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# A laboratory study of fluoride concentration in infant formulas marketed in Malaysia and estimation of daily intake

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**Abstract**

The window of maximum susceptibility for the development of dental fluorosis for anterior teeth is during the first two to three years of life. The primary source of fluoride intake for infants at this age is mainly from the diet including infant formula. Thus, the present work aimed to investigate the fluoride concentration in commercially available Malaysian infant formulas that required reconstitution before consumption. A total of 29 infant formulas available in the Malaysian market were reconstituted with deionised water, fluoridated tap water, and filtered tap water. The fluoride concentration of the infant formulas was analysed directly using a fluoride ion selective electrode. The daily fluoride intake estimation from the infant formulas was calculated using the median infant body weight and recommended volumes for formula consumption from newborn to > 12 months of age. Results showed that the fluoride concentration of the infant formulas when reconstituted with deionised water ranged between 0.009 to 0.197 mg/L that contributed to the estimated daily fluoride intake ranging from 0.005 to 0.100 mg (total intake per day) or 0.001 to 0.025 mg/kg (total intake per body weight/day). The fluoride concentration in the selected infant formulas was low, but after reconstitution with fluoridated tap water, the overall fluoride concentration in infant formulas sample significantly increased ($p < 0.001$). Nevertheless, the estimated daily fluoride intake from infant formulas alone did not exceed the lowest-observed-adverse-effect level (LOAEL) of fluoride at 0.10 mg/kg/day.



## Introduction

The use of fluoride in dentistry is effective in reducing the prevalence and severity of dental caries (O'Mullane *et al.,* 2016). However, excessive ingestion of fluoride during the maturation phase of dental tissues has been associated with an increased risk of dental fluorosis (Levy, 2003; Goodarzi *et al.,* 2016). Clinically, dental fluorosis appears from its mildest form as white spotting, chalky, and opaque areas on the enamel surfaces to brownish discolouration and surface pitting of the enamel in its severe form (Browne *et al.,* 2005). The window of maximum susceptibility for the development of dental fluorosis for permanent anterior teeth is during the first two years of life. During this risk period, the primary source of fluoride for infants is mainly from infant formula and baby foods such as porridges, pastas, and cereals (Buzalaf and Levy, 2011; Zohoori *et al.,* 2012).

The recommended upper limit of fluoride intake for infant is 0.1 mg/kg body weight (Opydo-Szymaczek and Opydo, 2011). Evidence from studies reported that when infant formula was reconstituted with distilled water, the fluoride content was low at between 0.014 to 0.702 mg/L (Cressey, 2010; Bussell *et al.,* 2016), depending on the source and the type of milk (Opydo-Szymaczek and Opydo, 2011). However, the level of fluoride in infant formulas increases when they are prepared using fluoridated water (Cressey, 2010; Zohoori *et al.,* 2012; Kaophun *et al.,* 2018). This may have a significant impact on the total fluoride intake, in particular, to fully formula-fed infants living in a fluoridated community (Hujoel *et al.,* 2009; Cressey, 2010). Studies on the level of fluoride in

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reconstituted infant milk in Malaysia, where most of the population lives in fluoridated areas, is very limited. In addition, little is known about the type of infant formulas available in the Malaysian market and its fluoride concentration. About four decades ago, a local study reported that the content of fluoride in an infant formula, when reconstituted with deionised water (0 mg/L), was low with a mean value of 0.087 ± 0.04 mg/L (Latifah and Razak, 1989). The study, however, did not investigate the effect of fluoride content in infant formula when reconstituted with fluoridated water, which is important considering that fluoride at levels ranging from 0.4 - 0.6 mg/L is added to municipal water supply in most areas in Malaysia. Since then, there have been no other additional local data on similar research.

Previous studies assessing the level of fluoride in infant formulas have mostly focused on cow-based and soy-based milks (Opydo-Szymaczek and Opydo, 2011; Nagata *et al.,* 2016). However, there are increasing trends of infants consuming goat-based and lactose-free formulas globally, with the same pattern being observed in the Malaysian market (Progressive Markets, 2018). Malaysian households have also started to follow the global trends of using domestic water filtration system (TechSci Research, 2018). There is strong evidence that some water filtration systems are able to remove a substantial amount of fluoride from the water, and if they are used to reconstitute infant formula, the fluoride concentration may be affected. In Malaysia, the commonly used domestic water filtration system uses reverse osmosis technology (TechSci Research, 2018). Evidence shows that water filtration systems using reverse osmosis technology significantly reduce the fluoride levels in drinking water up to 97.92% (Loh *et al.,* 2011; Mohd Kamil *et al.,* 2018).

