

Fluoride Intake of Infants from Formula

Claudia X Harriehausen*/ Fehmida Z Dosani**/ Brett T Chiquet***/ Michelle S Barratt****/
Ryan L Quock *****

Objective: This study aimed to assess fluoride intake in infants from formula reconstituted with water, with fluorosis risk in mind. **Study Design:** Data on water source, formula brand/type, volume of formula consumption and infant weight were collected for infants at two-, four-, six-, nine- and twelve-month pediatrician well child visits. Identified formula brands and water types were reconstituted and analyzed for fluoride concentration. Patient body mass and volume consumed/day were used to estimate fluoride intake from reconstituted formula. Descriptive statistics, one-way analysis of variance and chi-square tests were utilized. **Results:** All infants consumed formula reconstituted with minimally fluoridated water (0.0– 0.3 ppm). 4.4% of infants exceeded the recommended upper limit (UL) of 0.1mg/kg/day. Although mean daily fluoride consumption significantly differed among all groups, the proportion of infants at each visit milestone that exceeded daily fluoride intake of 0.1mg/kg/day was not statistically significantly different ($p>0.05$) for any age group. Predicted values calculated with optimally fluoridated water (0.7ppm) resulted in 36.8% of infants exceeding the UL. **Conclusions:** Optimally fluoridated water may increase fluorosis risk for patients younger than six months. Future investigation should include multiple sites and multi-year follow-up to assess actual fluorosis incidence.

Keywords: fluoride; fluorosis; formula; infant formula

INTRODUCTION

Fluoride has been established as a safe, effective agent in the prevention of dental caries and has dramatically reduced its incidence among children.¹ Excessive amounts ingested during tooth development can result in the development of dental fluorosis, a primarily cosmetic condition.^{2, 3} This developmental alteration of tooth enamel ranges from mild white spots to mottling or pitting.³

Although not the exclusive causal agent of fluorosis infant formula reconstituted with fluoridated water can be a source of systemic fluoride exposure during tooth development. Both powdered and liquid concentrate formula require the addition of water, while ready-to-feed formula does not. Fluoride content in the actual formula is minimal;⁴⁻⁶ the fluoride intake from powdered or concentrate liquid formulas that require reconstitution with water depends primarily upon the fluoride content of the water source. Most bottled waters typically have concentrations of fluoride lower than the recommended optimal levels (<0.7ppm); water filtration systems involving reverse osmosis also result in sub-optimal fluoride concentrations. Consequently, some infants might not be receiving the full benefit of the fluoride for caries prevention.⁷ However, consumption of infant formula reconstituted with optimally fluoridated water (0.7ppm) may increase a children's fluoride exposure, putting them at a higher risk of developing dental fluorosis.^{4-6, 8-11}

The United States Institute of Medicine (IOM) has established adequate intake (AI) and tolerable upper intake levels (UL) for all

*Claudia X Harriehausen, DDS, MSD, Private Practice .

**Fehmida Z Dosani, DDS, MSD, Assistant Professor, Department of Pediatric Dentistry, University of Texas School of Dentistry at Houston.

***Brett T Chiquet, DDS, PhD, Assistant Professor, Department of Pediatric Dentistry, University of Texas School of Dentistry at Houston.

****Michelle S Barratt, MD, MPH, Professor, Department of Pediatrics, University of Texas McGovern Medical School.

*****Ryan L Quock, DDS, Professor and Interim Chair, Department of Restorative Dentistry & Prosthodontics, University of Texas School of Dentistry at Houston.

Send all correspondence to:

Ryan Quock

Department of Restorative Dentistry and Prosthodontics

University of Texas School of Dentistry

7500 Cambridge

Houston, TX 77054 (USA)

Phone: (713) 486-4276, Fax (713) 486-4353

E-Mail: Ryan.Quock@uth.tmc.edu

age groups.¹² For infants aged birth to six months, the AI has been set at 0.01 mg/day based on the level of fluoride found in mother's milk. For infants aged seven to twelve months, an AI of 0.05 mg/kg/day serves as the recommendation. Moderate dental fluorosis marks the critical adverse effect for susceptible age groups such as infants, toddlers and children. The IOM identified a fluoride intake of 0.10 mg/kg/day as the lowest observed adverse effect level (LOAEL) for moderate enamel fluorosis in children from birth to eight years of age. The tolerable upper intake level (UL) was derived from the LOAEL for moderate fluorosis with an uncertainty factor of one. Based on a UL of 0.10 mg/kg/day of fluoride and a reference weight for infants ages birth through six months of 7 kg, the UL is 0.7 mg/day. For children ages seven through twelve months with a reference weight of 9 kg, the UL is 0.9 mg/day.¹²

Determining estimates of fluoride intake and exposure is especially important for infants and children with developing teeth because they are at higher risk of developing fluorosis. To date a number of studies have been completed in the United States to investigate the relationship between infant formula and fluorosis. However, no studies have been conducted in the Houston Metropolitan area, and feedback from patients indicates that the majority use bottled water, in contrast to data from the national Infant Feeding Practicing Survey (IFPS II) that reported that 70-75% of parents reconstitute formula with tap water.¹³ Therefore, the purpose of this study was to estimate fluoride intake of a specific population of infants aged two-, four-, six-, nine- and twelve-months and compare their fluoride intake to the recommended values set by the IOM for AI and UL. We hypothesized that 1) the mean daily fluoride intake for infants age two-, four-, six-, nine-, and twelve-months is equal among all the groups and 2) the proportions of infants age two-, four-, six-, nine-, and twelve-months that exceed the UL of 0.1mg/kg/day is equal among all the groups.

