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Analytical Studies of Enamel Fluorosis: Methodological Considerations

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INTRODUCTION

The epidemiology of enamel fluorosis has a rich history, going back to the 1902 clinical observations of a US Public Health Service physician stationed in Naples, Italy (1). The early investigation of enamel fluorosis led directly to significant progress in the area of dental public health (2–5). It serves as an illustration of the power of epidemiology to uncover important health relations and to establish the safety and efficacy of specific interventions well in advance of a complete understanding of the underlying mechanisms of action (6–8). Enamel fluorosis is a condition in which both exposure to suspected risk factors and host susceptibility may continually change during a temporally finite period of vulnerability limited to the early years of life. Recent epidemiologic investigations of enamel fluorosis highlight important methodological issues related to the identification of cases and controls, as well as to the analysis of risk factors for this condition. This paper focuses on these methodological issues, with special emphasis on the measurement of dependent variables.

BACKGROUND

Enamel fluorosis can be defined histologically as a subsurface hypomineralization of the dental enamel (9). It is known to be caused by the chronic ingestion of sufficiently high amounts of fluoride while formation of the enamel is occurring during the first years of life (9, 10). Clinically, enamel fluorosis is defined by the presence of characteristic enamel opacities (9, 10). The greater the amount of fluoride ingested the more severe are the clinical manifestations of enamel fluorosis (9, 10). In its mildest forms, enamel fluorosis appears as a few faint white flecks scattered across the dentition that would generally go unnoticed by all except a trained examiner. With increasing severity, the areas of white flecking or "snowflaking" become more pronounced and cover an increasingly greater proportion of the enamel, thus becoming noticeable to the casual observer. In its more severe forms, enamel fluorosis is characterized by dark brown staining and pitting of the enamel surface, with large enamel defects occurring in the most severe cases (9, 11). The public health importance of this condition results from the direct association between enamel fluorosis, a condition with the potential to have significant aesthetic impact, and exposure to fluoride-containing agents which reduce the risk of dental caries.

Enamel fluorosis was first described manifestationally as "mottled enamel" (12). It is a tribute to the early investigators that without the advantage of epidemiologic training they were able to identify the cause of mottled enamel as something in the drinking water drawn from certain geographically isolated deep wells (13, 14). Armed with this epidemiologically derived conclusion, researchers quickly identified the fluoride ion as the causative agent of mottled enamel via spectrographic and animal model studies (15–17).

In the process of reaching their conclusions, these early researchers made two key observations related to the development of enamel fluorosis that have had lasting methodological significance. The first of these observations was that individuals with enamel fluorosis did not necessarily demonstrate enamel fluorosis on all of their teeth; the specific teeth affected depended on these individuals' age at the time they were exposed to the suspected drinking water (13). The second observation, related to the first, was that because the specific tooth sites affected by mottled enamel varied, children with unerupted teeth could not be said with certainty to be free of mottled enamel, even if all of the visible enamel surfaces were free of the condition (18, 19). These observations, which should be considered in the planning and conduct of prevalence surveys, have special methodological significance for the con-

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Abbreviation: FRI, Fluorosis Risk Index.
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duct of epidemiologic investigations of enamel fluorosis risk factors.

If enamel fluorosis were the sole oral health outcome associated with the presence of fluoride ion in drinking water, the history of its discovery would have been little more than an interesting epidemiologic footnote, because significant concentrations of fluoride in water occur naturally only in isolated, geographically scattered areas (10). However, the additional observation by early researchers that the presence of fluoride ion in drinking water was also associated with a markedly lower prevalence of dental caries (16) added greatly to the importance of these early investigations. This observation led to Dean's classic investigations (16), which demonstrated that a 60 percent reduction in caries prevalence was seen in populations served by water supplies containing naturally occurring fluoride in a concentration of approximately 1 part per million. Of particular interest was the further observation that at this fluoride concentration, virtually no clinically noticeable enamel fluorosis was observed (16). In areas that had naturally occurring fluoride concentrations above 1.2 parts per million, Dean and his coworkers found that while there was a substantial increase in the prevalence and severity of enamel fluorosis, there was little further decline in the prevalence of dental caries (16). It was these observations that led to the concept of an "optimal" drinking water fluoride concentration, which has served as the basis for artificial water fluoridation efforts in the United States and elsewhere (4, 5).

The observation that the fluoride ion was associated with a decreased prevalence of dental caries led to the development of other products containing fluoride which were intended to be ingested, applied topically to the teeth, or both (4). These products have had a direct impact on the current prevalence of both caries and enamel fluorosis in the United States and elsewhere (2, 20–22).

There has been a marked decrease in the prevalence of dental caries in the United States and other Western countries since the advent of artificial water fluoridation and the introduction of other fluoride-containing products (23). For example, today, approximately 50 percent of US children aged 5–17 years are caries-free (21, 22). However, during this same period, the prevalence of enamel fluorosis has also increased markedly in both optimally fluoridated and *non*-fluoridated areas (2, 3, 20). After comparing the early findings of Dean and coworkers, circa 1940 (10, 19, 24, 25), with more recent studies that used the same index (26–29), an ad hoc committee of the US Public Health Service concluded that the prevalence of enamel fluorosis in the United States has increased by 63 percent (i.e., from 13.6 percent to 22.2 percent) in optimally fluoridated areas and by 600 percent or severalfold (i.e., from 0.9 percent to 6.4 percent) in nonfluoridated areas (3). However, in a subsequent review of additional investigations using different fluorosis indices, Clark (20) concluded that the prevalence of enamel fluorosis may be as high as 60 percent in some optimally fluoridated areas of North America and as high as 45 percent in some nonfluoridated areas. In addition, the proportion of the population with aesthetically more noticeable mild-to-moderate enamel fluorosis has, by conservative estimate, more than doubled in both optimally fluoridated and nonfluoridated areas during that same period (10, 19, 24–29).

