

Primary tooth fluorosis and fluoride intake during the first year of life

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Abstract – Objectives: Few studies in North America have assessed dental fluorosis of the primary dentition and few, if any, anywhere have assessed the relative importance in fluorosis etiology of fluoride intake during different time periods or from multiple sources. The purpose of this paper is to report on analyses relating estimated prenatal fluoride intake and fluoride intake during different parts of the first year of life to primary tooth fluorosis. **Methods:** As part of The Iowa Fluoride Study, subjects were recruited at birth and studied longitudinally. Trained examiners assessed dental fluorosis for children aged 4–7 years using the Tooth Surface Index of Fluorosis (TSIF) adapted for the primary dentition. Detailed parent questionnaires at childbirth were used to estimate prenatal fluoride intake and questionnaires sent at 6 weeks and 3, 6, 9, and 12 months were used to estimate fluoride intake during the first year of life (combined fluoride intake from water, food and beverage, supplements, and dentifrice). There were 504 children with prenatal and at least four of the five postnatal responses with complete data. **Results:** Fluorosis prevalence was 12.1%, occurring primarily on the second primary molars. Receiver operating characteristic (ROC) curves and logistic regression were used to assess the importance of different time periods' fluoride intake. In bivariate analyses, fluoride intake during each time interval was individually significantly related to fluorosis occurrence. For multivariate analyses, the period from 6 to 9 months was most important individually ($P=0.0001$), and no other period was jointly statistically significant. **Conclusions:** Results suggest that the middle of the first year of life is most important in fluorosis etiology for the primary dentition in this setting.

Key words: dental fluorosis; first year of life; fluoride intake; primary dentition

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A few studies of dental fluorosis of the primary dentition have been conducted in countries with predominantly low-to-moderate fluoride levels, like the US and most of Western Europe (1–6), and in settings with higher fluoride levels (7–11). Most studies have emphasized prevalence of fluorosis overall and by tooth type. The studies that have related primary tooth fluorosis results to fluoride intake generally have looked at various sources of fluoride intake in categories. For example, they have reported results in areas of high water fluoride levels versus optimal or low water fluoride levels (1–4, 10, 11). As expected, these studies have consistently shown more primary tooth dental fluorosis to be associated with higher water fluoride levels. Few

studies have assessed primary fluorosis in the absence of high-fluoridated water, yet primary fluorosis does occur in low fluoride or optimally fluoridated areas, although prevalence is low. For example, Leverett et al. (6) reported the prevalence of primary tooth fluorosis in a nonfluoridated area of the US to be between 2.3 and 4.3%, depending on dietary fluoride supplement use. However, no studies have related primary tooth dental fluorosis in these areas to estimated total fluoride intake prenatally and during early infancy when dental fluorosis could occur. Thus, little is known about risk factors for primary fluorosis in industrialized areas with approximately optimal water fluoride. Moreover, given multiple sources of fluoride in these areas,

it is important to assess fluoride intake from many sources in assessing primary tooth fluorosis etiology.

While fluorosis in the primary dentition has generally been reported to be less prevalent and less severe than fluorosis in the permanent dentition, there nonetheless appears to be a relationship between primary tooth and permanent tooth fluorosis in the same individuals. Milsom et al. (12) in a longitudinal study of 211 children found fluorosis of the primary second molars to be significantly predictive of fluorosis in the permanent incisors (relative risk = 1.86, 95% CI = 1.36, 2.54). The authors suggested that early identification of primary fluorosis could lead to interventions to reduce fluoride exposure and reduce the risk of fluorosis in the permanent teeth. Such interventions may not be of much practical value given tooth development and eruption patterns (13), and potential differences in fluoride exposures affecting the primary and permanent dentitions (13, 14). However, as has been discussed previously (10, 13), identification of consistent sources of fluoride early in life may be of value in reducing the risk of fluorosis in the permanent teeth.

The underlying cause of fluorosis – excessive fluoride ingestion during tooth development – is common to both primary and permanent tooth fluorosis. It is believed that the early maturation stage of enamel formation is the most critical stage for both primary and permanent tooth fluorosis (15–17), but that the highest risk for fluorosis occurs when there is fluoride exposure during both the secretory and maturation stages (18). Further, based on animal studies, somewhat elevated fluoride exposure over time appears to be associated with fluorosis more than isolated, acute high fluoride exposures (16). It has also been shown that the higher the chronic fluoride dose, the greater the risk for developing fluorosis, and the greater risk for severe fluorosis (1–4, 10, 11). Thus, relatively long term, slightly elevated fluoride exposures place individuals at higher risk for fluorosis.

