

## Estimation of Average Daily Intake of Fluorides Provided by Foods and Feeding Minutes According to Nutritional Recommendations of the Chilean Ministry of Health for Children from 0 to 12 Months

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### Abstract

Nutrition in the first 6 months is breastfeeding and/or formula milk. The last 6 months are food and milk. Estimation of intake by measuring fluoride (F<sup>-</sup>) in breast milk, formula milk and feeding in children from 7 to 12 months in kindergartens. F<sup>-</sup> was measured by microdiffusion and specific electrode. Intake with F<sup>-</sup> concentration and daily dose intake of fluoride (DDI-F) using children's weight.

With the percentage of exclusive breastfeeding, milk-formula consumption is inferred. With this combination, the intake of fluoride from milk is  $0.1980 \pm 0.0360$  and  $0.1653 \pm 0.0391$  mgF/day and the DDI-F is  $0.0363 \pm 0.0043$  and  $0.0293 \pm 0.0050$  mg F<sup>-</sup>/kg/day.

Daily intake of F<sup>-</sup> in children from 7 to 10 months' ranges from 0.394 to 0.433 mg/day. Intake for 10 to 12 months varies from 0.387 to 0.483 mg/day. DDI-F for 7 to 10 months is  $0.049 \pm 0.009$  mg F<sup>-</sup>/kg/day and for 10 to 12 months it is  $0.045 \pm 0.006$  mg F<sup>-</sup>/kg/day.

Kindergartens provide 3 of 4 meals in the diet. This 4<sup>th</sup> meal in children from 7 to 10 months is a bottle and dinner for 10 to 12 months. Water provides 150 ml for 7 to 10 months and 200 ml for 10 to 12 months. These contributions raise DDI-F for 7 to 10 months to  $0.076 \pm 0.007$  and for 10 to 12 months to  $0.070 \pm 0.006$  mg F<sup>-</sup>/kg/day. Breastfeeding with milk-formula between 0 and 6 months indicates that DDI-F are below optimal. DDI-F in children from 7 to 12 months fed in day care centers is lower than optimal and additional contributions raise it above that value. DDI-F in children from 7 to 12 months are above optimal and should not receive additional foods (toothpaste) for safe intake.

**Keywords:** Nutrition; Children from 0 to 12 Months; Intake of Fluoride; Daily Dose Intake of Fluoride; Kindergartens; Chile

### Abbreviations

F<sup>-</sup>: Fluoride; DDI-F: Daily Dose Intake of Fluoride; MINSAL: Ministry of Health of Chile; IFS: Iowa Fluoride Study; JUNJI: National Board of Kindergartens; JUNAEB: National School Aid and Scholarship Board; CESFAM: Family Health Centers; ILO: International Labour Organization; ETD-F: Total Daily Fluoride Excretion; RTD-F: Total Daily Fluoride Retention; UNICEF: United Nations Children's Fund; PAHO: Pan American Health Organization; PUC: Pontificia Universidad Católica de Chile; EFSA: European Food Safety Authority

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## Introduction

Excessive systemic fluoride ( $F^-$ ) consumption from birth to four years of age is a key factor in the development of dental fluorosis in permanent maxillary central incisors [1]. Levy, *et al.* [2] suggested that six to nine months is the determining period in dental fluorosis of the primary dentition. Warren, *et al.* [3] point out that the development of fluorosis in the late development of teeth is in utero until 11 months of age.

Children are at risk of dental fluorosis in both dentitions depending on the time and period of systemic exposure to  $F^-$ . Controlling  $F^-$  intake during enamel formation reduces the risk of dental fluorosis in both dentitions. The Iowa Fluoride Study (IFS) points to Total Fluoride Intake as the risk factor for dental fluorosis [4] and in babies it depends on: diet,  $F^-$  concentrations in breast milk and formula, foods/drinks during weaning, use of brushing and toothpaste.

Bhagavatula, *et al.* [5] points out that for the permanent dentition the window of susceptibility for the development of dental fluorosis is 2 to 8 years of life. From aesthetics, the permanent upper central incisors are the most affected and occurs during the first 3 years and several authors suggested monitoring  $F^-$  intake in children to minimize the risk of developing dental fluorosis in these teeth [6-8].

The most important intake of  $F^-$  occurs in early childhood while in the control of dental caries its use is throughout the life of the tooth and public policies must be cautious in this balance of controlling intake and reducing dental caries with this same agent [9].

Burt in 1992 [10] empirically determined that the “optimal” daily intake of  $F^-$  for children was between 0.05 to 0.07 mg  $F^-$ /kg body weight/day, a dose still accepted worldwide. It is based on the amount of  $F^-$  in the “average daily diet” consumed by children from 1 to 12 years according to McClure in 1943 [11].

The appearance of fluorosis varies according to the dose, thus Forsman in 1977 [12] states that 0.1 mg  $F^-$ /kg body weight/day is the level of exposure necessary to produce it, while Baelum, *et al.* in 1987 [13] report its appearance with an intake of less than 0.03 mg  $F^-$ /kg body weight/day of  $F^-$  per day, this situation remains unclear.

Bhagavatula *et al.* in 2017 [14] evaluated the association between  $F^-$  intake and dental fluorosis in permanent teeth in children aged 2 to 8 years and with a DDI- $F$  of 0.04 mg  $F^-$ /kg/day and indicated that the highest probability of fluorosis development was in 2<sup>nd</sup> premolars and 2<sup>nd</sup> molars.

