Risk Factors for Enamel Fluorosis in Optimally Fluoridated Children Born after the US Manufacturers' Decision to Reduce the Fluoride Concentration of Infant Formula

David G. Pendrys and Ralph V. Katz

This case-control study investigated risk factors for enamel fluorosis in optimally fluoridated children, born after the US infant formula industry voluntarily reduced the fluoride content of their products. Analysis was performed on 233 children, aged 10–14 years. Case-control status was determined using the Fluorosis Risk Index (FRI). Risk factor exposure was ascertained via a mailed questionnaire. Logistic regression analyses revealed a strong association between mild-to-moderate enamel fluorosis on early forming (FRI classification I) enamel surfaces and both fluoride supplement use (odds ratio (OR) = 5.95, 95% confidence interval (CI) 1.06–33.53), and early fluoride toothpaste use (OR = 6.35, 95% CI 1.21–33.40). The authors found a suggestive, but nonsignificant, association between fluorosis on these enamel surfaces and infant formula in the form of powdered concentrate (OR = 4.33, 95% CI 0.73–25.66). There was a strong association between mild-to-moderate fluorosis on later forming (FRI classification II) enamel surfaces and infant formula use in the form of powdered concentrate (OR = 10.77, 95% CI 1.89–61.25), fluoride supplement use (OR = 10.83, 95% CI 1.90–61.55), and early fluoride toothpaste use (OR = 8.37, 95% CI 1.68–41.72). No association was observed between the use of ready to feed infant formula and enamel fluorosis.

Prior to 1979, infant formula contained variable and often high concentrations of fluoride (24–26). Studies have suggested an association between the ingestion of formula manufactured before 1979 and enamel fluorosis (17, 27). Although US manufacturers of infant formula voluntarily agreed in 1979 to reduce and control the concentration of fluoride in their products (28), speculation has continued as to whether the concentration of fluoride remaining, when added to other ingested sources, may continue to represent an important source of total body intake (29). In particular, the concern has been raised that the dilution of concentrated formula with optimally fluoridated water, as directed for use, will result in a food source containing an above optimal concentration of fluoride (30, 31). Only recently have the children, who as infants would have ingested this reduced fluoride formula, reached an age where fluorosis can be properly assessed. Our recent report (21), of fluorosis risk factors in a population born after 1979 who had grown up in areas with low concentrations of fluoride in the water supply, found no association between infant formula use, in any form, and enamel fluorosis.

The dental profession has repeatedly warned against the use of fluoride supplements by children whose drinking water is optimally fluoridated, and no profes...
The purpose of this case-control study was to investigate risk factors for mild-to-moderate enamel fluorosis in an optimally fluoridated population of Connecticut children, born after US manufacturers of infant formula agreed to voluntarily reduce the fluoride concentration in their products. The primary goal of this investigation was to determine whether there was an association between enamel fluorosis and exposure during the first 8 years of life to three sources of non-waterborne ingested fluoride: infant formula, fluoride dentifrice, and fluoride supplementation.

MATERIALS AND METHODS

All sixth through eighth graders who were enrolled in participating school districts in five optimally fluoridated Connecticut communities at the time of the study (1994–1995) were invited to participate. All contacted school systems agreed to participate, and enrollment was with the enthusiastic cooperation of the participating school districts.

Fluorosis examinations were conducted in the subjects’ schools by two calibrated examiners who used portable dental chairs and headlights. Subjects’ teeth were dried with sterile cotton gauze for better visibility before the examination. Inter- and intra-examiner reliability examinations were randomly conducted daily throughout the data collection period.

Enamel fluorosis was measured using the fluorosis risk index (FRI) (46), which categorizes fluorosis cases and controls based on the presence of mild-to-moderate fluorosis on enamel surfaces that begin to form during defined developmental periods, either during the first year of life (FRI classification I enamel surface zones) or after the second year of life (FRI classification II enamel surface zones). Measuring fluorosis in this way is useful in risk factor investigations because enamel fluorosis observed at different enamel surface locations may be related to entirely different exposure profiles. A complete discussion of the FRI and its utility in analytical epidemiologic investigations has been presented elsewhere (46-49), as has its use in other investigations (12, 17, 21). Briefly, the questionnaire assessed the subject’s residency, frequency of toothbrushing, typical amount of toothpaste used during toothbrushing, and use of fluoride supplements during the first 8 years. Respondents also indicated whether they used either bottled water or a tap water filter for more than 2 of the first 8 years of the child’s life, at what age their child began to brush, and at what ages they helped their child brush.