MATERIALS AND METHOD

Institutional review board approval at the University of Texas Health Science Center at Houston was obtained for this study (HSC-08-15-0588). Subjects were recruited from infants (represented by parents/guardians) that presented to The University of Texas Physicians General Pediatrics Clinic at either their two-, four-, six-, nine-, or twelve-month routine well child visit. Only infants who were rated as class I by the American Society of Anesthesiologists Physical Classification system, who were primarily formula fed and who had English- or Spanish-speaking parents/guardians were included in this study. Parents/caregivers could only participate once in this study.

A survey (Figure 1) was completed by the parent/guardian of participating eligible children after the two-, four-, six-, nine-, or twelve-month well child visit to obtain the child's weight, brand and type of formula, water source used to reconstitute the formula and total volume of formula consumed by the infant over 24 hours. Subjects were de-identified for data analysis.

Based upon participant reporting, seven formula brands were purchased: powdered Similac Advance, powdered Similac Sensitive, powdered Similac Spit Up, liquid concentrate Similac Advance (Abbott Nutrition, Lake Forest, IL), powdered Enfamil Infant, powdered Enfamil Gentlease (Mead Johnson Nutrition, Glenview, IL), and powdered Gerber Soy (Gerber, Florham Park, NJ). Ready

Figure 1. Survey given to participating caregivers

Questionnaire

1. What is your child's age
 - a. 2 months
 - b. 4 months
 - c. 6 months
 - d. 9 months
 - e. 12 months
2. How much does your child weigh today during his/her well child visit?
 - a. _____ (kg or lb)
3. What type of formula does your child use?
 - a. Ready to feed
 - b. Powdered
 - c. Liquid concentrate
4. What brand of formula does your child use?
 - a. Similac Advance (Blue lid)
 - b. Similac Sensitive (Orange lid)
 - c. Similac Spit up (Green lid)
 - d. Enfamil Infant (Yellow lid)
 - e. Enfamil Gentlease (Purple lid)
 - f. Other: _____
5. What type of water do you use to mix with formula?
 - a. Tap
 - b. Filtered tap
 - c. Bottled
 - d. Vended water station
 - e. Well
6. If you use bottled water, what brand do you use? (Example: Ozarka, Dosani, Aquafina, Evian, Pure Life)
 - a. _____
7. If you use nursery water, what type do you use?
 - a. Nursery water with the PINK label
 - b. Nursery water with the PURPLE label
 - c. Gerber Pure Water
 - d. Other: _____
8. If you use filtered water, what type of filtration system do you have? (Example: Brita, PUR, Culligan, reverse osmosis)
 - a. _____
9. What zip code does your child live in?
 - a. _____
10. How much formula does your child drink during the day (in a 24 hour period)?
 - a. _____ (ounces)

to feed formula was not purchased because it does not need to be reconstituted with water and fluoride levels have been recently reported by brand (0.08-0.32ppm).¹³

For water source, survey participants could choose between bottled/spring/deionized, nursery, or tap water. For those using tap water, data collected in 2014 (the most recent year available at the time of the study) from the Drinking Water Quality Report City of Houston on fluoride concentrations by zip code were used to estimate the water fluoride concentration of patients living in the Houston Metropolitan area. The average concentration of community water was 0.27 ppm, which was rounded to the nearest tenth of a ppm to 0.3ppm. Nursery Water with Added Fluoride (DS Services of America, Atlanta, GA) was analyzed using the direct method.¹⁴ The total fluoride level of the Nursery Water was 0.3ppm. Distilled, spring, and purified bottled water samples were not obtained because there is recent literature reporting the fluoride levels of these types of waters. The reported fluoride levels are very low or insignificant ranging from 0.01-0.08.⁷ For the purpose of this study, deionized water was used to reconstitute the formula for patients using distilled, purified, and spring water.

A gravimetric preparation of water with fluoride concentration 0.7ppm was prepared in order to predict the fluoride intake of our patient population if they were to reconstitute infant formulas with the United States Department of Health and Human Services' recommended level for optimally fluoridated water.¹⁵

Six different powdered formulas and one liquid concentrate formula were reconstituted according to the manufacturer's instructions with water at 0.0, 0.3, and 0.7 ppm... All samples were analyzed for total fluoride content using the microdiffusion analysis method.¹⁴ Excel (Microsoft, Redmond, WA) was used to create a scatterplot for the 0.0, 0.3, and 0.7ppm coordinates and to obtain trendline formulae to predict the fluoride concentration formula reconstituted with any water with known ppm and these seven infant formulas.