The IFS reported that infants who consumed fluoridated water had an increased risk of developing incisor fluorosis and that in 1997 it was stated that exposure to  $F^-$  in childhood had a low probability of causing incisor fluorosis [15]. In 2006 the IFS indicated that “childhood exposure was a very strong predictor of fluorosis in incisor teeth when exposure occurred between 1 and 4 years [16], this led the researchers to point out that encouraging breastfeeding instead of formula milk was an effective way to reduce the risk of fluorosis in both dentitions [17].

Optimal  $F^-$  intake in children has been periodically reviewed due to dietary contributions and inadvertent toothpaste ingestion [18]. An increase in the prevalence of fluorosis in permanent teeth has been observed in the USA and Australia, even in areas with low  $F^-$  content in their water supplies, attributed to inadequate intake from supplements and the decision to set the “optimal” range of daily intake at the upper limit. Dietary  $F^-$  intake is a factor related to both caries prevention and the development of fluorosis and needs to be estimated from infant foods to plan an oral health program [19].

Until 1970, children were only exposed to fluoride through regular or artificial drinking water, but the situation changed when fluoridated toothpastes became available and dental professionals and patients/parents began using topical fluoride products and

supplements. Today, the main contribution to total daily fluoride intake comes from water, food, and fluoridated toothpaste [20]. Fluoridated toothpastes have been shown to prevent tooth decay, but their use in young children is a risk factor for the development of dental fluorosis [21].

The WHO [22] notes that the global prevalence of dental and skeletal fluorosis is not clear, but with data published between 1953 and 2000, it was estimated in 2006 that excessive concentrations of fluoride in drinking water have caused tens of millions of cases of dental and skeletal fluorosis worldwide. Children whose teeth and bones are developing are more susceptible and poor nutritional status aggravates this situation [23].

According to Burt in 1992 [10] the prevalence of fluorosis has increased in the United States over the past 30 to 50 years, with the greatest increase in areas with non-fluoridated waters. A Cochrane report [24] on dental fluorosis estimates that with 0.7 ppm F<sup>-</sup> in water, the percentage of mild fluorosis is 12%. Increasing to 40% when fluorosis of all levels is considered. In the US, the incidence of enamel fluorosis in adolescents has increased by approximately 41% because fluoride sources are now more widely available in various forms [25]. Moderate and severe forms of fluorosis are rare but increasing, but present with aesthetic and structural changes in 6-year-old permanent molars [26].

Studies of fluoride intake in infants in the United Kingdom in 2014 [27] have shown that diet is the sole source of intake for the majority of infants up to six months of age. Intake doses for infants aged 0 - 12 months living in areas with fluoridated and non-fluoridated water were  $0.107 \pm 0.054$  and  $0.024 \pm 0.015$  mg F/kg body weight/day, with the value in the fluoridated area being higher than the accepted maximum. Diet was the sole source of fluoride for 87% of infants and none used fluoride supplements [27]. In the United States during the first 12 months of age, up to 96% of total fluoride intake can be derived from diet and decreases to 53% by 24 months [28].

Childhood dental fluorosis worldwide and in Chile is increasing according to the latest reports. The intake of fluorides from food in children aged 0 to 12 months is unknown. This is the earliest observation that tends to explain the increase in fluorosis in the country because it represents almost all fluoride consumption in this period and could be a response to its increase.

Therefore, the hypothesis to the previous question is that the intake of foods with fluoride during the first year exceeds the maximum accepted levels that cause fluorosis and that range between 0.05 and 0.07 mg of fluoride per day per kg of body weight.

To do this, the following general objective is proposed: Estimate the average daily intake of fluorides and adapt it to what is recommended by the MINSAL for children aged 0 to 12 months residing in Chile.

## **Materials and Methods**

Non-experimental transactional study, supported by a descriptive field study, which is the best strategy to meet the specific objectives.

### **Sample and size**

The diet of children under one year of age varies widely in composition, consistency and quantity. Initially, the diet is based solely on milk, and then progresses to increasingly solid and complete foods as the child's physiological development allows.

According to MINSAL guidelines, infant feeding during the first 6 months is through breast milk and formula milk delivered at home and through private and state-run kindergartens dependent on the State. The last 6 months are through complementary feeding, including milk.

Complementary feeding in institutionalized kindergartens (JUNJI) is contracted through public tender through JUNAEB, which must cover between 60% and 75% of daily calories and 100% of daily protein required according to the child’s age. The feeding program is supervised at a national level, ensuring similar feedings for all kindergartens in Chile [29].

According to JUNJI [29] the food menus of the kindergartens are divided into three stages according to age: a) Nursery for children under 6 months (Breastfeeding Period), with children from 0 to 6 months, fed only with breast milk or formula. b) Nursery for children under 10 months (Transition Period), with children from 7 to 10 months, consuming all the food groups indicated by the MINSAL except legumes, eggs and fish. c) Nursery for children from 10 to 12 months (Maturation Period), consuming all the food groups indicated by the MINSAL.

Data management and statistical analysis: The fluoride concentration of each food group and kindergartens diets, sample and counter sample or duplicate average, in addition to the average and standard deviation for the entire food group and diets were reported. The Student t-test and analysis of variance were used to determine if there were statistically significant differences between the fluoride concentrations of the groups. These analyses were performed using the Statistical Package for Social Sciences 17.0 J (IBM, USA). The level of statistical significance was set at 5%.

Infant feeding in children from 0 to 6 months: F<sup>-</sup> intake during breastfeeding involves three instances:

a) Exclusive breastfeeding. In this instance, the concentration of fluoride in breast milk must be known. This concentration was calculated in 108 samples of breast milk from women between 18 and 45 years of age, from babies between 0 and 12 months of age.

