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Fluoride: Dose-Response Analysis For Non-cancer Effects

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Office of Water

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PREFACE

In March, 2006, the National Academy of Sciences National Research Council (NRC) released the Report: *Fluoride in Drinking Water: A Scientific Review of EPA's Standards*. The NRC (2006) noted that "in light of the collected evidence of various health endpoints and total exposure to fluoride, the committee concludes the EPA's MCLG of 4 mg/L should be lowered." They further suggested that, in order to develop an MCLG that is protective against severe enamel fluorosis, clinical stage II skeletal fluorosis and bone fractures, EPA should:

- Apply current approaches for quantifying dose-response where feasible,
- Consider susceptible populations,
- Characterize uncertainties and variability, and
- Provide better estimates of total exposure for individuals.

The Office of Water (OW) accepted the NRC (2006) findings as the summary of hazard for inorganic fluoride and focused on the dose-response analysis as they had recommended. The OW collected available dose-response data for severe dental fluorosis, clinical stage II skeletal fluorosis and skeletal fractures as they relate to fluoride exposure expanding on the data retrieved by NRC (2006) to include studies identified by literature searches that covered the time period from 2000 to 2010. Current methodologies (categorical regression and benchmark dose modeling) were applied in evaluating dose-response in order to identify an appropriate point of departure for severe dental fluorosis as the critical effect. The NRC analysis determined that severe dental fluorosis appears to occur at a lower dose than stage II skeletal fluorosis and/or bone fractures. This document presents the results of the dose-response analysis requested by NRC.

The objective of the OW effort was to identify a point of departure for the fluoride concentration in drinking water that would be protective for sensitive exposed populations (children) who are vulnerable to severe enamel fluorosis, and determine if that point of departure will also be protective against stage II skeletal fluorosis and bone fractures in adults. The OW analysis focused first on severe dental fluorosis based on the NRC analysis.

This document provides a detailed review of available dose-response data from published and peer-reviewed studies for the following endpoints as they relate to fluoride exposure from drinking water:

- Dental fluorosis
- Skeletal fluorosis
- Skeletal fractures

Detailed analyses of the suitability of studies that had been identified by NRC (2006) and those retrieved by the OW for dose-response analysis are included as separate documents to accompany this report. There are two separate collections of study evaluations, one covers dental effects (*Dental Fluorosis: Evaluations of Key Studies*, EPA Report No. 820-R-10-018) and the other skeletal effects (*Fluoride-Related Skeletal Effects: Evaluations of Key Studies*, EPA Report No. 820-R-10-017).

EPA identified a point of departure (POD) of 1.87 mg F/L for severe dental fluorosis based on benchmark dose modeling of the prevalence for severe dental fluorosis associated with specific drinking water fluoride concentrations as reported by Dean (1942). In that era, drinking water and the diet were the major sources of fluoride exposure. The POD is a lower confidence bound on the concentration in drinking water associated with severe dental fluorosis in 0.5% of the population studied. This POD was the lowest concentration that was statistically justified by the population size. This value is consistent with other analyses of the Dean (1942) data set that identify 2 mg/L as the threshold drinking water fluoride concentration for severe dental fluorosis (EPA, 1986, NRC 1993, 2006).

In this document drinking water intake information for ingestion of tap water delivered by public drinking water systems, as reported in the USDA 1977–1978 Food Consumption Survey, is used to estimate the fluoride dose from drinking water associated with the POD in children studied by Dean (1942). Data from a publication on dietary fluoride by McClure (1943) were used to estimate the dose from the diet for the children studied by Dean (1942). The combination of the drinking water and dietary estimates thus become the basis for the OW inorganic fluoride Reference Dose (RfD) estimate of 0.08 mg F/kg/day. The RfD is an estimate of the fluoride dose that will protect against severe dental fluorosis, clinical stage II skeletal fluorosis and skeletal fractures while allowing for a fluoride exposure adequate to protect against tooth decay for children and adults. Confidence in the RfD is considered to be medium.

The OW has also prepared and peer reviewed a second document that provides fluoride exposure estimates for the age groups susceptible to severe dental fluorosis. This second document, *Fluoride: Exposure and Relative Source Contribution Analysis* (EPA Report No. 820-R-10-015), can be accessed through the following url:
http://water.epa.gov/action/advisories/drinking/fluoride_index.cfm

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ACKNOWLEDGMENTS

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LIST OF ACRONYMS

ADA	American Dental Association
AI	Adequate Intake
BMD	Benchmark Dose
BMDL	Benchmark Dose lower 95% bound
BW	Body weight
CATMOD	Categorical modeling procedure of the SAS© Institute, Inc., of Cary, NC
CFI	Community Fluorosis Index
CSFII	Continuing Survey of Food Intake by Individuals
DDE	Developmental defects of enamel
dfs	Decayed or filled surfaces
dft	Decayed and filled primary teeth
DMF	Decayed, missing and filled
DMFS	Decayed, missing and filled permanent surfaces index
DMFT	Decayed, missing and filled permanent teeth index
DS	Decayed surface
DT	Decayed teeth
DWI	Drinking water intake
EDF	Environmental Defense Fund
F_{ei}	Community Index of Dental Fluorosis
F_{ti}	Severity index of dental fluorosis
FRI	Fluorosis Risk Index
FT	filled teeth
FS	filled surfaces
HR	Hazard ratio
IOM	Institute of Medicine
LOAEL	Lowest-Observed-Adverse-Effect Level
MCL	Maximum contaminant level
MCLG	Maximum contaminant level goal
MPFS	Mean percent of fluorosed surface
NF	Non-fluoridated
NIDR	National Institute of Dental Research
NIPDWR	National Interim Primary Drinking Water Regulations
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council

OR	Odds ratio
OHI-S	Simplified Oral Hygiene Index
OPF	Optimal fluoride level
OW	Office of Water
POD	Point of Departure
RfD	Reference dose
RMCL	Recommended Maximum Contaminant Level
RR	Relative Risk
SDWA	Safe Drinking Water Act
SMCL	Secondary Maximum Contaminant Level
TFI	Thylstrup and Fejerskov Index
TSIF	Tooth Surface Index of Fluorosis
UF_H	Human-to-sensitive-human intraspecies uncertainty factor
UF_A	Animal-to-human uncertainty factor
UF_S	Subchronic-to-chronic uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UL	Tolerable Upper Intake Level

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EXECUTIVE SUMMARY

In response to the 2006 National Research Council (NRC) report: *Fluoride in Drinking Water: A Scientific Review of EPA's Standards*, the U.S. EPA Office of Water (OW) began a reassessment of the dose-response associated with the effects of ingested fluoride on severe dental fluorosis and bone structure. This report is a culmination of that effort.

At low intake levels, fluoride has been shown to have therapeutic value in the prevention of dental caries; however, slightly higher levels, particularly in children during the period of enamel development can lead to dental fluorosis, a condition in which the enamel covering of the teeth fails to crystallize properly. Possible resulting problems include enamel defects ranging from barely discernable markings to brown stains and surface pitting. Prolonged high intake of fluoride, at any age, can result in skeletal fluorosis, a condition which may increase bone brittleness, and in a potential increase in risk of bone fracture. In high-dose cases, severe bone abnormalities can develop, crippling the affected individual.

After evaluation of the available literature, USEPA identified a study conducted by Dean (1942) using data from 1930 to early 1940 as that providing the most useful information on the association of fluoride intake from drinking water and the development of dental fluorosis in children. Although there are some limitations in Dean's study, its value lies in the fact that it is relatively free of confounding factors associated with the widespread use of fluoride-containing consumer products introduced after that time. Dean's study documented the prevalence and severity of dental fluorosis in 5824 children in 22 U.S. communities in 10 states where fluoride levels in drinking water ranged from 0.0 to 14.1 mg/L. The Dean study provides baseline data from which a statistically sound estimate of the threshold for severe dental fluorosis was derived. Severe dental fluorosis, a condition defined by Dean as pitting of the enamel of the teeth, was identified by the NRC (2006) as a condition that could lead to an increased risk for dental caries by diminishing the protective function of the enamel. The threshold for severe dental fluorosis in the children studied by Dean was derived statistically as the concentration in drinking water associated with the lower bound confidence limit for a prevalence rate of 0.5%; i.e., the concentration at which no more than 0.5% of exposed children in the susceptible age groups would develop any signs of severe dental fluorosis (pitting of one or more teeth). This threshold fluoride concentration (for Dean's study populations) is 1.87 mg/L.

Because data on drinking water intakes were not collected during Dean's study, OW used an indirect approach to estimate the dose resulting from the drinking water concentration associated with the calculated threshold for severe dental fluorosis. Data collected during the 1977/1978 Nationwide Food Consumption Survey on drinking water intakes and body weights of children were used to estimate fluoride doses in mg/kg/day for mean, 75^{th}, 90^{th}, and 95^{th} percentile tap water consumer groupings for children in the age categories 0.5 to 0.9 yr, 1 to 3 yr, 4 to 6 yr, 7 to 10 yr and 11 to 14 yr. This resulted in age- and drinking water consumption-specific dose estimates that ranged from 0.04 mg/kg/day to 0.19 mg/kg/day; for mean water consumption rates, the doses ranged from 0.04 to 0.09 mg/kg/day for the different age groups. These values were compared to the dose level of 0.05 mg/kg/day which had been recommended as an Adequate Intake (AI) by the Institute of Medicine (IOM, 1997) for optimal anticaries protection.

Any doses that were less than or equal to the 0.05 mg/kg/day were eliminated from consideration as the threshold dose for severe dental fluorosis. Doses greater than 0.05 mg/kg/day were considered as points of departure for the drinking water component of an oral Reference Dose (RfD) analysis. The OW selected 0.07 mg/kg/day as the contribution of drinking water to the RfD because it provided a reasonable difference in exposure (0.02 mg/kg/day) between it and the IOM (1997) AI of 0.05 mg/kg/day, considering day to day dietary variability and the uncertainties in the analysis.

Although 0.06 mg/kg/day was also one of the age-specific dose estimates derived from the Dean data, OW felt that a 0.01 mg/kg/day difference between the IOM recommendation and the dose corresponding to severe dental fluorosis was too small given the calculated range of dose estimates and uncertainties surrounding both the AI and the drinking water component of the RfD. Support for the OST estimate was provided in the data from a study of fluorosis conducted at the University of Iowa (Hong et al., 2006a) where no cases of severe dental fluorosis of the central incisors and first molars using a severe fluorosis scale based on staining and/or pitting of the enamel were noted among 579 children exposed to ≤ 0.06 mg/kg/day fluoride based on periodic exposure records provided by their parents during the period of tooth formation.

The drinking water oral RfD of 0.07 mg/kg/day was modified by OW to account for dietary fluoride intake at the time the children in the Dean (1942) study were exposed. The OW determined a dietary exposure component of 0.01 mg/kg/day based on a diet where solid foods had an average concentration of 0.5 ppm fluoride using caloric intakes, body weights, and representative dietary staples from McClure (1943). The dietary contribution of 0.01 mg/kg/day was added to the drinking water contribution of 0.07 mg/kg/day, resulting in a total oral RfD of 0.08 mg/kg/day. Confidence in the RfD is considered to be medium.

In evaluating the data available for skeletal effects of fluoride, OW did not identify any studies that were good candidates for dose- or concentration-response modeling. Unlike severe enamel fluorosis which showed a linear concentration-response, the skeletal effects display a biphasic relationship of fluoride exposure and its impact on bone strength which cannot be accommodated by currently available models. The available data led NRC and OW to conclude that exposure to concentrations of fluoride in drinking water of 4 mg/L and above are suggestive of, and appear to be positively associated with, an increased relative risk of bone fractures in susceptible populations when compared to populations exposed to 1 mg F/L. However, there are insufficient data to conclude that this increase in relative risk would also apply if comparisons were made to groups exposed to negligible fluoride concentrations or if comparisons were made based on total fluoride intake rather than on the basis of drinking water concentrations. A concentration of 4 mg/L in drinking water corresponds to a daily dose of 8 mg for person drinking 2 liters of water per day.

The NRC (2006) suggested that adults could be at risk for bone fractures at a fluoride drinking water concentration corresponding to a daily dose of 8 mg/day. The World Health Organization (2002) has chosen a higher value (≥14 mg/day) for an increased risk of bone fractures in some countries. The oral RfD of 0.08 mg/kg/day estimated by OW is equivalent to a daily dose of 5.6 mg for a 70 kg person. Compared to the NRC and WHO benchmarks, the OW proposed oral RfD of 0.08 mg/kg/day is consistent with the available data and is protective against a fluoride-related increased risk of bone fractures in adults.

1. Introduction

In 2006, the National Research Council (NRC) released the report: *Fluoride in Drinking Water: A Scientific Review of EPA's Standards*, the product of a three-year effort to examine the health effects of ingested fluoride, specifically that originating from drinking water sources. The development of the NRC (2006) report was funded by the U. S. EPA Office of Water (OW) in conjunction with the 2002/2003 review of the Maximum Contaminant Level Goal (MCLG) and the enforceable Maximum Contaminant Level (MCL) for fluoride established in 1986. This report builds on the foundation laid by NRC and focuses on examining available dose-response data for the critical noncancer effects of fluoride on teeth and bone identified by NRC (2006) as adverse health effects. This introduction provides the OW regulatory background on fluoride and summarizes the various events that preceded the NRC (2006) effort.

As summarized in 50 FR:20164, in December 1975 the U.S. EPA promulgated the National Interim Primary Drinking Water Regulations (NIPDWR) under Section 1412 of the Safe Drinking Water Act (SDWA). A Maximum Contaminant Level (MCL) for fluoride which ranged from 1.4 mg/L to 2.4 mg/L was promulgated depending on annual average ambient temperatures of the target area and became effective in June 1977. This range is twice that of the U.S. Public Health Service current recommendations for fluoridation of public water supplies, 0.7 mg/L to 1.2 mg/L (PHS 1962; see also CDC, 1995). Once effective, the MCL was challenged by the Environmental Defense Fund as not being sufficiently protective of human health. The Court of Appeals for the District of Columbia Circuit agreed with EPA [EDF v. Costle, 578 F.2d 337 (D.C. Cir. 1977) as cited in 50 FR: 20165].