Moreover, as recently reported, even small, short-term changes that reduce fluoride exposure can dramatically reduce the prevalence of fluorosis (19). The precise level or duration of fluoride ingestion that constitutes elevated risk is largely unknown for humans, and the timing of most critical period for development of permanent tooth fluorosis also has not been resolved (20–22). However, regardless of these issues, it must be remembered that development of dental fluorosis is a process that

mirrors tooth development, so that given a relatively consistent fluoride source and the sequence of tooth development, the development of fluorosis on the primary second molars may represent the beginning of a process that subsequently leads to fluorosis of the permanent first molars and permanent incisors (10, 12). Thus, identifying when this process begins may represent the earliest opportunity to appropriately address fluoride ingestion to prevent fluorosis in the permanent dentition. Therefore, the most critical period whereby fluorosis ingestion results in fluorosis of the primary teeth, at the beginning of the fluorosis development sequence, may also be the most appropriate time to target interventions to modify fluoride intake to reduce the risk of fluorosis in the permanent incisors.

The purpose of this paper is to report on analyses of the relationships between dental fluorosis occurrence and estimated prenatal (mother's) combined fluoride intake from water, dietary fluoride supplements, and fluoride dentifrice and infant's combined fluoride intake from water, foods/beverages, dietary fluoride supplements, and fluoride dentifrice among a birth cohort studied longitudinally.

Materials and methods

Subjects for this report have been participants in the Iowa Fluoride Study (13, 23–34) since birth. Briefly, using Institutional Review Board-approved procedures for informed consent, mothers were recruited from eight Iowa hospitals after childbirth and they provided information about their water sources (including beverages such as coffee made with water) and water intake, use of dietary fluoride supplements, and use and ingestion of fluoride dentifrices during pregnancy. From this information, prenatal daily combined fluoride intake was estimated. Demographic factors also were assessed at this time.

As described previously (32), dental fluorosis examinations were conducted using a portable chair and exam light by one of two trained and calibrated examiners. Teeth were examined using a mouth mirror and exam light, but the teeth were not dried. The Tooth Surface Index of Fluorosis (TSIF) was adapted for use with the primary dentition (35). Fluorosis was distinguished from other lesions, such as isolated nonfluoride opacities, through adaptation of Russell's criteria for differential diagnosis of fluorosis (36). Inter-examiner reliability was assessed by examinations of approximately 10% of

subjects by both examiners periodically throughout data collection, which took place from August 1997 through August 2000. Percent agreement and kappa statistics were computed at the subject, tooth, and surface levels. At the person level, percent agreement was 86.2% and kappa was 0.49; at the tooth level, percent agreement was 97.5% and kappa was 0.57; and at the surface level, percent agreement was 98.3% and kappa was 0.46.

Relevant to this report, structured questionnaires also were sent to mothers when their children were 6 weeks, and 3, 6, 9, 12, and 16 months old concerning the child's ingestion of water, beverages and foods made with water, other foods and beverages, dietary fluoride supplements, and fluoride dentifrice during the preceding time period. Questionnaires generally were received back several weeks thereafter. From these responses, estimates of fluoride intake from water (37), other foods/beverages, supplements (25), and dentifrice (24) were made separately and then combined. Individual water fluoride levels were used for fluoride intake estimates from water, while average product category fluoride levels from our own analyses and the literature were used for infant formulas, baby foods and cereals, soft drinks, ready-to-feed juices, and concentrates used for reconstitution (e.g. formula, juice, lemonade). Parent reports of the child's body weight (bw) at each questionnaire time point were used to calculate estimated fluoride intake in mg F/kg bw. Due to variations in response times, the fluoride intake estimates from the mailings were obtained at somewhat different time points (ages). In order to standardize these ages, linear interpolation was used to estimate the fluoride intake combined from the four sources for each day during the first year of life, and then results were summed over each time period and then averaged for the period, resulting in estimated mg F/kg bw per day. (The 16-month questionnaire was only used when needed to interpolate the 12-month estimate.) Analyses were conducted for those 504 children with complete fluoride intake data at baseline (prenatal) and all five questionnaires for the first year of life ($n = 469$) or with incomplete fluoride intake data for only one of the first five postnatal time periods ($n = 35$). Analyses were conducted both with fluoride variables only ($n = 504$) and adjusted for demographic covariates ($n = 465$). The covariates were gender, mother's age, father's age, mother's education, father's education, and family income. Education was coded as: 1, high school or less; 2, some college; 3, 4 years college graduate, and income (in US\$ 1000) was

coded as: 1, <10; 2, 10–19.999; 3, 20–29.999; 4, 30–39.999; 5, 40–49.999; 6, 50–59.999; 7, ≥ 60 .

