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Analysis of fluoride concentration in mother's milk substitutes

Análise da concentração de flúor em substitutos do leite materno

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ABSTRACT: The aim of the present study was to determine the fluoride concentration in some brands of mother's milk substitutes and evaluate the possibility of developing dental fluorosis by consuming these products. The products, all powdered, were divided into 3 groups: infant formulae (group I, n = 7), milk-based (group M, n = 8) and soy-based (group S, n = 3). Samples from 3 cans of different batches of each brand were reconstituted in deionized water and analyzed using the specific electrode method, after hexamethyldisiloxane (HMDS) facilitated diffusion. The fluoride content (mg F/L) of the products ranged from 0.044 to 0.326 (I), 0.014 to 0.045 (M) and 0.253 to 0.702 (S). There was significant difference in the fluoride content of cans from distinct batches ($p < 0.05$) in most of the brands. The reconstitution of all products in water with optimal fluoride concentration for consumption during the mineralization phase of the primary teeth could result in daily fluoride intake above 0.07 mg F/kg body weight/day. Therefore, the consumption of these products, especially when reconstituted with optimally fluoridated water, could increase the risk of developing dental fluorosis.

DESCRIPTORS: Fluorides; Dental fluorosis; Milk substitutes; Infant formula; Milk.

RESUMO: Este estudo teve como objetivos determinar os teores de flúor de algumas marcas comerciais de substitutos do leite materno e avaliar a possibilidade de desenvolvimento de fluorose dentária com o consumo destes produtos. Os produtos, todos em pó, foram divididos em três grupos: fórmulas infantis (grupo F, n = 7), leite (grupo L, n = 8) e leite de soja (grupo S, n = 3). As amostras de pó de três latas de lotes diferentes de cada marca comercial foram reconstituídas em água deionizada e analisadas pelo método eletrodo específico, após difusão facilitada por hexametildisiloxane (HMDS). O conteúdo de flúor (mg F/L) dos produtos variou de 0,044 a 0,326 (F), 0,014 a 0,045 (L) e 0,253 a 0,702 (S). Houve diferença significativa ($p < 0,05$) entre o conteúdo de flúor de latas de lotes diferentes do mesmo produto na maioria das marcas comerciais analisadas. A reconstituição de todos os produtos em água com concentração ótima de flúor, para o consumo de crianças na faixa etária de formação dos dentes decíduos, pode resultar em quantidades diárias de ingestão de flúor superiores a 0,07 mg F/kg peso/dia. Portanto, o consumo desses produtos, principalmente quando reconstituídos em água fluoretada poderia aumentar o risco de desenvolvimento de fluorose dentária.

DESCRITORES: Fluoretos; Fluorose dentária; Substitutos do leite; Fórmulas infantis; Leite.

INTRODUCTION

The widespread use of fluoride has caused a great impact on the control of the dental caries disease around the world[1,3,7,26]. However, if it is ingested in excess, fluoride can cause dental fluorosis, an enamel alteration that can occur during the mineralization phase of tooth formation. It generates a hypomineralized and porous enamel, clinically identified by white opaque spots or yellow and dark brown stains[17,26]. In public health, the concern is about the anterior teeth. Moderate or severe fluorosis generate aesthetic or functional problems.

It is considered that the daily intake of 0.05 to 0.07 mg F/kg body weight does not cause unaesthetic dental fluorosis, although it can cause the development of mild or very mild forms[5,7]. These authors agree that the estimations are still empiric, not considering the individual susceptibility to fluoride exposure.

Milk, human or not, raw or industrialized, is the most consumed food in the first years of life. The surveys related to breastfeeding reveal that in the sixth month of life, 45 to 64% of the Brazilian

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children do not consume breast milk anymore[2,19], which can be replaced by liquid or powdered cow's milk, or infant formulas[25,27].

In the Brazilian marketplace many domestically produced or foreign commercial kinds of powdered-milk and infant formulas can be found. None of these products show in their labels any information about fluoride content, as the fabricants are not obligated to do so. A previous study has reported that these Brazilian products may be important contributors to the total daily fluoride intake[6]. However, the study analyzed few brands, some of them hardly consumed by the majority of the Brazilian children due to their high cost. Therefore, it is also necessary to determine the fluoride content of products with more affordable prices. This was the purpose of this paper, as well as to evaluate the possibility of developing unaesthetic dental fluorosis based on the estimation of the daily fluoride intake by children up to 3 years-old when consuming these products.

