpastes marketed kids. In this study, the concentrations of total fluoride (TF = Total Soluble Fluoride + insoluble fluoride) and total soluble fluoride (TSF = Fluoride + FPO_3^{2-} ion) in dentifrices marketed to children purchased in Brazil and Mexico were evaluated. In addition, it was analyzed whether the guidelines established by both countries were being followed. The current resolutions of both countries only establish the concentration of 1,500 ppm of total fluoride as the maximum allowed limit, without considering the soluble fluoride, which is necessary for the anti-caries effect. Dentifrices marketed children were purchased in Brazil, a total of 24 brands (n = 2-3 / brand), and in Mexico, a total of 6 brands (n = 2-3 / brand), all dentifrices acquired were from different lots. The dentifrices were purchased in September/October 2019 and were analyzed while still fresh (October 2019), and those formulated with Na_2FPO_3 /CaCO3 were analyzed again in April 2021 to verify the stability of FST concentrations over the storage time. For toothpaste analysis, an amount of 100 mg (± 10) of toothpaste was resuspended in 10 mL of water, homogenized, and duplicates were used for TF analysis. The remaining suspension was centrifuged, and duplicates of the supernatant were used for TSF analysis. For dentifrices containing sodium monofluorophosphate (Na_2FPO_3), it was necessary to perform acid hydrolysis of the ion $FPO_3^{2-,}$ therefore, in the tubes for TF and TSF analysis, 2 M HCl was added, and after 1 hour at 45 ° C, the solutions were neutralized with 1 M NaOH and buffered with TISAB II. For the analysis of ionic fluoride (FI) in dentifrices formulated with Na_2FPO_3, duplicates of the supernatant were transferred to test tubes, and 1 M NaOH, TISAB II and 2 M HCl were added, in that order. For dentifrices containing sodium fluoride (NaF), the simplified technique was used to determine TF and TSF, in which acid hydrolysis is not required, therefore, only aliquots of the suspension were added to the tubes, to which TISAB II was added. Fluoride concentrations were analyzed with an ion-specific electrode, calibrated with fluoride standards, prepared under the same conditions as the samples. The results were expressed in ppm (μg F/g). In Brazil, 20.8% (5 out of 24) of the dentifrices contained MFP in its formulation, 2 of which had silica (SiO_2) as an abrasive, 3 had calcium carbonate ($CaCO_3$); and 79.8%, formulated with NaF and SiO_2 as an abrasive. On the other hand, in Mexico, all toothpastes purchased were formulated with NaF/SiO_2. The dentifrices acquired in both

countries had a TF concentration below 1,500 ppm F, with values from 476.0 ± 10.0 to 1,385.3 ± 3.2. The TSF found was very close to the TF found in dentifrices formulated with SiO_2 as abrasive, sold in Brazil or Mexico, and the dentifrices formulated with $CaCO_3$ as abrasive (purchased in Brazil) had insoluble F concentrations ranging from 17.0 to 27.7%. It was found that 70.9% of Brazilian and 83.4% Mexican dentifrices had a concentration greater than 1,000 ppm F, ranging from 1,005.1 ± 16.0 to 1,110.5 ± 57.8 in Brazilians and from 1,066.9 ± 6.3 to 1,398.8 ± 11.0 in Mexicans. The $Na_2FPO_3/CaCO_3$-based toothpastes were analyzed again in 2021, and it was found that all formulations showed a significant increase in the concentration of insoluble fluoride, with values ranging from 43.3% to 63.8%. Regarding the current resolutions, both countries only establish the concentration of 1,500 ppm of total fluoride as the maximum allowed limit, without mentioning the content of soluble fluoride, which is necessary for anti-caries effect. In conclusion, most of the dentifrices marketed to children in Brazil and Mexico have enough fluoride concentration available to promote the control of caries. The concentrations of total fluoride present in toothpaste are in accordance with the resolutions of both countries, however, they are not in accordance with the best scientific evidence available, as they do not consider the need for soluble fluoride in the formulation of toothpaste. All dentifrices containing $Na_2FPO_3/CaCO_3$ were not able to maintain a minimum concentration of soluble fluoride within the expiration date.

Key words: fluoride, dentifrice, toothpaste, dental caries.

LISTA DE ABREVIATURAS E SIGLAS

Abreviatura		Inglês	Português
Anvisa	–	National Health Surveillance Agency	Agência Nacional de Vigilância Sanitária
$CaCO_3$	–	Calcium Carbonate	Carbonato de Cálcio
F	–	Fluoreto	Fluoride
FPO_3^{2-}	–	Monofluorphosphate ion	Íon monofluorfosfato
TSF / FST	–	Total Soluble Fluoride	Fluoreto Solúvel Total
TF / FT	–	Total Soluble	Fluoreto Total
HCl	–	Hydrocloric acid	Ácido clorídrico
NaF	–	Sodium fluoride	Fluoreto de Sódio
Na_2FPO_3	–	Sodium Monofluorphosphate	Monofluorfosfato de Sódio
NaOH	–	Sodium Hydroxide	Hidróxido de Sódio
ppm	–	Parts per million	Partes por milhão
SiO_2	–	Silica	Sílica
TISAB	–	Total ionic strength adjustment buffer	Tampão de ajuste de força iônica

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1 INTRODUÇÃO

A escovação com dentifrício fluoretado é considerada a forma mais racional de uso de fluoreto, pois a partir dela combina-se a desorganização e remoção do biofilme dental pelo ato mecânico da escovação, concomitantemente à liberação de fluoreto (F) para o meio bucal (Cury e Tenuta, 2008; Tenuta et al., 2009). Dessa maneira, a presença de fluoreto pode atuar tanto na remineralização dental em locais onde o biofilme foi removido, como na redução da desmineralização onde houver biofilme remanescente que foi enriquecido por F durante a escovação dos dentes (Cury e Tenuta, 2014).