The changing market trend of infant formula and the use of different sources of water when preparing the formula warrant the need of regular monitoring of fluoride level to ensure safe dosage of fluoride level, particularly during infancy. Additionally, infant formula has evolved throughout the decades, and the newly added compositions may affect the bioavailability of fluoride. Thus, it is important that a new research is carried out to ascertain the fluoride level of milk consumed by infants. Hence, the objectives of the present work were to measure the fluoride concentration using ion selective electrode in selected infant formulas in Malaysia when prepared with different types of water, and to estimate the contribution of infant formulas to daily fluoride intake in Malaysian infants.

## Materials and methods

*Selection of samples*

Ethical approval to conduct the present work was obtained from the Medical Ethics Committee, Faculty of Dentistry, University of Malaya (DFCO1808/0069(L)). Infant formulas that were available in the Malaysian market were explored via an online platform that included major manufacturer's websites and online stores. The products were categorised into manufacturer target age group (starter: 0 - 6 months, follow-up: 6 - 12 months, and toddler: above 12 months) and milk type (cow-based, goat-based, soy-based, and lactose-free). Only powder-based infant formulas that required reconstitution with water prior to consumption were selected. Due to the wide age range (6 - 36 months) stated by some manufacturers for their products, samples in the follow-up and toddlers categories may come from this list. Ready-to-feed infant formula and brands with unclear age restriction or strictly formulated for toddlers three years and above were excluded. Following inclusion and exclusion criteria, 29 infant formula samples were purchased from retail physical stores and pharmacies in Klang Valley, Malaysia.

*Determination of fluoride concentration*

The standard protocol for direct analysis of fluoride measurement was used to determine the fluoride concentration in the milk samples (Martinez-Mier *et al.,* 2011). The concentration of fluoride ion was measured based on the readings recorded by a selective ion electrode (Orion$^{TM}$ Fluoride Electrode, Thermo Scientific Orion 9609BNWP). The ion reader and fluoride electrode were calibrated for reliability and validity using fluoride standard at 0.1 and 1.0 mg/L prior to usage.

Fluoridated water samples were obtained from various sites in the Petaling district and tested for fluoride concentration. Only tap water from a site that complied with the national standard of optimum fluoride concentration of 0.500 ± 0.010 mg/L was used for the reconstitution of infant formulas sample. The tap water with reverse osmosis filtration system (Coway, South Korea) was chosen due to its popularity and report as the most commonly used among Malaysians (TechSci Research, 2018).

All instruments in this experiment were washed using deionised water, and dried with a paper towel between measurements to prevent cross-contaminations between samples or standards. To ensure the accuracy of readings, recalibration was done after every six measurements.

*Preparation of infant formula samples*

Throughout the study, non-boiled water

(25 ± 1°C) was used in the preparation of infant formula to comply with the standard measurement using a selective ion electrode at room temperature (27 ± 2°C). Using non-boiled water would not pose any impact on the outcomes, as findings of an earlier pilot study showed no statistical difference in the concentration of fluoride in boiled and non-boiled water (Bussell *et al.,* 2016). The sample was prepared according to the manufacturers' recommendation with three types of water: (i) deionised water (0 mg/L, control), (ii) fluoridated tap water (0.50 mg/L), and (iii) filtered reverse osmosis water (0.051 mg/L). A volume of 20 mL TISAB II (total ionic strength adjustment buffer) was added to 20 mL of the reconstituted milk prior to the immersion of the electrode. The reading of fluoride concentration was recorded in triplicates, and the mean value was taken as the final fluoride concentration.