To estimate the daily consumption of F<sup>-</sup> in milligrams of breast milk, the volumes of breastfeeding indicated by the MINSAL were used. With these volumes and their frequency of use for each age (months), the total volume of daily intake was determined. This value was multiplied by the concentration of F<sup>-</sup> in breast milk in Chile of 0.04 ± 0.057 mg F<sup>-</sup>/L to obtain the daily intake of F<sup>-</sup> in milligrams.

b) The second instance considers exclusive milk-formula feeding. Using children’s weights from the MINSAL, maximum and minimum daily bottle volume obtained from Cesfam and milk manufacturers, and F<sup>-</sup> concentrations of milk-starting formula (0.053 ± 0.047 mg F<sup>-</sup>/Kg) and dispersion water, it was determined calculated the intake and daily dose of F<sup>-</sup> intake for each month.

c) Estimation from the combined consumption of breast milk with milk-formula in the daily intake of fluoride in babies from 0 to 6 months. Not all children receive breast milk, reports the MINSAL, regardless of the month of their birth, and to complete their diet they use formula milk. The percentage of exclusive monthly breastfeeding determines the consumption of formula milk and the percentages contributed by both milks. See figure 1.

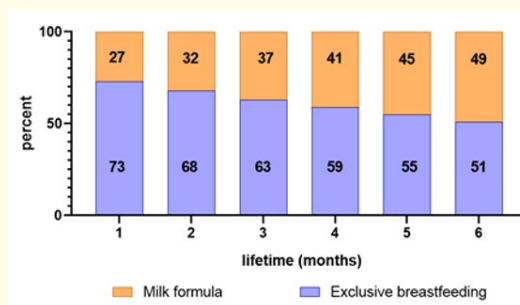


Figure 1: Monthly percentages that the types of milk provide each month in the milk diet in children from 0 to 6 months.

In the first month of life, the child receives 73% exclusive breastfeeding and 27% receives formula feeding, being this structure which is repeated the one that most resembles the diet that the child receives during his first semester. Using the data of the daily intake of fluoride from the combination of breast milk and formula, it is possible to calculate the DDI-F for these children. To calculate the daily dose of fluoride intake (DDI-F), the daily intake of F<sup>-</sup> must be divided by the average weight of boys and girls according to their age reported by the MINSAL [30] And it is expressed: Daily dose of fluoride intake (DDI-F) = mg F<sup>-</sup>/Kg/day.

Infant nutrition for children from 7 to 12 months: For this age, we use the food infrastructure of the JUNJI kindergartens, which delivers standardized foods to these children. These foods correspond to food minutes that the kindergartens deliver weekly and separated by age. The first corresponds to children from 7 to 10 months.

Children’s meals for children from 7 to 10 months: The following table shows the feeding plan for one day approved by JUNJI and delivered by the kindergarten for children from 7 to 10 months. See table 1.

	<b>Breakfast</b>	<b>Lunch</b>		<b>Afternoon Snack</b>	<b>Collations</b>
<b>Day</b>	<b>Liquid Portion</b>	<b>Main Dish</b>	<b>Dessert</b>	<b>Liquid Portion</b>	<b>Collations</b>
Monday	Monday Milk-based formula with corn cereal* 26% Fat Fortified Milk: 7.5% Corn Dextrinized Cereal: 5% 2% Vegetable Oil Volume: 220 mL *Purita Corn Cereal	Mixed vegetable and beef rice pureed soup. Mixed vegetables: 120g Beef: 40g Rice: 12g; Vegetable Oil: 2g Pureed soup. Volume: 200 mL Kiwi 100g Milk-based formula with rice cereal*, 26% Fat Fortified Milk: 7.5%. Dextrinized Rice Cereal: 5% Vegetable Oil at 2%. Volume: 220 mL	Kiwi 100g	Milk-based formula rice cereal* Milk 26%. Fortified Fat: 7.5%. Corn Dextrinized Cereal: 5%. Vegetable oil at 2%. Volume: 220 mL *Purita Rice Cereal	Apple Fruit Puree

**Table 1:** Daily kindergarten meal plan for children 7-10 months.

Table 1 shows one day of the week, the other days maintain the nutritional structure, but the foods that children receive change. The MINSAL recommends porridges in small quantities at the beginning of the feeding, increasing as the child accepts them. The quantities are referential and vary on different occasions. The food in this period is prepared with fluoridated drinking water, early carrier of fluoride [31].

The nutritional guides for kindergartens for children aged 10 to 12 months already indicate all the foods recommended by the MINSAL. The following table shows a daily menu for older children. See table 2.

**Food samples and sampling authorizations:** The samples in kindergartens are food minutes. The unit of analysis is the food and the random variable is the fluoride measurement. The foods are breakfast, main course, dessert, snack and collations for both ages. In 5 kindergartens during one week 100 samples of each food with 500 foods in total were collected. The JUNJI kindergartens are entities associated with the FONIS PROJECT, sampling two kindergartens in the commune of Recoleta and three in the commune of Cerro Navia.

	Breakfast	Lunch		Afternoon Snack	Collations
Day	Liquid Portion	Main Dish	Dessert	Liquid Portion	Collations
Monday	Rice Cereal Milk Formula. Milk 26% Fortified Fat Content: 7.5%. Dextrinated Rice Cereal: 5% Vegetable Oil: 2%. Volume: 220 mL. *Purita Rice Cereal	Pureed <b>Legume Soup with Lentils</b> . Lentils: 80g Vegetable Oil: 3g Pureed Soup: 200 mL	Apple compote 100g	Corn Cereal Milk Formula. Milk 26% Fortified Fat Content: 7.5%. Dextrinated Corn Cereal: 5% Vegetable Oil: 2%. Volume: 220 mL *Purita Corn Cereal	Banana Fruit Pureed. 100g

**Table 2:** Daily kindergarten feeding plan for children 10 to 12 months. In bold, the foods incorporated into this age group.