No entanto, para prover efeito anticárie, é essencial que o F esteja quimicamente solúvel na formulação do dentifrício para que possa ser liberado no meio bucal durante a escovação, e interferir no processo físico-químico de desenvolvimento da doença (Tenuta e Cury, 2013). De acordo com a melhor evidência científica disponível, um dentifrício fluoretado deve conter a concentração mínima de 1.000 ppm de F solúvel para fornecer efeito anticárie (Walsh et al., 2010). Essa concentração pode interferir no desenvolvimento de lesões de cárie (Wright et al., 2014), e tem sido demonstrada como eficaz tanto para dentes decíduos (Santos et al., 2013), como permanentes (Walsh et al., 2019). Um estudo realizado por Coelho et al. (2020), mostrou que há uma relação dose-efeito entre a concentração de fluoreto solúvel no dentifrício e a concentração que é biodisponibilizada no meio bucal quando o dente é escovado. Dessa maneira, é importante conhecer as formulações disponíveis no mercado, bem como recomendar as que promovam benefício anticárie à população.

Uma característica dos dentifrícios amplamente comercializados no Brasil (Ricomini Filho et al., 2012), em países da América Latina, a exemplo do Chile (Fernández et al., 2017), em muitos países em desenvolvimento ao redor do mundo (Van Loveren et al., 2005; Cury et al., 2006; Benzian et al., 2012; Soysa et al., 2018; Vorster et al., 2018) é que estes são formulados com carbonato de cálcio ($CaCO_3$) como agente abrasivo, o que reduz o custo da formulação, tornando o dentifrício mais acessível economicamente. Devido à presença do cálcio, estas formulações devem conter monofluorfosfato de sódio (Na_2FPO_3 ou NaMFP) para que não ocorra interação imediata do cálcio com o F, tornando-o insolúvel na formulação. Apesar de estável, o íon monofluorfosfato (FPO_3^{2-}) é susceptível a sofrer hidrólise com o tempo de armazenamento (Tabchoury e Cury, 1994; Conde et al., 2003), e o aumento da temperatura também contribui para o processo de hidrólise do FPO_3^{2-}, de forma que haja mais ligação do fluoreto com o cálcio do abrasivo, o que tornará o F insolúvel (Cury e Tenuta, 2014).

Por outro lado, outras formulações amplamente comercializadas contêm sílica (SiO_2) como abrasivo, possibilitando o uso de diferentes componentes fluoretados nas formulações de dentifrícios, sendo o fluoreto de sódio (NaF) comumente empregado. A sílica é um componente inerte, e o íon flúor não reage com ela, dessa forma, não há alteração da concentração de fluoreto solúvel ao longo do tempo (Cury et al., 2015). Entretanto, é valido ressaltar que o fluoreto solúvel disponibilizado das formulações de dentifrícios, seja como como íon fluoreto (F^-), em formulação à base de NaF/SiO_2, ou como íon monofluorfosfato (FPO_3^{2-}), em formulação à base de $Na_2FPO_3/CaCO_3$, possuem efeito anticárie semelhante (Cury e Tenuta, 2008).

Apesar da necessidade do dentifrício fluoretado serem iguais para crianças e adultos no que se refere a presença mínima de 1.000 ppm F solúvel (Santos et al., 2013; Walsh et al., 2010; Walsh et al., 2019), há uma grande diversidade de dentifrícios comercializados para o público infantil que não atendem esta concentração mínima. Estes dentifrícios se diferenciam dos convencionais pelo apelo comercial que costuma ser feito para o público infantil principalmente através do uso de personagens de desenhos animados e de filmes impressos nas embalagens. Estudos anteriores (Cury et al., 2010; Giacaman et al., 2013; Chávez et al., 2019) mostraram que estão presentes no mercado dentifrícios para crianças com baixa concentração de F (< 600 ppm F), o que pode comprometer o potencial anticárie (Santos et al., 2013).

Dessa maneira, dentifrícios comercializados para crianças podem apresentar concentração de fluoreto solúvel reduzida, seja pela menor quantidade de fluoreto adicionado ao produto, nos dentifrícios com baixa concentração de F (< 600 ppm F), ou pelo tipo de formulação, a exemplo de dentifrícios à base de $Na_2FPO_3/CaCO_3$. Ou seja, as formulações podem já ser fabricadas com baixa concentração de fluoreto, ou apresentarem baixa concentração de fluoreto solúvel por ter havido redução gradativa dessa concentração em formulações com $Na_2FPO_3/CaCO_3$. Sendo assim, os países devem ter resoluções que regulamentem tanto a concentração máxima de F que pode estar presente em uma formulação de dentifrício, bem como a concentração mínima de F solúvel necessária para que o dentifrício possua efeito anticárie. As atuais resoluções brasileiras (ANVISA, 2000) e mexicana (NMX-K-539-NYCE-2020), à semelhança do Mercosul (Mercosul, 2002) e União Europeia (União Europeia, 2008), apenas estabelecem que a concentração de F não deve ultrapassar 0,15% (1.500 ppm F; mg F/kg), no entanto, não faz menção a quanto de F deve ser solúvel no produto para que este tenha efeito anticárie. Em acréscimo, a resolução brasileira permite a utilização de diversos agentes fluoretados na formulação, inclusive sais de baixíssima solubilidade, como o fluoreto de cálcio (CaF_2), que não apresenta potencial anticárie.

A ausência de resolução atualizada que regulamente a concentração de F em dentifrícios a ser utilizada por toda a população, incluindo a infantil, possibilita que recomendações para uso de dentifrícios com baixa concentração de F por crianças continuem sendo implementadas. A exemplo da normativa mexicana para prevenção e controle de enfermidades bucais (NOM-013-SSA2-2015), a qual recomenda o uso de dentifrício contendo 550 ppm F para crianças menores de 6 anos, e informa que dentifrícios com concentração de 551 a 1.500 ppm F só devem ser utilizados por crianças maiores de 6 anos. Giacaman et al. (2013) descreveram situação no Chile, em que a regulamentação não era consistente com as melhores evidências disponíveis para o controle de cárie dental, enfatizando a necessidade de atualização. Essa discussão possibilitou que a as diretrizes fossem revistas, sendo que atualmente, dentifrícios comercializados para crianças de qualquer idade devem conter concentrações de F entre 1.000 e 1.500 ppm F (MINSAL, 2015).