Over the following eight years the MCL for fluoride continued to be discussed and debated frequently (50 FR: 20164). One of the most notable participants was the State of South Carolina which in 1981 petitioned EPA to delete fluoride from the Primary Drinking Water Regulations. South Carolina's contention was that dental fluorosis should be considered a cosmetic effect and not an adverse health effect. This position was supported by other states and several organizations including the American Medical Association and the American Dental Association. In addition, the Surgeon General at the time, C. Everett Koop, concurred that dental fluorosis, while not a desirable condition, was not an adverse health effect (Koop, 1984). Two years earlier in a letter to the EPA Deputy Administrator (Koop, 1982), Dr. Koop stated "I cannot condone the use of public water supplies that may cause undesirable cosmetic effects to teeth, just as I cannot condone the use of public water supplies below the optimum concentration because of a diminished protection against dental caries." In his 1984 letter (Koop, 1984) Dr. Koop reiterated his support of this position.

In 1985 EPA promulgated a Recommended Maximum Contaminant Level (RMCL; presently known as the Maximum Contaminant Level Goal, MCLG) of 4 mg/L (50 FR: 47142) based on a human study showing crippling skeletal fluorosis at exposures of 20 mg per day (Roholm, 1937). In 1986 EPA promulgated a MCL for fluoride of 4 mg/L and established a Secondary Maximum Contaminant Level (SMCL) of 2 mg/L (51 FR: 11396). National Secondary Drinking Water Regulations (NSDWRs or secondary standards) are non-enforceable guidelines regulating contaminants that may cause cosmetic effects (such as skin or tooth discoloration) or aesthetic effects (such as taste, odor, or color) in drinking water. EPA recommends secondary standards to

water systems but does not require systems to comply. However, states may choose to adopt them as enforceable standards.

In the early 1990s the National Research Council (NRC) was asked by the EPA to review the available data on the effects of ingested fluoride and the MCL of 4 mg/L. In its published results, NRC (1993) stated that the MCL of 4 mg/L was an appropriate interim standard but noted that further research in the areas of fluoride intake, enamel fluorosis, bone strength and fractures, and carcinogenicity was needed (NRC, 1993).

A decade later, in conjunction with the 2002/2003 EPA review of all drinking water regulations (67 FR: 19030; FR 68: 42908), the NRC was asked by the EPA to reevaluate the adequacy of the MCLG and SMCL for fluoride, focusing on health effects data published since 1993 and with a consideration of all oral sources of potential fluoride exposure (NRC, 2006). NRC (2006) concluded "In light of the collective evidence on various health end points and total exposure to fluoride, the committee concludes that EPA's MCLG of 4 mg/L should be lowered. Lowering the MCLG will prevent children from developing severe enamel fluorosis and will reduce the lifetime accumulation of fluoride into bone that the majority of the committee concludes is likely to put individuals at increased risk of bone fracture and possibly skeletal fluorosis, which are particular concerns for subpopulations that are prone to accumulating fluoride in their bones." The Committee encouraged EPA to update the risk assessment for fluoride applying current approaches for quantifying risk with consideration given to susceptible populations and the uncertainties and variability in the data (NRC, 2006).

This report provides a technical examination of the human dose-response data on dental fluorosis, skeletal fluorosis, and skeletal fractures. Critical studies were selected and, following a dose-response assessment, an oral Reference Dose is derived.

2. Summary of Hazard as Reported by NRC

NRC (2006) analyzed a large body of literature on fluoride, primarily papers published since the early 1990s, regarding the effects of fluoride on teeth; the musculoskeletal, reproductive, endocrine, gastrointestinal, renal, hepatic, and immune systems; and on the endpoints of developmental toxicity, neurotoxicity (including behavioral effects), genotoxicity, and carcinogenicity. Following this comprehensive analysis, NRC concluded that the biological tissues of most concern to fluoride exposures of 4 mg/L, the current MCLG, were the teeth and bones.

For the current RfD analysis, EPA obtained the critical references on dental fluorosis, dental cavities and bone fractures as related to fluoride exposure identified by NRC (2006). EPA also identified, retrieved, and reviewed relevant information for these effects as well as for skeletal fluorosis published during the period between 2000 to August 2010 that were not included in NRC (2006).

This section on the assessment of noncancer health effects of fluoride provides a summary of the hazard information for the following:

- Dental fluorosis
- The relationship between caries prevalence and the degree of dental fluorosis
- Skeletal fluorosis
- Bone fractures relative to fluoride exposure

A detailed discussion of the critical studies is presented in Section 3. The focus on the endpoints listed above is consistent with the NRC (2006) analysis of hazard and their charge to the OW.

2.1. Dental Enamel Fluorosis

2.1.1. Background

Fluoride has an affinity for the developing enamel because apatite crystals have the capacity to bind and integrate fluoride ion into the crystal lattice (Robinson et al., 1996). Apatite is a salt of calcium phosphate that co-crystallizes with hydroxyl, fluoride or chloride ions. Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is the primary calcium salt that is found in tooth enamel. The mineral formed in tooth enamel exposed to higher fluoride levels is fluoride containing carbonated apatite. Fluoride levels in subsurface fluorotic enamel are about 200 ppm rather than the 10-100 ppm fluoride in normal enamel. Precipitation of fluoride mineral salts at the surface of enamel results in high surface levels. This fluoride-substituted apatite has some increased resistance to bacterial acids that cause tooth decay. However, the primary function of fluoride in drinking water in reducing tooth decay is topical, primarily by the enhancement of remineralization (Fejerskov et al. 1994).

2.1.2. Measures of dental fluorosis

Excessive intake of fluoride during enamel development can lead to enamel fluorosis, a condition of the dental hard tissues in which the enamel covering of the teeth fails to crystallize properly, leading to defects that range from barely discernable markings to brown stains and

surface pitting. There are three main indices that have been used to label/grade the degree of enamel fluorosis:

- Dean's index (Dean, 1942)
- The Thylstrup-Fejerskov index (TFI; Thylstrup and Fejerskov, 1978); and
- The tooth surface index of fluorosis (TSIF; Horowitz et. al., 1984).

These indices are shown in the Tables 2-1, 2-2, and 2-3, respectively. Dean's scores are assigned on the basis of the severest form of fluorosis recorded for two or more teeth in each individual (Dean, 1942). The TFI index classifies fluorosis based on the facial surface of each tooth while the TSIF index ascribes a fluorosis score to each unrestored surface of each tooth. In the TFI and TSIF systems, fluorosis scores of 1 and 2 can be considered mild, 3 and 4 as moderate and 5-9 (TFI) or 5-7 (TSIF) as severe. Dean's index, defining severe fluorosis as a score of 4, is by far the most widely used in the examined literature.

Since there is not a one-to-one correlation among the various classification indices, careful consideration must be given when studies are reviewed to ensure a consistency of interpretation. In this report the presence of enamel pitting (discrete or confluent) will be defined as severe enamel fluorosis; the same position as that taken by NRC (2006).

Table 2-1. Clinical Criteria for Dean's Enamel Fluorosis Index

Diagnosis	Criteria
Normal (0)	The enamel presents the usual translucent semi-vitriform type of structure. The surface is smooth, glossy, and usually a pale creamy white color.
Questionable (0.5)	The enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flecks to occasional white spots. This classification is utilized when a definite diagnosis of the mildest form of fluorosis is not warranted and a classification of "normal" is not justified.
Very mild (1)	Small, opaque, paper white areas are scattered irregularly over the tooth but not involving as much as approximately 25% of the tooth surface. Frequently included in this classification are teeth showing no more than 1 to 2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars.
Mild (2)	The white opaque areas in the enamel of the teeth are more extensive but do not involve as much as 50% of the tooth.
Moderate (3)	All enamel surfaces of the teeth are affected, and surfaces subject to attrition show marked wear. Brown stain is frequently a disfiguring feature.
Severe (4)	All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be altered. The major diagnostic sign of this classification is the discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded appearance.

SOURCE: Dean (1942).

Table 2-2. Clinical Criteria and Scoring for the Thylstrup and Fejerskov Index (TFI) of Enamel Fluorosis	
Score	**Criteria**
0	Normal translucency of enamel remains after prolonged air-drying.
1	Narrow white lines corresponding to the perikymata (transverse ridges on the exposed surface of the surface of the enamel of permanent teeth).
2	Smooth surfaces: More pronounced lines of opacity that follow the perikymata. Occasionally confluence of adjacent lines. Occlusal surfaces: Scattered areas of opacity < 2 mm in diameter and pronounced opacity of cuspal ridges.
3	Smooth surfaces: Merging and irregular cloudy areas of opacity. Accentuated drawing of perikymata often visible between opacities. Occlusal surfaces: Confluent areas of marked opacity. Worn areas appear almost normal but usually circumscribed by a rim of opaque enamel.
4	Smooth surfaces: The entire surface exhibits marked opacity or appears chalky white. Parts of surface exposed to attrition appear less affected. Occlusal surfaces: Entire surface exhibits marked opacity. Attrition is often pronounced shortly after eruption.
5	Smooth and occlusal surfaces: Entire surface displays marked opacity with focal loss of outermost enamel (pits) < 2 mm in diameter.
6	Smooth surfaces: Pits are regularly arranged in horizontal bands < 2 mm in vertical extension. Occlusal surfaces: Confluent areas < 3 mm in diameter exhibit loss of enamel. Marked attrition.
7	Smooth surfaces: Loss of outermost enamel in irregular areas involving less than half of entire surface. Occlusal surfaces: Changes in morphology caused by merging pits and marked attrition.
8	Smooth and occlusal surfaces: Loss of outermost enamel involving more than half of surface.
9	Smooth and occlusal surfaces: Loss of main part of enamel with change in anatomic appearance of surface. Cervical rim of almost unaffected enamel is often noted.

SOURCE: Thylstrup and Fejerskov (1978).

Table 2-3. Clinical Criteria and Scoring for the Tooth Surface Index of Fluorosis (TSIF)	
Score	**Criteria**
0	Enamel shows no evidence of fluorosis.
1	Enamel shows definite evidence of fluorosis—namely, areas with parchment-white color that total less than one-third of the visible enamel surface. This category includes fluorosis confined only to incisal edges of anterior teeth and cusp tips of posterior teeth ("snowcapping").
2	Parchment-white fluorosis totals at least one-third, but less than two-thirds, of the visible surface.
3	Parchment-white fluorosis totals at least two-thirds of the visible surface.
4	Enamel shows staining in conjunction with any of the preceding levels of fluorosis. Staining is defined as an area of definite discoloration that may range from light to very dark brown.
5	Discrete pitting of the enamel exists, unaccompanied by evidence of staining of intact enamel. A pit is defined as a definite physical defect in the enamel surface with a rough floor that is surrounded by a wall of intact enamel. The pitted area is usually stained or differs in color from the surrounding enamel.
6	Both discrete pitting and staining of the intact enamel exist.
7	Confluent pitting of the enamel surface exists. Large areas of enamel may be missing and the anatomy of the tooth may be altered. Dark-brown stain is usually present.

SOURCE: Horowitz et al. (1984).

2.1.3. Adversity of dental fluorosis

There has been much debate over the past several decades whether enamel fluorosis is an adverse health effect (see Section 1 of this report for an overview). As noted in Section 1, NRC (2006) reached somewhat different conclusions from prior panels relative to the health impact of dental fluorosis. They concurred with previous panels that mild and moderate dental fluorosis are cosmetic; however, they felt that severe fluorosis had an adverse health impact because it damaged the enamel and reduced its efficacy in protecting the teeth from decay. Dental decay is the destruction of the outer coating of the tooth (enamel) through the action of bacteria in the dental plaque. If decay is untreated it spreads into the inner portion of the tooth causing a toothache and sometimes infection (an abscess). Although not all members of the panel agreed with the classification of severe fluorosis as an adverse health effect, all agreed that it should be avoided. Evidence cited by NRC in support of their conclusion included the following statements:

- "[T]he most severe forms of fluorosis manifest as heavily stained, pitted, and friable enamel that can result in loss of dental function" (Burt and Eklund, 1999).

- "The degree of porosity (hypermineralization) of such teeth results in a diminished physical strength of the enamel, and parts of the superficial enamel may break away . . . In the most severe forms of dental fluorosis, the extent and degree of porosity within the enamel are so severe that most of the outermost enamel will be chipped off immediately following eruption" (Fejerskov et al., 1990).

- "With increasing severity, the subsurface enamel all along the tooth becomes increasingly porous… the more severe forms are subject to extensive mechanical breakdown of the surface" (Aoba and Fejerskov, 2002).

- " . . the most severe forms of dental fluorosis might be more than a cosmetic defect if enough fluorotic enamel is fractured and lost to cause pain, adversely affect food choices, compromise chewing efficiency, and require complex dental treatment" (NRC, 1993).

NRC (2006) concluded that severe enamel fluorosis occurs in approximately 10% of children in communities with water fluoride concentrations at or near the current MCLG of 4 mg/L. An examination of the dose response of severe dental fluorosis is provided in Section 3 of this report. NRC (2006) recommended that "Additional studies, including longitudinal studies, of the prevalence and severity of enamel fluorosis should be done in U.S. communities with fluoride concentrations higher than 1 mg/L. These studies should focus on moderate and severe enamel fluorosis in relation to caries and in relation to psychological, behavioral, and social effects among affected children, their parents, and affected children after they become adults."

2.1.4. Relationship between severe dental fluorosis and dental caries

NRC (2006) considered the relationship between severe dental fluorosis and increased dental caries to be a plausible one. They found that there is some evidence that severe fluorosis can lead to a loss of the structural integrity of teeth, leading to an increase in dental caries. However, the evidence is mixed as will be discussed in Section 3 of this report.

2.2. Impact of Fluoride Exposure on Bone Structure

The two most studied effects of fluoride on the musculoskeletal system are skeletal fluorosis and bone fracture (NRC, 2006). As described in NRC (2006), fluoride in the bones of mammals exists in two forms. The less dominant form is a rapidly exchangeable form that associates with the surfaces of the hydroxyapatite crystals of the mineralized component of bone and does not require bone resorption for release to extracellular fluid. Hydroxyapatite is the mature form of a calcium phosphate insoluble salt that is deposited in and around the collagen fibrils in skeletal tissue. The predominant form of fluoride in bone resides within the hydroxyfluoroapatite crystals within the bone matrix rather than on its surfaces.