Total fluoride intake, in mg/kg/day, was calculated based on subject's weight, formula brand, water used, and daily volume consumed, and then compared to the AI and UL recommendations. Similar calculations were performed using 0.7ppm water to determine the proportion of infants ingesting fluoride who would exceed the UL if optimally fluoridated water was used to reconstitute the formula. Based on a sample size of n = 114, a one-way ANOVA has 80% power to detect an effect size of $f = .33$, which is a medium effect size by Cohen's guideline¹⁶ Descriptive statistics, one-way analysis of variance (ANOVA) and chi-square tests were used to analyze the data.

RESULTS

A total of 114 parents consented to participate and completed the survey portion of this project. Table 1 shows the reported infants' mean weights, brand and type of formula, water source used to reconstitute the formula, and total volume of formula consumed by the infant over 24 hours. The majority of infants consumed powdered formula (92.1 %), with fewer consuming liquid concentrate and ready to feed formula, (4.4% and 3.5% respectively). Ninety three percent of infants used bottled water to reconstitute the infant formula; 39.5% of infants consumed infant formula reconstituted with bottled water with little or no fluoride added (0.0ppm), 53.5% with Nursery Water with Added Fluoride (0.3ppm), 3.5% with tap water (0.3pm).

Six powdered formulas and one liquid concentrate formula were reconstituted with deionized (0.0ppm), Nursery Water (0.3ppm), and optimally fluoridated water (0.7ppm) and mean fluoride levels are shown on Table 2. Liquid concentrate had the lowest fluoride levels. Powdered Gerber Soy formula had relatively higher fluoride content when reconstituted, compared to the other six milk based formulas. A scatter plot graph (Figures 2 and 3) was created, which allowed extrapolation of a series of trendline equations (Table 2 and Figure 4).

The daily fluoride intake was extrapolated from the well child visit survey data gathered (weight, water used to reconstitute formula, and volume consumed/day). One hundred percent of infants between the ages of two and six months consumed fluoride above the AI (0.01mg/day). Of the nine- and twelve-month old infants, only 12.5% consumed fluoride above the AI (0.05mg/kg/day) from formula alone. Only 4.4% of infants aged two-, four-, and six-months consuming formula reconstituted with water at 0.3ppm exceeded the UL of 0.1mg/kg/day. No children aged nine- and twelve-months exceeded the UL. When water with 0.7ppm fluoride was used to predict fluoride concentrations if formula was reconstituted with optimally fluoridated water, 36.8% of infants would have reached the threshold for the UL (0.1mg/kg/day), with the two-, four-, and- six month infants being affected more (59.4%, 56.5%, and 33.3%, respectively) compared to nine- and twelve-month infants (14.3% and 9.1%, respectively).

ANOVA was used to compare the mean daily intake with predicted fluoride intake levels using optimally fluoridated water, and results were significantly different among all groups ($p=0.0029$, $p<0.001$, respectively) (Table 3). A post hoc Tukey showed that the two month old group actual mean intake values were significantly different from the nine and the twelve month olds ($p=0.017$, $p=0.04$, respectively). From the predicted intake values of formula reconstituted with water 0.7ppm F-, the two, and four month mean values were significantly different compared to the nine and twelve month ($p=0.006$, $p=0.004$ and $p=0.008$, $p=0.004$, respectively).

Chi-square analysis was used to compare the proportion of infants aged two-, four-, six-, nine-, and twelve months that exceeded a daily fluoride intake of 0.1mg/ kg/day. Proportions of subjects exceeding 0.1mg/kg/day were not statistically significant ($p=0.49$) for any age group when reconstituted with 0.0 or 0.3ppm fluoridated water, but were statistically significant ($p<0.001$) for the predicted fluoride values when formula was reconstituted with optimally fluoridated water (0.7ppm) as shown in Table 3.

Table 1. Descriptive information of the dataset.

Category*	Age							
	2m	4m	6m	9m	12m	Total		
Number	32	23	27	21	11	114		
Weight (Kg)	Mean	5.55	6.71	7.78	9.51	9.45	7.46	
	St. Dev.	0.95	0.83	1.13	1.49	1.11	1.87	
	Range	3.94-8.18	5.3-8.63	6.35-11.35	7.73-14.16	8.18-11.36	3.94-14.16	
Formula	Powdered Similac Advance	20	15	22	13	8	78	
	Powdered Similac Spit up	1	2	0	3	0	6	
	Powdered Similac Sensitive	5	0	1	1	1	8	
	Powdered Enfamil Infant	0	3	1	0	1	5	
	Powdered Enfamil Gentlease	2	1	0	0	0	3	
	Powdered Gerber Soy	1	1	2	1	0	5	
	Liquid Concentrate Similac Advance	1	0	0	3	1	5	
	Ready to Feed Similac Advance	1	0	1	0	0	2	
	Ready to Feed Enfamil Infant	0	1	0	0	0	1	
	Ready to Feed Similac Soy	1	0	0	0	0	1	
	N/A	2	1	1	0	0	4	
	Water	Deionized water	9	6	14	10	6	45
		Nursery water with added fluoride	20	15	12	10	4	61
		Tap water	1	1	0	1	1	4
Amount (ml)	Mean	856.69	974.59	948.64	848.75	659.5	883.55	
	St. Dev.	324.99	303.87	330.48	400.05	276.04	338.18	
	Range	295.74-1419.55	354.87-1419.55	295.74-1892.74	177.44-1656.14	177.44-1182.94	177.44-1892.74	