No Brasil, um estudo realizado por Santos et al. (2010) verificou se as recomendações fornecidas por órgãos governamentais brasileiros sobre higiene bucal em crianças eram consistentes, e se as possíveis inconsistências nessas recomendações estariam associadas a uma aparente falta de evidência científica. Informações conflitantes e inconsistentes como as descritas anteriormente, a exemplo da norma mexicana (NOM-013-SSA2-2015), quando disponibilizadas à população, podem gerar dúvidas, levar a utilizações inadequadas, ampliar as iniquidades em saúde bucal e colocar em questão a efetividade e segurança dessas práticas como medidas de saúde pública. Dessa forma, deve-se perspectiva de aproximar a pesquisa científica da prática clínica.

No entanto Brasil, não há estudos recentes que avaliem a concentração e estabilidade de fluoreto solúvel nos dentifrícios fluoretados disponíveis no mercado brasileiro que são comercializados para o público infantil. Da mesma maneira, não é conhecida a concentração de F solúvel nos dentifrícios comercializados no México que são comercializados para crianças. Sendo assim, o objetivo deste estudo foi avaliar a concentração e estabilidade de fluoreto solúvel em dentifrícios comercializados para crianças no Brasil e México, considerando seu potencial anticárie. Em acréscimo, foram analisadas as regulamentações vigentes em ambos os países sobre dentifrícios fluoretados.

2 ARTIGO: FLUORIDE CONCENTRATION IN CHILDREN'S DENTIFRICES MARKETED IN BRAZIL AND MEXICO, AND DISCUSSION ON CURRENT REGULATIONS *

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ABSTRACT

Fluoride toothpastes market to children should contain a minimum concentration of 1,000 ppm of fluoride (F), which must be chemically soluble to provide anti-caries effect. Therefore, we determined the concentrations of total fluoride (TF) and total soluble fluoride (TSF) in toothpastes marketed for children in Brazil and Mexico and analyzed the current regulations in force in both countries. Twenty-four brands were found and purchased in Brazil (19 formulated with NaF/SiO_2, three with $Na_2FPO_3/CaCO_3$ and two with Na_2FPO_3/SiO_2) and six in Mexico (all with NaF/SiO_2). TF and TSF concentrations were determined after the purchase (fresh samples) but fluoride stability in $Na_2FPO_3/CaCO_3$-formulations was checked after 18 months. The analyses were performed with an ion-specific electrode and the results expressed in ppm F (mg F/kg). The TF concentrations found ranged from 476.0 to 1,385.3 ppm F and they were close the declared by the manufactures (500 to 1,450 ppm F). The TF concentrations found were not greater than 1,500 ppm F, in accordance with the current regulations of both countries. However, toothpastes presenting TSF concentrations lower than 1000 ppm F were found either in low fluoride toothpaste (500 ppm F) formulated with NaF/SiO_2 as in fresh and aged $Na_2FPO_3/CaCO_3$-toothpastes, originally fabricated with 1,000-1,100 ppm of TF. In conclusion, although most toothpastes analyzed showed TSF concentration higher than 1,000 ppm F, the regulations in force in both countries allow products that are not in agreement with the best available evidence are available in the market.

Key words: fluoride, dentifrice, toothpaste, dental caries.

INTRODUCTION

The use of fluoride toothpaste has contributed to control dental caries worldwide (Whelton et al., 2019) and its benefit is strongly based on scientific evidence (Marinho et al., 2003). Furthermore, the use of fluoride toothpaste since the eruption of the first tooth in the child's mouth has been recommended (American Dental Association, 2014; Splieth et al., 2020) because the anticaries benefit can overcomes the risk of dental fluorosis (Tenuta and Cury, 2010; Tenuta et al., 2010; Onoriobe et al., 2014). For the balance between caries prevention and fluorosis risk, it has been recommended that toothpastes for children should contain from 1,000 to 1,500 ppm of total fluoride (TF). However, in most countries, toothpastes are considered as cosmetic products and the governmental regulations establish the maximum fluoride concentration allowed but not a minimum (Brazilian Health Surveillance Agency, 2000; Mercosur, 2002; European Union, 2008). Also, most regulations do not establish how much of the TF should be chemically soluble (TSF) in the formulation to guarantee the anticaries effect (Cury et al., 2015a).

Therefore, toothpastes market to children with TSF lower than 1,000 ppm F have been found in the market not because the formulation presents fluoride chemically unstable (Giacaman et al. 2013; Chávez et al., 2019) but mainly because is allowed by local the national regulations. The toothpastes market for children are usually formulated with sodium fluoride (NaF) and silica (SiO_2) as an abrasive (Giacaman et al. 2013; Chávez et al., 2019, Péres-Silva et al., 2021). As SiO_2 is an inert component, all F will be soluble, available as ion F (F^-). However, it possible to find toothpastes formulated with sodium monofluorophosphate (Na_2FPO_3) and calcium carbonate ($CaCO_3$) as abrasive. In this formulation, soluble F is mainly found as monofluorophosphate ion (FPO_3^{2-}). Although FPO_3^{2-} is stable in the formulation, it is susceptible to undergo hydrolysis over time, which favors reaction of F^- with the calcium from the abrasive, forming insoluble F salts (Tabchoury and Cury, 1994; Conde et al.,2003; Cury and Tenuta, 2014). Therefore, a reduced F concentration is even more critical for a Na_2FPO_3/$CaCO_3$-based toothpaste since the soluble F content will be increasingly reduced over time. Thus, while a 500 ppm F toothpaste formulated with silica is able to maintain all TF as TSF overtime, one formulated with abrasive containing Ca ($CaCO_3$ or $CaH_2PO_4.2H_2O$) will have lower TSF before the expire date of the product (Cury et al., 2015b).