Logistic regression and receiver operating characteristic (ROC) analyses were used to descriptively and inferentially assess the relationship between fluorosis (yes/no) and estimated combined fluoride intake during the different time periods. Using forward stepwise logistic regression, we tested whether the estimated fluoride intake rates for the 4-time periods defined by the five postnatal questionnaires (6 weeks to 3 months, 3–6 months, 6–9 months, and 9–12 months), and the prenatal intake were significant individual predictors of fluorosis (yes/no). We also tested, for each variable, whether the other variables contributed significantly useful information beyond that provided by the individual variable. These same tests were also performed while controlling for covariates.

An ROC curve is a plot of sensitivity versus (1-specificity) for each possible classification threshold for the predictive variable, and hence is a graphical way of displaying the complete performance of the predictor variable. For example, the 6–9-month fluoride intake for 25 of 61 fluorosis subjects and for 70 of 443 fluorosis-free subjects exceeds 0.10 mg F/kg bw. Hence if we use 0.10 as a threshold such that a subject with an intake value above 0.10 is classified as having fluorosis, then the sensitivity and (1-specificity) values corresponding to this threshold are $25/61 = 0.41$ and $70/443 = 0.16$, respectively. Thus, (0.16, 0.41) is one point on the ROC curve for the 6–9-month fluoride intake. The ROC curve for the 6–9-month fluoride intake is a plot of the (1-specificity, sensitivity) points corresponding to all possible threshold values, and is displayed in Fig. 1.

The ROC curve for a more accurate predictor variable will be closer to the top left corner (high sensitivity and high specificity) than a less accurate one, and hence will have a larger area under the curve (AUC) value. For example, the ROC curve and the AUC for the 6–9-month fluoride intake as a predictor of fluorosis (yes/no) are displayed in Fig. 1. The AUC for an ROC curve is always between 0 and 1. A non-informative predictor will have an ROC curve that lies along the diagonal and hence has an AUC of approximately 0.5, while a very informative predictor will have an AUC close to 1.0. Furthermore, for a randomly selected pair of subjects, one having fluorosis and one not having fluorosis, the AUC is an estimate of the probability that the predictive intake value for the fluorosis subject exceeds that of the fluorosis-free subject.

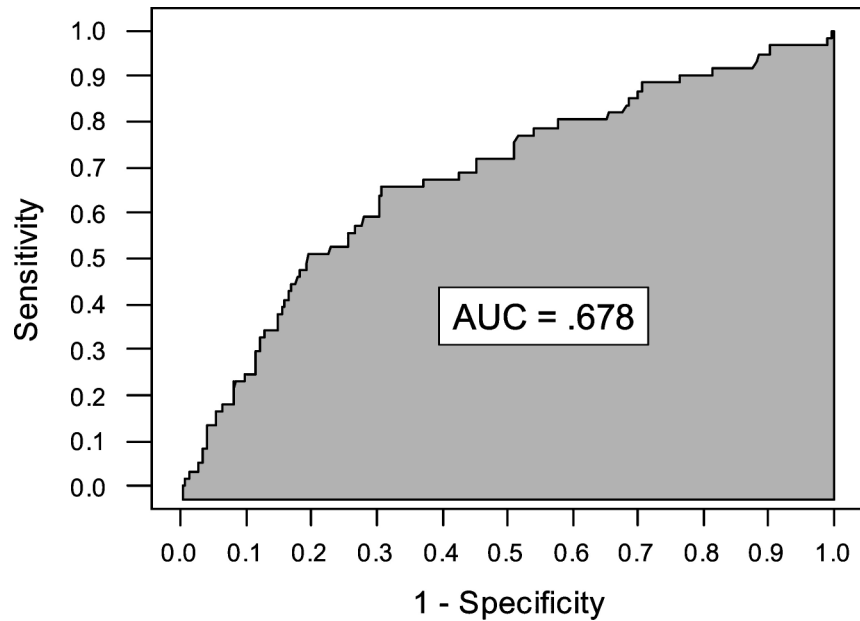


Fig. 1. ROC curve for 6–9-month total fluoride intake as a predictor of fluorosis with area under the curve shown by the shaded region.