MATERIALS AND METHODS

Sample preparation

Eight brands of powdered milk-based products (group M), seven of infant formulas (group I) and three of soy-based products (group S) were obtained from three cans of different batches (Table 1). All products made in the Brazilian territory, with affordable prices for the population in general and commercially available in Araçatuba city, São Paulo state, were selected for this study. Three powder samples of each can were reconstituted in 10 ml of deionized water according to the manufacturer's instructions.

Fluoride analyses

Previously to analysis, the samples were submitted to overnight hexamethyldisiloxane (HMDS) facilitated diffusion, a method developed by Taves[23] (1968) and modified by Whitford[26] (1996).

A small hole (± 2 mm) was burned into the lid of polystyrene Petri dishes (60 x 15 mm) and the inside periphery was ringed with petroleum jelly (Vaseline) for posterior seal. On the inside middle of the lid, 50 µl of 0.05 M sodium hydroxide were placed in 6 drops. A volume of 0.1 ml of the reconstituted samples was pipetted on the dish bottom plus 2.0 ml of deionized water. The dishes were closed and 2 ml of 3 M sulfuric acid saturate with HMDS were added to the bottom by injection through the hole on the lid and immediately sealed with Vaseline.

TABLE 1 - Products analyzed, fabricant and recommendations. Araçatuba, 2005.

Product Name	Fabricant	Group	Age for consumption (months)	Preparation: powder (g) + H_2O (mL)
Nan 1	Nestlé	I	0 to 6	30.80 g + 210 ml
Nan 2	Nestlé	I	6 to 12	32.34 g + 210 ml
Nan AR	Nestlé	I	0 to 12	31.71 g + 210 ml
Nestogeno 1	Nestlé	I	0 to 6	31.01 g + 210 ml
Nestogeno 2	Nestlé	I	6 to 12	32.90 g + 210 ml
Nestogeno plus	Nestlé	I	0 to 12	32.34 g + 210 ml
Nestogeno soja	Nestlé	I	6 to 12	33.60 g + 210 ml
Elegê Integral	Elegê	M	-	26 g + 200 ml
Itambé integral	Itambé	M	-	26 g + 200 ml
Maitá	Tangará	M	-	26 g + 200 ml
Ninho	Nestlé	M	-	26 g + 200 ml
Ninho +1	Nestlé	M	More than 12	29 g + 200 ml
Ninho +3	Nestlé	M	More than 36	29 g + 200 ml
Precioso	Itambé	M	-	26 g + 200 ml
Serra Branca	Kerry	M	-	30 g + 200 ml
SupraSoy	Josapar	S	-	26 g + 200 ml
Soymix	Svili	S	-	30 g + 200 ml
Soymilke	Olvebre	S	-	30 g + 200 ml

After overnight agitation (Orbital Gyratory shaker – NT-145, Nova Técnica, São Paulo, SP, Brazil), the lids were removed and the sodium hydroxide was buffered with 25 µl of 0.2 M acetic acid. The total volume was adjusted to 75 µl with deionized water.

Determination of fluoride content was performed in this final drop using an ion-specific electrode (Orion Research, Cambridge, MA, USA, model 9409) and a calomel reference electrode (Accumet model 13620-79) coupled with a microprocessor ionalyser (Orion Research, Cambridge, MA, USA, model 710A). Those were previously calibrated with five standards with known fluoride contents (0.01; 0.02; 0.04; 0.08; 0.16 µgF) prepared by serial dilution from a 100 mg F/L stock solution (Orion Research, Cambridge, MA, USA, # 940907) that were diffused in triplicate in the same way as the samples. The millivoltage potentials were converted to µgF using a standard curve with a coefficient correlation $r > 0.999$. All samples were analyzed in duplicate. The mean repeatability of the readings, based on the duplicated samples, was above 90%.

The Kruskal-Wallis test with $p < 0.05$ was used to statistically compare the fluoride concentrations among the cans of each product and the products of each group.

Estimations of fluoride intake

Mean fluoride concentration of the three cans of the analyzed products prepared with deionized water was used to estimate the possible fluoride intake for infants from one to thirty-six months-old based on the daily consumption as suggested by Woiski[27] (1994) and Vasconcelos[25] (1997). A simulation was also calculated with the products prepared in water with 0.7 mg F/L.

RESULTS

Mean fluoride content of the infant formulas are presented in Table 2.

According to the Kruskal-Wallis test, when the three brands of soy-based products were compared there was significant statistical difference ($p < 0.05$) between two of them: Soymix® and Soymilke®. When the different cans of the products were compared, there was significant statistical difference ($p < 0.05$) between cans of Nan 1®,

TABLE 2 - Fluoride content of each can described in Table 1, mean and standard deviation of the three cans. Araçatuba, 2005.