Thus, the Brazilian (ANVISA, 2000) and Mexican (NMX-K-539-NYCE-2020) regulations only establish the maximum concentration of F present in a toothpaste, which should not exceed 0.15% expressed in F (1,500 ppm F; mg F/kg). In addition, the regulations

do not mention the minimum concentration of soluble F that a toothpaste should contain and maintain until its expiration date. Therefore, it is mandatory that the regulations are revised in order that the fluoride dentifrices offered to the population, including the ones market for children, provide the anti-caries benefit based on the best evidence available.

Although there is great commercial interest in toothpastes marketed to children, there is no recent study evaluating the fluoride content of these toothpastes sold in Brazil and Mexico. Therefore, the aim of this study was to evaluate the concentrations of total fluoride and total soluble fluoride in toothpastes marketed to children in Brazil and Mexico, and the stability of soluble fluoride concentration in $Na_2FPO_3/CaCO_3$ formulations. In addition, the current regulations on fluoride dentifrice in both countries were analyzed.

METHODOLOGY

Sampling

The toothpastes marketed to children were purchased in Brazil and Mexico, and the information found on the packaging is described in Table 1. Twenty-four brands were found in the Brazilian market (Table 1; Codes A to X), being purchased in drugstores in in the cities of Piracicaba, Campinas, and Limeira, SP state. In Mexico, the six brands found were purchased in three supermarkets in Mexico City (Table 1; Codes Y to D1). Toothpaste tubes of different lots (n = 2-3) were used to determine the fluoride content in each formulation. All dentifrices were purchased between September and October in 2019, and the samples were analyzed shortly after the purchase (fresh samples). In April 2021, only $Na_2FPO_3/CaCO_3$-based toothpastes were evaluated again (aged samples).

Table 1. Dentifrices analyzed in the study and information provided by the manufacturers.

Code	Commercial name	Country	n	Formulation	TF declared	Lots	Expiration date
A	Bambinos 2 (2-5 anos)	Brazil	2	NaF/SiO_2	500	L000397 L000550	Jun/2021 Feb/2022
B	G.U.M. the Lion Guard	Brazil	2	NaF/SiO_2	995	17539 17431	Dec/2020 Dec/2020
C	Paw Patrol (3+ anos)	Brazil	3	NaF/SiO_2	1000	19114 19086 19114	Apr/2022 Mar/2022 Apr/2022
D	Sorriso Kids	Brazil	3	NaF/SiO_2	1100	9144BR122I 9115BR123C 9144BR122I	May/2021 Apr/2021 May/2021
E	Tandy	Brazil	3	NaF/SiO_2	1100	9148BR121C 9172BR122K 9011BR122K	May/2022 Jun/2022 Jan/2022
F	Colgate Smile (6+ anos)	Brazil	3	NaF/SiO_2	1100	(L)8247MX1136 (L)7285MX1136 (L)9038MX1116	Sep/2021 Oct/2021 Feb/2022
G	Oral-B Kids	Brazil	3	NaF/SiO_2	1100	83024354P0 83284354P2 82704354P0	Sep/2020 Oct/2020 Aug/2020
H	Oral-B Stages	Brazil	3	NaF/SiO_2	1100	91784354Q1 81514354Q2 81274354Q2	May/2021 Apr/2021 Apr/2021
I	Neutrocare	Brazil	3	NaF/SiO_2	1100	1921 1914 1802	Feb/2021 Jan/2021 Oct/2020
J	Peppa Pig (5+ anos)	Brazil	2	NaF/SiO_2	1100	48347 44416	May/2022 Oct/2021
K	Dentalclean (3+ anos)	Brazil	3	NaF/SiO_2	1100	43691 43542	Sep/2021 Dec/2021
L	Dentalclean (5+ anos)	Brazil	3	NaF/SiO_2	1100	43691 43542	Sep/2021 Dec/2021
M	Boni Kids	Brazil	3	NaF/SiO_2	1100	26106 13633 L1050078	Dec/2020 Mar/2020 Sep/2021
N	Hello Kitty	Brazil	2	NaF/SiO_2	1100	27229 27351	Jan/2021 Feb/2021
O	BITUFO Cocoricó	Brazil	3	NaF/SiO_2	1100	L8214AS L8281AS L8173AS	Aug/2021 Oct/2021 Jun/2021
P	Malvatrikids F-infantil	Brazil	2	NaF/SiO_2	1100	181413 190077	Nov/2021 Feb/2022

Continuation of Table 1. Dentifrices analyzed in the study and information provided by the manufacturers.

Code	Commercial name	Country	n	Formulation	TF declared	Lots	Expiration date
Q	Kid's CREST	Brazil	2	NaF/SiO_2	1100	7334GC 7334GC	Oct/2020 Oct/2020
R	Bambinos 3 (6+ anos)	Brazil	3	NaF/SiO_2	1100	L000559 L000469 L000613	Mar/2022 Oct/2021 Jun/2022
S	Dentil Kids Scooby-Doo	Brazil	3	NaF/SiO_2	1100	38516 44527 28867	Nov/2020 Oct/2021 Apr/2021
T	Dora a Aventureira	Brazil	2	$Na_2PO_3/CaCO_3$	900	11856119 11856119	Jun/2022 Jun/2022
U	Dentil Kids Zoo	Brazil	2	Na_2PO_3/SiO_2	1000	A99011 A99011	Jan/2022 Jan/2022
V	Dentil Kids	Brazil	2	Na_2PO_3/SiO_2	1100	1370018 28867	Jun/2022 Apr/2021
W	Tra Lá Lá Kids antiaçúcar	Brazil	3	$Na_2PO_3/CaCO_3$	1100	L290455 L290369 L290441	Apr/2022 Apr/2021 Mar/2022
X	Tra Lá Lá Kids	Brazil	2	$Na_2PO_3/CaCO_3$	1179	L290376 L290456	May/2021 Apr/2022
Y	G.U.M. the Lion Guard	Mexico	3	NaF/SiO_2	995	17430 17535 17535	Aug/2020 Nov/2020 Nov/2020
Z	Colgate Kids	Mexico	3	NaF/SiO_2	1085	8313BR122k 8324BR121K 8286BR122K	Nov/2021 Nov/2021 Oct/2021
A1	Colgate Smile	Mexico	3	NaF/SiO_2	1100	19023 MX1126 18362 MX1116 8292 MX1136	Jan/2022 Dec/2021 Oct/2020
B1	Oral-B Kids	Mexico	3	NaF/SiO_2	1100	90274354P0 90334354P0 83554354P2	Dec/2020 Jan/2021 Nov/2020
C1	Oral-B Stages	Mexico	3	NaF/SiO_2	1100	83614354Q0 83304354Q3 83624354Q0	Nov/2021 Oct/2021 Nov/2021
D1	Crest (Star Wars)	Mexico	2	NaF/SiO_2	1450	72694354P1 72694354P1	Aug/2020 Aug/2020