Thirty percent of the samples were randomly chosen and reanalysed for the reproducibility of the fluoride concentration following reconstitution with (i) deionised water ($n$ = 9), (ii) fluoridated tap water ($n$ = 9), and (iii) filtered reverse osmosis ($n$ = 9) water in triplicates. These 27 samples were selected to fulfil minimum recommendation for reproducibility test (Bujang and Baharum, 2017). The Intraclass Correlation Coefficient for the triplicate analysis was 0.995, and within-sample standard deviation was 0.005 mg/L, thus indicating excellent reproducibility of 99% for the fluoride concentration reading during measurement

*Data management and statistical analysis*

The daily fluoride intake estimation of the sample was calculated by multiplying the fluoride concentration in infant formulas with the manufacturer's recommended daily intake. The formula (Eq. 1) used to calculate daily fluoride intake was adopted from Noh *et al.* (2015):

$$\text{Daily fluoride intake (DFI) of the sample (mg)} = \text{Fluoride concentration of the sample (mg/L)} \times \text{daily intake of the sample (L)} \quad \text{(Eq. 1)}$$

Data from the present work were compared with the national recommendation of daily fluoride intake and tolerable upper fluoride intake level (NCCFN, 2017).

The daily fluoride intake estimation per body weight from different types of infant formulas was calculated based on age groups (0 to 6 months, 6 to 12 months, and 12 months and above) and the infant's median estimated weights (Bong *et al.,* 2015) using the formula shown in Eq. 2. The estimated value was then compared with the lowest-observed-adverse-effect level (LOAEL) of fluoride (mg/kg/day) as proposed by the Institute of Medicine (1997).

$$\text{Daily fluoride intake estimation per body weight (mg/kg)} = \text{Daily fluoride intake of the sample (mg)} \div \text{body weight (kg)} \quad \text{(Eq. 2)}$$

Data were analysed using Microsoft Excel (Microsoft Corp, USA) and SPSS version 23 (IBM Corp., Armonk, N.Y., USA). With an assumption that the initial fluoride concentration in the infant formulas was equivalent to the fluoride concentration in infant formulas after reconstitution with deionised water, a paired sample $t$-test with an $\alpha$ value of 0.05 was conducted to compare the fluoride concentration differences in infant formulas after reconstitution with different types of water, namely fluoridated tap water and filtered tap water. The overall mean fluoride concentration in infant formulas after reconstitution with these three types of water was then compared using One-way ANOVA and *post-hoc* tests.

## Results and discussion

*Fluoride concentration in infant formulas*

The 29 infant formulas (main samples) was further classified into different categories for data analysis purposes, which are manufacturer target age group and milk type ($n$ = 45). According to the age group, 21 samples were manufactured for infants of 12 months and above, 17 were for infants' age of 6 to 12 months, and seven were for newborn up to 6 months of age. Whereas for the milk type, there were 30 cow-based formulas, three soy-based, six goat-based, and six lactose-free samples (Figure 1). Only eight products were manufactured in Malaysia, and the remaining were imported from the Netherlands

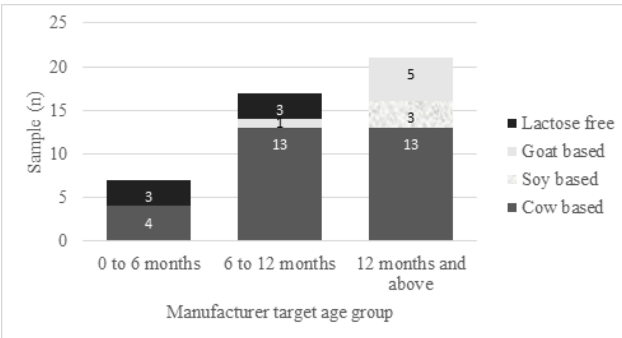


Figure 1. Summary of infant formula samples included in the present work. Note: The total number of infant formula presented here ($n$ = 45) is more than 29 due to overlapping categories for some samples. For examples, products that covered wide ranges of age such as 6 to 36 months were placed into two different age group categories.