STRATEGIES FOR INVESTIGATING ENAMEL FLUOROSIS RISK FACTORS

The search for the specific underlying causes of the observed increase in enamel fluorosis presents several methodological challenges. There are issues related to the study of enamel fluorosis that act as barriers to the conduct of sound research. Unlike the situation during the first half of the century, when fluoride in drinking water accounted for virtually all of the fluoride exposure in the general population, during the past 25 years the number of intended and unintended sources of ingested fluoride has grown considerably. Furthermore, the ages at which these different fluoride exposures may occur can vary markedly. Enamel fluorosis of the permanent dentition results from sufficient exposure to fluoride during a susceptible age window in which enamel formation occurs. This age window begins around birth and continues to approximately 8 years of age (for all teeth except the third molars or "wisdom teeth"). Within this broad window, specific types of teeth begin to form at different ages, and the amount of time necessary for them to form totally may vary. Therefore, the specific age window of susceptibility and/or maximum susceptibility will be different for different types of teeth and for different areas of enamel on the same tooth. Since specific fluoride exposures also occur at different ages, the fluorosis effect of a specific fluoride exposure on enamel may vary across the dentition. In addition, while enamel susceptibility to fluorosis occurs at a very early age, a complete diagnosis of the dentition for enamel fluorosis cannot be made until around the age of 12 years, when all of the permanent teeth (with the exception of the third molars) have erupted. Until a child reaches that age, neither the full extent of enamel fluorosis within the dentition nor its complete absence can be determined with confidence. For sound research to occur, all of these issues must be recognized and appropriate strategies must be employed to address them.

The findings of Dean et al., which established that at water fluoride concentrations of approximately 1 part per million the prevalence of fluorosis is relatively low (19), along with the observation that the greatest relative increase in the prevalence of enamel fluorosis during the past 50 years has occurred in *non*-fluoridated areas (2, 3, 20), suggest that sources of fluoride other than optimally fluoridated water must be the underlying cause of the increase in enamel fluorosis prevalence. It is also clear that whatever sources were responsible would need to have been relatively prevalent to have had such a dramatic effect on fluorosis prevalence.

One strategy that has been employed to understand the rise in fluorosis prevalence has been the construction of literature-based estimates of fluoride intake from individual and combined sources (30). Typically, for each individual fluoride source (e.g., fluoride toothpaste), this approach provides an estimated average and range of fluoride intake from that source across the population. An estimate of the average and range of total fluoride intake from all sources can also be obtained by this approach (30). While this strategy can be highly valuable in the process of hypothesis formation regarding specific fluorosis risk factors, analytical epidemiologic investigations are necessary to test those hypotheses.

The principal suspected sources for enamel fluorosis have included fluoride in infant formula, which in the past has been shown to be highly variable (30–32); fluoride toothpaste; fluoride supplements or vitamins (drops or tablets); and professional (dentist-applied) fluoride applications. Because fluoride-containing dental products are beneficial in the prevention of caries, and since the identification of any of these products as a fluorosis risk factor might well lead to its reduction in strength or its elimination, the importance of correctly identifying the true risk factors for enamel fluorosis is clear.

While prevalence studies can provide estimates of fluorosis and caries trends and help to generate hypotheses related to those trends, they cannot test specific hypotheses related to the trends. At the same time, because of the very long time period between exposure and ability to measure outcome, clinical trials are not especially practical for testing hypotheses related to enamel fluorosis. Therefore, the investigation of enamel fluorosis and its relation to the prevention of dental caries is an area where the use of observational analytical epidemiologic techniques in general, and case-control methodology in particular, is important.

Three important methodological areas related to the analytical investigation of enamel fluorosis risk factors are 1) choice of study design, 2) measurement of dependent variables, and 3) ascertainment of exposure history. All three of these methodological areas are influenced by the natural history of enamel fluorosis. Although the complete histopathology of enamel fluorosis is still not fully understood, it is generally accepted that fluorotic changes of the enamel can only occur during the development of the enamel (9, 33). Once the enamel has formed, it is no longer at risk for fluorosis. This period of enamel formation lies between birth and approximately the eighth year of life for the permanent dentition (with the exception of the third molars) (34–42). The development of enamel can be divided into three principal stages: the *secretory phase*, during which an organic matrix is formed; a *maturation phase*, in which most of the mineralization of the enamel occurs; and a *transitional phase*, which is between the other two phases (9, 33). There may be important differences in the susceptibility of an area of enamel to fluorotic change depending on which stage of enamel formation it is in at the time of a fluoride challenge (9, 33). However, it is important to understand that different teeth begin to develop at different ages and that they do not necessarily develop at the same pace (34–42). Moreover, all of the enamel covering an individual tooth does not form at the same time; rather, the development of the enamel progresses from the incisal edge or cusp tip cervically (toward the root) (34–42). Typically 6 or more years will elapse between the beginning of the formation of tooth enamel and the eruption of a tooth in the mouth. Permanent teeth do not begin to erupt until around the age of 6 years, and they continue to emerge over the next 6 years or so (34–42). Thus, children in these study populations need to be at least aged 12–13 years before the majority of their permanent teeth can be expected to be available for examination.

The fact that 6–12 years may pass between fluoride exposure and the first opportunity to assess enamel surfaces for fluorosis affects the choice of study design. A case-control design has several distinct advantages over either a prospective cohort design or a retrospective cohort design. A prospective cohort study of 12 years' duration presents several obvious and major practical problems related to the necessary follow-up time. These problems include: 1) loss to follow-up due to family movement and unwillingness to continue in the study; 2) exposure misclassification or loss of exposure group purity (for example, by a marked change in an early dietary pattern or movement from an optimally fluoridated area to a nonfluoridated area); 3) the prohibitive cost of conducting the necessary oral examinations at the end of the study at potentially widespread geographic points (due to family movement); and 4) the challenge of obtaining funding for a study of the required length and cost. A retrospective cohort inves-

tigation of enamel fluorosis risk factors would be difficult to conduct in the United States, since records would not exist regarding oral hygiene practices in general or fluoride toothpaste use in particular. Information on early diet would be, at best, limited to the recommendations of the child's pediatrician rather than specific to the actual diet. In addition to these practical problems, neither cohort design would be especially suited to simultaneous investigation of the multiple early sources of fluoride that have been hypothesized to be potential risk factors for enamel fluorosis. For these reasons, a case-control design, with its relatively low cost, short required study time, and ability to investigate multiple risk factors, has been the best choice with which to investigate risk factors for enamel fluorosis.