TF declared = Total fluoride declared by the manufacturer on label; ppm F = mg F/kg; NaF = sodium fluoride; Na_2FPO_3 = sodium monofluorophosphate; SiO_2= silica; $CaCO_3$ = calcium carbonate.

Determination of Fluoride Concentration

The concentrations of total fluoride (TF) and total soluble fluoride (TSF) in toothpastes were determined for all formulations ($Na_2FPO_3/CaCO_3$ and NaF/SiO_2). Fluoride concentration as MFP ion (FPO_3^{2-}), fluoride ion (FI) and the percentage of insoluble fluoride (% ins-F) was calculated only for $Na_2FPO_3/CaCO_3$-based toothpastes. Depending on the formulation, $Na_2FPO_3/CaCO_3$ or NaF/SiO_2, dentifrice samples were differently prepared.

For Na_2FPO_3-based toothpastes, fluoride analysis was carried out following the conventional protocol, as previously described by Cury et al., (2010). Briefly, from 90 to 110 mg of toothpaste was weighed (± 0.01 mg), and then the dentifrice sample was vigorously homogenized in 10.0 mL of purified water. Duplicates of 0.25 mL of the toothpaste slurry were transferred to test tubes for TF concentration analysis. The remaining suspension was centrifuged (3,000 *g* for 10 min at room temperature) and duplicates of 0.25 mL of the supernatant were transferred to test tubes test for TSF and FI determination. To TF and TSF tubes, 0.25 mL of 2 M HCl was added, and the samples were incubated for 1 h at 45ºC (water bath) to hydrolyze the FPO_3^{2-} ion. Then, the samples were neutralized with 0.5 mL of 1 M NaOH and buffered with 1.0 mL of TISAB II. For FI analysis, duplicates of 0.25 mL of the supernatant were transferred to tubes test, and 0.5 mL of 1 M NaOH, 1.0 mL of TISAB II, and 0.25 mL of 2 M HCl were added in this order to avoid FPO_3^{2-} ion hydrolysis. From these analyses, the concentrations of F as FPO_3^{2-} ion (FPO_3^{2-} = TSF - FI) and the percentage of ins-F were calculated [(TF found - TSF) x 100/(TF found)].

For the NaF-based toothpastes, a validated simplified protocol was used (Quiroz-Torres et al. 2020) discarded the unnecessary steps of acid hydrolysis. The same amount of toothpaste was weighed and homogenized in purified water. Duplicates of 1.0 mL of the toothpaste slurry were transferred to test tubes for TF analysis. After centrifugation, duplicates of 1.0 mL of the supernatant were transferred to tubes for TSF determination. TF and TSF were buffered with 1.0 mL of TISAB II.

Fluoride analysis

Fluoride analysis was carried out using an ion specific electrode (Thermo Scientific Orion model 9609BNWP, Orion Research, Cambridge, MA, USA) coupled to an ion analyzer (Thermo Scientific Orion EA-740, Orion Research, Cambridge, MA, USA). A calibration curve was made with F standards of different concentration prepared as the samples. For Na_2FPO_3-based toothpastes, standards ranged from 0.0625 to 2.5 μg F/mL in 0.25 M HCl, 0.25 M NaOH, and TISAB II 50% (v/v). For NaF-based toothpastes, standards ranged from 0.5 to 10.0 μg F/mL in TISAB II 50% (v/v). Results were expressed as ppm F (mg F/Kg).

Legislations about fluoride toothpastes

To analyze the legislations on fluoride toothpastes in both countries, a search was performed on the websites of Brazilian Ministry of Health and Mexican Official Journal of the Federation. The Table 2 describes the main data obtained, including the maximum fluoride allowed in .

Table 2. Specifications about Brazilian and Mexican resolutions on fluoride dentifrices.

Country	Resolution/Standard	Year	Supervisory entity	Classification	Fluoride concentration
Brazil	Resolution number 79, ANVISA	2000	ANVISA (Brazilian Health Surveillance Agency)	Cosmetic product	1,500 ppm (0.15%) of total fluoride as the maximum concentration allowed
Mexico	Standard NMX-K-539-NYCE-2020	2020	Normalización y Certificación NYCE, S.C.	Cosmetic product	1,500 ppm (0.15%) of total fluoride (as ion fluor) as the maximum concentration allowed

RESULTS

In Brazil sampling, a total of 24 different brands of dentifrices market to children were found, while in Mexico, only 6 brands (Table 1). Moreover, in Brazil, it was possible to observe different formulations, being 19 (79%) of the toothpastes formulated with NaF/SiO_2, 3 (13%) with $Na_2FPO_3/CaCO_3$, and 2 (8%) with Na_2FPO_3/SiO_2. Different from the Brazilian diversity, in Mexico all the 6 toothpastes were formulated with NaF/SiO_2.

Considering the total F declared by the manufacturers (Table 1), most of toothpastes, 3 from Mexico and 18 from Brazil in a total of 21 (70%), were formulated with 1,100 ppm F. The other six formulations in Brazil contained 500 (A), 900 (T), 995 (B), 1,000 (C, U), and 1,179 (X) ppm F. In Mexico, the other three formulations disclosed 995 (Y), 1,085 (Z) and 1,450 (D1) ppm F.