Table 1. Selected samples of infant formulas available in Malaysia ($n$ = 29), and the mean fluoride concentration (mg/L) after reconstitution with different types of wat

| Product ID | Product's Name | Manufacturer target age | Mean fluoride concentration (mg/L) | | |
|---|---|---|---|---|---|
| | | | **Deionised water, 0.000 mg/L** | **Fluoridated tap water, 0.500 ± 0.010 mg/L** | **Filtered tap water, 0.051 mg/L** |
| **Cow-based** | | | | | |
| C1 | Lactogen Comforts Follow Up Formula Step 2 | 6 to 36 months | 0.049 | 0.553 | 0.140 |
| C2 | Dumex Mamil Learning | 12 to 36 months | 0.026 | 0.483 | 0.085 |
| C3 | Anmum Infacare Rumusan Susulan Langkah 2 | 6 to 18 months | 0.028 | 0.517 | 0.087 |
| C4 | Frisolac Rumusan Susulan Langkah 2 | 6 to 36 months | 0.025 | 0.480 | 0.058 |
| C5 | Enfalac A+ Rumusan Susulan Langkah 2 | 6 to 18 months | 0.026 | 0.300 | 0.068 |
| C6 | Similac 2 Rumusan Susulan Advance Formula Langkah 2 | 6 to 18 months | 0.030 | 0.507 | 0.100 |
| C7 | Dumex Dupro 2 | 6 to 36 months | 0.023 | 0.557 | 0.087 |
| C8 | Pedia Sure Complete | 12 months and above | 0.036 | 0.507 | 0.100 |
| C9 | S26 Gold SMA Step 1 | 0 to 12 months | 0.037 | 0.530 | 0.090 |
| C10 | Dutch Baby | 6 to 12 months | 0.016 | 0.180 | 0.028 |
| C11 | Snow Neo Baby Rumusan Susulan Langkah 2 | 6 to 18 months | **0.110** | 0.483 | 0.093 |
| C12 | Novalac Novamil Susu Tepung Rumusan untuk Kanak-kanak Tanpa Sukrosa | 12 to 36 months | 0.033 | 0.443 | 0.054 |
| C13 | Dumex Bebelac Rumusan Susulan Langkah 2 | 6 to 36 months | **0.009** | 0.530 | 0.088 |
| C14 | Sustagen Junior 1+ Asli | 12 to 36 months | 0.026 | 0.453 | 0.096 |
| C15 | Morinaga BF-2 Rumusan Susulan | 6 to 36 months | 0.016 | 0.437 | 0.069 |
| C16 | Dumex Mamex Cherish Langkah 1 | 0 to 12 months | 0.037 | 0.480 | 0.076 |
| C17 | Nestle NAN Pro Langkah 1 | 0 to 12 months | 0.083 | 0.547 | 0.140 |
| C18 | Nutricia Aptamil Rumusan Bayi Langkah 1 | 0 to 6 months | 0.037 | 0.480 | 0.084 |

| | | | | | |
|---|---|---|---|---|---|
| | **Total mean (SD) for cow-based infant formulas** | | 0.037 ± 0.026 | 0.480 ± 0.085 | 0.088 ± 0.026 |
| **Soy-based** | | | | | |
| S1 | Dumex Dugro Soy | 12 months and above | 0.024 | 0.253 | 0.055 |
| S2 | Abbot Isomil Plus Rumusan Soya Yang Lengkap Tanpa Laktosa | 12 months and above | 0.018 | **0.120** | 0.029 |
| S3 | Biogreen O'Kid DHA Gold Organic Soya Milk Powder | 12 months and above | 0.197 | 0.757 | 0.280 |
| | **Total mean (SD) for soy-based infant formulas** | | 0.081 ± 0.103 | 0.377 ± 0.338 | 0.121 ± 0.138 |
| **Goat-based** | | | | | |
| G1 | Karihome Rumusan Susulan Susu Kambing Langkah 2 | 6 to 36 months | 0.130 | 0.627 | 0.200 |
| G2 | Biolife Green Food Purenat Pure Goat Milk Powder | 12 months and above | 0.052 | 0.497 | 0.071 |
| G3 | Khalish Goats Milk Date Fruit and Cocoa | 12 months and above | 0.063 | **0.640** | 0.110 |
| G4 | Wildan ADEK Vanilla | 12 months and above | 0.067 | 0.557 | 0.110 |
| G5 | Lazz Susu Kambing Pracampuran | 12 months and above | 0.020 | 0.537 | 0.110 |
| | **Total mean (SD) for goat-based infant formulas** | | 0.077 ± 0.044 | 0.583 ± 0.058 | 0.134 ± 0.054 |
| **Lactose-free** | | | | | |
| L1 | Nestle NAN Rumusan Khas Tanpa Laktosa | 0 to 12 months | 0.018 | 0.473 | 0.071 |
| L2 | Similac LF Rumusan Khas Tanpa Laktosa | 0 to 12 months | 0.025 | 0.580 | 0.099 |
| L3 | Dumex Mamex LF | 0 to 12 months | 0.028 | 0.227 | 0.042 |
| | **Total mean (SD) for lactose-free infant formulas** | | 0.024 ± 0.005 | 0.427 ± 0.160 | 0.071 ± 0.026 |
| | **MEAN FLUORIDE CONCENTRATION** | | | | |
| | Mean ± SD (mg/l)[b] | | 0.045 ± 0.041 | 0.474 ± 0.140 | 0.094 ± 0.050 |
| | Mean fluoride changes (mg/l) | | | 0.430 | 0.049 |
| | *p* value[a] | | | $p < 0.001$ | $p < 0.001$ |