ISSUES PERTAINING TO DEPENDENT VARIABLES

The natural history of enamel fluorosis also influences how it is measured. Beginning with an index developed by Dean at the time of his classic investigations, a series of different fluorosis indices have been developed (19, 43–45). Most of these indices use the individual as the unit of measure. For example, Dean's index identifies six levels of fluorosis severity (normal, questionable, very mild, mild, moderate, and severe), categorizing a subject on the basis of the two most severely affected teeth in the mouth (19). Indices of this sort are ideal for prevalence surveys, as they allow for a relatively precise estimate not only of the number of individuals with enamel fluorosis but also of the distribution of fluorosis severity within a population. The use of the individual as the unit of study works well in prevalence surveys, where the usual question of interest is what proportion of the population has been affected, and where there is no attempt to link the presence of fluorosis with a specific past exposure.

However, analytical risk factor investigations place different demands on an index related specifically to the natural history of enamel fluorosis. In an analytical investigation of the relation between enamel fluorosis and specific past exposures, the use of the individual as the unit of measure may be too broad. Whereas different areas of the dentition are at changing levels of susceptibility to enamel fluorosis at different chronologic ages during the child's development, exposure to different specific fluoride sources also changes with chronologic age during the child's development. Areas of enamel that are at a particular stage of formation at the time of a specific fluoride exposure may be at considerably different risk than areas of enamel that either are at a different stage of development or have not yet begun to form. Depending on which areas of enamel are measured, the likelihood of an association with an exposure might vary considerably. Therefore, grouping all enamel surface areas together for determination of case status could potentially lead to considerable nondifferential misclassification bias, increasing the likelihood in a case-control study of masking any true associations (46). This suggests that an approach to the measurement of enamel fluorosis which identifies a unit of measure more precise than the entire dentition, and that links this unit of measure to the age period in which development occurs, would have utility in these investigations.

It may be possible to modify other indices to achieve this purpose; however, to date only one index designed specifically to address this fluorosis measurement question has been introduced—the so-called Fluorosis Risk Index (FRI) (45). Some aspects of this index and findings from investigations in which it has been used are discussed below, not to justify or critique that particular index but rather to illustrate the broader methodological issues involved.

The FRI divides the enamel surfaces of the dentition into zones that can be grouped together on the basis of their age of development. In this way, the FRI assigns enamel surface zones to two groups: those that begin to form during the first year of life (so-called FRI classification I enamel surface zones) and those that do not begin to form until after the second year of life (so-called FRI classification II enamel surface zones). Enamel surface zones that cannot be assigned with confidence to either classification group on the basis of the dental developmental literature are left unassigned. These unassigned enamel surface zones do not directly contribute to the identification of a subject as either a case or a control, but they have indirect influence in that the presence of fluorosis on such a surface renders a subject ineligible to be a control under either of the two FRI classifications. An analysis of data from two recent investigations (47, 48) suggests that less than 5 percent of all potential fluorosis cases are lost due to leaving certain enamel surface areas unclassified.

A benefit of this approach to fluorosis measurement is the enhanced ability to demonstrate temporal relations between exposure and enamel surfaces affected. Making a distinction between early- and later-forming enamel surfaces is a useful strategy for dealing with the fact that both age of enamel development and age at exposure can vary markedly. The teeth that begin to form during the first year of life or shortly thereafter are the anterior teeth—those that are arguably of the greatest aesthetic concern—and the first molars. Formation of the posterior teeth, with the exception of the first molars, does not begin until after the second year. The fluorosis risk potential of age-specific fluoride exposures can be evaluated in terms of these age-dependent

groupings of tooth enamel. Fluoride exposures occurring during the first 2 years of life have long been of special interest (49–53). Exposure to infant formula is virtually limited to the first year (30, 47, 48, 54), and other important oral health-related behaviors are often first introduced during this period (55). These exposures would have a direct effect on enamel surfaces that began to form during the first year. Depending on the stage of formation (secretory, transition, or maturation phase) during which enamel is the most susceptible to fluorotic changes, exposures that occur later in the development of the enamel may pose the greatest risk (33). An examination of potential associations between fluorosis on these early-forming enamel surfaces and both early and later exposures provides the best opportunity to epidemiologically identify the importance of the different phases of enamel development. This information is useful in the process of evaluating the relative benefit-risk of a specific fluoride exposure at a specific dose, at a specific age.

Including enamel surfaces that begin to form during the second year in this early group would offer the potential advantage of increasing the number of available surfaces contributing to a subject's fluorosis status. However, balanced against that strategy must be a recognition of the limitations of the enamel development literature with regard to the exact timing of enamel formation, as well as consideration of individual biologic variability. Both of these considerations would increase the risk of misclassifying surfaces into this early group that may not truly begin to form until after the second year. Importantly, not including these surface zones does not decrease the ability to assess the effect of exposures occurring during the second year, which can be evaluated on the basis of surface zones that began to form during the first year and which would be expected in the main to be still in the secretory phase.

Using the FRI, subjects receive a case-control designation for both the FRI classification I and the FRI classification II enamel surface zones. A subject might meet the criteria for identification as a fluorosis case based on examination of the classification I surface zones but not meet the case criteria based on the classification II surface zones (or vice versa). Data from three recent investigations of enamel fluorosis risk factors serve to illustrate the significance of this point (47, 48, 56). Figure 1 shows the combined total percentages of subjects from these case-control investigations who were identified as a case under at least one of the two FRI classifications. The figure shows that only 48 percent of these cases were identified as cases under both of the FRI classifications. The remainder of the cases met the case criteria for only one of the two classifications. The potential for nondifferential misclassification bias is evident were subjects to be classified collectively as cases based on the presence of fluorosis anywhere in the dentition.