Hence, the AUC of the ROC curve provides an intuitive summary measure of the predictor variable. This measure conveys the practical usefulness of the predictor variable, which complements the *P*-value provided by the logistic regression analysis. Hanley and McNeil (38) provide an excellent discussion of the interpretation of the ROC AUC.

The ranking of the corresponding AUCs indicates the relative importance of estimated prenatal fluoride intake and fluoride intake during different time periods. We also computed the AUC for the *composite intake*, defined as, $b_1x_1 + b_2x_2 + \dots + b_5x_5$, where x_1 – x_5 are the four time-period intakes and the estimated prenatal intake and b_1 – b_5 are the regression coefficient estimates resulting from including x_1 – x_5 as independent variables in a logistic regression analysis.

To help convey the practical significance of the intake variables, specificity is tabulated for sensitivity values of approximately 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 for 6–9-month fluoride intake.

Results

Table 1 summarizes the baseline demographic characteristics of the 504 subjects. More than half of the mothers and nearly two-thirds of the fathers were aged 30 years or more. Almost half of mothers and fathers were college graduates, while 87% had family incomes at baseline of US\$ 20,000 or more.

Fluorosis prevalence results for the 504 subjects in this report were very similar to those for the full sample examined ($n = 698$) (32). Among the 504, 61

(12.1%) had fluorosis present on one or more of their primary teeth. Nearly all fluorosis was very mild (TSIF score = 1), with only one individual having higher TSIF scores. The mean number of teeth affected for the entire sample was 0.45 and among those with any fluorosis it was 3.70. The mean

Table 1. Characteristics of the sample (at baseline)

| Variable | Category | Percentage |
|----------------------|--------------------------|------------|
| Child's sex | Male | 47.4 |
| | Female | 52.6 |
| Mother's age (years) | 17–24 | 14.9 |
| | 25–29 | 34.7 |
| | 30–34 | 30.6 |
| | 35–45 | 19.8 |
| Father's age (years) | 20–24 | 6.7 |
| | 25–29 | 27.6 |
| | 30–34 | 34.3 |
| | 35–60 | 31.4 |
| Mother's education | Up to high school | 17.9 |
| | Some college | 35.7 |
| | College graduate or more | 46.4 |
| Father's education | Up to high school | 27.4 |
| | Some college | 28.1 |
| | College graduate or more | 44.5 |
| Family income (US\$) | <20,000 | 12.9 |
| | 20,000–39,999 | 36.2 |
| | >40,000 | 50.9 |
| Mother's race | White | 98.4 |
| | Other | 1.6 |
| First child | Yes | 42.1 |
| | No | 57.9 |

numbers of surfaces affected were 0.63 and 5.21, respectively. Fluorosis occurred bilaterally in one or both arches most of the time, so that usually (74%) an even number of teeth were affected per person (30% had two, 28% had four, 11% had six, and 5% had eight affected).

The primary second molars were most commonly affected, with each tooth's fluorosis prevalence about 9%, compared with about 1–2% for each first molar and less than 1% for each canine and incisor. Among the second molar teeth with fluorosis, the buccal surface was affected in nearly 99% of cases, with the occlusal (27%) and lingual (23%) surfaces affected to lesser degrees. On the buccal surface of the second molars, the most common location was near the gingival margin, with 71–87% of affected teeth exhibiting fluorosis on the gingival third of the buccal surface. The middle and occlusal thirds of the buccal surface were each involved about 26–44% of the time in affected teeth (depending on the specific tooth).

Figure 2 summarizes the estimated mean daily fluoride intake in mg F/kg bw for water (both alone and when used in preparation of other beverages and foods), other foods and beverages, supplements, and dentifrice (adapted from Levy et al. (30)) for the different time periods. Mean estimated fluoride intake from water was the largest component for the different time periods. Total estimated daily fluoride intake from water/kg bw was fairly steady from 6 weeks to 9 months (0.042–0.045 mg F/kg bw), and substantially lower thereafter (0.034 mg F/kg bw). As a proportion of estimated combined intake, there was a decline in the contribution from water with increasing age (from about 80% down to 65%).