[a] = paired sample *t*-test; [b] = Comparison between the three types of water used for infant formula reconstitution (One-way ANOVA and *post-hoc* tests). Fluoridated tap water *vs* deionised water ($p < 0.001$); fluoridated tap water *vs* filtered tap water ($p < 0.001$); and filtered tap water vs deionised water (p = 0.092).

(*n* = 4), Thailand (*n* = 3), Singapore (*n* = 3), New Zealand (*n* = 2), Indonesia (*n* = 2), and the Philippines (*n* = 2). Japan, Brunei, France, Spain, and the USA each supplied one product.

The fluoride concentration of Malaysia infant formulas was determined low after reconstitution with deionised water, ranging from 0.009 to 0.197 mg/L (Table 1). The findings are similar to those reported by Clifford *et al.* (2009) in Australia (0.040 to 0.140 mg/L) and Zohoori *et al.* (2012) in United Kingdom (0.020 to 0.180 mg/L). In contrast, studies conducted in Thailand and Brazil reported higher fluoride concentration in infant formulas with mean of 0.308 mg/L (Kaophun *et al.,* 2018) and 0.310 mg/L (Nagata *et al.,* 2016), respectively. These studies were reported using microdiffusion method for fluoride analysis (Nagata *et al.,* 2016; Kaophun *et al.,* 2018).

As for higher amount of fluoride in infant formulas in studies using the microdiffusion method as compared to the direct method, Martinez-Mier *et al.* (2009) reported that both techniques were reliable for fluoride analysis. The possible explanation of the differences in fluoride concentration observed from the different methods used is the potential precipitation of the fluoride after the infant formulas are reconstituted with water. In addition to that, the fluoride ion-selective electrode used in the direct technique measures both fluoride concentration and fluoride ion activities (Martinez-Mier *et al.,* 2009). The direct addition of TISAB in the direct technique may also affect fluoride activities by the adjustment of its ionic strength, thus resulting in reduced fluoride concentration readings (Martinez-Mier *et al.,* 2009).

While the majority of the samples had low fluoride concentration, soy-based infant formulas had the highest fluoride concentration (0.197 mg/L) when reconstituted with deionised water (Table 1). Similar results were reported on infant formula samples in other countries such as Australia (Clifford *et al.,* 2009), United States (Siew *et al.,* 2009), and Brazil (Nagata *et al.,* 2016). Authors explained the higher fluoride level was due to the presence of endogenous fluoride in the soy extract (Nagata *et al.,* 2016). The soy extract consists of phytates and tricalcium phosphates that bind fluoride which may cause and intrinsic fluoride to arise (McKnight-Hanes *et al.,* 1988).