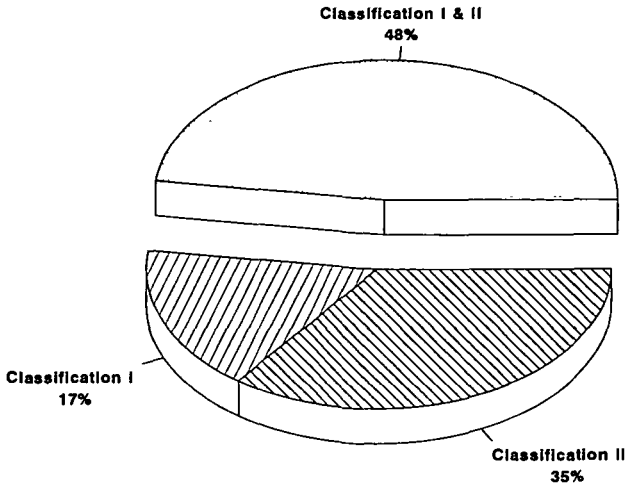

FIGURE 1. Percentages of subjects diagnosed as being cases (n = 946) with mild to moderate enamel fluorosis, stratified by Fluorosis Risk Index (FRI) classification (47, 48, 56). FRI classification I, fluorosis on early-forming (during the first year of life) enamel surfaces only; FRI classification II, fluorosis on later-forming (after the second year of life) enamel surfaces only; FRI classifications I and II, fluorosis on both early-forming and later-forming enamel surfaces.

The practical effect of this is illustrated in table 1, which shows data drawn from two of the risk factor investigations cited above (47, 48). These studies investigated enamel fluorosis risk factors in an optimally fluoridated population and a nonfluoridated population born prior to the reduction in the fluoride supplement protocol for the first 2 years of life and the infant formula industry's voluntary reduction in the fluoride content of their products, both of which occurred around 1979 (57–59). The table shows that, for the use of milk-based infant formula, there was a pronounced difference between the odds ratio estimates for enamel fluorosis on early-forming, FRI classification I enamel surface zones (odds ratio = 3.3) and the estimates for fluorosis on later-forming, FRI classification II enamel surface zones (odds ratio = 1.4). Had fluorosis cases been identified on the basis of the entire dentition (i.e., as illustrated by combining FRI classification I and classification II enamel surfaces), this important association would have been significantly masked. These findings are consistent with the expectation that whereas an exposure occurring throughout the entire developmental period of the permanent teeth would place all enamel at relatively similar risk, an exposure occurring only during a specific and limited time period would place different enamel surfaces at potentially markedly different risks of

TABLE 1. Adjusted odds ratio estimates for mild-to-moderate enamel fluorosis, stratified by Fluorosis Risk Index (FRI) classification, in two studies

Study and variable	FRI classification I		FRI classification II		FRI classifications I and II combined	
	Adjusted odds ratio	95% confidence interval	Adjusted odds ratio	95% confidence interval	Adjusted odds ratio	95% confidence interval
Pendrys et al., 1994 (47) (optimally fluoridated Connecticut children born between 1975 and 1979)						
Use of milk-based infant formula*						
Never†	1.0		1.0		1.0	
Use at ages 10–24 months	3.3	1.4, 8.1	1.4	0.5, 4.1	1.8	0.5, 5.8
Pendrys et al., 1996 (48) (nonfluoridated Connecticut and Massachusetts children born between 1980 and 1983)						
Fluoride supplementation during year 1‡						
No†	1.0		1.0		1.0	
Yes	1.2	0.7, 2.1	1.7	0.8, 3.5	1.5	0.8, 3.1
Fluoride supplementation during years 3–8‡						
None†	1.0		1.0		1.0	
All 6 years	2.6	1.3, 5.0	5.1	2.1, 9.8	3.4	1.4, 8.1

* Use of milk-based infant formula as the main source of food. Based on logistic regression analyses adjused for age, sex, median household income, current town of residence, examiner, exposure to infant formula during other age periods, amount of toothpaste typically used, and fluoride supplement use. Adapted from Pendrys et al., 1994 (47).

† Reference group.

‡ Based on logistic regression analyses in which each fluoride supplementation period (i.e., year 1 and years 3–8) was adjusted for supplementation during the other period, sex, median household income, dental examiner, race/ethnicity, breastfeeding, usual amount of toothpaste used when brushing, usual daily frequency of tooth-brushing, and age at which tooth-brushing began. Adapted and modified from Pendrys et al., 1996 (48).

enamel fluorosis, dependent on the stage of development during the exposure.

The findings related to fluoride supplementation during the first year of life illustrate a second important point. They indicate that fluoride supplementation during the first year was only weakly associated with enamel fluorosis on either early-forming or later-forming enamel surfaces, when adjusted for later supplementation. The table illustrates that a weak association would have been observed (odds ratio = 1.5) were enamel fluorosis case status measured on the basis of the entire dentition (i.e., FRI classifications I and II combined). However, this summary finding could not exclude the real possibility that, in fact, a true strong association existed between exposure to fluoride supplementation during the first year and fluorosis on the aesthetically important enamel surface areas that begin forming during the first year, but this true association was being masked by the inclusion of cases based on fluorosis on later-forming enamel surface areas. This is the actual situation illustrated in table 1 regarding infant formula use. That is, one could not rule out first-year fluoride supplementation as an important risk factor on the basis of whole-mouth summary data alone.

The findings related to fluoride supplementation throughout ages 3–8 years illustrate an important related point. The table shows that fluoride supplementation throughout this period was associated both with fluorosis on enamel surface areas that began forming during that period and fluorosis on enamel surface areas that began to form 2 years before that exposure period. In the case of this exposure, a summary value based on the entire dentition would have suggested an important association; however, the observation that there was a moderately strong association specifically between this later supplement exposure and enamel fluorosis on the earliest-forming enamel surfaces would have been absent. Specific risk factor information of this kind contributes to a further understanding of the histopathology of enamel fluorosis, and can be an important aid in the difficult process of deciding the best use and timing of specific caries prevention agents in the context of total fluoride intake.