**Collection and transport of samples:** Due to their condition, they must be refrigerated ( $\pm 4^{\circ}\text{C}$ ) in the kindergarten and during transport. In 2 refrigerated boxes separated by age, each bottle, previously weighed in mg, identified with the type of food and date of collection. The samples were transported to the laboratory and stored until analysis.

**Fluoride analysis and processing of food samples:** Fluoride concentrations in food samples were obtained using the microdiffusion technique, potentiometric method, specific electrode and an ionometer that provides values in millivolts (mV). The volumes of the samples and the concentrations of the reagents used in the microdiffusion analysis of fluorides for foods are shown in the table 3.

Sample	Volume or mass Sample Microdiffusion	Distilled Water	H <sub>2</sub> SO <sub>4</sub> 3M HDMS	[NaOH] 0,05N	[Acetic Acid] 0,1M	mL Final Well
Food or Menus	1mL or 1,0g	2,0 mL	1 mL	50 $\mu\text{L}$	50 $\mu\text{L}$	100 $\mu\text{L}$

**Table 3:** Concentration, volume of reagents and samples in the fluoride microdiffusion technique.

HDMS: Hexamethyldisiloxane; H<sub>2</sub>SO<sub>4</sub>: Sulfuric Acid; NaOH: Sodium Hydroxide.

The measured values of samples and the fluoride calibration curve are transformed into concentration by means of a logarithmic regression mV vs F concentration. The concentrations are expressed as mg F/kg of sample or /1L or ppm of F [32], adjusting to the MINSAL indications for each food to standardize the results.

## Results and Discussion

**Fluoride concentration in breast milk:** Breast milk samples were collected from women between 18 and 45 years of age, from municipalities with fluoridated drinking water and who did not consume fluoridated supplements, except toothpaste, and from children between 0 and 12 months of age. The average concentration in breast milk was  $0.049 \pm 0.0571$  mg F/1000 L, with the fluoride concentration measured in each month of lactation. Table 4 shows the fluoride content in breast milk between 1 and 6 months.

When showing the concentration of fluoride in the first 6 months of breastfeeding, which is the critical period according to the WHO for infant nutrition, we find low values that increase from the 4<sup>th</sup> month of breastfeeding and subsequently decrease slowly.

Age (months)	Number of Samples	Average (mg F <sup>-</sup> /L milk)	Standard Deviation (mg F <sup>-</sup> /L milk)	Median (mg F <sup>-</sup> /L milk)
1	15	0,033	0,030	0,021
2	8	0,035	0,009	0,045
3	10	0,034	0,025	0,021
4	10	0,060	0,060	0,032
5	22	0,051	0,054	0,034
6	9	0,049	0,029	0,040

**Table 4:** Fluoride content in breast milk per month of breastfeeding between 1 to 6 months.

Fluoride intake in exclusively breastfed children: To estimate the intake of fluoride in breast milk, the recommendations of the WHO [33] and the MINSAL [34] were used. The maximum and minimum daily frequencies of breastfeeding and the maximum and minimum volumes were used to determine the maximum and minimum total volume ingested per day according to the age of the child. These values multiplied by the concentration of F<sup>-</sup> in breast milk provide the daily intake of F<sup>-</sup> in months 1 TO 6. See table 5.

Age (months)	Max. daily breast-feedings	Min. daily breast-feedings	Max. breast-feeding volume (mL)	Min. breast-feeding volume (mL)	Max. daily volume (mL)	Min. daily volume (mL)	[F <sup>-</sup> ] Breast milk (mg/L)	Max. intake (mg F <sup>-</sup> /day)	Min. intake (mg F <sup>-</sup> /day)
1	12	8	90	60	1080	480	0,021	0,0223	0,0099
2	8	6	150	120	1200	720	0,045	0,0540	0,0324
3	8	6	150	120	1200	720	0,021	0,0252	0,0151
4	7	6	180	120	1260	720	0,032	0,0404	0,0231
5	7	6	180	120	1260	720	0,034	0,0429	0,0245
6	6	5	240	180	1440	900	0,040	0,0577	0,0361

**Table 5:** Fluoride intake from breast milk in children aged 1 to 6 months.

Max.= Maximum; Min.= Minimum.

Fluoride intake through breast milk varies from a maximum of 0.058 mg F<sup>-</sup>/day at 6 months to a minimum of 0.010 mg F<sup>-</sup>/day at 1 month of age. The maximum average intake is 0.0404 ± 0.0235 mg F<sup>-</sup>/day and the minimum intake is 0.0132 ± 0.0095 mg F<sup>-</sup>/day and with these fluoride intake data the fluoride intake dose can be known.

The daily dose intake of fluoride (DDI-F) from breast milk in children aged 1 to 6 months includes the daily intake and average weights of children according to age, this was obtained from MINSAL [30]. The DDI-F for children aged 1 to 6 months is shown in table 6.

The DDI-F from breast milk ranges from a maximum of 0.0022 to a minimum of 0.0104 mg F<sup>-</sup>/kg/day. The DDI-F averages from 1 to 6 months do not exceed the “optimal” daily intake dose.