Response to the exposure questionnaire was mailed to the parents of the identified case and control subjects. This questionnaire has been used and described in three previous investigations (12, 17, 21). Briefly, the questionnaire assessed the subject’s residency, frequency of toothbrushing, typical amount of toothpaste used during toothbrushing, and use of fluoride supplements during the first 8 years. Respondents also indicated whether they used either bottled water or a tap water filter for more than 2 of the first 8 years of the child’s life, at what age their child began to brush, and at what ages they helped their child brush. Parents were offered $20 for return of the completed questionnaire. Two mailings followed the initial mailing.

Incomplete questionnaires were returned to the parent with a letter identifying the specific areas requiring completion. Only responses from parents/guardians who had lived with their child during the first 8 years of the subject’s life were accepted for analysis.
A randomly drawn reliability sample, blocked on the mailing round on which the questionnaire was returned, was mailed a second questionnaire one month after completion of the third mailing round. None of the parents or subjects were informed of the subject’s case or control status until after the return of the reliability sample.

The fluoridation status of subjects who had lived any of their first 8 years in other than one of the study communities was determined by the 1992 Fluoridation Census (51). All analyses were limited to subjects who had lived only in optimally fluoridated communities throughout the first 8 years of their life.

All data were entered into an IBM-compatible computer and analyzed using SPSS for Windows (52) and Epidemiological Graphics, Estimate, and Testing (EGRET) (53) statistical packages. All descriptive and inferential analyses were conducted separately on the basis of FRI classification I or FRI classification II enamel surface zones, respectively. Basic descriptive and univariate statistics, as well as Mantel-Haenszel odds ratios (53, 54) to the extent allowed by cell size, were used in the construction of the multivariate analyses. Unconditional logistic regression analyses were used to develop a model of exposures associated with mild-to-moderate enamel fluorosis. The regression coefficient-generated odds ratio was used to estimate relative risk for each factor, adjusted for all other factors in the model (52, 53, 55, 56). Tests for trend and departure from linearity were performed where appropriate (53, 57). Infant formula use, use of fluoride toothpaste, and fluoride supplement use during the first 8 years were the independent variables of principal interest. Socioeconomic status was measured, as in three previous studies, by the median household income of subjects’ census tract data (58). Ninety-five percent confidence intervals were generated for all adjusted odds ratios.

RESULTS

Table 1 describes the process of fluorosis identification and fluoride history ascertainment. The table shows that 867 subjects (95 percent of those enrolled and 14 percent of those eligible by grade level) were examined for fluorosis. Intra- and inter-examiner agreement on case versus control status was 100 percent and 86 percent, respectively (Cohen’s kappa = 1.0 and 0.70, respectively (59)).

Based on these examinations, 360 cases and controls for mild-to-moderate fluorosis were identified, all of whose parents/guardians were sent fluoride history questionnaires. A 91 percent response rate yielded 326 completed questionnaires after three mailing rounds. Response rates were similar regardless of case/control status. A 16 percent reliability sample showed an average agreement between the first and repeat questionnaire responses of 87 percent, which was similar regardless of case/control subject status.

A total of 163 cases and controls using FRI classification I, and 188 cases and controls using FRI classification II were available for subsequent analyses after exclusions due to residence history, informant status, and year of birth. These subjects ranged in age from 10 years to 14 years (mean = 12.9 years), and 56 percent of the subjects were female. Ninety-one percent of the subjects were white, which was reflective of the town populations. Nonwhite subjects were primarily black and Hispanic (3 percent each, respectively). All of the children were born after 1979 (i.e., 1980–1983), and all lived in optimally fluoridated areas throughout the entire first 8 years of life. Eighty-two percent had lived in optimally fluoridated areas throughout the first 8 years of their life.