2.2.1. Skeletal fluorosis

Skeletal fluorosis, a bone and joint condition, occurs following prolonged exposure to high concentrations of fluoride (see Section 3 of this report for a discussion of critical studies). As summarized in NRC (2006), skeletal fluorosis is categorized into one of four stages: a preclinical stage and three clinical stages that increase in severity. The most severe stage (clinical stage III) historically has been referred to as the "crippling" stage. At stage II, mobility is not significantly affected, but it is characterized by sporadic pain, stiffness of joints, and osteosclerosis of the pelvis and spine. As NRC has noted, very few epidemiological studies of skeletal fluorosis in the United States have been documented, especially when the source is restricted to water consumption alone. In a retrospective study involving 170,000 radiological examinations of people in Texas and Oklahoma where many communities had water fluoride levels above 4 mg/L, Stevenson and Watson (1957) diagnosed only 23 cases of fluoride osteosclerosis in people consuming water with 4 to 8 mg F/L and no cases in people exposed to lower concentrations of fluoride in drinking water. The paper stated, without providing details, that these 23 individuals did not have unusual amounts of arthritis or back stiffness given their age (44 to 85), but 11 did have bone density of an extreme degree and nine had more than minimal calcification of pelvic ligaments (four had Grade 2 and five had Grade 4 calcification in either the sacrotuberous or sacrospinous ligaments). Based on the information presented, it is reasonable to assume that no cases of stage III skeletal fluorosis existed. However, NRC concluded that, based on the publication contents, it was not possible to determine if there were any cases that could be characterized as stage II skeletal fluorosis.

NRC (2006) concluded that on the basis of existing epidemiologic literature, stage III skeletal fluorosis appears to be a rare condition in the U.S. and that the occurrence of stage II skeletal fluorosis at drinking water fluoride levels of 4 mg/L could not be determined. To fill this data gap, they recommended that a systematic study of stage II and stage III skeletal fluorosis be conducted to clarify the relationship of fluoride ingestion, fluoride concentration in bone, and clinical symptoms.

2.2.2. Fluoride and bone fractures

With respect to bone fractures, NRC (2006) notes that inducing a permanent alteration of skeletal mass in adults is difficult because bone has an innate mechanism for self correction. This mechanism involves the formation of bone by osteoblasts and the resorption of bone by

osteoclasts. Fluoride is known to stimulate osteoblast proliferation and may also affect oseoclastogenesis.

There have been numerous clinical trials of fluoride compounds used in the treatment of osteoporosis in conjunction with hormone and calcium supplements. As noted by NRC (2006) the evidence is convincing that the effect of fluoride, at therapeutic doses, is an increase in bone density with 30 mg/day the lowest dose of sodium fluoride to show a clear increase in bone density. According to NRC, the measurement of bone strength in humans is not easy to determine, but animal studies provide some help with this determination.

Some animal studies report a biphasic effect of fluoride on bone strength (Beary, 1969; Rich and Feist, 1970; Turner et al., 1992). When the concentrations of fluoride in rat bone were less than 1200 mg/kg, Turner et al. (1992) found that bone strength was increased but at concentrations of 6000 to 7000 mg/kg bone strength was decreased. In studies conducted by Yan et al. (2007), fluoride exposure (50 or 100 ppm in drinking water for three weeks) led to dose-dependent increases in proximal tibia trabecular and vertebral bone mass density in C57BL/6J mice but not in C3H/HeJ mice. Osteoclast potential, in situ trabecular osteoclast numbers, and serum markers for osteoclastogenesis were observed in the latter strain, but not in C57BL/6J mice, suggesting genetically controlled strain differences.

Rabbit studies which provide a better comparison to humans due to a similar bone resorption physiology, suggest that a high concentration of fluoride in drinking water (100 mg F/L) might diminish bone strength through direct changes on bone mineral and mineralization resulting in denser bone and increased hardness (Turner et al., 1997; Chachra et al., 1999). However, the two rabbit studies tested only one fluoride concentration, 100 mg/L, equivalent to about 8 mg/kg/day, and it is not known whether such effects occur in humans (NRC, 2006, p. 143).

NRC (2006) concluded that "the weight of evidence supports the conclusion that lifetime exposure to fluoride at drinking water concentrations of 4 mg/L and higher is likely to increase fracture rates in the population, compared with exposure to fluoride at 1 mg/L, particularly in some susceptible demographic groups that are prone to accumulating fluoride into their bones." The committee found "that the available epidemiologic data for assessing bone fracture risk in relation to fluoride exposure around 2 mg/L is suggestive but inadequate for drawing firm conclusions about the risk or safety of exposures at that concentration." Accordingly, the committee recommended a more complete analysis of communities consuming water with fluoride at 2 and 4 mg/L. See Section 3 for a detailed discussion of some of the available studies.

3. Selection of Critical Studies for Dose-Response Analysis of Fluoride Drinking Water Data

Recent U.S. epidemiological studies evaluating the relationship between the concentration of fluoride in drinking water and the prevalence and severity of dental fluorosis, dental caries, and stage II skeletal fluorosis are complicated by several confounding factors, including the widespread use of fluoride-containing dentifrices and mouth rinses, the use of fluoride supplements in early childhood, and the potential presence of fluoride in processed foods and beverages (a result of the use of fluoridated water in the preparation of these products). Consequently, total fluoride intake can be difficult to quantify in dose-response analyses based on water intake data from these studies. In contrast, epidemiological studies conducted in the U.S. prior to the introduction of such fluoride products can be expected to be relatively free of these confounding factors. Therefore, historical epidemiological studies, if conducted according to standardized and acceptable protocols, are preferred for evaluating the potential effects of ingested fluoride.

3.1. Dental Fluorosis

Not all epidemiological studies of dental fluorosis are equally useful for dose-response modeling. The NRC (2006) notes that, in the evaluation of severe dental fluorosis, "it is more informative to know the proportion of a population who have any teeth with dark staining and pitting than the proportion of all teeth or all tooth surfaces that have these most severe manifestations of enamel fluorosis." Epidemiological studies that have focused on individual subject effects are therefore more relevant to evaluating severe dental fluorosis than those studies designed to focus on individual teeth or tooth surfaces. Furthermore, because "teeth most frequently affected by enamel fluorosis are posterior teeth" (NRC, 2006); studies examining only anterior teeth may not provide a complete picture as to the severity of fluorosis in the study population. The NRC (2006) cites Den Besten (1999) in noting that "Because the severity of fluorosis is related to the duration, timing, and dose of fluoride intake, cumulative exposure during the entire maturation stage, not merely during critical periods of certain types of tooth development, is probably the most important exposure measure to consider when assessing the risk of fluorosis." The cumulative effects of fluoride exposure would be expected to be seen most clearly in children 12-14 yrs old at a time when most of the permanent dentition is fully erupted (Dean, 1942); consequently, studies evaluating younger age groups may not provide sufficient data for a dose-response analysis.

Other studies that may not be ideal for use in fluorosis dose-response modeling include the following: 1) those in which fluorosis is indicated only as being present or absent; 2) those in which fluorosis is expressed as a mean index value for the entire population; 3) those in which the percent occurrence of both moderate and severe fluorosis are combined; and 4) those in which the exposure groups encompass fairly wide ranges of fluoride concentrations and/or are open-ended (e.g., highest exposure group $\geq$ 2 mg F/L). Such data cannot be used to identify the threshold for severe fluorosis.

In evaluating studies on dental fluorosis, other factors, which must be taken into consideration include: 1) the effects of climate and varying drinking water intake rates; 2) the possible

presence of excessive amounts of dietary fluoride; 3) low levels of calcium intake in the study population (which may enhance fluoride uptake from drinking water and other sources); 4) the use of surface water or very shallow wells in which the fluoride levels may fluctuate randomly or seasonally; and 5) various sources of industrial pollution, such as coal burning, which may increase exposures to fluoride.

The NRC (2006) also cautions that not all enamel defects are caused by fluoride. Citing Curzon and Spector (1977) and Cutress and Suckling (1990), NRC states that "Mottling unrelated to fluoride has been suggested to be due to malnutrition, metabolic disorders, exposure to certain dietary trace elements, …or physical trauma to the tooth." Furthermore, there is some evidence that "hypobaric hypoxia that occurs at high altitudes is associated with bilaterally symmetrical and diffuse disturbances in enamel mineralization that may be mistaken for fluorosis."

3.1.1. Critical study for severe dental fluorosis

All the factors mentioned above must be taken into consideration when identifying epidemiological studies that may be useful for evaluating dose-response relationships for dental fluorosis and, in particular, for severe dental fluorosis defined by discrete areas of pitting in the enamel.

The NRC (2006) examined available data on the prevalence of severe enamel fluorosis (Dean's Index) in the U.S. and found a clear correspondence with increasing water fluoride concentrations (Fig. 3-1). NRC (2006) noted, however, that "Because of the wide variability in the methods and populations, and the lack of independence when a given study provided more than one result, the estimates were not subjected to formal statistical analysis." The trend shown in Fig. 3-1 suggests a threshold for severe fluorosis at about 2 mg F/L.

The available epidemiological studies that included data on dental fluorosis and dental caries were carefully evaluated by the authors of the current report and screened to identify those studies that might provide the most useful quantitative data to further define the threshold for severe fluorosis. The conclusion reached was that one of the most relevant studies was that conducted by Dean (1942). Dean (1942) reported the results of surveys documenting the prevalence and severity of dental fluorosis in 22 U.S. communities in 10 states where fluoride levels in drinking water ranged from 0.0 to 14.1 mg/L (Table 3-1). Dean (1942) is one of the earliest studies of this type using a standardized protocol for reporting dental fluorosis. Dean's work was done in the late 1930's and the early 1940's and is relatively free of confounding factors associated with the widespread use of fluoride-containing consumer products introduced after that time. A total of 5824 children were examined for dental fluorosis in the Dean (1942) study. The children were primarily in the age range of 9 to 14 yrs old and/or in school grades 2-12 (about 6–17 yrs old). The dental fluorosis status of each participant in the study was recorded according to Dean's Index of Fluorosis (see Section 2.1.2 for description), a categorical scoring system in which 0 represents no evidence of fluorosis; 0.5, questionable; 1, very mild; 2, mild; 3, moderate; and 4, severe fluorosis (pitting required). A child was classified based on the severest form of dental fluorosis on two or more teeth. The frequency of occurrence for each score was computed within each study population (Table 3-1). The requirement for two or more teeth to exhibit externally apparent pitted enamel increases confidence in the classification for severe dental fluorosis.

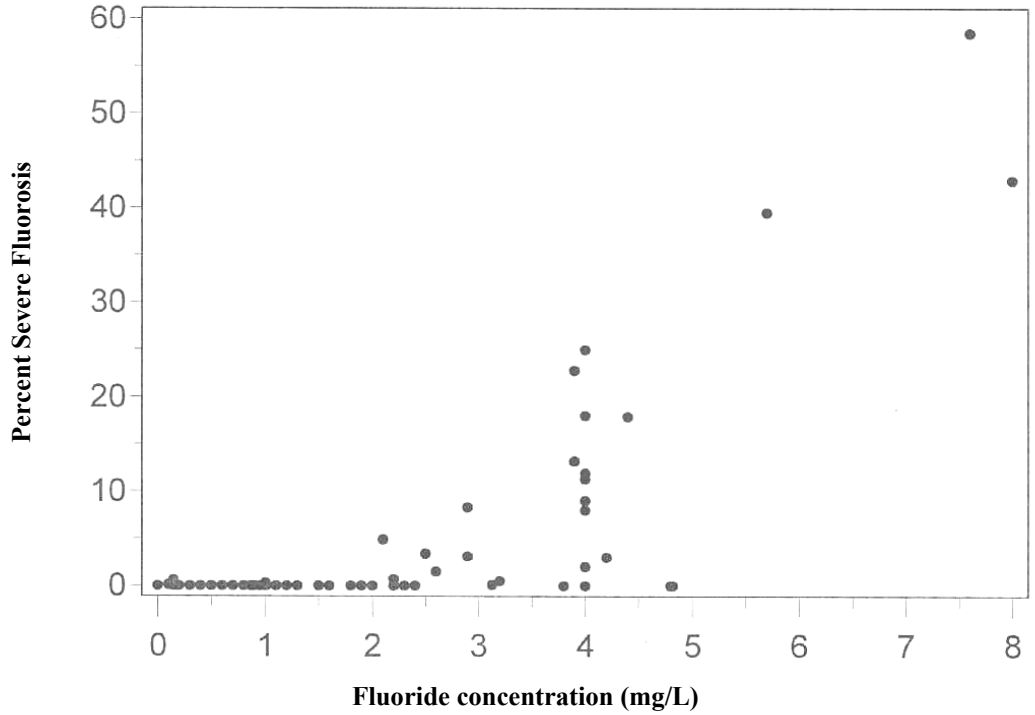

Figure 3-1. Relationship between severe fluorosis and fluoride in drinking water (U.S. studies) (NRC, 2006).

The strengths of this study lie in:

- Its large scale, and wide range of fluoride concentrations and consistency of results across several different communities
- The fact that the same individuals were involved in the measurements of fluorosis and fluoride in drinking water
- Inclusions of several geographic areas across the U.S.
- An acceptable standardized method was used to categorize fluorosis
- A clear dose-response was observed for severe dental fluorosis
- A requirement for continuous residence in the community
- For the most part, the children examined were mostly in the most appropriate age group, such that the majority of their permanent teeth had erupted. The towns where some children were younger than 12 years were the high fluoride towns where severe fluorosis was most likely to occur making the number of erupted of permanent teeth less important to the detection of severe fluorosis on at least 2 teeth.