Estimated mean fluoride intake from other dietary sources (ready-to-feed formulas, infant foods, juices, etc.) was the next largest component at all ages, and increased substantially from about 0.007 mg F/kg bw for 6 weeks to 3 months to 0.014 mg F/kg bw later. Proportional contribution was greatest at 9–12 months at about 27%. Mean fluoride intake from supplements declined steadily from about 0.004 mg F/kg bw at 6 weeks to 3 months down to 0.001 mg F/kg bw at 9–12 months, and proportional contribution declined from about 7–2%. Fluoride dentifrice use began at 3 months by a few children and estimated mean intake increased substantially at 9–12 months, to 0.003 mg F/kg bw, constituting about 6% of combined fluoride intake. Estimated combined daily prenatal fluoride intake from water, foods and beverages, dietary fluoride supplements, and fluoride dentifrice averaged 1.47 mg (SD = 1.35), with a median of 1.23 mg. The range was from 0 to 13.95 mg, with 10th percentile of 0.18 mg, 25th of 0.46 mg, 75th of 2.10 mg, 90th of 2.87 mg, and 95th of 3.81 mg.

Figure 3 summarizes the distribution for estimated combined daily fluoride intake from the four sources. The 10th, 25th, and 50th (median) percentiles were low for the first period, then increased substantially, and declined or leveled off at 9–12 months at about 0.02 mg F/kg bw (10th), 0.03 mg F/kg bw (25th) and 0.05 mg F/kg bw (50th), respectively. In contrast, the 75th percentile stayed fairly level at 0.083–0.091 mg F/kg bw through 6–9 months and then declined to 0.07 mg F/kg bw, while the 90th percentile was at about 0.12–0.14 mg F/kg bw and declined to 0.10 mg F/kg bw at 9–12 months. The means increased slightly to about 0.06 mg F/kg bw

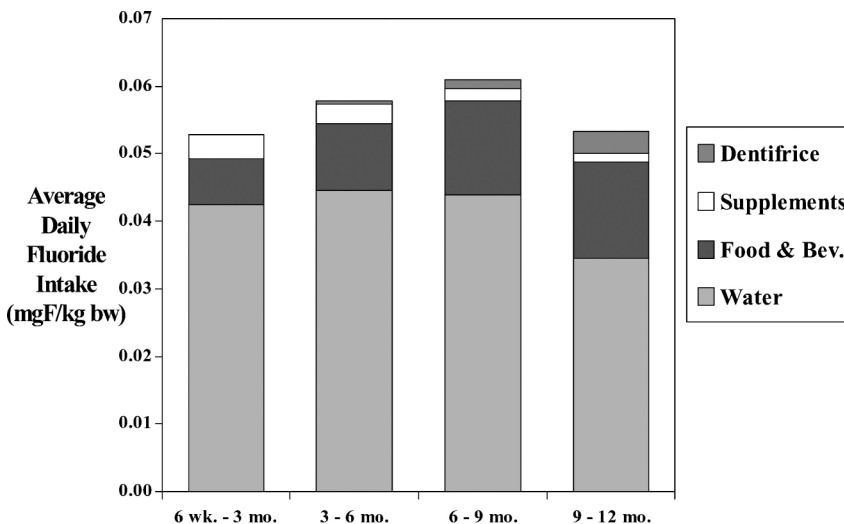


Fig. 2. Components of average daily fluoride intake (n = 504).

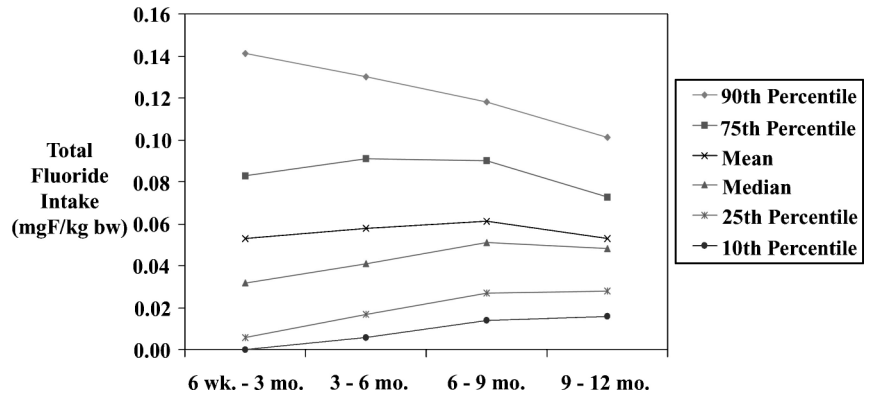


Fig.3. Percentile for average daily fluoride intake (mg F/kg bw) (n = 504).

at 3–6 and 6–9 months, and then declined thereafter. The distribution of fluoride intake narrowed with increasing age.