Age (months)	Average Weight (Kg)	Max. Daily F <sup>-</sup> Intake (mg F <sup>-</sup> /day)	Min. Daily F <sup>-</sup> Intake (mg F <sup>-</sup> /day)	Max. Daily DDI-F (mg F <sup>-</sup> /Kg/day)	Min. Daily DDI-F (mg F <sup>-</sup> /Kg/day)
1	4,5	0,0223	0,0099	0,0050	0,0022
2	5,2	0,0540	0,0324	0,0104	0,0062
3	6,0	0,0252	0,0151	0,0042	0,0025
4	6,7	0,0404	0,0231	0,0060	0,0034
5	7,1	0,0429	0,0245	0,0060	0,0035
6	7,6	0,0577	0,0361	0,0076	0,0047
Average	6,1833	0,0404	0,0235	0,0065	0,0038
Standard Deviation	0,585	0,0115	0,0075	0,0012	0,0008

**Table 6:** Estimation of daily dose intake fluoride (DDI-F) from breast milk in children aged 0 to 6 months.

Max.= Maximum; Min.= Minimum.

When comparing the maximum daily dose and the minimum daily intake dose of F<sup>-</sup> during the period analyzed, these values indicate that no dose exceeds the suggested dose in children, which ranges from 0.05 to 0.07 mg F<sup>-</sup>/kg of body weight/day [10].

Estimated daily intake of F<sup>-</sup> in infants fed exclusively with milk formulas: This intake is in children fed with milk formulas from birth to 6 months. The average and standard deviation of fluoride from 60 samples of milk formulas is 0.054 ± 0.051 mg of F<sup>-</sup>/Kg. The formulas were dispersed in drinking water that has a concentration of 0.613 ± 0.046 mg of F<sup>-</sup>/L. The estimate of average bottle volumes by age were obtained from the MINSAL, milk formula producing laboratories and CESFAM. Table 7 provides values for estimating fluoride intake based on information provided by the mentioned institutions.

Age (months)	Daily Bottles Max.	Daily Bottles Min	Water (mL)	Formula Milk (g)	Max. Daily Volume (mL)	Min. Daily volume (mL)	[F <sup>-</sup> ] For- mula Milk (mg F <sup>-</sup> / Kg)	[F <sup>-</sup> ] Water (mg F <sup>-</sup> /L)	Max. Daily F <sup>-</sup> (mg F <sup>-</sup> / day)	Min. Daily F <sup>-</sup> (mg F <sup>-</sup> / day)
1	7	6	105	18	735	630	0,054	0,671	0,500	0,429
2	6	5	140	23	840	700	0,054	0,671	0,571	0,476
3	6	5	160	23	960	800	0,054	0,671	0,652	0,543
4	5	4	180	27	900	720	0,054	0,671	0,611	0,489
5	4	4	190	27	760	760	0,054	0,671	0,516	0,516
6	3	2	190	27	570	380	0,054	0,671	0,387	0,258
Average									0,5395	0,4518
SD									0,0858	0,0935

**Table 7:** Estimated maximum and minimum fluoride intake (mg F<sup>-</sup>/day) from bottles with formula milk in children aged 1 to 6 months. SD: Standard Deviation.

Max.= Maximum; Min.= Minimum.



In the sixth month, the number of bottles decreased due to complementary feeding and the intake for that month also decreases. The highest fluoride intake from formula occurs in the third month and the highest fluoride intake comes from drinking water. Fluoride intake from formula milk varied from 0.258 mg/day to 0.652 mg/day. With these intake values it is possible to know the dose of fluoride intake from formula milk.

Daily dose of fluoride intake (DDI-F) from formula milk: Known the fluoride intake from formula milk dispersed with drinking water in children from 1 to 6 months and with the average weight of girls and boys provided by the MINSAL [30] THE DDI-F is estimated.

The DDI-F for boys and girls from 1 to 6 months who use exclusive formula milk can be seen in table 8.

Age (months)	Average Weight (Kg)	Max. Daily F <sup>-</sup> Intake (mg F <sup>-</sup> /day)	Min. Daily F <sup>-</sup> Intake (mg F <sup>-</sup> /day)	Max. Daily DDI-F (mg F <sup>-</sup> /kg/day)	Min. Daily DDI-F (mg F <sup>-</sup> /kg/day)
1	4,5	0,500	0,429	0,111	0,095
2	5,2	0,571	0,476	0,110	0,092
3	6,0	0,652	0,543	0,109	0,091
4	6,7	0,611	0,489	0,091	0,073
5	7,1	0,516	0,516	0,073	0,073
6	7,6	0,387	0,258	0,051	0,034
Average				0,0908	0,0763
SD				0,0224	0,0209

**Table 8:** Estimation of the daily dose of fluoride (DDI-F) from formula milk in children from 0 to 6 months. In bold color, the DDI-F values that exceed established range of 0.05 to 0.07 mg F/kg body weight/day. SD: Standard Deviation.

*Max.= Maximum; Min.= Minimum.*

The estimation of F intake doses from formula feeding from the first month to the fifth month indicates that average maximum and minimum doses exceed the established optimal range that is still considered valid. Only the sixth month is below the established minimum level and this is explained because children begin to receive complementary feeding.

Estimation of Fluoride intake from breast milk and formula by month of feeding. In their first 6 months of life, infants receive a percentage of breast milk supplemented with another percentage of milk formula to complete their daily diet. These milk combinations reflect the nutrition that the child receives during the first six months of life, where the proportion of breast milk decreases over time. Table 9 shows the percentages of fluoride intake from breast milk and formula by month of feeding.

Fluoride intake increases with age and combined consumption of both milks, with a greater share of formula milk. From the sixth month, children begin to receive complementary feeding. With this intake and the average weights of boys and girls, the DDI-F is calculated. See table 10.