The key point is that while studies which have used whole-dentition fluorosis measures have produced use-

ful fluorosis risk information (52, 60–65), risk factor investigations which characterize enamel fluorosis more precisely, rather than globally on the basis of the entire dentition, will have the greatest potential to provide the deepest insights into the specific underlying etiology of the increasing prevalence of enamel fluorosis. To maximize the utility of this approach, the clinical examiner must resist the temptation to allow a first-impression global assessment to bias his or her diagnosis of specific surface zones. An initial clinical impression may at times be difficult to avoid, depending on the specific clinical presentation. To the extent that the examiner is influenced by this impression in the diagnosis of individual sites, a bias will be introduced into the process, and the two fluorosis status classifications will become more similar, diminishing the utility of the process and creating a situation more akin to that of whole-mouth scores. However, the clinical examiner who understands and accepts the rationale underlying the approach sufficiently to learn and use it may be less likely to make the conflicting assumption that there is a whole-mouth generality to the presence or absence of fluorosis throughout the dentition. Furthermore, the number of actual diagnostic sites examined with this approach makes it less likely that an examiner will remember that a specific site is a member of a particular development-time classification group; this reduces the likelihood that examiner knowledge of the fluorosis status of a site in a particular group will influence his or her diagnostic judgment about other sites within that group. Nevertheless, the potential for an initial clinical impression to occur and to influence specific diagnostic decisions exists and must be recognized. (This problem may occur to some extent in any given study.)

Another inherent challenge to this method is the need for the examiner to visually divide the tooth surface into four zones. As with the above issue, the more an examiner deviates from an accurate division of these zones the greater will be the potential for misclassification of findings into the wrong age-dependent classification. A protection against this misclassification is the presence of unclassified zones which reflect enamel development-time uncertainty and visually separate the age-dependent classified zones on the enamel surface. For example, no FRI classification I enamel surface zone lies directly adjacent to an FRI classification II enamel surface zone. Thus, any misclassifications that occur will probably move a diagnostic finding from a specific classification group into the group of unclassified surface zones or vice versa. While the goal is to avoid *any* misclassification, this type will affect the analysis less than the misclassification of a finding from one FRI classification (I or II) to the other, which would involve a visual measurement error on the order of half a tooth surface.

Despite the challenges inherent in using a method of this type, the approach does appear to possess the ability to differentiate fluorosis on the basis of its location on specific age-dependent enamel surface areas, as illustrated in figure 1 and table 1. However, the FRI should be seen as representing only one attempt to address these issues. Opportunities may well arise to improve on the general method, especially as our knowledge base concerning the timing of enamel formation, specific mechanisms of enamel fluorosis, and the role of specific risk factors grows. The methodological considerations presented here would be expected to apply equally to the investigation of other developmental defects of the dentition.

In a case-control investigation of enamel fluorosis, the severity of the fluorosis becomes part of the case definition. That is, a threshold of fluorosis severity must be established in order to differentiate cases from noncases. For example, in the case of the FRI, only enamel surfaces with fluorosis of mild-to-moderate severity or worse contribute to the categorization of a subject as a case. While it is certainly possible to establish different levels of severity stringency within the original case definition, creating two or more case groups, this will generally be a secondary analysis.

As with any case-control investigation, careful construction of criteria for the selection of the control group is a critical methodological step. In this regard, it is important in enamel fluorosis risk factor investigations to differentiate between *noncases*—subjects who fail to meet the case definition (e.g., fluorosis of mild or greater severity)—and *fluorosis-free subjects*—those who show no evidence of enamel fluorosis whatsoever. The latter group is a subset of the noncases.

Practically speaking, enamel surfaces that are visible for clinical examination could be graded as follows: 1) having fluorosis present at or above the threshold severity; 2) having no fluorosis of any severity; or 3) being questionable, in the sense that the enamel cannot be diagnosed with confidence as *either* showing fluorosis at or above the threshold severity or being fluorosis-free. The "fluorosis present" category could be further subdivided to differentiate the more severe fluorosis cases from other cases, using an ordinal logistic regression model (66, 67). However, in the decision to do this, the potential gain in information must be balanced against a likely decrease in examiner reliability. This strategy would probably be most worthwhile when applied to study populations in which a sizable portion of subjects with fluorosis showed signs of more severe fluorosis.

Depending on the threshold severity chosen as part of the case definition in the investigation, the "questionable" category can cover considerable diagnostic

ground, ranging from the slightest visible white flecks to areas of severity just beneath the criteria for a positive diagnosis in the judgment of the examiner. Although Dean's index contained a "questionable" category (19), the two most frequently used fluorosis indices, developed recently, do not (43, 44). The absence of a "questionable" category in a prevalence survey helps to prevent confusion as to how to interpret such a category in terms of fluorosis prevalence. Subjects not meeting the criteria of the lowest fluorosis severity level are diagnosed as fluorosis-free. However, the inclusion of a diagnostic category of "questionable" in the examination protocol of an analytical investigation is an important methodological element with which to reduce the misclassification of cases and controls that would otherwise occur were an examiner forced to diagnose all enamel surface areas as having fluorosis either present or absent. Misclassification of this type would be expected to drive obtained odds ratio estimates toward the null (46). This effect is illustrated in table 2, which is adapted from a study by Pendrys et al. (47). This table shows the adjusted odds ratios from multiple logistic regression for inappropriate fluoride supplement use during the first 4 years of life and for frequent tooth-brushing during the first 8 years. It gives these values using three methods of case-control selection. In method A, the method actually used in the study analyses, the so-called "questionable" surfaces do not contribute to a subject's categorization as a case, but their presence does eliminate the subject as a control. In method B, the questionable surfaces are considered fluorosis-free and thus would contribute toward a subject's categorization as a control. In method C, these questionable surfaces are considered fluorosis-positive, and thus they contribute toward a subject's categorization as a case. Data on two early fluoride exposures are shown to illustrate this point. The inappropriate use of fluoride supplementation by children living in optimally fluoridated areas has never been a recommended practice, because of the very high likelihood of the practice's leading to far-above-optimal fluoride ingestion, with the consequent high risk of enamel fluorosis (57, 68, 69). It can therefore serve as a "gold standard" in assessing the effects of different methods of managing the questionable surfaces. The estimated odds ratio of 5.36 associated with inappropriate fluoride supplementation obtained using method B (i.e., with questionable surfaces considered fluorosis-free) is approximately half that obtained using method A (odds ratio = 11.47). The effect of using method B on the estimated odds ratio associated with early frequent tooth-brushing is a reduction in the obtained adjusted odds ratio estimate from a statistically significant value of 2.80 to a nonsignificant value of 1.94. Considering these questionable surfaces positive for fluorosis reduces the estimated odds ratios for these two variables even further. While it is true that more flexible case or control inclusion criteria will allow a larger total yield of cases and controls from a population sample examined and thus offer potentially greater statistical power, the effect of misclassification