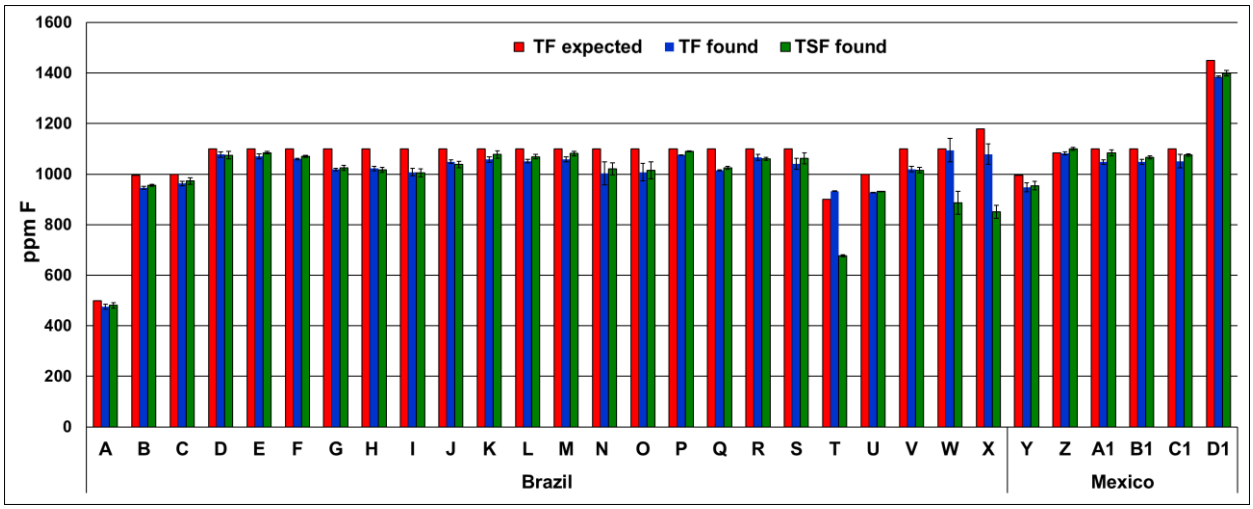

Figure 1. Concentrations of total fluoride (TF) expected (declared by the manufacturer), total fluoride (TF) and total soluble fluoride (TSF) in toothpastes purchased in Brazil and in Mexico (ppm F). Analyses were performed in Sept-Oct 2019 with fresh samples.

The TF concentration found in all products ranged from 476.0 to 1385.3 ppm F, being in accordance with what was declared by the manufacture (Figure 1). All concentrations were also in accordance with Brazilian and Mexican regulations, presenting a TF concentration that did not exceed 1,500 ppm F (Table 2).

The TSF concentration in fresh samples of NaF-based toothpastes ranged from 480.7 to 1,398.8 , while for Na_2FPO_3-based toothpastes, from 677.4 to 1,015.7 (Figure 1). Most of

toothpastes market to children in Brazil, 17 (70.9%), and in Mexico, 5 (83.4%), had a concentration of soluble F greater than 1,000 ppm, ranging from 1,005.1 to 1,110.5 in Brazilians and from 1,066.9 to 1,398.8 in Mexicans.

Na_2FPO_3-based toothpastes presented lower TSF concentration than TF found (Table 3) when $CaCO_3$ was the abrasive used in the formulation (T, W, X), which was not observed for the silica-containing (U, V). Fresh samples of the Na_2FPO_3-based toothpastes T, W, and W presented 27.4, 19.0 and 21.2% of insoluble fluoride, respectively, with TSF concentration lower than 886.5 ppm F.

Table 3. Total fluoride (TF) concentration expected, TF and total soluble fluoride (TSF), soluble fluoride found as monofluorophosphate ion (FPO_3^{2-}) and F ion (FI), and percentage of insoluble F (%Ins-F) in fresh samples of Na_2FPO_3-based toothpastes purchased in Brazil.

Code	TF expected	ppm F found as		ppm F found as		%Ins-F
		TF	TSF	FPO_3^{2-}	FI	
T	900	932.7 ± 0.6	677.4 ± 3.5	597.0 ± 5.5	80.4 ± 2.1	27.4 ± 0.4
U	1000	928.5 ± 0.0	932.2 ± 0.0	904.4 ± 0.2	28.8 ± 1.2	0.4 ± 0.0
V	1100	1019.7 ± 10.5	1015.7 ± 10.4	985.4 ± 9.2	30.4 ± 1.2	0.4 ± 0.0
W	1100	1094.7 ± 46.2	886.5 ± 44.6	759.7 ± 66.4	126.8 ± 21.8	19.0 ± 0.7
X	1179	1078.8 ± 40.8	850.5 ± 25.5	720.4 ± 35.9	126.5 ± 8.1	21.2 ± 4.2

The aged samples of Na_2FPO_3-$CaCO_3$ toothpastes presented an increased amount of insoluble fluoride after the 18-month period (Figure 2). The aged samples of toothpastes T, W, and W presented 63.7, 43.6, and 49.2% of insoluble fluoride, respectively, with TSF concentration lower than 667.7 ppm F, reaching to 345.8 ppm F in toothpaste T.