*Proportions of type of formula and water source reported by parents in each age group were not statistically significant.

Table 2. Fluoride concentration of infant formulas reconstituted with water at different levels

Formula			Trendline equation**
	Deionized Water	Nursery Water	
Powdered Similac Advance	0.05	0.425	$y = 0.9973x + 0.0776$
Powdered Similac Spit Up	0.13	0.49	$y = 0.9919x + 0.1527$
Powdered Similac Sensitive	0.05	0.41	$y = 1.0365x + 0.0678$
Powdered Enfamil Infant	0.09	0.4	$y = 1.0209x + 0.0914$
Powdered Enfamil Gentlease	0.09	0.4	$y = 0.9541x + 0.0986$
Powdered Gerber Soy	0.175	0.51	$y = 0.9284x + 0.1955$
Liquid Concentrate Similac Advance	0.035	0.23	$y = 0.4865x + 0.0528$
Control	0	0.3	n/a

*Results reported in parts per million (ppm). Samples tested in duplicate; averages shown.

**Trendline equation to determine total fluoride concentration of reconstituted formula in ppm (y) at water fluoride concentration (x).

Figure 2. Scatter Plot Graph.

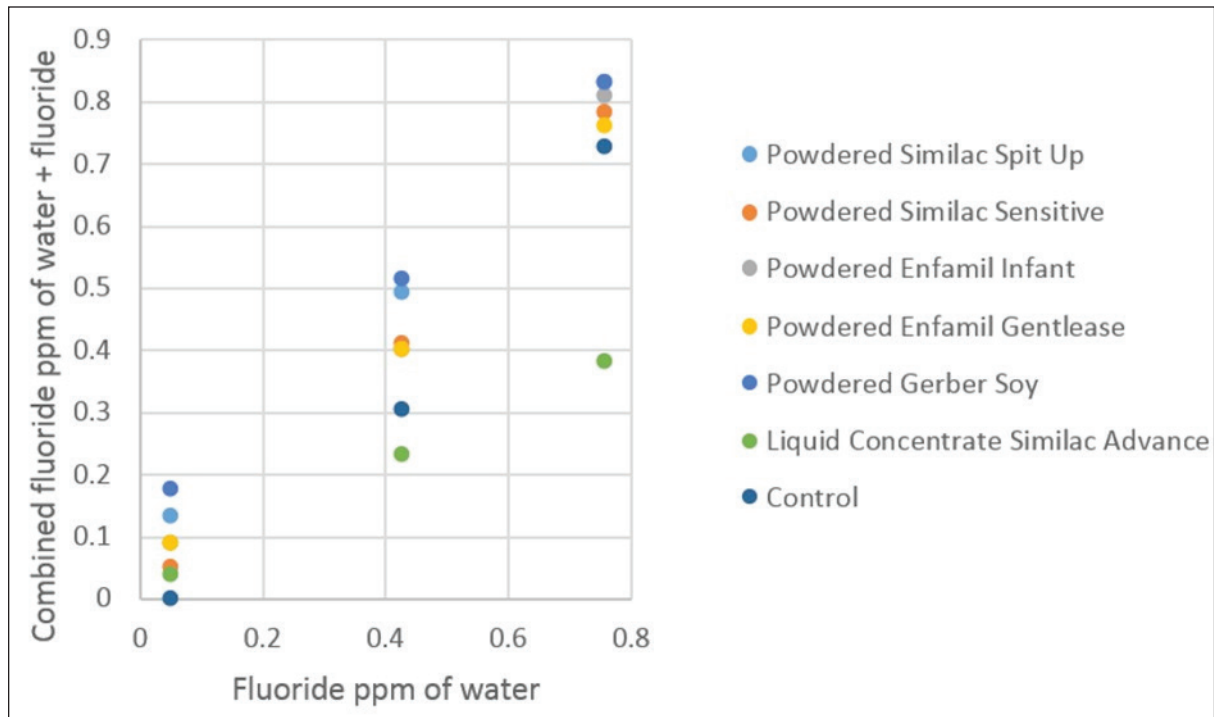


Figure 3. Application of trend line equations to predict total ingested fluoride based on formula used, daily consumption, and known fluoride concentrations. Here, equation for powdered Similac infant formula is used as an example. Goal is to solve for the maximum recommended fluoride concentration in the water source to prevent exceeding the UL.