- Fluoride concentrations in the drinking water were the average of 12 monthly samples for 18 of the 22 towns. The 4 concentrations based on a single measurement were the 4 localities with the highest fluoride concentrations
- Home units for water treatment that might remove fluoride were not widely available
- Other potential sources of fluoride exposures, such as dentifrices and supplements, were not an issue

Table 3-1. Percent Distribution of Fluorosis in Populations Studied by Dean (1942)

Town	No.	Age (yr)	F (mg/L)[a]	Dean's Index					
				0	0.5	1	2	3	4
Waukegan, IL	423	12-14	0.0	97.9	1.9	0.2	0.0	0.0	0.0
Michigan City, IN	236	12-14	0.1	97.5	2.5	0.0	0.0	0.0	0.0
Zanesville, OH	459	12-14	0.2	85.4	13.1	1.5	0.0	0.0	0.0
Lima, OH	454	12-14	0.3	84.1	13.7	2.2	0.0	0.0	0.0
Marion, OH	263	12-14	0.4	57.4	36.5	5.3	0.8	0.0	0.0
Elgin, IL	403	12-14	0.5	60.5	35.3	3.5	0.7	0.0	0.0
Pueblo, CO	614	12-14	0.6	72.3	21.2	6.2	0.3	0.0	0.0
Kewanee, IL	123	12-14	0.9	52.8	35.0	10.6	1.6	0.0	0.0
Aurora, IL	633	12-14	1.2	53.2	31.8	13.9	1.1	0.0	0.0
Joliet, IL	447	12-14	1.3	40.5	34.2	22.2	3.1	0.0	0.0
Elmhurst, IL	170	12-14	1.8	28.2	31.8	30.0	8.8	1.2	0.0
Galesburg, IL	273	12-14	1.9	25.3	27.1	40.3	6.2	1.1	0.0
Clovis, NM	138	9-11	2.2	13.0	16.0	23.9	35.4	11.0	0.7
Colorado Springs, CO	404	12-14	2.6	6.4	19.8	42.1	21.3	8.9	1.5
Plainview, TX	97	9-12	2.9	4.1	8.3	34.0	26.8	23.7	3.1
Amarillo, TX	289	9-12	3.9[b]	3.1	6.6	15.2	28.0	33.9	13.2
Conway, SC	59	9-11	4.0	5.1	6.7	20.4	32.2	23.7	11.9
Lubbock, TX	189	9-12	4.4	1.1	1.1	12.2	21.7	46.0	17.9
Post, TX	38	grade 4-6[c]	5.7[d]	0.0	0.0	0.0	10.5	50.0	39.5
Chetopa, KS	65	grade 3-12[c]	7.6[d]	0.0	0.0	9.2	21.5	10.8	58.5
Ankeny, IA	21	grade 2-12[c]	8.0[d]	0.0	0.0	0.0	9.5	47.6	42.8
Bauxite, AK	26	14-19	14.1[d]	0.0	0.0	3.9	3.9	38.5	53.8

SOURCE: Modified from Dean (1942).

[a] Analytical technique used to measure F in water samples was that of Elvove (1933), who utilized a colorimetric method employing a zirconium-alizarin reagent. Dean (1942, p. 29) reported that the sensitivity of the method "may be considered as about 0.1 parts per million."

[b] "Subject to a possible correction to 4.2 mg F/L during susceptible period of age group examined" (no other explanation given by Dean, 1942).

[c] Grades 4–6 include ages of approximately 10–12 years; grades 3–12 include ages of approximately 8–17 years and grades 2–12, ages 7–17 years.

[d] Single determination, all others arithmetical mean of 12 consecutive monthly samples.

The Dean (1942) study also has a number of weaknesses; they include:

- No information was provided on the occurrence of dental caries in the study populations
- No information was provided on potential confounding factors, such as unique dietary intakes of fluoride

- Variability in examiner reliability was not addressed
- The size of the study populations at several of the higher water fluoride levels was relatively small
- Only white children were included (schools were segregated); therefore, ethnicity or racial differences in susceptibility were not addressed
- Differences in dental hygiene, dietary intakes, body weights and puberty/hormonal condition (e.g., age of menarche) could complicate extrapolation of results to present day populations
- Not all climate zones within the U.S. were included
- The data were not analyzed statistically
- Data on drinking water intakes were not collected
- The specific teeth responsible for the fluorosis scores were not identified
- The analytical method used to analyze for fluoride in drinking water was not as sensitive and free from interfering substances as more modern methods using spectrophotometry or a fluoride ion-specific electrode

The analytical technique used to measure fluoride in the Dean study was the zirconium-alizarin method with visual color comparison to standard solutions (Elvove, 1933). Although this method is no longer used, according to Megregian and Maier (1952) the regent was sensitive to small increments of fluoride over a range of 0.0 to 3.0 ppm, the critical range for assessing the threshold for severe fluorosis, and within this range the response was consistent with Beer's law. The sensitivity of the method was reported to be about 0.1 ppm in Dean (1942); and a concentration as low as 0.1 ppm is given in the tabulated summary of the data (Table 1 in Dean, 1942); however, in an earlier paper describing the method, Elvove (1933), reported a sensitivity of 0.2 ppm.

The analytical method has been reported to be sensitive to interfering substances such as aluminum, bicarbonate and sulfate (Megregian and Maier, 1952). However, examination of water quality data from the same time period for several of the key towns used in Dean's study indicated that potentially interfering substances were not at concentrations that would cause analytical problems (Dean and Elvove, 1936, 1937). The fluoride concentrations presented by Dean were, for all but four towns, averages of 12 consecutive monthly samples which, to some degree, adjusted for seasonal variation and potential errors in any one analysis. In a few cases, later studies of the water from the same towns found fluoride levels reasonably similar to those listed by Dean (1942). Therefore, in the absence of any evidence to suggest otherwise, it is assumed here that the sensitivity of the method is 0.1 ppm as reported by Dean (1942).

Because of its size and comprehensive nature, the Dean study provides baseline data from which a statistically-sound estimate of the threshold for severe fluorosis (Dean's Index score of 4) can be derived. The results shown in Table 3-1 indicate a dose-response relationship for both the moderate and severe levels of fluorosis, with the threshold for severe fluorosis occurring at approximately 2 mg F/L drinking water (see Section 4 for dose-response analysis).

3.1.2. Supplemental U.S. studies on dental fluorosis

There are a number of other U.S. epidemiological studies that provide information on the occurrence and severity of dental fluorosis that can be used to supplement and compare with the Dean (1942) data. The most relevant of these are summarized below.

Galagan and Lamson (1953): Galagan and Lamson (1953) evaluated the occurrence and severity of dental fluorosis in 726 children (9-16 yr-olds) residing in six Arizona towns where the average annual temperature was 70°F. Only children who had consumed water from the common municipal supply continuously from birth through their ninth year were included in the study. The fluoride levels in the water supplies of the towns ranged from 0.4 to 1.2 mg/L. Dean's scoring system was used to categorize fluorosis. Table 3-2 shows that prevalence and severity of fluorosis was higher in the study populations from towns with higher levels of fluoride in drinking water. Notably, severe fluorosis occurred in 1 of 95 children (1%) examined in Chandler where the fluoride level was 0.8 mg/L, and in 2 of 70 children (2.9%) examined in Florence where the fluoride level was 1.2 mg/L. Galagan and Lamson (1953) reported that their study populations may have had an additional source of fluoride through consumption of beans, a dietary staple. The beans are normally boiled for long periods of time in water, which may have resulted in the absorption of fluoride from the water. Galagan and Lamson (1953), however, did not estimate the amount of fluoride that may have been ingested through this pathway.

Because the Galagan and Lamson (1953) study used a very similar protocol to that used by Dean (1942), it is a useful source for additional dental fluorosis data for communities in hot arid climate zones. The results differ from those of Dean (1942) in that there were three cases of severe dental fluorosis in towns where the fluoride drinking water concentration was ≤ 1.2 mg/L even though continuous residence was a requirement for inclusion in the study.

Table 3-2. Percent Distribution of Fluorosis in Arizona Populations Studied by Galagan and Lamson (1953)

Town	No.	F (mg/L)	Dean's Index					
			0	0.5	1	2	3	4
Yuma	82	0.4	64.6	31.7	2.4	1.2	0	0
Tempe	113	0.5	52.2	38	8.8	0.9	0	0
Tucson	316	0.7	38	45.3	12	3.2	1.6	0
Chandler	95	0.8	42.1	38.9	9.5	6.3	2.1	1.1
Casa Grande	50	1.0	14	38	30	18	0	0
Florence	70	1.2	24.3	20	25.7	14.3	12.9	2.9

SOURCE: Modified from Galagan and Lamson (1953).

Richards et al. (1967): In the early 1960's, the California Department of Public Health began a 5-year program to evaluate the correlation between fluoride concentration in drinking water, dental caries, dental fluorosis, and temperature. Dental caries and fluorosis were evaluated in 9000 children, 12–14 years old, from 83 towns, representing 6 different fluoride concentration ranges and three different temperature zones in the states of California, Texas, Colorado, Arizona, New Mexico and Illinois. The full data set from the published paper showing the results by temperature zone are presented in Section 3.1.4.1. The combined results for all temperature ranges, re-grouped into three fluoride concentration ranges, are shown in Table 3-3.

Table 3-3. Percentage of Children by Fluorosis Diagnosis in the Study of Richards et al. (1967)			
Degree of Fluorosis	**Fluoride Concentration**		
	≤0.7 ppm (N = 3818)[a]	**0.8-1.3 ppm (N = 2334)[a]**	**>1.8 ppm (N =1088)[a]**
< Moderate	99.95	97.77	80.88
Moderate	0.05	2.14	13.42
Severe	0	0.09	5.70

SOURCE: Modified from Richards et al. (1967).
[a]Number of children for whom the diagnosis was made.

Eklund et al. (1987): Dental fluorosis and coronal caries were evaluated in adult lifelong residents (age ~30–60 years old) of two neighboring New Mexico towns (Eklund et al., 1987). One town (Lordsburg, N = 164), had naturally fluoridated drinking water containing 3.5 mg F/L. The second town (Deming, N = 151) had naturally fluoridated drinking water containing 0.7 mg F/L. Subjects in both study populations were those who had been born in the community and had consumed city water during their first six years of life as well as through most or all of their adulthood. Fluorosis severity was evaluated using Dean's 1942 classification scheme, but the qualitative description of severe fluorosis specified if the pitting was discrete or confluent (the latter was considered "very severe" and given a score of 5). Residents of Lordsburg had much more severe and very severe fluorosis (76.2 %) than residents of Deming (0%), as shown in Table 3-4. The study authors noted that the two populations, living only 60 miles apart, have very similar socioeconomic and cultural characteristics (74-89% Hispanic), suggesting that any non-drinking water fluoride exposures such as dietary fluoride were similar. Furthermore, the age of the population would probably have precluded the use of fluoride supplements or dentifrices during the most sensitive period for dental fluorosis; therefore, it is likely that the results reported are due primarily to fluoride in drinking water.

Table 3-4. Percent Distribution of Fluorosis in New Mexico Populations Studied by Eklund et al. (1987)									
Town	**F (mg/L)**	**No.**	**Dean's Index**						
			0	**0.5**	**1**	**2**	**3**	**4**	**5**
Deming	0.7	151	68.9	15.2	11.3	1.3	3.3	0	0
Lordsburg	3.5	164	0	0	0.6	0.6	22.6	38.4	37.8

Szpunar and Burt (1988): In studies conducted on schoolchildren aged 6–12 yrs in four Michigan communities, Szpunar and Burt (1988) reported that water fluoride levels up to 1.2 mg/L were not associated with any cases of severe fluorosis, and, in fact, the highest TSIF score recorded was 2.

Driscoll et al. (1983); Horowitz et al. (1984); Driscoll et al. (1986); Heifetz et al. (1988); Selwitz et al. (1995); and Selwitz et al. (1998): This series of studies began as a cross-sectional survey of dental fluorosis and dental caries in 807 schoolchildren, ages 8–16 yr old, residing in seven Illinois communities where the water supplies contained natural fluoride at levels of 1.06, 2.08, 2.84, 2.89, 3.77, 3.84, or 4.07 mg/L (Driscoll et al., 1983). The recommended optimal level of fluoride in drinking water for that geographic area was reported by the study authors to be 1 mg/L, and the communities were grouped together according to whether their fluoride level was optimal (1 mg/L), 2x optimal (2 mg/L), 3x optimal (3 mg/L) or 4x optimal

(4 mg/L). Dean's Index was used to assess fluorosis. The percent distributions of children with fluorosis in each fluoride group are shown in Table 3-5.

The prevalence of dental fluorosis was characteristically low in the optimal fluoride area. Substantial increases in fluorosis occurred in the above-optimal fluoride areas, with the condition being most pronounced in the 4x optimal area (3.77–4.07 mg F/L). A clear dose-response can be seen in the occurrence of severe fluorosis. The children in this study were examined in 1980; therefore, they were born in the years of 1964 to 1972. The extent to which they might have been exposed to non-drinking water fluoride was not evaluated for the study populations in total.

Eight children living in an optimal fluoride area exhibited moderate to severe fluorosis, and the study authors questioned the parents of these children to determine if there had been any other sources of fluoride exposure. The questions covered such factors as erroneous residence history, prolonged absence from the community, use of water from sources other than the community supply, consumption of high-fluoride infant formula, use of dietary fluoride supplements, and ingestion of unusual amounts of fluoride dentifrices. The information collected on other possible sources of fluoride exposure could not account for the moderate to severe fluorosis seen in this group of children.

Table 3-5. Percent Distribution of Fluorosis in Illinois Populations Studied by Driscoll et al. (1983)

Fluoride Level (mg/L)	N	Dean's Index					
		0	0.5	1	2	3	4
1.06	336	56.0	29.5	7.4	4.8	1.8	0.6
2.08	143	18.2	28.7	23.1	16.8	8.4	4.9
2.84–2.89	192	22.9	26.0	15.1	19.8	7.8	8.3
3.77–4.07	136	12.5	15.4	16.9	25.0	7.4	22.8

In a continuation of this study, Driscoll et al. (1986) compared the occurrence of dental fluorosis in one of the seven Illinois towns (Kewanee; fluoride level in drinking water 1.06 mg/L), with that in four towns in Iowa where the fluoride levels were negligible (<0.3 mg/L). The results are shown in Table 3-6. Fluorosis was clearly more prevalent in Kewanee, Illinois.

Table 3-6. Percent Distribution of Fluorosis in Midwest U.S. Populations Studied by Driscoll et al. (1986)

Fluoride Level (mg/L)	N	Dean's Index					
		0	0.5	1	2	3	4
<0.3[a]	316	93.0	4.1	1.9	1.0	0	0
1.06[b]	336	56.0	29.5	7.4	4.8	1.8	0.6

[a]Belle Plaine, Durant, Marengo, and Missouri Valley, Iowa.
[b]Kewanee, Illinois.