Logistic regression results are given in Table 2. In terms of individual importance based upon the logistic regressions, the prenatal, 6–9-month and 9–12-month intake were most important, with *P*-values of 0.009, 0.0001 and 0.0001, respectively. In terms of the AUCs, the 6–9-month intake was the most important (AUC = 0.678), followed by the 9–12-month intake (AUC = 0.639). Note that the AUC for the composite intake (AUC = 0.690) is not much more than for the 6–9-month intake, showing that the model with the 6–9-month intake as the only independent variable is improved very little by including the other variables in the model. Although

Table 2. Logistic regression results

| | | <i>n</i> = 504 | | |
|------------------------|--|-----------------|-------|------------|
| Predictor | | <i>P</i> -value | AUC | Odds ratio |
| A | Prenatal intake (mg F/day) | 0.0088* | 0.591 | 1.26 |
| B | 6-Week to 3-month combined intake (units are tenths of mg F/kg bw/day) | 0.0448* | 0.590 | 1.54 |
| C | 3–6-Month combined intake (units are tenths of mg F/kg bw/day) | 0.0015* | 0.623 | 2.16 |
| D | 6–9-Month combined intake (units are tenths of mg F/kg bw/day) | 0.0001* | 0.678 | 3.90 |
| E | 9–12-Month combined intake (units are tenths of mg F/kg bw/day) | 0.0001* | 0.639 | 4.24 |
| Composite intake (A–E) | | 0.0002* | 0.690 | |

The response variable is fluorosis (yes/no). The predictor is the only independent variable in each model, except for the composite intake model which includes all five of the predictors as independent variables.

Table 3. Logistic regression tests for significance of the other four predictors

| | Reduced model predictor | <i>P</i> -value (<i>n</i> = 504) |
|---|-------------------------|-----------------------------------|
| A | Prenatal intake | 0.0015* |
| B | 6–3-Month total intake | 0.0004* |
| C | 3–6-Month total intake | 0.0059* |
| D | 6–9-Month total intake | 0.3369 |
| E | 9–12-Month total intake | 0.0511 |

A significant *P*-value indicates that the full model containing all five predictors is more predictive than the reduced model containing only a single predictor. This is a *df*₄ test.

intakes for the different time periods were moderately correlated, there was much variation in individual intake amounts and changes in water sources, so that multicollinearity was not a problem. The correlations of fluoride intake between time points varied from 0.16 to 0.81.

Table 3 shows the logistic regression results testing, for each variable, whether the other variables contribute significantly useful information beyond that provided by the individual variable. For example, we test for the importance of the 6–9-month intake by testing the null hypothesis that the model that contains all five variables is not more useful than the model containing only the 6–9-month intake, versus the alternative hypothesis that the larger model is more useful. The *P*-value of 0.3369 shows that the model that contains only the 6–9-month intake is not significantly improved by including the prenatal, 6-week to 3-month, 3–6-month, and 9–12-month intakes. In contrast, the prenatal, 6-week to 3-month, and 3–6-month intake models are significantly improved by the addition of the other predictors, while the 9–12 month intake (*P* = 0.0511) tends toward significant improvement with the addition of the other predictors.

Furthermore, when we do a forward stepwise logistic regression using a 0.05 significance entry level with the five fluoride intake variables, the final

model contains only the 6–9-month intake. Tests for two-way interactions between the 6–9-month intake and each of the other fluoride intake variables, one at a time with both main effects and interaction included, showed no significant interactions.

Results were very similar for the covariate adjusted model, with four of the five fluoride intake points individually significantly associated with fluorosis and the 6-week to 3-month intake approaching significance ($P=0.094$). Again the 6–9-month intake was the best predictor of fluorosis ($P=0.0001$). Logistic regression tests for the significance of the other four variables showed no significant improvement in prediction beyond that provided by the 6–9-month intake ($P=0.35$), almost significant improvement in prediction beyond that provided by the 9–12-month intake ($P=0.06$), and significant improvement in prediction beyond that provided by either the prenatal ($P=0.005$), 6-week to 3-month ($P=0.002$), or 3–6-month ($P=0.007$) intakes. Stepwise logistic regression again found the 6–9-month intake to be the only significant main effect, with no significant two-way interactions with any of the other intake variables.