TABLE 2. Effect of three methods of managing "questionable" enamel surface zones on adjusted* odds ratios for mild-to-moderate enamel fluorosis associated with early frequent tooth-brushing and inappropriate fluoride supplement use among optimally fluoridated Connecticut children born between 1975 and 1979†

Variable	Method A: questionable surfaces eliminate subject as a control but do not contribute to subject's categorization as a case (actual method used in analysis (47))		Method B: questionable surfaces considered fluorosis-free (i.e., contribute to control group)		Method C: questionable surfaces considered to have fluorosis (i.e., contribute to case group)	
	Adjusted odds ratio	95% confidence interval	Adjusted odds ratio	95% confidence interval	Adjusted odds ratio	95% confidence interval
Inappropriate fluoride supplement use						
Never	1.00§		1.00§		1.00§	
Use at ages 1–4 years	11.47	2.68, 49.05	5.36	2.08, 13.80	4.94	1.38, 17.76
Frequent tooth-brushing‡						
Never	1.00§		1.00§		1.00§	
Birth to age 8 years	2.80	1.15, 6.81	1.94	0.98, 3.85	1.16	0.62, 2.16

* Adjusted for age, sex, median household income, current town of residence, amount of toothpaste typically used, use of infant formula, and dental examiner.
† Adapted from Pendrys et al., 1994 (47).
‡ Brushed teeth more than once per day.
§ Reference group.

of fluorosis cases and controls on odds ratio estimates, illustrated in the table, cautions against this practice. An additional strategy would be to include subjects who have been diagnosed as having questionable signs of enamel fluorosis as a distinct diagnostic category (or multiple categories) in an ordinal logistic regression model (66, 67). This approach has the advantage of providing the potential to allow additional insights into specific exposure-fluorosis relations, but the complexity of analysis will be increased and the findings may become more challenging to interpret.

Another consideration in distinguishing true fluorosis controls from noncases is the inevitable question of masked enamel surfaces, surfaces that are not visible for diagnosis. Masking of surfaces can be due to several causes, but most often it is due to the noneruption of teeth or to the presence of orthodontic appliances or restorations. The possibility exists that these unseen surfaces would be diagnosed as being other than fluorosis-free were they visible. Therefore, among subjects diagnosed as fluorosis-free except for the masked surfaces, the potential exists for misclassification of subjects as controls because of the absence of information on these remaining surfaces. The eruption of teeth is age-related (34–36), which is why Dean argued that subjects in fluorosis investigations should be at least 12 years of age (19). Figure 2 illustrates the potential for subject fluorosis status misclassification were control status based solely on the earliest-forming and -erupting enamel surfaces. This figure represents all of the subjects from three recent case-control investigations (47, 48, 56) who would have been declared fluorosis-free on the basis of the early-erupting enamel surfaces. The figure shows that 41 percent of the subjects who would have been declared fluorosis-free on the basis of early-erupting enamel surfaces would not have been declared fluorosis-free on the basis of the later-erupting enamel surfaces.

Even when appropriately aged subjects are recruited, surfaces may still be masked because of orthodontic appliances, restorations, or the incomplete eruption or noneruption of some of the later-erupting teeth. Any masked surface areas present the theoretical possibility that fluorosis is present but not visible for observation. The most conservative approach would therefore be to exclude subjects with any masked surfaces. However, an analysis of data from two recent fluorosis risk factor investigations in which 86 percent of the subjects were aged 12 years or older (47, 48) suggests that subjects with masked surfaces, who would otherwise have been categorized as controls on the basis of visible enamel surfaces, appear to have about the same exposure history as control subjects for whom all enamel surfaces are visible (table 3). The

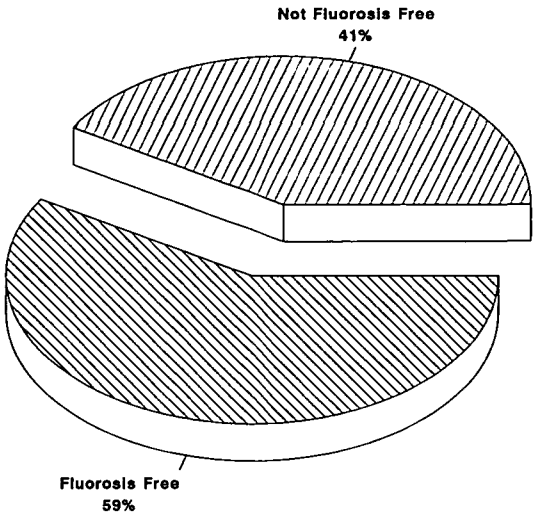

FIGURE 2. Proportions of subjects whose later-erupting (after the second year of life) tooth enamel surfaces were diagnosed as either fluorosis-free or other than fluorosis-free, among all subjects diagnosed as having fluorosis-free early-erupting (during the first year of life) enamel surfaces (47, 48, 56). The total number of controls based on early-erupting enamel surfaces was 413.

inclusion of these subjects with masked surfaces in the control group did not in general strongly affect odds ratio estimates; some estimates decreased and others actually increased. However, the odds ratio estimates associated with two of the variables, use of soy-based infant formula (FRI classification I analysis) and frequent tooth-brushing (FRI classification II analysis), were both sufficiently reduced to no longer achieve statistical significance. In general, the magnitude of the decreases in the odds ratio estimates was somewhat greater than that of the increases. Therefore, caution is advised when one is considering whether to include subjects with masked surfaces in a control group. Appropriate subgroup analyses will probably be helpful for determining the homogeneity of the unmasked and masked groups.