Trendline Equation $Y=0.9973X + 0.0776$
 Y = Total fluoride per mg/liter of reconstituted formula
 X = mg/L of water source
 UL = 0.1 mg/kg/day

Calculate maximum fluoride intake per day for weight
 0.1mg/kg X ___ kg = ___mg

Calculate maximum Y (formula fluoride concentration per liter)
 ___ mg ÷ ___ L = ___ mg/L

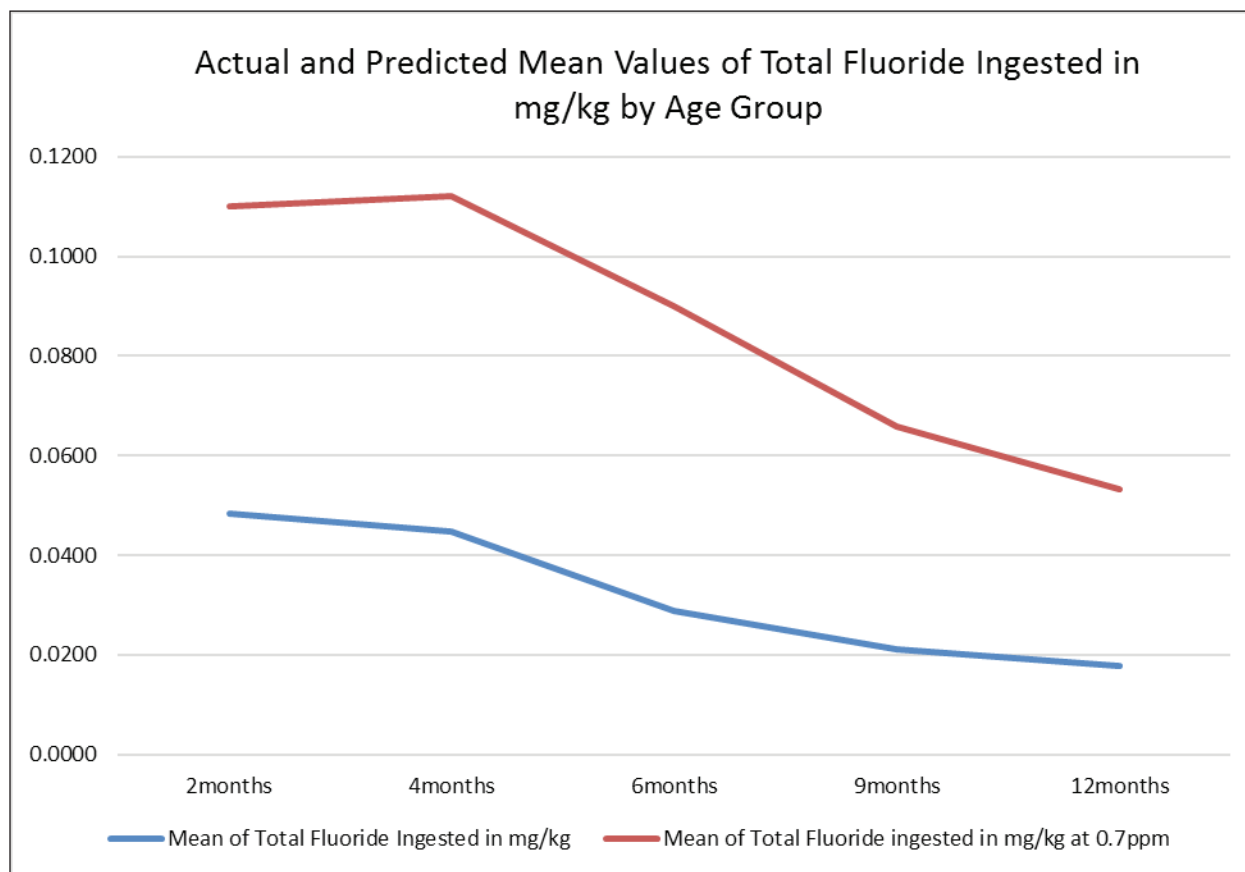
Solve for X (concentration of water source)
 $X = \frac{Y - 0.0776}{0.9973}$
 $X = \frac{\text{___ mg/L} - 0.0776}{0.9973}$
 $X = \text{___ mg/L (ppm)}$ Water source should not exceed this amount in order to reduce risk of fluorosis. Practitioners can assist parents in selecting the appropriate water source for formula reconstitution.