In 1988, Heifetz et al. reported on a 5-yr follow-up study that was conducted on 8–10 yr old and 13–15 yr old children residing in the same seven Illinois towns studied by Driscoll et al. (1983, 1986, and others). The study population was divided into three cohorts:

- Cohort 1 (13–15 year olds in 1980) whose developing teeth were at risk for dental fluorosis from 1965–72;

- Cohort 2 (8–10 year olds in 1980 and 13–15 year olds in 1985) who were at risk from 1970–77; and
- Cohort 3 (8–10 year olds in 1985) who were at risk from 1975–82.

Heifetz et al. (1988) used the Driscoll et al. (1983) data for the 13–15 yr old children in Cohort 1. The children in Cohorts 2 and 3 were examined in 1985. The tooth surface index of fluorosis (TSIF) was used to evaluate the occurrence and severity of dental fluorosis; therefore, the data are not exactly comparable to the results of the Driscoll et al. (1983 and 1986) studies which used Dean's scoring system (i.e., the Heifetz et al. results are expressed in the terms of tooth surfaces rather than individuals). In the TSIF scoring system, a score of 5 and above includes pitting of the enamel. The results are shown in Table 3-7.

Table 3-7. Percent Distribution of TSIF Scores for all Permanent Tooth Surfaces in Populations Studied by Heifetz et al. (1988)

Group	No.	TSIF Score							
		0	1	2	3	4	5	6	7
8–10 yr-olds – 1980									
Optimal[a]	113	81.2	14.8	2.3	1.6	0.0	0.1	0.0	0.0
2x Optimal	61	53.0	33.0	6.9	6.8	0.2	0.2	0.0	0.0
3x Optimal	82	48.5	30.6	10.9	8.1	0.5	1.0	0.1	0.3
4x Optimal	59	30.3	28.5	17.1	19.7	0.3	2.8	0.1	1.2
8–10 yr-olds – 1985									
Optimal[a]	156	72.0	20.6	5.6	1.8	0.0	0.1	0.0	0.0
2x Optimal	102	48.0	30.4	11.6	8.7	0.0	1.3	0.0	0.0
3x Optimal	112	48.0	29.4	12.3	8.2	0.2	1.5	0.0	0.4
4x Optimal	62	24.2	32.2	18.7	19.7	0.6	3.1	0.1	1.4
13–15 yr-olds – 1980									
Optimal[a]	111	88.6	9.1	1.5	0.8	0.0	0.0	0.0	0.0
2x Optimal	39	61.7	25.4	7.8	5.0	0.0	0.1	0.0	0.0
3x Optimal	50	54.0	21.6	13.7	9.6	0.2	0.7	0.0	0.1
4x Optimal	34	36.9	25.6	16.7	18.6	0.3	1.3	0.1	0.5
13–15 yr-olds – 1985									
Optimal[a]	94	70.6	21.6	4.9	2.8	0.1	0.0	0.0	0.0
2x Optimal	23	33.5	32.5	18.6	13.8	0.3	1.3	0.0	0.0
3x Optimal	47	30.8	34.9	18.2	13.6	0.3	1.2	0.1	0.9
4x Optimal	29	22.5	30.8	18.8	22.1	0.5	3.9	0.0	1.5

[a] "Optimal" is defined by the study authors as 1 mg F/L for the study region (Midwest U.S.).

Heifetz et al. (1988) reported that the study populations in the 2x optimal fluoride group (1.95–2.08 mg F/L) appeared to be approaching a critical threshold for producing severe fluorosis in that 7.6% of labial surfaces of maxillary anterior teeth of 13–15 years olds examined in 1985

exhibited severe fluorosis. The study authors noted that beginning in the early 1970's, there were other possible sources of exposure to fluoride, including commercial infant formula, processed foods, fluoride dentifrices, and fluoride supplements.

A ten-year follow-up study of 8–10 yr old and 14–16 yr old children from these same towns was conducted by Selwitz et al. (1995). The evaluations took place in 1990, and the TSIF method was used to evaluate fluorosis (Note: information on the percentage distribution of individuals in each fluorosis category, rather than tooth surfaces, was not available from the study authors). The TSIF scores for the 8–10 yr olds are shown in Table 3-8 and those for the 14–16 yr olds are shown in Table 3-9, with comparisons to the results from the 1980 and 1985 studies.

Table 3-8. Comparison of TSIF Scores and Mean Percent Fluorosed Surfaces for 8–10 yr old Children in Illinois Communities (Selwitz et al., 1995)

Group	No.	No. Surf.	% Distribution of TSIF Scores					Percent Surfaces Fluorosed[b]	MPFS[c]	P value
			0	1	2	3	4-7			
1980										
Optimal[a]	113	3505	81.2	14.8	2.3	1.6	0.1	18.8	18.2	–
2x Optimal	61	1807	53.0	33.0	6.9	6.7	0.4	47.0	47.3	<0.001[d]
3x Optimal	82	2447	48.5	30.6	10.9	8.1	1.9	51.5	52.4	<0.001[d]
4x Optimal	59	1765	30.3	28.5	17.1	19.7	4.4	69.7	69.2	<0.001[d]
1985										
Optimal[a]	156	5220	72.0	20.5	5.6	1.8	0.1	28.0	28.9	–
2x Optimal	102	3121	48.0	30.4	11.6	8.7	1.3	52.0	52.8	<0.001[d]
3x Optimal	112	3426	48.0	29.4	12.3	8.2	2.1	52.0	50.9	<0.001[d]
4x Optimal	62	1880	24.2	32.2	18.7	19.7	5.2	75.8	77.1	<0.001[d]
1990										
Optimal[a]	167	4867	81.4	14.4	2.9	1.3	0.0	18.6	17.8	–
2x Optimal	76	2071	45.0	24.7	14.2	14.7	1.4	55.0	55.6	<0.001[d]
3x Optimal	69	1984	45.3	25.1	14.5	12.2	2.9	54.7	55.2	<0.001[d]
4x Optimal	57	1570	38.4	24.9	15.3	18.3	3.1	61.6	59.8	<0.001[d]

[a]"Optimal" is defined by the study authors as 1 mg F/L for the study region (Midwest USA).
[b]Percent of surfaces fluorosed across all subjects.
[c]Mean percent of fluorosed surfaces per subject.
[d]Difference from optimal; significant, P<0.002, adjusted α level for multiple comparisons using the Bonferroni procedure.

In children residing in areas with optimal water fluoride levels depicted in Table 3-8 and Table 3-9, the proportion of fluorosed tooth surfaces increased significantly from 1980 to 1985, but then declined by 1990 to the levels previously observed in 1980. In children residing in areas with above optimal fluoride levels, fluorosis remained stable or showed no sustained increase from 1980 to 1990.

Table 3-9. Comparison of TSIF Scores and Mean Percent Fluorosed Surfaces for Children in Illinois Communities (Selwitz et al., 1995)										
Group	No.	No. Surf.	% Distribution of TSIF Scores					Percent Surfaces Fluorosed[b]	MPFS[c]	P value
			0	1	2	3	4-7			
1980 (Children 13-15 yr old)										
Optimal[a]	111	7340	88.6	9.1	1.5	0.8	0.0	11.4	11.1	–
2x Optimal	39	2540	61.7	25.4	7.8	5.0	0.1	38.3	38.4	<0.001[d]
3x Optimal	50	3341	54.0	21.6	13.7	9.6	1.0	46.0	45.5	<0.001[d]
4x Optimal	34	2265	36.9	25.6	16.7	18.6	2.2	63.1	63.5	<0.001[d]
1985 (Children 13-15 yr old)										
Optimal[a]	94	5480	70.6	21.6	4.9	2.8	0.1	29.4	30.5	–
2x Optimal	23	1492	33.5	32.5	18.6	13.8	1.6	66.5	67.2	<0.001[d]
3x Optimal	47	3115	30.8	34.9	18.2	13.6	2.5	69.2	69.1	<0.001[d]
4x Optimal	29	1843	22.5	30.8	18.8	22.1	5.9	77.5	77.8	<0.001[d]
1990[e] (Children 14-16 yr old)										
Optimal[a]	91	6064	84.7	13.4	1.6	0.2	0.1	15.3	14.9	–
2x Optimal	29	1883	52.5	22.9	13.1	11.0	0.5	47.5	48.9	<0.001[d]
3x Optimal	48	3134	53.3	21.0	12.4	10.3	3.0	46.7	45.4	<0.001[d]
4x Optimal	20	1275	33.3	20.8	18.0	24.8	3.1	66.7	67.6	<0.001[d]

[a]"Optimal" is defined by the study authors as 1 mg F/L for the study region (Midwest USA).
[b]Percent of surfaces fluorosed across all subjects.
[c]Mean percent of fluorosed surfaces per subject.
[d]Difference from optimal; significant, P<0.002, adjusted α level for multiple comparisons using the Bonferroni procedure.
[e]Children in 1990 were closer in age to 14-16 than to 13-15 yr.

In 1998, Selwitz et al. compared the fluorosis data from surveys conducted in Kewanee, IL (fluoride level 1.0 mg/L) with those from two communities in Nebraska (Holdrege and Broken Bow) where the fluoride levels were negligible (<0.3 mg/L). The dental examination in all three communities took place in 1990. The results are shown in Table 3-10. The percent of tooth surfaces fluorosed across all subjects was similar in the three communities for the 8–10 yr olds (17.7–18.5%). For the 13–16 yr olds, the total percent fluorosed was higher in Kewanee (15.1%) than in the two Nebraska communities (2.1 and 9.2%). The mean percent of fluorosed tooth surfaces per person, adjusted for age and use of dietary fluoride supplements, for all study participants was similar in the three communities (17.6% in Kewanee, 12.3% in Holdrege, and 13.1% in Broken Bow (98% CI); more than 80% of tooth surfaces in all participants were fluorosis-free. The study authors concluded that in comparison with studies undertaken in the previous decade, the difference in dental fluorosis prevalence between fluoridated and non-fluoridated communities had narrowed considerably.

Table 3-10. Comparison of TSIF Scores and Percent Fluorosed Surfaces for Children in Kewanee, IL and two Communities in Nebraska (Selwitz et al., 1998)									
Group	F (mg/L)	No.	No. Surf.	% Distribution of TSIF Scores					% Surface Fluorosed[b]
				0	1	2	3	4-7	
8-10 yr olds									
Kewanee	1[a]	167	4867	81.4	14.4	2.8	1.3	0.0[c]	18.5
Holdrege	<0.3	104	2956	81.7	12.6	3.4	2.3	0.1	18.4
Broken Bow	<0.3	47	1424	82.3	15.2	2.2	0.3	0.0	17.7
13-16 yr olds									
Kewanee	1[a]	93	6203	85.0	13.1	1.6	0.3	0.1	15.1
Holdrege	<0.3	24	1447	97.9	1.9	0.2	0.0	0.0	2.1
Broken Bow	<0.3	60	3748	90.9	8.1	0.7	0.4	0.0	9.2

[a]Fluoride level in water supply in Kewanee is considered optimal for Midwest USA.
[b]Percent of surfaces fluorosed across all subjects.
[c]Two surfaces were affected.

Jackson et al. (1995): Dental fluorosis and caries prevalence in children aged 7–14 yr (born between 1978 and 1985) from three communities in Indiana having different levels of fluoride in drinking water (0.2 mg/L; 1.0 mg/L; and 4.0 mg/L) were compared using the TSIF index and Dean's scoring system for fluorosis (Jackson et al., 1995). The children included in the study had to meet the criterion of lifetime residency in the communities (e.g., born to parents who were residents of the communities and not being absent from the communities for more than 2 weeks in any one year). The examinations were conducted in February of 1992. As shown in Table 3-11, the prevalence of fluorosis increased with increasing fluoride concentration in drinking water, and the prevalence of severe fluorosis was 11.3% at 4 mg/L based on Dean's score of 4, and 19.8% based on the TSIF Index (score of ≥5). Considering the age of the children, it is likely that these populations were exposed to fluoride toothpaste during early childhood. The study authors also reported that fluoride supplements were consumed by 57.9% of the subjects in the 0.2 mg F/L group; 19.8% in the 1.0 mg F/L group, and 8.9% in the 4.0 mg F/L group.

Table 3-11. Percent Distribution of Fluorosis in Children from Three Indiana Communities with Different Levels of Fluoride in Drinking Water							
Fluoride Level (mg/L)	**No.**	**Dean's Fluorosis Scoring System**					
		0	**0.5**	**1**	**2**	**3**	**4[a]**
0.2	124	85.5	0	13.7	0.8	0	0
1.0	116	61.2	0	31.9	6.9	0	0
4.0	97	10.3	1.0	26.8	18.6	32.0	11.3
Fluoride Level (mg/L)	**No.**	**TSIF Score**					
		0	**1**	**2**	**3**	**4**	**5–7[b]**
0.2	126	81.8	15.1	3.2	0	0	0
1.0	117	54.7	34.2	9.4	0.9	0.9	0
4.0	101	7.9	22.8	16.8	25.7	6.9	19.8

SOURCE: Jackson et al. (1995).

[a]Score of 4 includes pitting.
[b]Scores of 5 and above include pitting.

Jackson et al. (1999): In December 1994, children representing the same age groups (7–14 yr) and from the same three communities were examined for dental fluorosis. Fluorosis was scored using the TSIF index. As in the previous study, the children had to meet the criterion of lifetime residency in the study communities. The prevalence of fluorosis increased by about 14%, 20% and 6 % in the 0.2, 1.0 and 4.0 mg F/L communities, respectively. However, the prevalence of severe fluorosis decreased from 18% in 1992 to 9% in 1994 for children 7–10 yr old. For children 11–14 yr old, the prevalence of severe fluorosis decreased from 25 to 8%. Although increases occurred in prevalence of fluorosis in the 0.2 or 1.0 mg F/L communities, these were mainly confined to the TSIF categories 1 and 2, and no children in either community exhibited severe fluorosis at either time period. The study authors did not suggest a reason for the difference in severe fluorosis for the children examined in 1992 compared to those examined in 1994.