Further considerations include the number of masked surfaces in the study sample and the specific reasons that surfaces are masked. For example, the occlusal surfaces of the permanent first molar teeth account for 40 percent of the FRI classification I surface zones. The presence of either sealant or a restoration may make determination of the fluorosis status of the surface impossible. However, in a situation where a restoration has been done and part of the enamel surface is masked, categorization of an otherwise fluorosis-free restored surface as fluorosis-free might be desirable in order to maximize both the numbers in the control group and the representativeness of the control group. Since the appearance of any sign of fluorosis would place such a surface in a "questionable"

TABLE 3. Adjusted odds ratio estimates for mild-to-moderate enamel fluorosis, by Fluorosis Risk Index (FRI) classification, in analyses excluding and including subjects with masked enamel surfaces from the control group*

Variable	FRI classification I				FRI classification II			
	Masked subjects excluded		Masked subjects included		Masked subjects excluded		Masked subjects included	
	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval
Inappropriate fluoride supplement use†								
Never use‡	1.00		1.00		1.00		1.00	
Use at ages 1–4 years	11.47	2.68, 49.05	9.89	2.72, 35.95	19.28	2.22, 167.1	18.11	3.60, 91.16
Use of milk-based infant formula§								
Never use‡	1.00		1.00		1.00		1.00	
Use at ages 10–24 months	3.34	1.38, 8.07	2.97	1.30, 6.76	1.43	0.51, 4.09	1.82	0.80, 4.13
Use of soy-based infant formula§								
Never use‡	1.00		1.00		1.00		1.00	
Use at ages 10–24 months	7.16	1.35, 37.89	3.26	0.83, 12.77	1.09	0.19, 6.29	1.42	0.32, 6.28
Frequent tooth-brushing¶								
Never use‡	1.00		1.00		1.00		1.00	
Birth to age 8 years	2.80	1.15, 6.81	3.41	1.46, 7.79	2.63	1.03, 6.73	2.12	0.97, 4.65
Appropriate fluoride supplementation#								
Never use‡	1.00		1.00		1.00		1.00	
Use at ages 2–8 years	2.25	1.08, 4.69	2.32	1.12, 4.78	7.97	2.98, 21.33	5.36	2.24, 12.85
Tooth-brushing history**								
Began after year 2, once per day‡	1.00		1.00		1.00		1.00	
Began during years 1–2, more than once per day	2.56	1.34, 4.88	2.55	1.34, 4.86	4.23	1.72, 10.41	4.46	2.23, 8.94

* Adapted from two studies (47, 48).

† Use by children living in optimally fluoridated communities. Based on logistic regression analyses adjusted for age, sex, median household income, current town of residence, clinical examiner, exposure to infant formula, tooth-brushing frequency, amount of toothpaste typcally used, and fluoride supplement use during other age periods. Adapted from Pendrys et al., 1994 (47).

‡ Reference group.

§ Use of such formula as the main source of food. Based on logistic regression analyses adjusted for age, sex, median household income, current town of residence, clinical examiner, exposure to infant formula during other age periods, tooth-brushing frequency, amount of toothpaste typically used, and fluoride supplement use. Adapted from Pendrys et al., 1994 (47).

¶ Brushing more than once per day. Based on logistic regression analyses adjusted for age, sex, median household income, current town of residence, clinical examiner, exposure to infant formula, tooth-brushing frequency, amount of toothpaste typically used, and fluoride supplement use. Adapted from Pendrys et al., 1994 (47).

Based on logistic regression analyses adjusted for sex, race/ethnicity, median household income, dental examiner, breastfeeding, age at which tooth-brushing began, tooth-brushing frequency, amount of toothpaste typically used, and fluoride supplementation during the first year of life. Adapted from Pendrys et al., 1996 (48).

** Based on logistic regression analyses adjusted for sex, race/ethnicity, median household income, dental examiner, breastfeeding, amount of toothpaste typically used, and fluoride supplementation. Adapted from Pendrys et al., 1996 (48).

category, the likelihood of misclassifying a surface that once had fluorosis (and was replaced by a restoration) as fluorosis-free would be low, except for a situation in which most or all of the surface was covered by the restoration. A subgroup analysis would again be useful in this situation to help one determine the appropriateness of including these surfaces as fluorosis-free.

Masking of surfaces in the examples given in table 3 resulted primarily from unerupted teeth and the presence of orthodontic appliances. Little masking was due to missing teeth, which is reflective of the overall US child population today (21). Therefore, these observations would not necessarily apply to a population in which tooth loss was a significant cause of masked surfaces.

ISSUES PERTAINING TO INDEPENDENT VARIABLES

During the past 50 years, there has been a dramatic increase in the number of sources of ingested fluoride (2, 4). These sources include those in which fluoride was intended for ingestion (for example, fluoridated water) and those that were never intended to be a source of ingested fluoride, such as fluoride toothpaste. It has been recognized for some time that a multivariate approach to the analysis of fluorosis risk factor data is essential, because of the potentially complex relation between different fluoride exposures and the modifying effects of demographic and socioeconomic factors (2). Nevertheless, risk factor studies presenting only unad-

justed bivariate findings continue to appear in the literature. The need for multivariate-adjusted analyses is illustrated in the findings of a study which reported on risk factors for enamel fluorosis in an optimally fluoridated Connecticut middle school population, all of whom were born prior to the US infant formula industry's voluntary decision to reduce and control the concentration of fluoride in infant formula (47). Early frequent tooth-brushing and inappropriate fluoride supplementation of these optimally fluoridated children were both shown to be important risk factors for enamel fluorosis in this population (47). Bivariate analyses also revealed both of these exposures to be important confounding variables for the use of infant formula as the main source of food. Specifically, children given infant formula were both *less* likely to have been frequent tooth-brushers and *less* likely to have been inappropriately supplemented, as compared with non-formula users. Table 4, which shows crude odds ratio estimates as well as odds ratio estimates adjusted for early frequent tooth-brushing and inappropriate supplementation, illustrates the effect of this confounding on the estimated risk associated with infant formula use. One can see that for both milk- and soy-based formula, when data are adjusted for frequency of tooth-brushing and fluoride supplement use, the odds ratio estimates increase dramatically and achieve statistical significance. In this case, the use of bivariate analyses alone would have led to the spurious conclusion that there was no important, statistically significant association between enamel fluorosis and infant formula ingestion. Equally important, failure to adjust for other fluoride exposures could lead to a false conclusion that an exposure or exposure time is important in the development of enamel fluorosis. For example, in two separate investigations of risk factors for enamel fluorosis in nonfluoridated communities, an apparently important association between fluoride supplementation during the first year of life and fluorosis, based on an unadjusted bivariate analysis, disappeared when this factor was adjusted for history of fluoride supplementation at a later age and other relevant variables (48, 54). Since most fluoride-containing products are caries-preventive or therapeutic agents, and since identification of a product as a fluorosis risk factor could well lead to its modification or elimination, the importance of avoiding spurious risk-factor findings is self-evident. Therefore, bivariate analyses should only be used as an aid in understanding potential relations between different fluoride exposures and as a preliminary step in the construction of the principal multivariate analyses, which should include all past fluoride exposures as well as relevant demographic and socioeconomic variables.