Table 3. ANOVA and Chi-Square Statistical Analysis

Category		2 months	4 months	6 months	9 months	12 months	p-value
Mean values of Total Fluoride Ingested (mg/kg/day)	Average	0.049	0.045	0.029	0.021	0.018	0.003
	Variance	0.0012	0.0011	0.0010	0.0006	0.0003	
Mean values of Total Predicted Fluoride ingested (mg/kg/day) at 0.7ppm	Average	0.110	0.112	0.090	0.066	0.053	<0.001
	Variance	0.0033	0.0016	0.0018	0.0012	0.0009	
Total Fluoride Ingested	>0.1mg/kg	3 (9.4%)	1 (4.3%)	1 (3.7%)	0 (0%)	0 (0%)	0.49
	<0.1mg/kg	29 (90.6%)	22 (95.7%)	26 (96.3%)	21 (100%)	11 (100%)	
Total Fluoride Ingested at 0.7ppm	>0.1mg/kg	19 (59.4%)	13 (56.5%)	9 (33.3%)	3 (14.3%)	1 (9.1%)	<0.001
	<0.1mg/kg	13 (40.6%)	10 (43.5%)	18 (66.7%)	18 (85.7%)	10 (90.9%)	

*Significant p-values (p<0.05) bol

Figure 4. Actual and predicted mean values of total fluoride ingested in mg/kg by age group.



DISCUSSION

In this study fluoride intake from infant formula in two-, four-, six-, nine-, and twelve-month old infants was estimated. All infants consumed infant formula with sub-optimally fluoridated water (0.0 – 0.3 ppm), resulting in an overall low risk for dental fluorosis. Mean daily fluoride intake was significantly different for each group ($p=0.003$); on the basis of this, the first hypothesis that daily fluoride intake would be equal among all groups is rejected. By contrast, the second hypothesis that the proportions of infants that exceed a daily fluoride intake of 0.1mg/kg/day would be equal among all the groups is accepted, because there were no significant differences found ($p=0.49$).

A small number of infants under the age of six months (4.4%) did exceed the UL (0.1mg/kg/day), indicating that some infants aged birth to six months may be exposed to a risk of dental fluorosis from infant formula even when it is reconstituted with low levels of fluoride in the water. At low fluoride levels, the greater concern was that while infants aged two-, four-, and six-months met the adequate intake for caries reduction, infants aged nine- and twelve-months did not meet the AI from formula alone, as previously reported.⁶ It is important to note that from birth to six months of age, the majority of nutrition is provided by formula for those subjects not being breastfed.

However, predicted fluoride ingestion of this patient population using optimally fluoridated water (0.7ppm) indicated that more infants are likely to exceed AI and UL (with infants aged two-, four-, and six-months being affected the most), thereby placing infants

at greater risk for dental fluorosis, which is consistent with other studies.^{4, 6, 17, 18}

Fluoride levels of bottled water, infant targeted bottled water with added fluoride (Nursery Water) or tap water in the Houston Metropolitan area were below the recommended levels of 0.7ppm. Previous studies reported the range of fluoride concentrations for Nursery Water with Added Fluoride to be 0.435 – 0.623 ppm.^{4, 7, 10} However, in this study the fluoride level was only 0.3 ppm. Other types of bottled waters do not show the fluoride content in their labels, and these types of bottled water contain minimal or insignificant amounts of fluoride;⁷ patients that consume primarily bottled water may not receive the full benefits of fluoridated water for caries prevention.

Only 3.5% of this patient population used tap water to reconstitute infant formula, contrary to the national Infant Feeding Practices Survey II (IFPS II) conducted from 2005-2007, which reported that 70-75% of mothers used tap water to reconstitute infant formula.¹³ The average concentration of Houston community water was 0.27 ppm fluoride.¹⁹ However, tap water fluoride concentrations in Houston can range from 0.18 to 1.02 ppm depending on zip code.²⁰ Therefore, one cannot assume that patients drinking tap water are consuming water with optimally fluoridated levels (0.7ppm), and fluoride exposure from formula reconstituted with tap water may be unpredictable.

The vast majority of this patient population used powdered infant formula (92.1%). These findings are consistent with the IFPS II findings, which reported about 90% of mothers used powdered

formula for the first year of life.¹³ In this study fluoride concentrations of powdered soy formula were higher compared to the other five powdered milk-based formulas, as previously reported.²¹⁻²⁴ Soy-based formulas have phytates and tricalcium phosphate, which both easily bind to fluoride, increasing the fluoride levels in these type of formulas.²¹

This study did not attempt to prove that fluoride exposure from formula is causative of fluorosis and simply looked at risk based on the IOM's recommended levels. Previous studies examining causation have varying results. A systematic review conducted in 2009 concluded that the evidence that fluoride in infant formula caused enamel fluorosis was negligible, noting that none of the individual reports included a statistical assessment of whether the fluoride in infant formula was responsible for fluorosis.²⁵ However, a longitudinal study in 2010 found that children (mean age nine) with mild dental fluorosis in the permanent dentition (97%) had significantly greater cumulative fluoride intake between the ages of three to nine months from reconstituted powdered infant formula and other beverages with added water than did those without fluorosis. These findings support the suggestion that fluorosis can be lessened by avoiding consumption of large amounts of fluoride from reconstituted powdered infant formula.⁹