### Table 1. Ascertainment of cases and controls for mild-to-moderate enamel fluorosis and fluoride exposure questionnaire data among Connecticut children born between 1980 and 1983

<table>
<thead>
<tr>
<th>Subject category</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case/control ascertainment</td>
<td></td>
</tr>
<tr>
<td>Total no. of subjects examined for enamel fluorosis*</td>
<td>867</td>
</tr>
<tr>
<td>Diagnosed as other than a definite case or control†</td>
<td>507</td>
</tr>
<tr>
<td>Diagnosed as a fluorosis case or control‡</td>
<td>360</td>
</tr>
<tr>
<td>Fluoride exposure history ascertainment</td>
<td></td>
</tr>
<tr>
<td>Unable to contact with questionnaire</td>
<td>3</td>
</tr>
<tr>
<td>Parents of cases/controls sent questionnaires</td>
<td>357</td>
</tr>
<tr>
<td>Questionnaires returned</td>
<td>326</td>
</tr>
<tr>
<td>Excluded on the basis of mixed or nonfluoridated residence history, excluded informant, or born before 1980§</td>
<td>93</td>
</tr>
<tr>
<td>Total cases and controls in analysis</td>
<td>233</td>
</tr>
</tbody>
</table>

* Study was open to all middle-school-aged children enrolled in participating school districts.
† Includes subjects diagnosed as questionable for mild-to-moderate enamel fluorosis and subjects whose enamel surfaces were masked by the presence of orthodontic appliances or restorations.
‡ Subjects who were either a case or control for enamel fluorosis under at least one of the two Fluorosis Risk Index classifications.
§ Only parents/guardians who lived with their child during the first 8 years of the child’s life were considered acceptable informants.
least one surface zone more severely affected with enamel pitting or staining.

An analyses of crude odds ratios suggested a strong association between enamel fluorosis and a history of infant formula use in the form of powdered concentrate, as the main infant food source, especially during the last quarter of the first year of life. As the main source of food was highly correlated across the different quarters of the first year, only the last quarter was included in the regression models. However, initial analyses indicated that there was no difference in the risk of enamel fluorosis associated with whether the formula was milk-based or soy-based. Therefore, these types of formula were combined in the final multivariate analyses. All of the respondents indicated that they had helped their child brush during at least part of the first 8 years. Therefore, parental help with toothbrushing was not found to be useful as a covariate. However, this information was used in the determination of age at which toothbrushing began, which was defined as either the reported age when brushing began or the age when the parent first began to help the child brush, whichever came first. Preliminary analyses further suggested that the amount of toothpaste usually used and the daily frequency of toothbrushing were strongly associated with enamel fluorosis. A clear independent relation between the age at which brushing began and enamel fluorosis was not found. However, this variable was left in the model as a covariate for the other toothbrushing variables. Overall, 27 percent of the subjects were inappropriately supplemented sometime during the first 8 years. The analyses of crude odds ratios suggested that supplementation during the first 2 years was strongly associated with enamel fluorosis.

Table 3 presents crude and logistic regression-derived adjusted odds ratio estimates with 95 percent confidence intervals for mild-to-moderate enamel fluorosis for both FRI classifications. The logistic regression-derived odds ratio estimates are adjusted for the other variables in the table as well as for age, sex, median household income, examiner, and age at which toothbrushing began. The analyses revealed no significant interactions between any of the variables in the model.

Table 3 shows that a history of infant formula use in the form of powdered concentrate as the main source of food during the last quarter of the first year conveyed adjusted odds ratios of 4.33 and 10.77, for mild-to-moderate enamel fluorosis on FRI classification I and classification II enamel surface zones, respectively, compared with subjects who did not use formula as the main source of food during this period. This result was statistically significant for fluorosis on the FRI classification II enamel surface zones. The test for trend was borderline statistically significant for the FRI classification I enamel surfaces \( p = 0.05 \) and highly significant for the FRI classification II enamel surfaces \( p = 0.002 \). The test for deviation from linearity was nonsignificant for both FRI classification analyses.