Hong et al. (2006a): As part of the Iowa Fluoride Study, 628 children aged 8–10 yr (mean 9.3 yr; born in March 1992–February 1995) were evaluated for fluorosis of the permanent maxillary central incisors and first molars. The fluoride intake of 405 of these same children had been followed from birth through 36 months by means of questionnaires their parents completed every 3–4 months. Daily fluoride intake was estimated from water, beverages, and selected foods, fluoride supplements and dentifrice. Fluorosis was evaluated using the Fluorosis Risk Index; this index considers fluorosis as severe when there is pronounced staining and/or pitting of the enamel. A case of incisor fluorosis was defined as having an FRI of 2 or 3 on both maxillary central incisors; a case of first molar fluorosis was defined as having FRI of 2 or 3 on at least two first molars. Hong et al. (2006a) reported that six individuals (1.5%) showed signs of severe fluorosis (FRI of 3). Four individuals were listed as having severe fluorosis on the maxillary central incisors, and all of them had high levels of fluoride intake (>0.06 mg/kg/day) at either 0–12 months or 12–36 months. Two subjects with severe fluorosis on the first molars recorded both moderate (0.04–0.06 mg/kg/day) and high (>0.06 mg/kg/day) fluoride intake over the first three years.

3.1.3. Supplemental non-U.S. studies on dental fluorosis

A relatively large number of studies have evaluated the prevalence of dental fluorosis and dental caries in populations outside the United States. In many cases these studies are not directly comparable to U.S. populations because of intrinsic differences in socioeconomic characteristics, dietary habits, dental hygiene practices, climatic conditions and other potential sources of confounding factors. In some cases where these populations were not exposed to fluoride from commercial dental products and the primary source of fluoride exposure was through drinking water, the results of the studies can be useful for comparison with U.S. studies. Additional studies are discussed below.

Forsman (1974): The occurrence of dental fluorosis (Dean's index) and dental caries (DMFT or DMFS scores) was studied in residents (mostly school children) in three communities in southern Sweden (Gadderås, Påskallavik and Billesholm). In Gadderås, 39 individuals (2–35 yrs old) were examined; 28 were born in the town and 15 were less than 15 yrs old. A new water supply containing approximately 10 mg F/L went into use in 1946 and all homes were connected by 1950. In Påskallavik, 190 children born in 1955–1966 were examined, 61 of whom were born in the town. The water source had a fluoride level of 7–10 mg/l from mid-1956 to beginning 1965; prior to 1956, private wells with low fluoride content were used, and after 1965 the water source was changed to one with a fluoride content of 2.0–2.5 mg/l. For the purposes of this study, Forsman (1974) considered the fluoride level to be ~ 10 mg/L. In Billesholm, of the 300 children examined 133 were born in the district and had always lived there. Water was obtained from two deep wells; from 1957 to 1969 fluoride level varied between 4 and 7 mg/L, but mostly was around 5.5 mg/L. From 1969 to 1973 the fluoride content was 1–3 mg/L or less. For purposes of this study, the fluoride level was considered by Forsman (1974) to be ~ 5 mg/L. Other sources of fluoride exposure, such as dietary intake, were not evaluated. The control population (160 children) came from areas of Kronoberg County with stable water sources containing 0.9 to 1.7 mg F/L. This group is classified as ~1 mg F/L.

Fluorosis data for the permanent teeth of school children born and reared in the three study areas were presented in graphical form (see Fig. 3-2). In general, the data indicate increasing severity of fluorosis with increasing fluoride exposure. In Gadderås (~10 mg F/L), severe fluorosis was seen in approximately 64% of the study population. Of the 26 children born in Påskallavik between 1957 and 1961, and exposed to ~10 mg F/L for 4 years or more, all but one had moderate to severe fluorosis. Of the 12 children born in Påskallavik in 1962–64 (exposed to ~10 mg F/L up until 1965 when the water source was changed to one with 2–2.5 mg F/L) only about 18% exhibited severe fluorosis. Forsman (1974) reported that the difference between the two groups was significant at the 1% level. In Billesholm (~5 mg F/L), about 22% of the children exhibited severe fluorosis. Specific information on the distribution of fluorosis scores in the control areas (~1 mg F/L) were not reported by Forsman, however, it was noted that in earlier studies no cases of fluorosis more severe than Grade 2 were found in one of the control populations (Kronoberg County, N = 160).

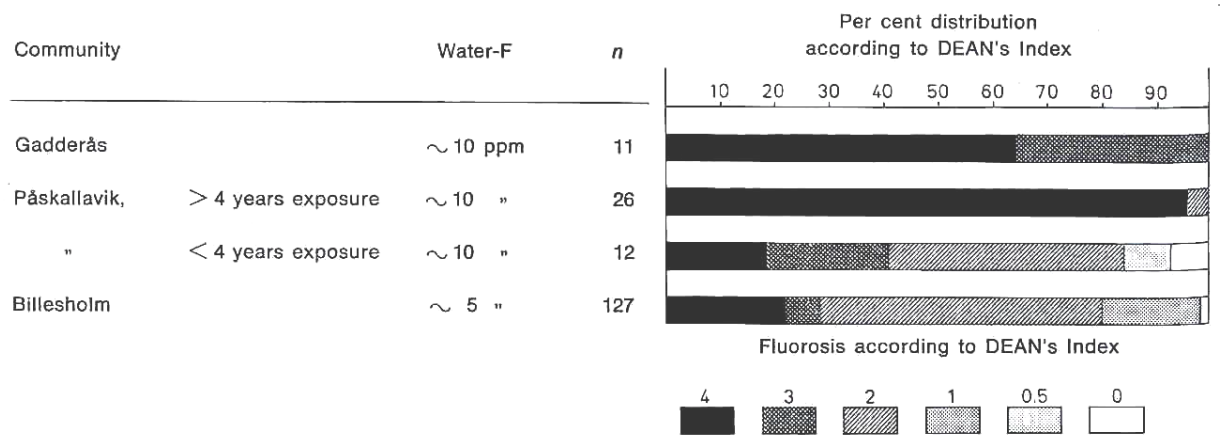

Figure 3-2. Fluorosis in permanent teeth of school children born and reared in three study areas in Sweden (Forsman, 1974).

In examining the occurrence of fluorosis in individuals in Gadderås whose exposure to 10 mg F/L drinking water began at different time periods after birth, Forsman (1974) found a clear concordance between the teeth affected by fluorosis relative to the F concentration during the period when permanent teeth were undergoing mineralization. All children born in Gadderås after 1950 (when all homes were receiving drinking water with 10 mg F/L) had Grade 3 or Grade 4 fluorosis on all permanent teeth. Children who began drinking water with 10 mg F/L at an age of about 5 years and above showed evidence of fluorosis only on the late mineralizing teeth.

Forsman (1974) also reported that fluorosis continued to develop in children after they moved away from the area with a 10 mg F/L to low fluoride areas. In one case, a 3-yr-old who had moved to a district with <0.2 mg F/L still developed Grade 3 or 4 fluorosis on all teeth except the second molars which were graded as having a fluorosis score of 2. In two other cases, 7-yr-old children, who had moved to a low fluoride district, developed Grade 3 or 4 fluorosis on all teeth except the third molars. Forsman (1974) considered these to be cases of continuous exposure to fluoride as a result of fluoride from tissue depots after the external exposure had ceased.

Mann et al. (1987): The prevalence and severity of dental caries and fluorosis was studied in a community in the Gaza Strip characterized by drinking water with 5 mg F/L (Mann et al., 1987). The study population consisted of 182 adolescents (90 boys and 92 girls; 15–16 years old) residing since birth in the same village. Dental fluorosis was determined according to Dean's index. Thirty-eight boys and 8 girls exhibited severe fluorosis, resulting in a population prevalence rate of 25%.

Chen (1989): Dental fluorosis and caries prevalence were evaluated in children 6 to 16 yrs old (2,669 boys and 2,438 girls) residing in 14 communities in Shenkang Hsiang province, Taiwan (Chen, 1989). Chen (1989) reported that the recommended range of optimal water fluoride concentrations used in the study was 0.4–0.5 mg/L for the tropical zones of Taiwan and 0.6–0.7 mg/L for the subtropical zone, and is based on zone-specific water consumption rates. The study author noted that the great majority of the population acquire their drinking water from shallow wells, and the rest from deep-wells.

The study communities were divided into six exposure categories based on water fluoride concentrations, and the range of concentrations in each of the exposure groups was as follows: 0.21–0.25 mg F/L (negligible); 0.43–0.48 mg F/L (optimal); 0.75–0.98 mg F/L (2x optimal); 2.40 mg F/L (4x optimal); 2.84–3.24 mg F/L (5x optimal); and 4.69 mg F/L (7x optimal). Beginning in June 1981 a communal water supply (non-fluoridated, with a fluoride level of 0.09 mg/L) was available to the population and was supplied to all the schools in the province. Dental fluorosis was scored using TSIF and the Dean Fluorosis Index. The prevalence and severity of fluorosis was distinctly greater in all areas with higher than optimal fluoride levels (Table 3-12). Severe fluorosis occurred in 0.2% of the children in the 2x optimal group, 0% in the 4x group, 0.3% in the 5x group, and in 1.3% in the 7x group.

Table 3-12. Percent Distribution of Fluorosis in Taiwanese Populations Studied by Chen (1989)

Fluoride Level (mg/L)[a]	N	Dean's Index					
		0	0.5	1	2	3	4
0.21-0.25	851	89.7	4.9	4.1	1.3	–	–
0.43-0.48	1660	86.2	7.3	3.6	2.7	0.1	–
0.75-0.98	849	67.7	12.2	13.3	5.6	0.8	0.2
2.40	420	43.8	17.1	18.6	16.2	4.3	–
2.84-3.24	912	27.7	16.6	18.1	31.6	5.7	0.3
4.69	380	12.1	7.6	18.2	48.2	12.6	1.3

[a]According to Chen (1989), the recommended range of optimal water fluoride concentrations is 0.4–0.5 mg/L for the tropical zones of Taiwan and 0.6–0.7 mg/L for the subtropical zones.

Chen (1989) did not evaluate other fluoride exposure factors, such as dietary contributions to fluoride intake and the use of fluoride dentifrice and supplements; therefore, it is unclear to what extent the observed fluorosis was due to fluoride in drinking water alone. Furthermore, because the study population was living in a tropical area, fluoride exposure was likely elevated due to higher drinking water consumption rates when compared to more temperate regions. Other factors complicating the interpretation of Chen's data is the young age of some of the subjects (as young as 6 yrs old), and the introduction of a very low fluoride drinking water source in 1981. Both of these factors may have contributed to overall lower fluorosis prevalence and severity scores and thereby confounded interpretation of the Chen (1989) results.

Thaper et al. (1989): The prevalence and severity of dental fluorosis was studied in 16 rural communities in the State of Rajastan, India, by Thaper et al. (1989). The study population consisted of 792 children 6–10 years old. Dental fluorosis was scored according to Dean's Index. At mean fluoride levels of 1.04 mg/L and 2.4 mg/L, there were no cases of severe fluorosis in the permanent teeth. At 3.91 mg F/L, 3.65–16.10% of the examined permanent teeth exhibited severe fluorosis; at a mean fluoride level of 6.0 mg/L, 10.32–14.57% exhibited severe fluorosis. The number of individuals with severe fluorosis was not reported.

Cortes et al. (1996): The authors examined a total of 457 school children (6–12 years old) residing in three regions of Brazil for dental fluorosis (and dental caries): Olho D'Agua with 2–3 mg F/L drinking water, Vitoria with 0.7 mg/L, and Maceio with less than 0.01 mg/L. Participating schools were selected for similarities in socioeconomic profiles, although Olho D'Agua was a more rural community while Maceio and Vitoria were more urban (no other information was

provided on the study populations). Only exposure to fluoride in the drinking water was considered in the study. Photographs were taken and used for assessment of the degree of fluorosis of the upper central incisors. The TFI was used to score fluorosis. In reporting the results, Cortes et al. (1996) combined the data for TFI scores of 1 and 2, for scores of 3 and 4, and for all scores of 5 and above (Table 3-13). Because only the upper central incisors were scored, it is uncertain as to how representative the results are for the entire dentition of the children examined. Nevertheless, the data show a trend towards increasing severity of fluorosis with increasing concentration of fluoride in the water (data not analyzed statistically).

Table 3-13. Distribution of Fluorosis Scores[a] in Brazilian School Children Studied by Cortes et al. (1996)

TFI Score	Maceio (0.01 mg F/L)		Vitoria (0.7 mg F/L)		Olho D'Agua (2–3 mg F/L)	
	No.	Percent	No.	Percent	No.	Percent
0	148	92.5	96	47.8	8	8.3
1-2	12	7.5	95	47.3	28	29.2
3-4	-		9	4.5	42	43.8
≥5	-		1	0.5	18	18.8

[a]Upper central incisors only were scored.

Grobler et al. (2001): In studies conducted in South Africa, Grobler et al. (2001) found that 0.8% of a study population of children (10–15 yrs old) whose drinking water contained 0.48 mg F/L exhibited severe fluorosis (Dean's Index), and in a population whose drinking water contained 3.0 mg F/L 30% exhibited severe fluorosis (Table 3-14). It was reported that fluoride exposure was not affected by dietary habits or the use of fluoride supplements or dentifrices, but it was noted that the area is hot and dry which may have resulted in increased drinking water intake (the average annual maximum temperatures for Sanddrif and Kuboes were reported to be ~25°C or 77°F; the temperature for Leeu Gamka was not given).

Table 3-14. Percent Distribution of Fluorosis in S. African Populations Studied by Grobler et al. (2001)

Town	No.	F (mg/L)	Dean's Index					
			0	0.5	1	2	3	4
Sanddrif	47	0.19	38.3	14.9	25.5	17.0	4.2	0
Kuboes	115	0.48	40.0	9.5	33.9	10.4	5.2	0.8
Leeu Gamka	120	3.0	0.8	4.1	15.8	18.3	30.8	30.0

3.1.4. Environmental, physiological and genetic factors affecting dental fluorosis

In addition to fluoride levels in drinking water, various other environmental and physiological factors may affect the occurrence and severity of dental fluorosis, or produce changes in enamel mineralization which may resemble fluoride-induced dental fluorosis. These include climate and altitude of place of residence; dietary habits; and nutritional status (vitamin and essential mineral deficiencies and exposure to certain minerals); physiological state (e.g., acid-base balance) and certain pathological conditions.