The risk of developing dental fluorosis from consumption of formula alone is not known. During the first years of life, children are exposed to other sources of fluoride, such as toothpaste, beverages and solid food; therefore, it is difficult to isolate infant formula as the only causative agent for the development of dental fluorosis. The American Dental Association (ADA) recommends using optimally fluoridated water to reconstitute powdered and liquid concentrate formula, while being cognizant of the potential risk of developing mild dental fluorosis.²⁶

Theoretically, in order for dental fluorosis to manifest, sufficient levels of fluoride must be introduced over time to developing tooth buds during amelogenesis (enamel formation). Various dietary sources, such as infant formula, can contribute to fluoride distribution during development. Generally speaking, it is thought that the development of all permanent teeth (except third molars) occurs before the age of nine years²⁶ – susceptibility for fluorosis practically ends after this time. Elevated fluoride intake in the first three years of life has been linked to the prevalence of fluorosis.²⁷ First molars, which are typically the first permanent teeth to erupt, experience crown completion (including enamel formation) during the first 26-27 months of life,²⁸ with initiation of calcification beginning at birth.²⁹ Given this understanding, it is reasonable that the patient population in this study, infants in the first twelve months of life, have some level of susceptibility for dental fluorosis.

In addition to factors such as dietary fluoride exposure and stage of tooth development, excretion of systemic fluoride likely also plays a role in fluoride availability to developing teeth. The majority of fluoride is excreted in urine; urinary pH has been linked to level of fluoride excreted (higher urinary pH favors excretion of fluoride, lower urinary pH favors crossover of fluoride back to plasma).³⁰ The combination of sufficient levels of both fluoride exposure and retention coinciding with amelogenesis can lead to disruption of that process. The resultant increased enamel porosity is evident clinically in the white opacities characteristic of fluorosis.³¹

When there is a concern of fluorosis, it may be prudent to use water with relatively low fluoride content for infants under the age of six months, as they are likely to meet AI without exceeding the UL. For infants aged seven to twelve months, we continue to endorse the ADA recommendations to use of optimally fluoridated water for reconstituting powdered or liquid concentrate infant formulas to maximize the effect of fluoride in decreasing the prevalence of caries, as consumption of formula reconstituted with low fluoride water does not meet AI in most cases. Ready-to-feed formula, or liquid or powdered concentrate formula reconstituted with water that is either fluoride-free or has low concentrations of fluoride are options when fluorosis is a concern.

The trendline equations and scatter plot graph (Table 2 and Figure 2) were designed to assist healthcare professionals predict the amount of fluoride that an infant can potentially ingest based on the type of powdered or liquid concentrate formula and the source of water use to reconstitute the infant formula. Practitioners will be able to utilize these equations with the patient's weight and volume of formula consumption to assist parents in deciding which type of water they should use when preparing infant formulas (Figure 3).

One limitation of this study is that there was one site of survey administration. Consequently, we were not able to compare the results with other patient populations. Community tap water and nursery water fluoride levels may also vary over time and therefore fluoride intake from formula reconstituted with water from these sources may not be predictable. Additionally, the cross-sectional design of the study does not allow inferences to be made regarding the causation of dental fluorosis. Lastly, the results were limited to the estimation of fluoride intake from infant formula, which, while it represents a significant proportion of infant nutrition, does not include fluoride exposure from other sources found in older children such as high fluoride foods and ingested toothpaste. Healthcare professionals should be aware that children are exposed to multiple sources of fluoride during the period of tooth development. Reducing fluoride intake from reconstituted formula alone will not completely eliminate the risk of fluorosis.

CONCLUSIONS

1. This study found that all infants consumed infant formula with low fluoridated levels in the water (0.0– 0.3 ppm), resulting in a low risk for dental fluorosis.
2. Most infants in this study aged nine and twelve months that reconstitute infant formula with water less than 0.7 ppm fluoride will not meet the recommended adequate intake from infant formula alone.
3. Significantly more infants, particularly those under six months old, will exceed the UL when consuming formula reconstituted with 0.7 ppm water, increasing their risk of developing dental fluorosis.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. Bhavini Acharya and Gisela Velasquez for their mentorship, and Mr. Stanley Cron for his assistance with the statistical analysis for this project.