TABLE 4. Crude and adjusted odds ratio estimates for infant formula use and mild-to-moderate enamel fluorosis on FRI* classification I enamel surface zones†

Use of infant formula‡	Crude OR*	95% CI*	Adjusted OR§	95% CI
None¶	1.00		1.00	
Milk-based formula	1.96	0.91, 4.24	2.77	1.29, 5.97
Soy-based formula	2.38	0.58, 10.60	3.80	1.04, 13.82

* FRI, Fluorosis Risk Index; OR, odds ratio; CI, confidence interval.

† Data were adapted from Pendrys et al., 1994 (47).

‡ Use of formula as the main source of food during the age period 10–24 months.

§ Adjusted for fluoride supplement use and frequency of tooth-brushing.

¶ Reference group.

Obtaining a fluoride exposure history may be susceptible to the "good parent" effect, where a parent's recall of past exposure events is biased by her/his notion of what a good parent would have done. For example, parents' notions of the specific tooth-brushing behaviors a good parent would have had her/his child adopt may affect their responses to questions on tooth-brushing history. For this reason, questions should be presented as neutrally as possible, allowing for several choices. If this effect were to occur in a nonsystematic fashion, the outcome would be nondifferential misclassification, with a resultant movement of estimated odds ratios toward the null. However, if a particular biased response were differentially associated with an exposure that was a true fluorosis risk factor, the potential for an observed spurious association would exist. On the other hand, if no true association existed between the spurious factor and fluorosis, adjustment for the true risk factor in a multivariate analysis would reveal a true lack of association between the spurious factor and fluorosis. This is therefore another important reason for utilizing multivariate analyses.

TESTING FOR BIAS

One methodological concern that is generic to all case-control investigations is whether parental awareness of a child's condition, in this case opacities on the child's teeth, could lead to recall bias when the parent is completing a questionnaire dealing with early fluoride exposures. A useful way to test for this bias in studies of enamel fluorosis risk factors is to make use of the presence of enamel opacities due to causes other than early exposure to fluoride. Fluoride-induced opacities and opacities due to other causes often appear similar to the untrained observer, including dentists, and some international researchers have expressed the view that a clinical distinction between the two is not possible (70, 71). However, in the United States, a set of very specific clinical criteria by which to make this differentiation have been accepted

TABLE 5. Relation between the presence of nonfluoride opacities among subjects otherwise diagnosed as fluorosis-free and history of having been exposed to fluoride sources shown to be associated with mild-to-moderate enamel fluorosis among children who grew up in nonfluoridated areas*

Nonfluoride opacities	Total no. (fluorosis-free)	Fluoride supplementation during years 2–8		Began tooth-brushing during years 1–2, typically brushing more than once per day	
		No.	%	No.	%
Absent	184	146	79.3	69	37.5
Present	14	10	71.4	4	28.6

* Adapted from Pendrys et al., 1996 (48).

and successfully used in fluoride investigations for nearly 40 years (72, 73). The practical value of this is that, were there a recall bias due to parental awareness of opacities on the child's teeth, the expectation would be that *among fluorosis-free subjects* one would see a greater apparent association with fluoride exposures among subjects *with* nonfluoride opacities than among subjects *without* nonfluoride opacities. Table 5 illustrates the use of this kind of analysis in a recent case-control investigation in which examiner diagnosis of fluorosis status was not revealed to subjects' parents until after they had completed a fluoride history questionnaire (48). While the number of fluorosis-free subjects with nonfluoride opacities is relatively small, it is clear that the proportion of these subjects with a history of fluoride exposure is no greater than that for fluorosis-free subjects without nonfluoride opacities. Therefore, in this illustration, this type of analysis failed to reveal any evidence of recall bias associated with parental awareness of the presence of opacities on the child's teeth.

SUMMARY

The epidemiology of enamel fluorosis has important public health implications, because enamel fluorosis is a side effect of fluoride exposures, virtually all of which are either directly or indirectly related to attempts to prevent or treat dental caries. Dental caries in turn continues to be an important public health concern. For example, an estimated 84 percent of 17-year-old children in the United States have either caries or teeth that were restored because of caries (21). An estimated 96 percent of US adults have either coronal caries or restorations due to coronal caries, while the prevalence of US adults with root surface caries or restorations due to root surface caries is 21 percent (74). Dental caries continues to be a major cause of adult tooth loss in the United States and around the world (75–81). Since the identification of a specific fluoride exposure as a risk factor for enamel fluorosis may well lead to either the modification or elimination of that exposure, with a consequent potential increase in risk of caries in the population, the need to avoid falsely identifying fluoride interventions as risk factors is clear. At the same time, failure to accurately identify true risk factors for enamel fluorosis could lead to the needless reduction or elimination of beneficial fluoride regimens that are not of themselves important fluorosis risk factors, in a misguided attempt to address the fluorosis problem. Thus, the need to accurately identify true risk factors is also clear. To accomplish this goal, careful attention must be paid to methodology when planning and conducting investigations of fluorosis risk factors. This paper has attempted to identify and discuss several of the key methodological issues related to the epidemiologic investigation of suspected risk factors for enamel fluorosis.

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