*Impact of water fluoride concentration on the fluoride content of infant formulas*

After reconstitution with fluoridated tap water, the overall fluoride concentration in infant formulas sample significantly increased ($p < 0.001$) (Table 1). The results have confirmed the existing evidence that the fluoride concentration in infant formulas after reconstitution depends on the fluoride concentration in the water that is used to reconstitute them (Levy, 2003; Siew *et al.,* 2009; Cressey, 2010; Mahvi *et al.,* 2012; Zohoori *et al.,* 2012; Noh *et al.,* 2015; Nagata *et al.,* 2016; Bussell *et al.,* 2016; Kaophun *et al.,* 2018). Apart from that, the evidence suggests that the use of water filters may affect the fluoride concentration in the water supply (Mohd Kamil *et al.,* 2018). In the present work, reverse osmosis water filter was found to remove fluoride by 0.449 mg/L (89.8%) from the initial fluoride concentration of 0.500 mg/L. This finding is concordant with the study by Levy (2003) who affirmed that fluoride is removed by reverse osmosis water filters. After reconstitution with filtered tap water, the overall fluoride concentration in infant formulas sample increased in comparison to samples in the control group (deionised water). However, the difference was not statistically significant ($p = 0.092$). Since almost all infant formulas reconstituted with filtered tap water had fluoride concentration below 0.200 mg/L (Table 1), the effect of filtered tap water to the fluoride concentration in infant formulas was similar to the sub-optimal or non-fluoridated water (Fomon and Ekstrand, 1999).

The mean fluoride concentration in infant formulas according to milk type and target age group is shown in Table 2. The results showed that the highest mean fluoride concentration after reconstitution with fluoridated tap water was goat-based formula manufactured for 6 months and above (0.627 mg/L). As goat-based formula was not available for infants less than 6-month-old for this age group, cow-based formulas had the highest mean fluoride (0.510 mg/L). There was limited evidence to compare the findings of goat-based formulas. This potentially could be due to the low availability of goat-based formulas in the market, which only become popular lately in some countries including Malaysia (Progressive Markets, 2018). The available evidence from a study in Thailand reported that the mean fluoride of this milk type ranged from 0.235 to 0.243 mg/L (Kaophun *et al.,* 2018).

No clear difference in fluoride content was observed between imported and locally produced products (Table 2). The findings are expected as major international manufacturers voluntary reduce the level of fluoride in infant formula powder in the United States (Pendrys and Katz, 1998), Europe, Australia, and New Zealand (Do *et al.,* 2012) since 1980 to 1990s as a global effort to prevent dental fluorosis. The availability of infant formula products from major global manufacturers such as Dutch Lady, Nestle, and Danonne in Malaysia may explain the low fluoride level found in the present work, which is consistent with international findings.

Table 2. Mean fluoride concentration (mg/L) in infant formulas according to milk type and product origin, after reconstitution with different types of water.

| Milk type | No. of sample ($n$) | Mean fluoride concentration (mg/L) | | |
|---|---|---|---|---|
| | | Deionised water (control – 0.000 mg/L) Mean ± SD | Fluoridated tap water (0.500 ± 0.010 mg/L) Mean ± SD | Filtered tap water (reverse osmosis – 0.051 mg/L) Mean ± SD |
| **0 to 6 months** | | | | |
| Cow-based | 4 | **0.049** ± 0.023 | **0.510** ± 0.036 | **0.098** ± 0.029 |
| Lactose-free | 3 | 0.024 ± 0.005 | 0.427 ± 0.179 | 0.071 ± 0.029 |
| **6 to 12 months** | | | | |
| Cow-based | 13 | 0.038 ± 0.029 | 0.470 ± 0.111 | 0.087 ± 0.030 |
| Goat-based | 1 | **0.130** | **0.627** | **0.200** |
| Lactose-free | 3 | 0.024 ± 0.005 | 0.427 ± 0.179 | 0.071 ± 0.029 |
| **12 months and above** | | | | |
| Cow-based | 13 | 0.034 ± 0.025 | 0.481 ± 0.067 | 0.087 ± 0.022 |
| Soy-based | 3 | **0.081** ± 0.103 | 0.377 ± 0.338 | **0.121** ± 0.138 |
| Goat-based | 5 | 0.066 ± 0.040 | **0.574** ± 0.060 | 0.120 ± 0.048 |
| **Product origin** | | | | |
| Local | 8 | 0.034 ± 0.023 | 0.499 ± 0.137 | 0.086 ± 0.028 |
| Imported | 21 | 0.048 ± 0.046 | 0.465 ± 0.142 | 0.097 ± 0.056 |
| $p$-value | | 0.423 | 0.566 | 0.618 |

*Contribution of infant formulas to daily fluoride intake in Malaysian infants*

The mean estimated daily fluoride intake of Malaysian infants calculated from the above-mentioned equations was determined within the range of 0.015 to 0.459 mg (Table 3). The daily fluoride intake in the present work was estimated solely from infant formulas. Since there was no recent available data on the feeding pattern among Malaysian infants, the calculation of the estimated daily intake of fluoride was based on the manufacturers' feeding recommendation and the median body weight of infants in Malaysia (Bong *et al.,* 2015).