REFERENCES

1. CDC: Centers for Disease Control. Recommendations for using fluoride to prevent and control dental caries in the United States, MMWR Recommendations and Reports 2001, 50:1-42.
2. Browne D, Whelton, H., O' Mullane, D. Fluoride metabolism and fluorosis. *J Dent*; 33(3):177-186. 2005.
3. Mascarenhas AK. Risk factors for dental fluorosis: a review of the recent literature. *Pediatr Dent*;22:269-277. 2000.
4. Buzalaf MA, Damante CA, Trevizani LM, Granjeiro JM. Risk of fluorosis associated with infant formulas prepared with bottled water. *J Dent Child (Chic)*;71:110-113. 2004.
5. Fomon SJ, Ekstrand J, Ziegler EE. Fluoride intake and prevalence of dental fluorosis: Trends in fluoride intake with special attention to infants. *J Public Health Dent*; 60:131-139. 2000.
6. Siew C, Strock S, Ristic H, et al. Assessing a potential risk factor for enamel fluorosis: A preliminary evaluation of fluoride content in infant formulas. *J Am Dent Assoc*; 140:1228-1236. 2009.
7. Quock RL, Chan JT. Fluoride content of bottled water and its implications for the general dentist. *Gen Dent*; 57:29-33. 2009.
8. Do LG, Levy SM, Spencer AJ. Association between infant formula feeding and dental fluorosis and caries in Australian children. *J Public Health Dent*; 72:112-121. 2012.
9. Levy SM, Broffitt B, Marshall TA, Eichenberger-Gilmore JM, Warren JJ. Associations between fluorosis of permanent incisors and fluoride intake from infant formula, other dietary sources and dentifrice during early childhood. *J Am Dent Assoc*; 141:1190-1201. 2010.
10. Steinmetz JE, Martinez-Mier EA, Jones JE, et al. Fluoride content of water used to reconstitute infant formula. *Clin Pediatr (Phila)*; 50:100-105. 2011.
11. Zohoori FV, Moynihan PJ, Omid N, Abuhaloob L, Maguire A. Impact of water fluoride concentration on the fluoride content of infant foods and drinks requiring preparation with liquids before feeding. *Community Dent Oral Epidemiol*; 40:432-440. 2012.
12. IOM: Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride., Washington: National Academic Press, 288-313. 1997.
13. CDC: Centers for Disease Control and Prevention. Infant Feeding Practices Study II. At www.cdc.gov/ifps/results/index.htm#ch3 Accessed on October 21, 2016.
14. Martinez-Mier EA, Cury JA, Heilman JR, et al. Development of gold standard ion-selective electrode-based methods for fluoride analysis. *Caries Res*; 45:3-12. 2011.
15. U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation. U.S. Public Health Service recommendation for fluoride concentration in drinking water for the prevention of dental caries. *Public Health Reports* 2015, 130:1-14.
16. Cohen J. Statistical power analysis for the behavioral sciences. Edited by Hillsdale, N.J., L. Erlbaum Associates, 1988, p.pp. xxi, p 567.
17. Cressey P Dietary fluoride intake for fully formula-fed infants in New Zealand: Impact of formula and water fluoride. *J Public Health Dent*; 70:285-291. 2010.
18. Opydo-Szymaczek J, Opydo J. Dietary fluoride intake from infant and toddler formulas in Poland. *Food Chem Toxicol*; 49:1759-1763. 2011.
19. Department of Public Works and Engineering. 2014 Drinking Water Quality Report. City of Houston. Edited by Houston, Texas.
20. Jadav UG, Acharya BS, Velasquez GM, Vance BJ, Tate RH, Quock,RL. Survey of fluoride levels in vended water stations. *Gen Dent*;62(5):47-50. 2014.
21. Adair SM, Wei SH. Supplemental fluoride recommendations for infant based on dietary fluoride intake. *Caries Res*; 12:76-82. 1978.
22. Johnson J, Jr., Bawden JW: The fluoride content of infant formulas available in 1985. *Pediatr Dent*; 9:33-37. 1987.
23. McKnight-Hanes MC, Leverett DH, Adair SM, Shields CP: Fluoride content of infant formulas: soy-based formulas as a potential factor in dental fluorosis. *Pediatr Dent*; 10:189-194. 1988.
24. Nohno K, Zohoori FV, Maguire A. Fluoride intake of Japanese infants from infant milk formula. *Caries Res*; 45:486-493. 2011.
25. Hujoel PP, Zina LG, Moimaz SA, Cunha-Cruz J. Infant formula and enamel fluorosis: a systematic review. *J Am Dent Assoc*; 140:841-854. 2009.
26. Berg J, Gerweck C, Hujoel PP, et al. American Dental Association Council on Scientific Affairs Expert Panel on Fluoride Intake From Infant F, Fluorosis. Evidence-based clinical recommendations regarding fluoride intake from reconstituted infant formula and enamel fluorosis: a report of the American Dental Association Council on Scientific Affairs. *J Am Dent Assoc*; 142:79-87. 2011.
27. Hong L, Levy SM, Warren JJ, Broffitt B, Cavanaugh J. Fluoride intake levels in relation to fluorosis development in permanent maxillary central incisors and first molars. *Caries Res*;40(6):494-500. 2006.
28. Moorrees CF, Fanning EA, Hung EE JR. Age variation of formation stages for ten permanent teeth. *J Dent Res*;42:1490-1502. 1963.
29. Ash MM, Nelson SJ. Wheeler's dental anatomy, physiology, and occlusion. W.B. Saunders, Philadelphia; 32, 45, 53.
30. Angmar-Mansson B, Whitford GM. Environmental and physiological factors affecting dental fluorosis. *J Dent Res*;69:706-713. 1990.
31. Fejerskov O, Manji F, Baelum V. The nature and mechanisms of dental fluorosis in man. *J Dent Res*;69:692-700. 1990.