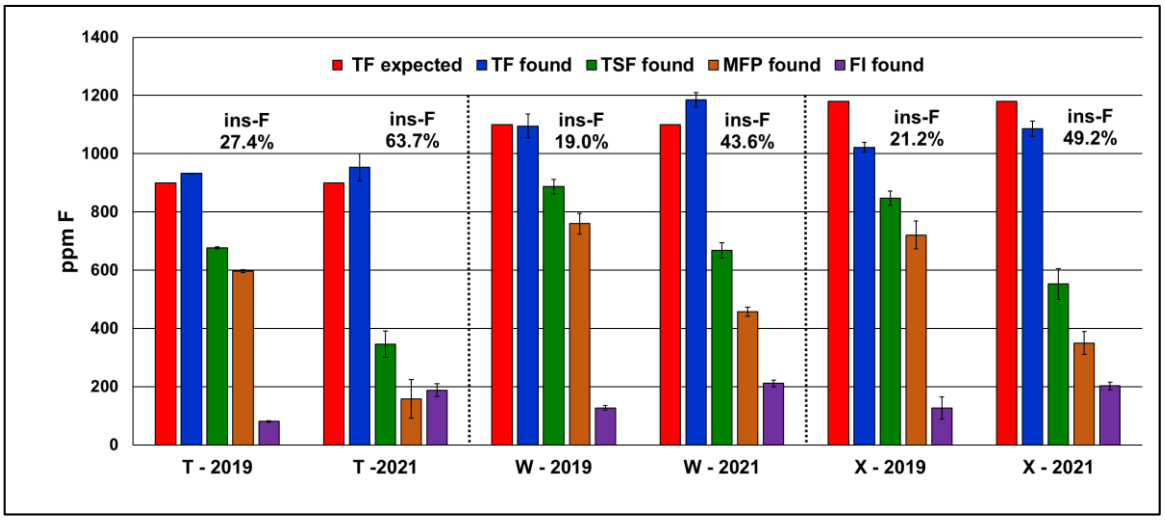

Figure 2. Concentrations of total fluoride (TF) expected (declared by the manufacturer), total fluoride (TF), total soluble fluoride (TSF), FPO_3^{2-} ion, fluoride ion (FI) and percentage of insoluble fluoride (% ins-F) found in $Na_2FPO_3/CaCO_3$-based toothpastes fresh (2019) and after 18-month storage at room temperature (2021).

DISCUSSION

In this study, the concentrations of total fluoride (TF) and total soluble fluoride (TSF) present in toothpastes market to children in Brazil and Mexico were evaluated. In Brazil, the fluoride concentration declared by the manufactures ranged from 500 to 1,179, while in Mexico from 995 to 1,450 ppm F. All the toothpastes presented TF concentration similarly to the F content described by the manufacturer. Among the toothpastes formulated with SiO_2 as abrasive (NaF/SiO_2 and Na_2FPO_3/SiO_2), the TSF concentration was similar to the TF concentration found. On the other hand, the toothpastes formulated with Na_2FPO_3 and $CaCO_3$ as abrasive presented lower TSF concentration compared to the TF concentration. In these brands, the % of insoluble fluoride increased from 27.4 % in fresh samples to 63.7% after 18 months of storage. Considering all the dentifrices evaluated, 70.9% of the Brazilians and 83.4% of the Mexicans presented a TSF concentration greater than 1,000 ppm F, the minimum concentration necessary to provide anti-caries effect.

In Brazil, it was found a great diversity of brands and formulations of dentifrices market to children when compared to Mexico (Table 1). This difference may be explained by the variety of companies present in the Brazilian market. While in Mexico, the brands available are from multinational companies, in Brazil a great diversity of local producers contribute to

different brands and formulations available in the market. All the Mexican toothpastes and most of the Brazilian presented NaF-based formulations with SiO_2 as abrasive and showed TSF concentration similar to the TF found (Figure 1). As silica is an inert abrasive, the soluble fluoride content in this type of formulation remains stable in the product over time.

Differently from NaF-based, Na_2FPO_3-based toothpastes were only found in the Brazilian market. In those that were formulated with Na_2FPO_3/SiO_2 (codes U and V), the TF and TSF concentrations were similar (Table 3), and the TSF concentration corresponded to the sum of fluoride as FPO_3^{2-} and as FI. As expected, SiO_2 as abrasive does not interfere with the soluble fluoride content. On the other hand, the dentifrices formulated with $Na_2FPO_3/CaCO_3$ (codes T, W and X) presented 19.0% to 27.4% of insoluble fluoride (Table 3) in fresh samples, which shows a considerable reduction of TSF concentration when compared to the TF found. It is well that in $Na_2FPO_3/CaCO_3$ formulation, the FPO_3^{2-} is susceptible to undergo hydrolysis, releasing the F^- that reacts with the Ca^{++} from the abrasive, making the F insoluble (Cury and Tenuta, 2014). Although these formulations had declared from 900 to 1,179 ppm F, the TSF concentration found ranged from 677.4 to 886.5 ppm F.

In addition to the fresh samples of $Na_2FPO_3/CaCO_3$ dentifrices (codes T, W and X) that were performed in 2019 (September and October), new samples were collected from the same tubes in 2021 (April) to evaluate again the F content (Figure 3). After the 18-months period, it was possible to observe that the TSF concentration drastically reduced in the samples, from 345,8 to 667,8 ppm F, and the percentage of insoluble fluoride ranged from 43.6% to 63.7%, representing around 50% of the F content in the formulation. Some dentifrices were close to or 1 year from the expiration date (Table 1). The prolonged storage period favored continuous FPO_3^{2-} hydrolysis, increasing the insoluble F content (Tabchoury and Cury, 1994; Conde et al., 2003; Cury et al., 2015b).

Although there is a great commercial interest and appeal with advertisements in toothpastes marketed for children, they should contain the same conventional fluoride concentration as the ones sold for the general public. Regardless of the target audience, a toothpaste must contain a minimum concentration of 1,000 ppm of soluble F to provide anti-caries effect (Walsh et al., 2010; Santos et al., 2013). An interesting result was that most toothpastes were formulated with 1100 ppm F (Table 1), which could favor the minimum concentration necessary to be effective. However, we still found in the Brazilian market a toothpaste with low fluoride concentration (A). Another concern is related to $Na_2FPO_3/CaCO_3$

toothpastes containing around 1,000 ppm F, since the soluble fluoride content is reduced in recently acquired toothpastes, with the concentration decreasing over time. Therefore, it would be necessary to increase the concentration of TF in the formulation, considering that part of fluoride would be insoluble. This already occurs in $Na_2FPO_3/CaCO_3$ toothpastes sold for the general public, since these formulations usually contain 1450/1500 ppm F. Another solution would be for companies to invest in the development of more stable $Na_2FPO_3/CaCO_3$ formulations that do not compromise the soluble fluoride content.