Age (months)	Max. intake F of BM (mg F/day)	Min. intake F of BM (mg F/day)	Max. intake F of FM (mg F/day)	Min. intake F of BM (mg F/day)
1	0,0163	0,0072	0,1350	0,1158
2	0,0373	0,0224	0,1770	0,1476
3	0,0169	0,0101	0,2152	0,1792
4	0,0242	0,0138	0,2444	0,1956
5	0,0240	0,0137	0,2270	0,2270
6	0,0294	0,0184	0,1896	0,1264
Average	0,0247	0,0143	0,1980	0,1653
SD	0,0072	0,0050	0,0360	0,0391

**Table 9:** Maximum and minimum fluoride intake from breast milk (BM) and formula milk (FM) per month of feeding.

Max.= Maximum; Min.= Minimum; BM= Breast Milk; FM= Formula Milk; SD= Standard Deviation.

Age (months)	Average weight (kg)	Max. intake F (mg F/day)	Min. intake F (mg F/day)	Max. DDI-F (mgF/Kg/day)	Min. DDI-F (mgF/kg/day)
1	4,5	0,1513	0,1230	0,0336	0,0273
2	5,2	0,2143	0,1700	0,0412	0,0327
3	6	0,2321	0,1893	0,0387	0,0316
4	6,7	0,2686	0,2094	0,0401	0,0313
5	7,1	0,2510	0,2407	0,0354	0,0339
6	7,6	0,2190	0,1448	0,0288	0,0191
Average	6,1833	0,227	0,1795	0,0363	0,0293
SD	0,585	0,0369	0,0392	0,0043	0,0050

**Table 10:** Estimated daily intake and daily dose of fluoride intake (DDI-F) from the percent combination of breast milk and formula in children aged 0 to 6 months.

Max.= Maximum; Min.= Minimum; SD= Standard Deviation.

The estimation of the DDI-F from the combined consumption of breast milk with milk formula in children aged 0 to 6 months indicates that the average maximum and minimum doses do not exceed the established limits and the monthly doses do not exceed these limits [10]. The DDI-F in the sixth month is lower than the average and is explained by the change in diet.

The CDC in 2022 in the USA [35] indicates that exclusive breastfeeding decreases from 62.6% in the 1st month TO 24.9% in the 6th. In Spain the INE [36] indicates for exclusive breastfeeding at 45 days of 66.16% which decreases to 28.53% at 6 months. In their first months, children are exclusively or partially breastfed with breast milk. Those breastfed with breast milk have low levels of fluoride intake. Babies partially or totally breastfed with formula receive much higher levels of fluoride intake. In Chile the highest % of intake is due to the fluoride content of the water (0.671 mg/L) and also considers the fluoride in formula milk [37]. Breast milk has fluoride concentrations ≈0.006 mg/L and infants with this milk have an DDI-F < 0.001 mg/kg/day [38]. Our estimates of DDI-F with exclusive breast milk are higher with values between 0.0038 and 0.0065 mg F/kg/day.

The fluoride concentration in the water used to disperse formula milk is the largest fluoride affluent. An infant with formula milk and water of 0.8 mg F/L ingests 0.137 mg F/kg/day which compared to breast milk intake only receives 0.001 mg F/kg/day. In Chile, drinking water with 0.671 mg F/L and 0.054 mg F/kg in formula milk, a 4.5 kg infant would receive between 0.095 to 0.111 mg F/kg/day. With breast milk, he would have a DDI-F of 0.0022 to 0.0050 mg F/kg/day. This reaffirms the influence of water fluoride on the diet of children under 6 months.

The upper intake level (UL) for fluoride has been set in infants younger than 6 months, WHERE the UK Department of Health sets it at 0.22 mg F/kg/day and the US IOM SETS it at 0.1 mg/kg/day [38]. The average DDI-F in children 0-6 months, on combined breast milk and formula, is between 0.031 and 0.038 mg F/kg/day, well below the UK and US ULs.

Fluoride intake from food minutes in children in JUNJI kindergartens: In JUNJI kindergartens, the fluoride concentration of breakfast, main course, dessert, snack and collations was measured. Its standard deviation and median were calculated for a total of 500 food samples. The fluoride concentration is transformed into mg of fluoride by multiplying by the mass of the food and these are the mg of fluoride intake contributed by each meal. Table 11 shows the contributions to fluoride intake by meals for both ages.

Child's Age						
All Kindergartens	7 - 10 months			10 - 12 months		
Food mg F	Average	SD	Median	Average	SD	Median
Breakfast	0,131	0,022	0,134	0,139	0,028	0,136
Main Dish	0,104	0,021	0,108	0,100	0,021	0,100
Dessert	0,018	0,013	0,020	0,016	0,014	0,012
Snack	0,135	0,022	0,140	0,133	0,023	0,134
Collations	0,017	0,013	0,016	0,016	0,014	0,017
Daily Intake mg F	0,404	0,061	0,401	0,404	0,051	0,397

**Table 11:** Average daily fluoride intake in milligrams for breakfast, main course, dessert, snack and collations in children aged 7 to 10 and 10 to 12 months and daily fluoride intake from the minutes.

*SD= Standard Deviation.*

The mg of fluoride provided by breakfast and the main dish show significant differences with  $p = 0.017$  and also between snack and main dish with  $p = 0.018$ . There are no differences between breakfast and snack and between dessert and snack. The standard deviation is low, due to the standardization in the preparation, masses of authorized foods and control by the JUNJI. The median is similar to the mean of fluoride in the foods confirming that these values are part of the same sample. The nutritional guidelines in older children vary in some foods, but their nutritional composition is identical to that of younger children. The fluoride intake in children aged 10 to 12 months is similar to that of 7 to 10 months because the foods incorporated into their diet do not affect the intake of fluoride because they have equivalent concentrations or if they are higher, such as fish or shellfish, or they are not part of the diet and if they are present their frequency of use is low.