Table 3 shows that a history of usually brushing with more than a pea size amount of toothpaste with a frequency of more than once per day conveyed statis-
TABLE 3. Crude and adjusted* odds ratio (OR) estimates with 95% confidence intervals (CI) for mild-to-moderate enamel fluorosis, stratified by the Fluorosis Risk Index (FRI) classification, by history of infant formula use, early toothbrushing habits, fluoride supplementation, bottled water or tap filter usage, and ethnicity, for Connecticut children born between 1980 and 1983 who grew up in optimally fluoridated communities.

<table>
<thead>
<tr>
<th>Variable</th>
<th>FRI classification I</th>
<th>FRI classification II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 112)</td>
<td>Controls (n = 51)</td>
</tr>
<tr>
<td>Formula use at months 10–12‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formula used</td>
<td>64</td>
<td>38</td>
</tr>
<tr>
<td>Ready to feed</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Liquid concentrate</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>Powdered concentrate</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Early fluoride toothpaste use§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pea size amount, once/day</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Pea size amount, &gt;once/day</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>&gt;Pea size amount, once/day</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>&gt;Pea size amount, &gt;once/day</td>
<td>61</td>
<td>25</td>
</tr>
<tr>
<td>Supplemented year 1–2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>90</td>
<td>49</td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Bottled water/tap filter use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>106</td>
<td>43</td>
</tr>
<tr>
<td>Yes†</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>102</td>
<td>43</td>
</tr>
<tr>
<td>Nonwhite</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

* Odds ratio estimates for each variable are adjusted for all of the other variables in the table, age, sex, median household income, age at which toothbrushing began and dental examiner.
† Reference category.
‡ Milk and soy formulas combined. Test for trend was borderline statistically significant for the FRI classification I enamel surfaces (p = 0.05) and highly significant for the FRI classification II enamel surfaces (p = 0.002). Test for deviation from linearity was nonsignificant for both FRI classification analyses.
§ Amount of toothpaste used and daily frequency of toothbrushing during the first 8 years. Test for trend was of borderline significance for the FRI classification I analysis (p = 0.05) and highly significant for the FRI classification II analysis (p = 0.003). Test for deviation from linearity was again nonsignificant for both FRI classification analyses.
¶ Used more than 2 of the 8 survey years.

Etc.
frequency of use per se. Tests for trend suggest a linear dose-response related to these habits. These findings are consistent with our previous findings in US fluoridated and nonfluoridated populations, which indicated a strong association between enamel fluorosis and early fluoride toothpaste use (17, 21).

Our findings are consistent with a growing number of other investigations conducted in the United States and elsewhere, with varying study designs and quality of method, that have reported a relation between early fluoride toothpaste use and enamel fluorosis (11-23). The findings of this investigation add to the evidence suggesting the need to reduce the ingestion of fluoride via toothpaste by pre-school age children. Specifically, our findings support the current dental public health recommendation that parents supervise their preschool child during toothbrushing to ensure that only a pea size amount of toothpaste is used and to encourage the child to expectorate the toothpaste used (60). Our findings also support the recommendation that a lower fluoride toothpaste be marketed specifically for use by pre-school age children (61).

Our finding of a strong association between mild- to-moderate enamel fluorosis and the inappropriate use of fluoride supplement during the first 2 years by children living in optimally fluoridated areas is consistent with our previous findings in a fluoridated population (17). These findings reinforce the continued need to educate dentists, pediatricians, and other medical practitioners as to the proper use of these agents, in particular the importance of not prescribing fluoride supplement to children who reside in optimally fluoridated areas.

The findings of this investigation suggest that the use of infant formula in the form of powdered concentrate as the main source of food, at age 10 months and older, continues to be an important enamel fluorosis risk factor for children who live in optimally fluoridated areas. Only one subject was exposed beyond the first year. An association was also seen between enamel fluorosis and powdered concentrate use during months 4-6 and 7-9; however, the strength of the associations were weaker and not statistically significant. The data further suggest that there may continue to be an association between enamel fluorosis and the use of formula in the form of liquid concentrate. However, the odds ratio point estimates were lower compared with those observed for powdered concentrate, and the findings were not statistically significant. Importantly, the data did not suggest an association between ready to feed formula and enamel fluorosis. Fourteen percent of the powdered concentrate used was reported as having been soy-based; however, there was also no association between enamel fluorosis and whether the formula was milk- or soy-based per se.