The estimated daily fluoride intake (mg) from 0 to 6 month infants who were fully fed with infant formulas was found to be similar to the recommended daily fluoride intake and the tolerable upper intake level (0.021 to 0.459 mg/day). On the other hand, the daily fluoride intake estimation (mg) in infant formulas for 6 to 12 months (0.015 to 0.388

Table 3. Mean estimated daily fluoride intake (mg) from infant formulas according to the type and target age of milk.

| Milk type | No. of sample ($n$) | Recommended daily fluoride intake (mg/day)[a] | Tolerable upper fluoride intake level (mg/day)[b] | Mean estimated daily fluoride intake (mg) | | |
|---|---|---|---|---|---|---|
| | | | | Deionised water (control – 0.000 mg/L) ± SD (mg) | Fluoridated tap water (0.500 ± 0.010 mg/L) ± SD (mg) | Filtered tap water (reverse osmosis – 0.051 mg/L) ± SD (mg) |
| **0 to 6 months** | | | | | | |
| Cow-based | 4 | 0.01 | 0.70 | **0.044** ± 0.021 | **0.459** ± 0.032 | **0.088** ± 0.026 |
| Lactose-free | 3 | | | 0.021 ± 0.005 | 0.384 ± 0.161 | 0.064 ± 0.026 |
| **6 to 12 months** | | | | | | |
| Cow-based | 13 | 0.50 | 0.90 | 0.026 ± 0.022 | 0.311 ± 0.088 | 0.057 ± 0.020 |
| Goat-based | 1 | | | **0.080** | **0.388** | **0.123** |
| Lactose-free | 3 | | | 0.015 ± 0.005 | 0.265 ± 0.092 | 0.044 ± 0.015 |
| **12 months and above** | | | | | | |
| Cow-based | 13 | 0.70 | 1.30 | 0.018 ± 0.009 | **0.268** ± 0.073 | 0.055 ± 0.030 |
| Soy-based | 3 | | | **0.043** ± 0.049 | 0.210 ± 0.148 | **0.066** ± 0.064 |
| Goat-based | 5 | | | 0.032 ± 0.035 | 0.240 ± 0.124 | 0.056 ± 0.050 |

[a] = Recommended daily fluoride intake (NCCFN, 2017); [b] = Tolerable upper fluoride intake level (NCCFN, 2017).

Table 4. Mean estimated daily fluoride intake per body weight (mg/kg) from infant formulas.

| Milk type | Median body weight of infants (kg)[a] Male Female | Lowest-observed-adverse-effect level of fluoride (mg/kg/day)[b] | Mean estimated daily fluoride intake per body weight (mg/kg) | | |
|---|---|---|---|---|---|
| | | | Deionised water (control – 0.000 mg/L) ± SD (mg/kg) | Fluoridated tap water (0.500 ± 0.010 mg/L) ± SD (mg/kg) | Filtered tap water (reverse osmosis – 0.051 mg/L) ± SD (mg/kg) |
| **0 to 6 months** | | | | | |
| Cow-based | 3.09<br>3.02 | 0.100 | **0.014** ± 0.007<br>**0.014** ± 0.007 | 0.149 ± 0.010<br>**0.152** ± 0.011 | 0.028 ± 0.008<br>**0.029** ± 0.009 |
| Lactose-free | 3.09<br>3.02 | | 0.007 ± 0.002<br>0.007 ± 0.002 | 0.124 ± 0.052<br>0.127 ± 0.053 | 0.021 ± 0.008<br>0.021 ± 0.009 |
| **6 to 12 months** | | | | | |
| Cow-based | 7.45<br>6.96 | | 0.004 ± 0.003<br>0.004 ± 0.003 | 0.042 ± 0.012<br>0.045 ± 0.013 | 0.008 ± 0.003<br>0.008 ± 0.003 |
| Goat-based | 7.45<br>6.96 | | 0.011<br>**0.012** | 0.052<br>**0.056** | 0.017<br>**0.018** |
| Lactose-free | 7.45<br>6.96 | | 0.002 ± 0.001<br>0.002 ± 0.001 | 0.036 ± 0.012<br>0.038 ± 0.013 | 0.006 ± 0.002<br>0.006 ± 0.002 |
| **12 months and above** | | | | | |
| Cow-based | 9.00<br>8.49 | | 0.002 ± 0.0010<br>0.002 ±0.0011 | 0.030 ± 0.008<br>**0.032** ± 0.009 | 0.006 ± 0.003<br>0.006 ± 0.004 |
| Soy-based | 9.00<br>8.49 | | **0.005** ± 0.0055<br>**0.005** ± 0.0058 | 0.023 ± 0.016<br>0.025 ± 0.017 | 0.007 ± 0.007<br>**0.008** ± 0.008 |
| Goat-based | 9.00<br>8.49 | | 0.004 ± 0.0039<br>0.004 ± 0.0041 | 0.027 ± 0.014<br>0.028 ± 0.015 | 0.006 ± 0.006<br>0.007 ± 0.006 |