DDI-F in JUNJI Kindergartens in children aged 7 to 12 months: With the fluoride intake in children in kindergartens, their Daily Dose of Fluoride Intake can be calculated. With the average weight of the two age groups, its value was calculated for both ages. The DDI-F obtained for both ages from the minutes of the children can be seen in table 12.

Age Group (months)	Daily F <sup>-</sup> Intake (mg)	Weight of Age Groups (Kg)	Dose Daily Intake F <sup>-</sup> (mg F <sup>-</sup> /kg/day)
7 to 10	0,404 ± 0,061	8,250 ± 0,245	0,049 ± 0,009
10 to 12	0,404 ± 0,051	9,050 ± 0,163	0,045 ± 0,006

**Table 12:** Daily dose of fluoride intake for children from 7 to 10 months and 10 to 12 months from the nutritional minutes of the JUNJI kindergartens.

The DDI-F for both ages from daily foods are similar and are in the range reported by Burt (1992) [10] of 0.05 and 0.07 mg F<sup>-</sup>/kg of body weight/day which provides a recommended dose of fluoride intake, guaranteeing safe nutrition from the FOOD in children FROM 7 months of age who receive in JUNJI kindergartens.

Estimation of the total daily dose of fluoride (DDI-F) in children aged 7 to 12 months: JUNJI contributes to the daily nutrition of children with 3 of the 4 main meals. The 4th main meal was not analyzed for project reasons. This last meal was estimated using literature information on diet in children from 6 months of age. The literature on food quantity and frequency [39-41] and feeding guidelines from 6 months of age [42,43] allow to establish the 4th meal outside of kindergarten.

For children aged 7 to 10 months, complementary feeding is a bottle equivalent to breakfast. For 10 to 12 months, the 4th meal is dinner equivalent to the main dish. The estimate for total intake also includes drinking water. The MINSAL recommends between 150 to 200 mL separate from food, estimating the smallest volume for the younger population and the largest volume for older children. Fluoride intake for children aged 7 to 10 months would be a 220 mL bottle providing 0.133 ± 0.022 mg F<sup>-</sup>/day and 150 mL of drinking water providing 0.092 ± 0.007 mg F<sup>-</sup>/day. And intake for children aged 10 to 12 months is a 200 mL dinner of soup-puree with 0.104 ± 0.021 mg F<sup>-</sup>/day and 200 mL of water with 0.123 ± 0.009 mg F<sup>-</sup>/day.

Considering the additional fluoride contributions for children aged 7 to 12 months to complete the total fluoride intake, plus the contribution from the daycare feeding minutes, the daily dose of total fluoride intake (DDI-F) can be estimated in children under one-year-old, as shown in table 13.

Age Group (months)	F <sup>-</sup> Kindergarten (mg/day)	Bottle/Dinner F <sup>-</sup> (mg/day)	F <sup>-</sup> Water (mg/day)	F <sup>-</sup> Total (mg/day)	Weight of Age Groups (Kg)	Daily Dose Intake F <sup>-</sup> (mg F <sup>-</sup> /kg/day)
7 to 10	0,404 ± 0,061	0,133 ± 0,022	0,092 ± 0,007	0,629 ± 0,061	8,250 ± 0,245	0,076 ± 0,007
10 to 12	0,404 ± 0,051	0,104 ± 0,021	0,123 ± 0,009	0,631 ± 0,051	9,050 ± 0,163	0,070 ± 0,006

**Table 13:** Estimation of the daily dose of total fluoride intake in children 7 to 12 months.

These results indicate that the total intake doses of fluoride (DDI-F) for children from 7 to 12 months are “above the upper limit of what is recommended according to the range of values reported by Burt 1992 [10], which oscillates between 0.05 and 0.07 mg F<sup>-</sup>/kg body weight/day”. Studies report that the daily intake of fluoride is variable, indicating that it varies according to the different sources of exposure [44]. The impact of fluoride in children is best estimated by dose (mg F<sup>-</sup>/kg/weight/day), duration of exposure, and age.

The general equation for dietary intake is: Dietary intake =  $\Sigma$  (food additive concentration in food x food consumption) [45]. This equation was used to calculate the daily fluoride intake from foods in the kindergarten daily menu. Fluoride concentration was measured in all foods and multiplied by the amount of food to obtain milligrams of fluoride. Total fluoride intake was obtained by summing the fluoride contributions from each food.

The European Community has not established tolerable upper intake levels (UL) for fluoride in infants aged 6 to 12 months, as infants receive different feeding patterns [46]. Casaglia, *et al.* in 2021 [47], determined the recommended daily allowance for fluoride (RDA-F) and by extrapolation obtained a standard dietary pattern that allowed them to estimate the average daily intake of fluoride from food. The RDA-F (mg F/day) in children aged 7 to 12 months varies between 0.20 to 0.90 and the estimated fluoride intake varies between 0.166 to 0.254 mg F/day. With these values if we associate the DDI-F in children aged 7 to 10 months is 0.028 mg F/kg/day and for 10 to 12 months is 0.025 mg F/kg/day, both DDI-F are within the range established as recommended, these values being much lower than those obtained by us in children of similar ages.