Table 4 displays sensitivity, specificity, and 1-specificity values for various levels of the 6–9-month intake for the complete data. For example, choosing to classify a subject as having fluorosis if the subject's 6–9-month intake exceeds the threshold of 0.09 mg F/kg bw results in a sensitivity of 51% (i.e. 51% of the true fluorosis subjects are correctly classified as having fluorosis) and a specificity of 81% (i.e. 81% of the fluorosis-free subjects are correctly classified as not having fluorosis.) We note that (sensitivity, 1-specificity) points corresponding to all possible thresholds are plotted in Fig. 1, thus, the 10 pairs of (sensitivity, 1-specificity) values in

Table 4. Sensitivity, specificity, and 1-specificity for various levels of 6–9-month total fluoride intake ($n=504$)

| Average fluoride intake (6–9-month) (mg F/kg bw) | Sensitivity | Specificity | 1-specificity |
|--|-------------|-------------|---------------|
| 0.141 | 0.11 | 0.96 | 0.04 |
| 0.119 | 0.21 | 0.92 | 0.08 |
| 0.108 | 0.31 | 0.88 | 0.12 |
| 0.100 | 0.41 | 0.84 | 0.16 |
| 0.094 | 0.51 | 0.81 | 0.19 |
| 0.075 | 0.61 | 0.70 | 0.30 |
| 0.054 | 0.70 | 0.55 | 0.45 |
| 0.039 | 0.80 | 0.42 | 0.58 |
| 0.025 | 0.90 | 0.24 | 0.76 |
| 0.002 | 1.00 | 0.00 | 1.00 |

Table 4 can be found in the plot in Fig. 1. However, Fig. 1 does not provide the corresponding threshold values.

Discussion

This study assessed longitudinal fluoride ingestion and its relationship to dental fluorosis in the primary dentition. Previous studies of fluorosis risk factors have generally only assessed fluoride ingestion retrospectively and often included only a limited number of fluoride sources. In addition, many of these studies assessed fluoride ingestion only at a single time point, or assessed past fluoride ingestion in general, without reference to specific ages or time periods. In some studies, particularly in others limited to the primary dentition, only water fluoride concentrations were assessed at the community level with no individual ingestion data collected. Thus, the approach used in the present study offered many methodological advantages over previous studies of fluorosis risk factors in that fluoride ingestion was assessed at the individual level, included a number of fluoride sources, and was assessed longitudinally at multiple time points, near the time of the exposures, thus reducing recall bias.

The present study found that fluoride ingestion during each time period studied (prenatal, 6-week to 3-month, 3–6-month, 6–9-month, and 9–12-month) was individually significantly associated with dental fluorosis in the primary dentition, with the 6–9-month period of fluoride ingestion most strongly associated with fluorosis prevalence. Main effects and interaction effects for the other time periods were not jointly significant.

While some studies (39) have implied that fluorosis occurs only when a threshold level of fluoride ingestion is exceeded during an age-related 'window' of risk, others (22) suggest that there is essentially no defined 'window' because fluorosis is really the result of long-term fluoride ingestion. Cumulative ingestion of fluoride may begin in the prenatal period, and fluorosis occurs for a given tooth not only due to ingestion during the 'most critical' early maturation stage of enamel development, but is also dependent on earlier fluoride ingestion during the secretory stage (18). According to this view, many time periods may be critical in fluorosis development, rather than ingestion only during a specific time period.

The results of this study point strongly to the 6–9-month period being most important, which