Products	1st can (mg F/L)	2nd can (mg F/L)	3rd can (mg F/L)	Mean (mg F/L) ± Standard Deviation of the 3 cans
Nan 1	0.100 †	0.478 †	0.402	0.326 ± 0.169
Nan 2	0.348 †	0.127 †	0.161	0.212 ± 0.101
Nan AR	0.025 †	0.032	0.143 †	0.067 ± 0.056
Nestogeno 1	0.050 †	0.055	0.155 †	0.087 ± 0.050
Nestogeno 2	0.023	0.096 †	0.013 †	0.044 ± 0.038
Nestogeno plus	0.245	0.264 †	0.115 †	0.208 ± 0.068
Nestogeno soja	0.084	0.085	0.085	0.085 ± 0.003
Elegê Integral	0.017	0.052 †	0.011 †	0.027 ± 0.019
Itambé integral	0.017	0.017	0.018	0.017 ± 0.001
Maitá	0.029	0.037 †	0.015 †	0.027 ± 0.009
Ninho	0.024 †	0.012 †	0.013	0.016 ± 0.005
Ninho +1	0.074 †	0.024 †	0.036	0.045 ± 0.022
Ninho +3	0.036	0.037 †	0.031 †	0.035 ± 0.003
Precioso	0.019	0.020	0.019	0.019 ± 0.001
Serra Branca	0.011 †	0.015	0.016 †	0.014 ± 0.003
Soymix *	0.755	0.613	0.737	0.702 ± 0.071
SupraSoy	0.268	0.363	0.372	0.334 ± 0.050
Soymilke *	0.287 †	0.212 †	0.261	0.253 ± 0.035

*$p < 0.05$ for different soy-based products. †$p < 0.05$ for different batches of the same brand.

Nan 2®, Nestogeno 1®, Nestogeno 2®, Nestogeno Plus®, Nan AR®, Elegê®, Maitá®, Ninho®, Ninho +1®, Ninho + 3®, Serra Branca and Soymilke®.

Table 3 shows estimative values of the fluoride intake by children up to thirty-six months of age when consuming the products prepared with deionized and 0.7 mg F/L water.

DISCUSSION

Mother's milk is the most complete food in the first months of life[11,13,25] and, as the World Health Organization (WHO) recommends[10], it should be exclusively consumed at least in the first four months of life, continuing as a food supplementation until two years of age or more. However, for reasons like transmissible diseases, medication use, the mother returning to work and other reasons, in the age of three months, 32 to 45% of Brazilian children do not consume breast milk anymore[2,19]. In the first months of life, 58% of children had already been fed with other kinds of milk[16].

As a mother's milk substitute, infant formulas are recommended for the first semester of the baby's life. However, due to their high cost, fluid or powdered cow's milk is also used, diluted at 2/3[25,27], and then continued, for older children, without dilution[27]. Those people who are allergic to milk based products can alternatively consume soy-based products[25].

Considering that excessive fluoride intake, especially between the sixth and the ninth month of age, can lead to the development of fluorosis in primary teeth[14], and between the fifteenth and the thirtieth month, on the anterior permanent teeth[8], the concern about the fluoride content of these products is justified.

Eight powdered brands of milk, seven infant formulas and three soy-based products were analyzed in this study (Table 1) and the fluoride concentrations (Table 2) were similar to those found by Buzalaf *et al.*[6] (2001). However, this previous study analyzed few brands, some of them hardly consumed by the majority of the Brazilian children due to their high cost. The present study was conducted with more economically accessible brands and more than one batch of each brand was analyzed.

Most of the fabricants put in the label indications about age of consumption and about preparation of the product. These instructions may or may not be followed by the person who takes care of the child. Sometimes a higher amount of the powder is used. Otherwise, there is no indication about the type of water that should be used. Therefore, the water used may come from different sources: public drinking water, from wells or bottled mineral waters, with different fluoride concentrations[1,4,9,24]. In Brazil, a concentration of 0.6 to 0.8 mg F/L for public water is recommended[7,22]. When prepared with fluoride-free water, only the soy-based product Soymix® led to a daily fluoride intake above the limit considered safe as to the risk of enamel fluorosis development in primary teeth. However, when reconstituted in fluoridated drinking water (0.7 mg F/L), preparations with all products presented fluoride contents above the limit considered safe during primary teeth formation. The same can happen when children in the age of permanent incisor teeth formation consume Soymix® reconstituted in fluoridated drinking water.

One could think about asking mothers to avoid the use of fluoridated water to reconstitute these products, but this recommendation seems to be inappropriate for the majority of the Brazilian population, since the public drinking water is more accessible because of cost and availability.