[a] = Median body weight of infants in Malaysia (Bong *et al.,* 2015); [b] = Lowest-observed-adverse-effect level of fluoride (Institute of Medicine, 1997).

mg/day) and 12 months and above (0.018 to 0.268 mg/day) was determined less than the recommended daily fluoride intake. Similar findings were reported from a study on infant milks in the United Kingdom (Bussell *et al.,* 2016). The low level of daily fluoride intake determined in infants aged 6 months and above in the present work is not a major concern for fluoride deficiency as the total fluoride ingestion for infants of this age group includes both dietary and non-dietary sources (Zohoori *et al.,* 2012). Hence, in addition to formulas, foods and water also serve as fluoride sources to this group of infants (Yanagida *et al.,* 2019). Following ingestion, the bioavailability of fluoride has been reported to be generally high (Spak *et al.,* 1982). However, it is known that other diet constituents can influence the degree of fluoride absorption and retention. When consumed with water in a soluble form such as sodium fluoride, the absorption of fluoride is nearly complete. However, when it is ingested with milk, baby formula, or foods, especially those with high concentrations of calcium or certain other divalent or trivalent ions that form insoluble compounds, the absorption of fluoride may be reduced by 10 to 25% (Spak *et al.,* 1982; Institute of Medicine, 1997).

The mean estimated daily fluoride intake per body weight was calculated in the range of 0.002 to 0.152 mg/kg (Table 4). The findings showed that none of the infant formulas exceeded the lowest-observed-adverse-effect level of 0.10 mg/kg/day, except for 0 to 6 month infants who were fully fed with infant formulas reconstituted with fluoridated tap water. However, considering that mineralisation of dental tissues of the permanent dentition starts at about 11 months, it is less likely to affect development of the permanent teeth. This holds true among infants with mix-feeding methods as human milk have very low fluoride concentrations (< 0.5 μM), even when a mother drinks fluoridated water due to the limited transfer from plasma to breast-milk (Sener *et al.,* 2007). Nevertheless, the excessive fluoride intake during 0 to 6 months may interfere with the mineralisation of the primary dentition, thus probably causing dental fluorosis in the deciduous teeth (Cressey, 2010; Nagata *et al.,* 2016).

## Conclusion

In general, the concentration of fluoride in the selected infant formulas available in the Malaysian market was low, and this concentration was affected by the type of water used for reconstitution. The estimated daily fluoride intake from infant formulas alone was also found not to exceed the lowest-observed-adverse-effect level of fluoride except for the 0 to 6 month-old infants who were fully fed with infant formulas reconstituted with fluoridated tap water. These findings suggest that the fluoride intake from powder-based infant formulas in Malaysia is low and thus, the potential association with dental fluorosis in the permanent dentition upon consumption of these formulas is very unlikely. Our results indicate that there is no justification to produce a national guideline on infant formula preparations for Malaysian infants, in particular to those living in fluoridated areas. Nevertheless, health care professionals should continue providing advice to parents and child carers that reconstituting infant formula with fluoridated water will not cause any harm to their children's teeth development.

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