3.1.4.1. The effect of climate

In 1962 the Public Health Service published revised Drinking Water Standards for fluoride which took into account differences in water consumption rates in different climates (PHS, 1962). The recommended fluoride levels for drinking water ranged from 0.7 mg/L for warmer climates to 1.2 mg/L for colder climates (based on the annual average of the maximum daily air temperatures). These standards, still in effect today (CDC, 1995), were the result of earlier studies which indicated that the prevalence and severity of fluorosis in populations residing in hot climates was higher than that for comparable populations in cooler climates having similar fluoride levels in drinking water (Galagan and Lamson, 1953, see Section 3.1.2 for discussion). In the Galagan and Lamson (1953) study, children aged 9–16 yr residing in six Arizona towns were examined for dental fluorosis. These Arizona communities were reported to have a mean annual temperature of 70°F. Galagan and Lamson (1953) compared their results to those reported by Dean (1942) for towns with similar fluoride levels in drinking water but with lower mean annual temperatures. The Dean communities used in the comparison had a mean average annual temperature of 50°F according to Galagan and Lamson (1953). The Galagan and Lamson communities exhibited both a higher prevalence and increased severity of fluorosis compared to the communities studied by Dean (1942). Selected data from these two studies are shown in Table 3-15. The mean annual temperatures for the study localities are also included in the table. The studies selected for Table 3-15 were conducted in the period between 1942 and 1953. This minimizes the impact of the increased exposure to fluoride from dental products on the trends observed.

Table 3-15. Prevalence of Fluorosis in Children Living in Hot Climates Compared with Children Living in Temperate Climates

F (mg/L)	% with Fluorosis	% Distribution of Fluorosis (Dean's Index)				Location	Mean Annual Temp. (°F)	Reference
		1	2	3	4			
0.2	1.5	1.5	0	0	0	Zanesville, OH	55.7[b]	Dean (1942)
0.3	2.2	2.2	0	0	0	Lima, OH	50.0[c]	Dean (1942)
0.4	4	2.4	1.2	0	0	Yuma, AZ[a]	72.2[d]	Galagan and Lamson (1953)
0.4	6.1	5.3	0.8	0	0	Marion, OH	52.1[d]	Dean, 1942
0.5	10	9	1	0	0	Tempe, AZ[a]	68.6[d]	Galagan and Lamson (1953)
0.5	4.2	3.5	0.7	0	0	Elgin, IL	53[d]	Dean (1942)
0.6	6.5	6.2	0.3	0	0	Pueblo, CO	52.6[d]	Dean (1942)
0.7	17	12	3	2	0	Tucson, AZ[a]	67.4[d]	Galagan and Lamson (1953)
0.8	19	9	6	2	1	Chandler, AZ[a]	67.6[d]	Galagan and Lamson (1953)
0.9	12	10.6	1.6	0	0	Kewanee, IL	50.9[d]	Dean (1942)
1.0	48	30	18	0	0	Casa Grande, AZ[a]	71.0[d]	Galagan and Lamson (1953)
1.2	56	26	14	13	3	Florence, AZ[a]	69.3[d]	Galagan and Lamson (1953)
1.2	15	14	1	0	0	Aurora, IL	49.4[d]	Dean (1942)
1.2	32	30	2	0	0	E. Moline, IL	50.9[d]	Dean (1946)
1.2	33	29	4	0	0	Maywood, IL	50.1[d]	Dean (1946)

[a]Studies that were conducted on populations living in hot climates are highlighted.
[b]Yearly average based on monthly means of daily averages for Muskingum County, OH, 1961-1990, http://www.worldclimate.com/cgi-bin/data.pl?ref=N39W081+1302+339417C..
[c]Yearly average based on monthly means of daily averages for Allen County, OH, 1961-1990, http://www.worldclimate.com/cgi-bin/data.pl?ref=N40W084+1302+334551C..
[d]As reported in Galagan and Lamson (1953).

The results presented in Table 3-15 support the conclusion that fluorosis prevalence and severity is generally greater in populations living in hot climates compared to those in cooler climates for similar fluoride drinking water levels. However, there are limitations to this conclusion because the comparison is based on water concentration and lacks information on other sources of fluoride exposure.

Galagan and Lamson (1953) suggested that the higher temperatures in hot climates, as well as the increased amount of sunshine each day (radiant heat), contributed to the increased fluorosis by causing an increase in drinking water intake, resulting in an increase in fluoride intake. Temperature-related increases in total fluid and water consumption were documented by Galagan and Vermillion (1957) for children (under age 1 to age 10 yr) residing in two communities in California. The study showed that for every degree increase in daily maximum temperature between 50 and 100°F, water intake increased, on average, by 0.062 ounces per pound of body weight. This relationship was described by the equation:

Water intake (in ounces per pound) = 0.0062 x temperature (in °F) – 0.038

The correlation coefficient for this relationship was not reported.

Water accounted for 43% of the total daily intake and average water intake ranged from 18 mL/kg [0.27 fluid ounces (0.008 mL) per day per pound of body weight (0.454 kg) at a mean daily maximum temperature of 50ºF] to approximately 38 mL/kg [0.58 fluid ounces per day per pound of body weight) at a mean daily maximum temperature of 100ºF (estimates based on graphical presentation of the data)]. These data indicate that water intake can more than double under extremely hot conditions; consequently, fluoride intake from drinking water would increase proportionally.

In the early 1960's, the California Department of Public Health began a 5-year program to evaluate the correlation between fluoride concentration in drinking water, dental caries, dental fluorosis, and temperature (Richards et al., 1967). Dental caries and fluorosis were evaluated in 9000 children, 12–14 years old, from 83 towns, representing 6 different fluoride concentrations and eight different temperature-range zones in the states of California, Texas, Colorado, Arizona, New Mexico and Illinois. The findings for the relationship between fluorosis status, fluoride concentration and ambient air temperature for 7140 children are shown in Table 3-16. The results suggest that the likelihood for severe fluorosis increases as air temperature increases. Use of ranges for the fluoride concentration reduces the confidence in this conclusion because the increases in moderate and severe fluorosis at the higher temperatures could also be the result of having more systems delivering water at the high end of the concentration range at the higher temperatures than at the lower temperatures.

Angmar-Månsson and Whitford (1990) have argued that temperature-related increases in drinking water consumption may no longer be relevant for assessing fluoride intake in the U.S. and other western countries because of changes in life style and dietary habits. These authors note that water intake rates in hot climates may be affected by the increased use of air-conditioning in homes, schools and public places, and fluoride intake may be reduced because of the increased consumption of beverages other than drinking water that may contain little fluoride. In recent years the consumption of bottled water and the use of home water filtration systems, may have

further reduced fluoride intake through tap water. In addition, time spent indoors by children may have increased due to the increase in television and computer use. In a study of the 2592 school children in 16 towns in Texas, Butler et al. (1985) found that children from homes with air conditioning had a non-significant but lower prevalence of dental mottling (odds ratio 0.58, 95% CI 0.40–0.85) compared to children whose homes were not air conditioned.

Table 3-16. Percentage of Children by Fluorosis Diagnosis for Each Fluoride-Temperature Zone (Richards et al., 1967)

Fluorosis Score	Fluoride Concentration (ppm)					
	≤0.15	0.2-0.4	0.5-0.7	0.8-1.0[b]	1.1-1.3	≥1.8
≤65°F	**Zone 1 (N = 330)[a]**	**Zone 4 (N = 169)**	**Zone 7 (N = 340)**	**Zone 10 (N = 316)**	**Zone 13 (N = 302)**	**Zone 16 (N = 306)**
Normal	97.3	71.6	44.7	40.0	33.1	11.1
Questionable	2.4	26.0	40.9	39.2	41.1	23.5
Very mild	0.3	2.4	13.5	18.0	22.5	29.5
Mild	–	–	0.9	2.8	3.3	15.7
Moderate	–	–	–	–	–	15.0
Severe	–	–	–	–	–	5.2
66°–79°F	**Zone 2 (N = 707)**	**Zone 5 (N = 709)**	**Zone 8 (N = 688)**	**Zone 11 (N = 548)**	**Zone 14 (N = 508)**	**Zone 17 (N = 553)**
Normal	96.1	74.2	26.6	22.8	26.6	14.8
Questionable	3.5	19.5	42.9	44.3	32.7	18.4
Very mild	0.4	6.2	28.6	26.6	28.1	27.8
Mild	–	0.1	1.9	5.8	9.6	20.8
Moderate	–	–	–	0.5	2.8	12.8
Severe	–	–	–	–	0.2	5.4
≥80°F	**Zone 3[c] (N = 209)**	**Zone 6 (N = 335)**	**Zone 9 (N = 331)**	**Zone 12 (N = 350)**	**Zone 15 (N = 310)**	**Zone 18 (N = 229)**
Normal	52.6	32.2	18.1	18.3	8.4	8.3
Questionable	46.9	44.8	51.1	26.0	29.0	18.8
Very mild	0.5	20.0	26.0	37.7	37.5	25.3
Mild	–	3.0	4.2	15.1	17.4	27.9
Moderate	–	–	0.6	2.9	7.4	12.7
Severe	–	–	–	–	0.3	7.0

[a]N = number of children for whom diagnosis was made.
[b]Given as 0.8–0.7 ppm in paper and presumed to be a typographical error.
[c]Fluoride concentration 0.2 ppm.

3.1.4.2. The effect of altitude

According to Angmar-Mansson and Whitford (1990) there is evidence that hypobaric hypoxia that occurs at high altitudes is associated with bilaterally symmetrical and diffuse disturbances in enamel mineralization that may be mistaken for fluorosis. In addition to hypoxia per se, other physiological effects, including alterations in growth and development, acid-base status, hormonal balance, hematocrit, hemodynamics, and the function of the renal and cardiovascular systems may contribute to the disturbances in enamel mineralization observed in populations living at high altitudes (Angmar-Mansson and Whitford, 1990).

Several studies have examined the occurrence of dental fluorosis in populations living at different altitudes and exposed to different levels of fluoride in their drinking water. Manji et al. (1986) recorded the occurrence of dental fluorosis in children 11–15 years old living at different altitudes (sea level, 1500 m and 2400 m) in towns in Kenya with different fluoride drinking water levels (<0.5 mg/L and 0.5–1.0 mg/L). Fluorosis was measured using the TFI scoring system. In the low fluoride areas (0.5 mg F/L) the percentage of children exhibiting dental fluorosis was 36.4, 78.0 and 100.0 at sea level, 1500, and 2400 m, respectively (statistical significance not reported). In the high fluoride areas, the percentages were 71.2 at sea level and 93.8 at 1500 m (no study population at 2400 m). The severity of fluorosis for each tooth type increased significantly with increases in altitude for both the low and high fluoride areas ($p<0.001$; data shown graphically in study report).

The percent distribution of children for each grade of fluorosis for each study area was not included in the study report; however, Manji et al. (1986) reported that in the low fluoride areas less than 2% of the children had more than 50% of their teeth with TFI scores of ≥ 3 at sea level, compared with 10% at 1500 m and 60% at 2400 m. Similarly, in the high fluoride areas less than 2% of the children had more than 50% of their teeth with TFI scores of ≥ 3 at sea level, compared to over 20% at 1500 m. Manji et al. (1986) excluded temperature as a factor in the differences seen between the study populations, and noted that, based on the mean annual maximum air temperatures, there was an inverse relationship between temperature and prevalence and severity of dental fluorosis. The authors did not feel that dietary differences between the study populations contributed to the altitude-related differences in fluorosis, although specific information on non-drinking water fluoride intake was not reported. Manji et al. (1986) concluded that children living at high altitudes were more susceptible to dental fluorosis.

Yoder et al. (1998) examined the occurrence of dental fluorosis in school children (ages 9–19 yr) in three communities in Tanzania located at different altitudes (100 m, 840 m, and 1463 m) and with different levels of fluoride in their drinking water (mean concentrations of 0.046 ±0.047, 5.72 ±4.71, and 0.18 ±0.32 mg F/L, respectively; (significantly different at $p<0.0001$, based on analysis of covariance). The corresponding mean TFI scores were: 0.01 ±0.07, 4.44 ±1.68, and 4.39 ±1.52, and the percentages of teeth showing severe fluorosis were 0, 48.6 and 54.9%, respectively. The percentage of severely fluorosed teeth in subjects from the highest altitude community was greater than in those from the middle altitude community even though the drinking water concentration at the high altitude locality was lower.

Statistical analysis implicated altitude as a risk factor for severe fluorosis. The study authors, however, did not exclude the possibility that other factors, such as the use of a fluoride-containing food additive, the presence of other elements in the diet (aluminum and magnesium), nutritional factors (malnutrition, insufficient milk consumption, ingestion of tea), or genetic differences, contributed to the observed increased occurrence and severity of fluorosis in the high altitude community in spite of the relatively low water fluoride level. Urinary fluoride levels in that community were normal, suggesting that fluoride intake was not excessive.

In studies conducted in the mountainous areas of Uganda, Rwenyonyi et al. (1999) compared the occurrence of dental fluorosis among 10–14-yr-old children in two fluoride districts (0.5 mg/L and 2.5 mg/L) while controlling for other factors related to fluorosis. The altitudes of the two study

areas with a fluoride level of 0.5 mg/L were 900 and 2,200 m; in the two study areas with 2.5 mg F/L, the altitudes were 1,750 and 2,800 m. Fluorosis was evaluated using the Thylstrup and Fejerskov index. In the 0.5 mg F/L areas, the percentage of children with fluorosis (TFI score ≥ 1 for at least one tooth) was 25% at 900 m and 45% at 2200 m (significantly different at $p = 0.006$). In the 2.5 mg F/L areas, the percentage of children with TFI ≥ 1 was 69% at 1750 m and 86% at 2800 m (significantly different at $p = 0.0003$). The severity of fluorosis also increased with increases in altitude as shown by the increased percentage of children having TFI scores of 3 or higher. The prevalence of severe fluorosis (TFI ≥5) was estimated from the graphical presentation of the data to be 4% at 900 m and 7% at 2200 m where the fluoride level was 0.5 mg/L, and 26% at 1750 m and 38% at 2800 m where the fluoride level was 2.5 mg/L. However, these differences in percent occurrence of TFI ≥5 were not significant in either the low fluoride area ($\chi^2 = 1.02$, d.f. = 1, $p = 0.313$) or in the high fluoride area ($\chi^2 = 3.49$, d.f. = 1, $p = 0.062$).