generally corresponds to the early maturation stage of the primary second molars (40). The results do not seem to support the long-term, cumulative ingestion concept. If the cumulative ingestion concept were supported, we would have expected that the 'best' regression model would have included at least two time periods and that additional time periods would have improved the predictive ability of the 'most important' period. However, we believe that it is premature to dismiss the importance of the other time periods (birth to 6 and 9 months and later) based on our results. First, the substantial, individual biologic variation in tooth development and eruption time suggests that some children would have their 'most critical' periods earlier or later. Secondly, the study cohort as a whole had quite low levels of breast-feeding after 6 weeks of age, probably contributing to the lower level of across-subject variation in estimated fluoride intake from 6 months onwards (Fig. 3). Most importantly, the metabolism of fluoride in young children is known to be such that substantial proportions of ingested fluoride are retained in the developing bone, available for release into the bloodstream at times of lower fluoride intake in order to maintain the prevailing steady-state level (18). Thus, fluoride intake during different periods certainly has the potential to contribute to fluorosis etiology. For these reasons, we recommend additional study of the relative importance of different time periods of fluoride intake in the development of fluorosis. In particular, future studies should consider not only fluorosis etiology on the primary second molars, but also other primary teeth. In the present study, the overwhelming majority of teeth affected were primary second molars, with very few anterior teeth affected, so that the timing of fluoride exposures to fluorosis occurrence on other teeth could not be assessed. Given that in the present study estimated fluoride ingestion did not change dramatically, on average, during the first year of life (Figs. 2 and 3), it may be that fluorosis in primary anterior teeth is not largely dependent on postnatal exposures; however, further study is needed to support this hypothesis.

It is also important to acknowledge that conclusions drawn from these results are most pertinent to those situations where levels of fluoride intake are similar. Specifically, the majority of dental fluorosis in this study was found on second molars, while prenatal fluoride intake and intake during the first few months of life generally was relatively low. In some studies outside the US with higher ambient fluoride levels, dental fluorosis has been prevalent

on all primary teeth, not just second molars. Had the fluoride intake in our study been much greater prenatally and in the first 6 months of life when the other primary teeth do so much of the mineralization and are thus at risk for dental fluorosis, another pattern of fluorosis and different relationships with periods of fluoride intake would be expected.

It is important to note that fluoride ingestion very early in life may be important not only for primary tooth fluorosis, but also for fluorosis development in early erupting permanent teeth. Thus, in the context of preventing permanent tooth dental fluorosis, interventions to reduce fluoride ingestion may be especially prudent before the end of the first year of life and prior to the early maturation stage of the permanent incisors.

Perhaps more important from the perspective of preventing dental fluorosis on the esthetically important permanent maxillary incisors is that the 6–9-month age range may be a critical time to monitor fluoride intakes since dietary habits and dentifrice use patterns may become established at about this age. For example, during this age period the typical infant diet moves away from primarily breast milk or formula, and items such as baby foods and fruit juices, both often significant sources of fluoride (28, 29, 41), become major components of the diet. Moreover, as the primary teeth begin to erupt at this age, it is also advantageous to counsel parents about the appropriate use of fluorides for caries prevention. Thus, assessing risk for both caries and fluorosis at this early age (6–9 months) may be critical to achieve the optimal balance of fluoride exposures for each individual child. Unfortunately, assessment of individual risk is, at best, difficult for clinicians or researchers to do validly and reliably.

Despite the methodological advantages over many previous studies, the present study did have several limitations. The study sample was not fully representative of any defined population group, and was disproportionately white and of higher socioeconomic status (SES). Those remaining in the study were of higher SES than those declining to join or leaving earlier. The data on fluoride intake were obtained from parents' responses to the periodic questionnaires and were not directly validated. Estimates of fluoride intake from infant formulas, baby foods and cereals, soft drinks, ready-to-feed juices, and concentrates used for reconstitution used average values for fluoride content based on the literature, our analyses, and our prevalence of use data. Prenatal estimates assessed combined fluoride intake from water itself, water added to beverages,

dentifrice, and dietary supplements only, without including other nonwater foods and beverages. Recall bias was possible over the 6-week to 3-month intervals of interest. Even though we think the fluoride intake estimates are good ones, they were based on assessment at only five points during the year and do not specifically allow study of different time periods. Also, they do not account fully for weekly or monthly variations in intake. In addition, it was not feasible in the longitudinal study to utilize duplicate diet plating for dietary fluoride estimates or other methods of direct observation of supplement, dentifrice, and dietary fluoride intake.

Although the Iowa Fluoride Study thus far has been limited to primary tooth fluorosis, additional investigation into the etiology of permanent tooth fluorosis, both as a continuation of the present study and in other studies, is needed. Given greater complexity of diets, varied use of therapeutic fluoride products, and the longer duration of fluoride ingestion affecting tooth development in the permanent dentition, such studies will require complex analytic strategies. The analytic approach used in the present study appears to be promising for use in future studies of fluorosis in the permanent dentition; however, other approaches also should be explored.

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