These results do not necessarily reflect the real amount of fluoride absorbed by these children, since the fluoride bioavailability of these foods seems to be reduced[21]. Because of that, and the fact that it seems to be conditioned by individual susceptibility to fluoride exposure[26], one cannot affirm that the daily intake of these products, reconstituted in fluoridated drinking water, is in effect responsible for the development of dental fluorosis. However, regular consumption associated to other sources of fluoride intake, like other foods and dentifrices[15,18], can significantly contribute to the total fluoride intake, increasing the risk of dental fluorosis.

Other researches have already evaluated the fluoride content of powdered milk and powdered milk substitutes[6,12,20]. But the present study found a great variation of fluoride concentration ($p < 0.05$) among different cans (batches) of the same brand. During the manufacturing process, the products are dehydrated and ingredients such as vitamins and mineral salts are added, which can be combined with fluoride. This explains the fluoride variation found among the cans, especially in the infant formulas. Therefore, it is necessary to standardize the fluoride content of these products, as well as to divulgate these contents on the label.

CONCLUSIONS

Just one of the soy-based products presented a fluoride content above the limit that could prob-

TABLE 3 - Estimative values of fluoride intake by children up to thirty-six months of age (mg F/kg body weight) from the consumption of the analyzed products. Araçatuba, 2005.

Age (months)	1[a]		6[b]		12[c]		18[d]		24[d]		36[e]	
Products	H_2O deion.	H_2O 0.7 mgF/L	H_2O deion.	H_2O 0.7 mgF/L	H_2O deion.	H_2O 0.7 mgF/L	H_2O deion.	H_2O 0.7 mgF/L	H_2O deion.	H_2O 0.7 mgF/L	H_2O deion.	H_2O 0.7 mgF/L
Nan 1	0.065	0.205	0.033	0.103	*	*	*	*	*	*	*	*
Nan 2	*	*	0.021	0.091	0.013	0.056	*	*	*	*	*	*
Nan Anti-reg	0.013	0.153	0.007	0.077	0.004	0.047	*	*	*	*	*	*
Nestogeno 1	0.017	0.157	0.009	0.079	*	*	*	*	*	*	*	*
Nestogeno 2	*	*	0.004	0.074	0.003	0.046	*	*	*	*	*	*
Nestogeno Plus	0.042	0.182	0.021	0.091	0.013	0.056	*	*	*	*	*	*
Nestogeno Soja	*	*	0.009	0.079	0.005	0.049	*	*	*	*	*	*
Elegê	0.005	0.134	0.003	0.073	0.002	0.045	0.002	0.047	0.002	0.042	0.001	0.026
Itambé	0.003	0.133	0.002	0.072	0.001	0.044	0.001	0.047	0.001	0.041	0.001	0.026
Maitá	0.005	0.134	0.003	0.073	0.002	0.045	0.002	0.047	0.002	0.042	0.001	0.026
Ninho	0.003	0.133	0.002	0.072	0.001	0.044	0.001	0.047	0.001	0.041	0.001	0.026
Ninho +1	*	*	*	*	0.003	0.046	0.003	0.049	0.003	0.043	0.002	0.027
Ninho +3	*	*	*	*	*	*	*	*	*	*	0.001	0.026
Precioso	0.004	0.133	0.002	0.072	0.001	0.045	0.001	0.047	0.001	0.041	0.001	0.026
Serra Branca	0.003	0.132	0.001	0.071	0.001	0.044	0.001	0.047	0.001	0.041	0.001	0.026
Soymix	0.140	0.280	0.070	0.140	0.043	0.087	0.046	0.091	0.041	0.081	0.025	0.050
SupraSoy	0.067	0.207	0.033	0.103	0.021	0.064	0.022	0.067	0.019	0.060	0.012	0.037
Soymilke	0.051	0.191	0.025	0.095	0.016	0.059	0.017	0.062	0.015	0.055	0.009	0.034

* not in the age bracket recommended by the fabricant. a: 900 ml of daily intake; b: 800 ml of daily intake; c: 650 ml of daily intake; d: 750 ml of daily intake; e: 500 ml of daily intake.

ably lead to the development of fluorosis in primary teeth even if reconstituted in fluoride-free water. All the products showed a fluoride content above the limit that could probably lead to the development of unaesthetic fluorosis in primary teeth, and one of the soy products could probably lead to the development of fluorosis in permanent incisor teeth if reconstituted in fluoridated water (0.7 mg F/L). One should note that these conclusions are based on the total fluoride content of the analyzed products, whose consumption was assumed to be in a regular way.

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