It is important to consider that these children, born between 1980 and 1983, would have been ingesting infant formula after the 1979 decision by formula manufacturers in the United States to voluntary reduce the concentration of formula in their products. That this reduction occurred is supported by laboratory analyses of infant formula fluoride concentration conducted in the years following the manufacturers' decision (31, 62). A subgroup analysis of our current data indicates that the observed associations with powdered formula were independent of the age of the subjects. This finding is evidence that the observed effect was not a result of a "carry over" effect, with the oldest subjects (born in 1980) somehow being exposed to the pre-reduction, higher fluoride containing formula. The finding of no association between ready-to-feed formula and enamel fluorosis provides additional evidence that the association with powdered concentrate was related to exposure to infant formula following the industry's voluntary reduction in the fluoride concentration of their products.

These findings of continued fluorosis risk associated with infant formula use are in contrast to those from our recent risk factor investigation in a nonfluoridated population, which was also a post-1979 birth cohort. That investigation found no association between enamel fluorosis and infant formula use in any form (21). Taken together, the findings of that study and the current investigation are consistent with the hypothesis that the addition of relatively low concentrations of fluoride from concentrated infant formula to optimally fluoridated water may produce a liquid with an above optimal fluoride concentration, with the potential to contribute to the development of enamel fluorosis (30, 31). The statistically significant test for trend supports this hypothesis, suggesting that the more optimally fluoridated water needed to prepare the formula for use (with powdered concentrate requiring dilution with more water than liquid concentrate), the greater the associated enamel fluorosis risk. However, to our knowledge, this is the first epidemiologic investigation of an optimally fluoridated population to report findings of enamel fluorosis risk associated with infant formula produced after the industry's voluntary reduction of fluoride content. Therefore, further study will be needed to fully define the fluorosis risk potential of infant formula, as currently manufactured in its different forms, across different populations. However, for the present at least, these findings suggest that in optimally fluoridated areas, the most prudent action by parents who wish to give their children formula, may be to use the ready-to-feed varieties. Alternately, these

parents could dilute formula concentrate with bottled water instead of tap water. However, care would need to be exercised to be sure that the bottled water used contained a low fluoride concentration.

While also not statistically significant, the data in table 3 suggest that nonwhites had about half the risk of becoming fluorosis cases compared with whites. This finding is consistent with the statistically significant findings in our previous study in a nonfluoridated population, where it was found that whites had a three- to fourfold increase in the risk of being a fluorosis case compared with nonwhites. The numbers of enrolled minorities were not sufficient to allow further analysis by specific minority group. Future research, with significant minority representation, will be needed to clarify these suggestive findings.

The association between infant formula ingestion during the first year of life and fluorosis on areas of the enamel that do not begin to form until after the second year of life (i.e., FRI classification II enamel surface zones) supports the suggestion that an early exposure to a fluoride source may have the potential to effect enamel surfaces that do not begin to form until after that exposure has occurred, due to the early uptake and later release of fluoride from bone during development (63). This association was observed in a fully adjusted analysis, and its relation to other exposures was carefully analyzed to minimize the likelihood that the association was due to confounding. Nevertheless, it is always possible that this association was due to the confounding effects of some other, yet unknown and unmeasured, factor. For this reason, further studies will be needed to verify this suggested temporal relation and to clarify under what early fluoride exposure conditions it may occur. This finding serves to illustrate the potentially complex relation between age at the time of fluoride exposure and susceptibility of specific enamel sites.

All of the cases reported in this study of children who had grown up in optimally fluoridated communities possessed fluorosis of at least mild-to-moderate severity, with nine subjects showing signs of more severe fluorosis. The classic findings of Dean et al. (64) demonstrated that at water fluoride concentrations of approximately 1 ppm fluoride (so-called optimum), dramatic reductions in caries prevalence were observed without esthetically noticeable fluorosis occurring (64). In contrast, evidence today suggests that the prevalence of enamel fluorosis has been increasing among children in optimally fluoridated as well as nonfluoridated areas in the United States (1, 2).

REFERENCES

Acknowledgments

This work was supported by grant nos. R29-DE08939 and DE9400110592, awarded by the National Institute of Dental Research.

The authors wish to thank Laura Byrne for her assistance with data management.