Zohoori, *et al.* in 2014 [27] estimated the DDI-F in children aged 1 to 12 months living in fluoridated (0.97 mg F/L) and non-fluoridated (0.19 mg F/L) areas, considering food/drinks consumed, toothpaste and toothbrushing. The DDI-F in children from fluoridated areas was  $0.107 \pm 0.054$  mg F/kg/day and in non-fluoridated areas was  $0.024 \pm 0.055$  mg F/kg/day. Children with fluoridated water receive a much higher DDI-F than the optimal range suggested by Burt 1992 [10].

Zohoori, *et al.* in 2019 [31], DDI-F study, total daily fluoride excretion (ETD-F) and total daily fluoride retention (RTD-F) in 31 children from fluoridated areas ( $0.86 \pm 0.23$  mg F/L) and non-fluorinated ( $0.12 \pm 0.09$  mg F/L) from 6 to 12 months of age. The DDI-F was evaluated from food and toothpaste intake, dietary diary, toothpaste use, and food fluoride analysis. ETD-F was estimated by collection and analysis of fluoride in urine and feces. The RTD-F (mg F/kg/day) was estimated by subtracting the ETD-F from the DDI-F. DDI-F was lower in the non-fluoridated region ( $p < 0.001$ ) in children with milk-formula, with no differences with children with breast milk. Significant differences in DDI-F between children with milk-formula in the fluoridated zone and children with breast milk from both zones ( $p < 0.001$ ). No differences in DDI-F in children with breast milk from both areas. ETD-F influenced by type of feeding, with greater excretion in children with milk-formula ( $p < 0.001$ ). RTD-F was lower in non-fluoridated areas ( $p < 0.001$ ) but higher in children fed milk-formula ( $p = 0.001$ ). It was concluded that children aged 6 to 12 months retain a relatively large proportion of the daily dose of fluoride, approximately  $> 60\%$ . This should be considered in prevention programs based on the use of fluoride and whose objectives are to maximize caries prevention and minimize the risk of dental fluorosis.

Fluoride contribution of toothpaste to intake: Casaglia, *et al.* in 2021 [47], reports fluoride intake from toothpaste in children aged 6 to 12 months and Zohoori, *et al.* in their estimates of DDI-F, includes the ingestion of fluoridated toothpaste.

Casaglia, *et al.* [47] report the intake of fluoride from toothpastes of 500, 600 and 1000 ppm, considering the amount used, percentage ingested and bibliographic information on the amount of paste used. A paste intake of 60% is reported in children aged 6 to 12 months, with dentifrices applied at 0.420 to 0.588g, and daily fluoride intake from dentifrice with 1000 ppm is reported to be 0.125 to 0.492 mg/day.

In Chile, the MINSAL Zero Program in 2019 recommends using fluoridated toothpaste between 1000 and 1500 ppm as a cavity prevention strategy, noting that the paste should always be dispensed per adult and for 0 to 2 years old, an amount the size of the tooth should be used grain of rice or "painting the tip of the brush." In our country there are no toothpastes with less than 1000 ppm of fluoride. With the information from Casaglia, *et al.* 2021, daily fluoride intakes of 0.125 to 0.492 mg/day with 1000 ppm toothpastes and if we

add this intake to our estimate, our total intake range would be 0.755 to 1.122 mg F<sup>-</sup>/day. With an average weight in children from 7 to 12 months of 8.65 kg, the DDI-F value would be 0.087 to 0.130 mg F<sup>-</sup>/Kg/day, which significantly exceeds the recommendations of Burt from 1992. Therefore, it is not we recommend the use of fluoridated toothpaste in children under one year of age.

## **Conclusion**

### **Conclusions for infant feeding in children aged 0 to 6 months:**

- a) Estimation of the DDI-F during the dairy period from 0 to 6 months the feeding includes three instances: exclusive breastfeeding, exclusive feeding with milk-formula and combined feeding of breast milk with formula milk.
- b) The DDI-F for breastfeeding has values lower than the “optimal” ones. In exclusive feeding with formula milk, the average maximum and minimum doses exceed what was established by Burt
- c) 1992. With breast milk supplemented with formula milk, estimates of fluoride intake indicate average maximum and minimum doses below the recommended optimum.
- d) We consider the third stage the closest to nutritional reality during the first 6 months and is the one that combines feeding with exclusive breast milk combined with formula milk.
- e) DDI-F values in children up to 6 months when compared to the tolerable upper intake level (UL) for fluoride are very low compared to the limits established by the US and the UK.
- f) The DDI-F results reinforce the strong idea of favoring exclusive breastfeeding since this ensures the greatest number of children with the least fluoride consumption during the dairy period.

### **Conclusions for infant feeding in children aged 7 to 12 months:**

- a) Feeding in kindergartens differentiates two periods, transition and maturation, with feeding being different in types of food, but the same in types of meals.
- b) DDI-F for children from 7 to 10 months fed in JUNJI kindergartens is  $0.049 \pm 0.009$  mg F<sup>-</sup>/kg/day for and in children from 10 to 12 months it is  $0.045 \pm 0.006$  mg F<sup>-</sup>/kg/day.
- c) Fluoride intake from the 4<sup>th</sup> meal outside kindergarten plus drinking water intake in children aged 7 to 10 months raises their DDI-F to  $0.076 \pm 0.007$  mg F<sup>-</sup>/kg/day. In children 10 to 12 months it is  $0.070 \pm 0.006$  mg F<sup>-</sup>/kg/day considering dinner plus drinking water consumption.
- d) Fluoride intakes for children aged 7 to 12 months are above the upper limit of what is recommended and there should be no additional contributions (fluoridated toothpastes) to ensure that food intake is safe.

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### **Conflict of Interest**

The authors of the publication declare that they have no conflict of interest of any kind.

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