All the evaluated dentifrices presented TF concentration lower than 1,500 ppm F, being in accordance with the current Brazilian (ANVISA, 2000) and Mexican (NMX-K-539-NYCE-2020) regulations that establishes that the maximum concentration of fluoride in dentifrices should not exceed 1,500 ppm F. Different from the Brazilian, the Mexican standard declares that the concentration refers to fluoride ion, however, the presence of FPO_3^{2-} ion present in Na_2FPO_3-based toothpastes, marketed for the general public, is also a soluble fluoride source that should be included in the Mexican standard. Unfortunately, the current Brazilian resolution, which is similar to European Union (European Union, 2008) and Mercorsur (Mercosur, 2002), and Mexico standard do not specify how much of the total fluoride present in the formulation should be maintained soluble in a toothpaste formulation to be anti-caries effective.

The lack of regulations that state a minimum concentration of soluble fluoride in toothpastes impacts not only Brazil and Mexico, being a worldwide problem signaled by the World Dental Federation (FDI, 2018). FDI advocates the use of toothpaste with a fluoride concentration between 1,000 to 1,500 ppm, with a minimum of 800 ppm of soluble fluoride. The concentration of 800 ppm F can be justified since $Na_2FPO_3/CaCO_3$ toothpaste would hardily maintain a concentration higher than 1,000 ppm F close to the expiration date. Therefore, it is expected that $Na_2FPO_3/CaCO_3$ formulations containing 1,450/1,500 ppm F, present the minimum concentration of 1,000 ppm of soluble F in recently acquired toothpastes (fresh sample), and the concentration of 800 ppm F after two years from product manufacture (aged sample), that could be a feasible concentration to be obtained even by small producers. This discussion is relevant, since $Na_2FPO_3/CaCO_3$ toothpastes are more affordable when compared to those with SiO_2 as abrasive, having a high social impact in developing countries, where $Na_2FPO_3/CaCO_3$ toothpastes are widely available in the market (Cury et al., 2020).

The absence of updated regulations establishing the minimum concentration of F in dentifrices, enables dentifrices with low F concentration continue to be recommended. Like the

Mexican guideline for prevention and control of oral diseases (NOM-013-SSA2-2015), in which the use of toothpaste containing 550 ppm F is recommended for children under 6 years old, and also informs that toothpaste with a concentration of 551 to 1,500 ppm F should only be used by children over 6 years old. Despite of this recommendation, low fluoride dentifrices (< 600 ppm F) were not found in the largest supermarkets of Mexico City. The use of low-F formulations has been raised as an alternative to reduce the risk of fluorosis. However, the use of low-F dentifrices by preschoolers did not reduce the risk of caries in the primary dentition and did not decrease the risk of fluorosis in permanent teeth (Santos et al., 2013). Therefore, fluoride toothpastes with conventional concentration (1,000 to 1,500 ppm F) should be recommended to children, using an age-related amount of toothpaste for tooth brushing till age of six (Toumba et al., 2019).

Brazil and Mexico are still before legislations that only take into consideration the maximum fluoride concentration (0,15%) that a toothpaste should contain, without considering the need of soluble fluoride to provide the anti-caries effect. This fact differs from other legislations, as in United States (US Food and Drug Administration, 2013) and Madagascar (Bureau des Normes de Madagascar, 2018). Although it has been pointed out in previous studies the need of change in the Brazilian regulation on fluoride toothpaste (Cury et al. 2015; Cury et al., 2020), the Brazilian regulation remain unchanged. Although the Mexican standard on fluoride toothpaste was recently update (NMX-K-539-NYCE-2020), it should also mention the need of soluble F to provide the anti-caries effect, not only as F ion, but considering monofluorophosphate ion (FPO_3^{2-}) too. However, the regulations of both countries must establish the minimum concentration of soluble F that a toothpaste should contain to provide anti-caries effect to the entire population, irrespective of age. Therefore, the present study reinforces the attention for the lack of requirement on the necessity of a minimum fluoride concentration in the resolution of Mercosur countries and in other Latino American countries.

In conclusion, toothpastes marketed for children in Brazil and Mexico are diverse in terms of brand, formulation, and the fluoride content. All toothpastes presented TF concentration lower than 1500 ppm F, being in accordance with current regulations of both countries. Most of the Brazilians and Mexicans toothpastes presented TSF concentration greater than 1,000 ppm F, the minimum concentration necessary to provide anti-caries effect. $Na_2FPO_3/CaCO_3$ toothpastes presented reduced TSF concentration, highlighting the need for improvements in formulations. In addition, the regulations of both countries should be revised,

requiring the minimum concentration of 1,000 ppm of soluble fluoride to provide anti-caries effect not only to children, but to the entire population.

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CONCLUSÃO

Com base nas análises realizadas em dentifrícios comercializados para crianças no Brasil e México, conclui-se que:

- Os dentifrícios comercializados para crianças no Brasil e México diferem em marcas, composição, concentração de fluoreto e biodisponibilidade (fluoreto solúvel total).
- A maioria dos dentifrícios adquiridos no Brasil e México apresentam concentração mínima de 1.000 ppm de fluoreto solúvel na formulação, o qual proporciona benefício anticárie.
- As formulações à base de $Na_2FPO_3/CaCO_3$ apresentam redução na concentração de fluoreto solúvel, sendo agravada pelo tempo de armazenamento, evidenciando a necessidade de melhorias neste tipo de formulação.
- Todos os dentifrícios adquiridos no Brasil e México apresentam concentração menor que 1.500 ppm F de fluoreto total, limite máximo que é estabelecido pelas regulamentações vigentes de ambos os países.
- As regulamentações de ambos os países devem ser revistas, exigindo que os dentifrícios possuam concentração mínima de fluoreto solúvel para prover efeito anticárie.

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ANEXOS

Anexo 1: verificação de originalidade e prevenção de plágio

Dissertação Ademir Filho

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