Rwenyonyi et al. (1999) noted that besides altitude, fluoride exposure level, use of infant formula, vegetarian diets, and the storing of water in clay pots "had independent significant explanatory effects in the linear regression analysis." Water storage was associated with reduced odds ratios in the low fluoride area, whereas the use of infant formula was a significant risk indicator in the high fluoride areas. The study authors concluded that most of the variance in the prevalence and severity of dental fluorosis was explained by the fluoride intake from liquid, but altitude remained a significant risk indicator after controlling for the effect of other potential confounding factors by multiple and logistic regression analyses.

The areas studied by Rwenyonyi et al. (1999) are at altitudes of 900–2800 m. Only a few studies conducted in the U.S. have been at altitudes falling within this range; these include Clovis, NM (1299 m), Pueblo, CO (1462 m) and Colorado Springs, CO (1900 m). All three cities were included in Dean's 1942 survey. At that time, the drinking water fluoride level in Clovis was 2.2 mg/L; that at Pueblo was 0.6 mg/L and that at Colorado Springs was 2.6 mg/L. Table 3-17 compares the percent occurrence of severe fluorosis in the Ugandan and U.S. communities. Comparable information was not available for the studies conducted in Kenya by Manji et al. (1986) and those conducted in Tanzania by Yoder et al. (1998).

Table 3-17. Occurrence of Severe Fluorosis in High Altitude Areas in Uganda and the U.S.

Town	Fluoride (mg/L)	No.	Altitude (m)	Percent Severe Fluorosis
Mpondwe, Uganda	0.5	81	900	4[a]
Kyabayenze, Uganda	0.5	82	2200	7[a]
Pueblo, CO	0.6	614	1462	0[b]
Mutolere/Kagera, Uganda	2.5	163	1750	26[a]
Kabindi, Uganda	2.5	155	2800	38[a]
Colorado Springs, CO	2.6	404	1900	1.5[b]
Clovis, NM	2.2	138	1299	0.7[b]

SOURCE: Dean (1942); Rwenyonyi et al. (1999).
[a]Percent of children with severe fluorosis (TFI ≥5).
[b]Based on Dean's index of fluorosis; "severe" defined as Dean's score of 4.

Because of the relatively low percentages of severe fluorosis seen in the U.S. studies conducted at comparable altitudes, the evidence suggests that factors other than altitude are the primary cause of

the higher rates of severe fluorosis seen in the Rwenyonyi et al. (1999) study. This, however, does not exclude the possibility that some degree of the "fluorosis" seen in the high-altitude U.S. study populations may have been due to hypobaric hypoxia which, as NRC (2006) notes, may be mistaken for fluoride-induced fluorosis.

3.1.4.3. Physiological/nutrition factors

The occurrence and severity of fluorosis may vary among individuals and populations exposed to the same levels of fluoride in environmental media. Such differences can be due to factors which enhance fluoride retention in the tissues, or produce alterations in enamel mineralization that may be indistinguishable from those produced by fluoride. Included among the physiological/nutritional variables that can affect fluorosis are: calcium deficiency; exposure to minerals such as strontium and aluminum; protein malnutrition; metabolic or respiratory acid-base abnormalities; certain pathological conditions and exposure to drugs early in childhood. These are discussed in this section.

Minerals: Studies on laboratory animals have indicated that calcium in the diet inhibits GI tract absorption of fluoride (Whitford, 1994); however, fluoride uptake into enamel is independent of calcium uptake and the effects of fluoride on calcium homeostasis are not necessarily a factor in enamel fluorosis (Aoba and Fejerskov, 2002). Accordingly, low levels of calcium in the diet may increase the rate of absorption of ingested fluoride, and thereby favor the development of dental fluorosis with increased calcium intake leading to decreased fluoride absorption. On the other hand, oral intake of calcium will not directly alter the effects of absorbed fluoride on enamel development.

Certain forms of "mottled" enamel may be caused by exposure to excessive amounts of trace minerals, even in the absence of significant exposure to fluoride. Curzon and Spector (1977) surveyed 1313 children 12–14 years old in seven towns in Wisconsin where the drinking water contained low levels of fluoride (1.0–1.2 mg/L) but variable and sometime elevated levels of strontium (0.022–33.9 mg/L). Mottling of the dental enamel of the teeth was found to increase in prevalence and severity as the strontium concentration increased. No such relationship was observed with fluoride concentrations varying in the narrow range of 1.0 to 1.3 mg /L.

Other minerals may also produce similar effects. Rozier (1994) cites a study by Butler et al. (1985) which suggested that exposure to zinc in drinking water may contribute to dental mottling. In the Butler et al. (1985) study, 2592 school children in 16 towns in Texas were examined for dental mottling. Three of the towns had relatively high levels of zinc in the drinking water. Butler et al. (1985) reported that zinc was a predictor for mottling, but that the association was not strong. The odds ratio was 2.18 (95% CI 1.26 to 3.78). Butler et al. (1985) state that animal studies have shown that exposure to zinc, as well as to strontium and chromium, can cause dental mottling.

As a result of studies conducted in Tanzania, Yoder et al. (1998) suggested that elevated levels of aluminum and/or magnesium in magadi (a lake-shore salt deposit used in cooking as a food tenderizer and to shorten cooking time) may have contributed to the severe dental fluorosis seen in children (9–19 yrs old) exposed to only a very low level of fluoride in drinking water (0.18 mg/L) in the town that had a prevalence of 54.9% severe dental fluorosis. Magnesium reportedly affects enamel formation in laboratory animals (Angmar-Månsson et al., 1984);

however, similar effects have not yet been reported in humans. Aluminum is known to cause ostemalacia as a result of aluminum-induced phosphate depletion, and Yoder et al. (1998) were of the opinion that a similar mechanism might affect teeth. The effect of aluminum on dental enamel mineralization in humans is not known.

Acid-base disturbances: Angmar-Månsson and Whitford (1990) reviewed data documenting the effects of acid-base imbalances on dental fluorosis. The rate at which fluoride is excreted by the kidneys is affected by urinary pH. The renal clearance rate is depressed by acidosis, and enhanced by alkalosis. Consequently, soft and hard tissue levels of fluoride may be increased under conditions of acidosis and decreased under conditions of alkalosis. Significantly though, Angmar-Månsson and Whitford (1990) reported that animal studies have shown that both acidosis and alkalosis, in the absence of fluoride, may adversely affect the mineralization of the enamel in a manner resembling that of fluorosis. Fluoride supplementation appeared to attenuate the effects caused by acidosis and enhance the effects caused by alkalosis.

Angmar-Månsson and Whitford (1990) describe several variables which may affect acid-base balance; these included the acid load of the diet, certain drugs, certain metabolic or respiratory disorders, altitude (see Section 3.1.4.2), and physical activity. Of these, dietary factors were considered the most important and high protein diets in particular were associated with moderate to high levels of acidosis.

The extent to which changes in acid-base balance may affect the occurrence and severity of dental fluorosis in human populations has not been fully documented; however, infant formula based on cow's milk reportedly can cause some degree of systemic acidosis and an acidic urine, and there are reports that dental fluorosis is higher in formula-fed infants than in those fed breast-milk. This difference, however, may be due to high levels of fluoride in infant formula prepared with tap water compared with that in breast milk. Whitford (1990) noted that fluoride levels in human breast milk are only 0.4 times the concentration in maternal plasma. As an example, Whitford estimated that an infant's fluoride intake through consumption of 800 mL of breast milk containing 0.4 μmol F/L (0.0076 mg/L) would be 0.006 mg, vs. 0.80 mg from consuming the same volume of formula prepared with water containing 1 mg F/L.

In older children the increased retention of fluoride under acidotic conditions may be of greatest concern only when fluoride intake is excessive, although further research is needed to fully document such effects.

Pathological conditions: Primary diabetes insipidus; nephrogenic diabetes insipidus, diabetes mellitus; acute glomerulonephritis, pyelonephritis, renal tubular acidosis, and nephrotic syndrome are disorders affecting urinary flow rate which can result in abnormal increases in the consumption of water (Angmar-Månsson and Whitford, 1990). As noted by Angmar-Månsson and Whitford (1990), over one million children in the U.S. may be affected by one of these conditions, and it is possible that these disorders may contribute to higher levels of dental fluorosis in these children because of increased water consumption.

Because fluoride is excreted primarily through the kidney, individuals with kidney disease and reduced glomerular filtration are likely to have increased plasma fluoride levels (NRC, 2006), which, in turn, may result in increased tissue levels of fluoride. Such individuals may be more

susceptible to the adverse effects of fluoride including dental fluorosis in children and skeletal fluorosis in adults (see Section 3.3).

Exposure to drugs: Tredwin et al. (2005) have reviewed the various minerals and drugs that induce disorders of the teeth. Included among these are chemicals that cause extrinsic tooth discoloration, such as chlorhexidine (an antimicrobial), iron salts, essential oils and co-amoxiclav (a combination of amoxicillin and clavulanic acid). In an efficacy study of chlorhexidine mouthrinses, Lorenz et al. (2006) reported that 40 of 68 test subjects using a chlorhexidine mouth rinse for 21 days exhibited signs of discoloration of teeth or tongue.

Hong et al. (2004) conducted a prospective study on 490 children from birth to 5 years of age, using a series of parent questionnaires to assess fluoride intake and amoxicillin intake. Amoxicillin use for 6 weeks to 3 months and 3 months to 6 months significantly increased the risk for fluorosis of primary second molars in bivariate analyses. After controlling for fluoride intake, the adjusted risk of fluorosis was not significant for amoxicillin use. The study authors concluded that amoxicillin could play a contributory role in the development of primary tooth fluorosis. In a continuation of these studies, Hong et al. (2005) used relative risk (RR), Mantel-Haenszel stratified analyses, and multivariate logistics regression to examine the relationship between amoxicillin use and dental fluorosis in the early erupting permanent teeth of 579 children who were participants in the Iowa Fluoride study. The children were followed from birth to age 32 months, and were assessed for fluorosis at age 9 years. Amoxicillin use from 3–6 months significantly increased the risk of fluorosis on the central maxillary incisors (RR = 2.04; 95% CI = 1.49–2.78). After adjusting for fluoride intake and otitis media, the risk of fluorosis was still statistically significant (RR = 1.85, 95% CI = 1.20–2.78).

As reviewed by Tredwin et al. (2005), other drugs that have been reported to induce discoloration of the teeth include the antibiotics tetracyclines, minocycline (semi-synthetic tetracycline derivative), and ciprofloxacin. The first causes yellowish to brown or gray discoloration; the second causes grey-green or blue-green discoloration, and the third causes a greenish discoloration.

3.1.4.4. Genetic factors

Genetic factors may produce conditions that mimic dental fluorosis or cause an increased susceptibility to dental fluorosis. The NRC (2006) notes that a genetic condition called amelogenesis imperfecta can be mistaken for fluorosis. The condition results in defective development of dental enamel, marked by a brown color of the teeth due to improper differentiation of the ameloblasts. This genetic condition reportedly occurs at a rate of 0.007% to 0.14%, depending on the population studied.

Racial differences in susceptibility to dental fluorosis have been reported in several studies. Russell (1962) evaluated dental fluorosis in 337 white and 82 African-American children who had been born in and spent at least their first seven years in Grand Rapids, MI. The fluoride concentration in the water supply during this time was reported to be very strictly controlled at 1 mg/L. For children 12–14 years old, Russell (1962) reported that the prevalence of very mild and mild fluorosis was 7.7% in white children and 14.1% in African-American children, suggesting a slightly higher susceptibility in the latter group (data not evaluated statistically). For

all age groups the percentages were 7.1% for white children and 15.9% for African-American children.

In an epidemiological study conducted in 1980–81 in 16 towns in Texas, Butler et al. (1985) found that the odds ratio for African-American children to develop dental fluorosis was 2.3 (95% CI = 1.4–3.7) when compared to the prevalence rates seen in Hispanic and non-Hispanic white children. All 2592 children studied were lifetime residents of the communities and were enrolled in grades 2–6 (ages 7–13) or grades 9–12 (ages 14–19) at the time of the study. Fluoride concentrations in the drinking water of the communities studied ranged from 0.2–3.3 ppm.

Williams and Zwemer (1990) evaluated the prevalence of dental fluorosis (TSIF scoring system) in 374, 12–14-year-old school children living in an urban or rural area of Georgia. The participants included 217 county children (100 Afro-Americans and 127 whites) and 157 city children (102 Afro-American and 55 whites). City residents had a life-long exposure to drinking water with fluoride levels of 0.9–1.2 mg/L, whereas the fluoride level in the drinking water of the county children ranged from 0.2 to 0.9 mg/L. Chi-square analysis revealed a statistically significant association between higher TSIF scores and urban residence**.** However, the association with gender, race, dietary habits, toothpaste ingestion or fluoride supplement use (information obtained from parental questionnaires) was not significant. The prevalence of moderate (TSIF score 4) and severe (TSIF score 5) dental fluorosis was 9.6% and 4.5% in the city (N = 157) and 0.5% and 0.9% in the county, respectively.

In order to evaluate the existence of susceptibility or tolerance genes in humans, Liu et al. (2006) analyzed leukocyte gene expression (using the gene chip HG-U133A) in 30 children, 10–12 years old, in populations from two residential areas of China with different levels of fluoride in their drinking water (1.1–2.0 mg F/L in one village and 0.76 mg F/L in another). Comparisons were made between three groups of 10 children each, all selected at random. Two groups were from the town with 1.1–2 mg F/L; in one group all the children showed signs of fluorosis, and in the other group none of the children exhibited fluorosis. The third control group of ten came from the town with only 0.76 mg F/L.

The data were analyzed with Affymetrix Microarray Suite 5.0. The change in the p-value was calculated by the Wilcoxon's signed rank text. The signal log ratio (SLR) algorithm was used to estimate the magnitude and direction of the change in the transcript, when two arrays were compared. The robustly up-regulated or down-regulated genes were selected that conformed to all of the following criteria: present in the experimental sample, increase or decrease in expression and $SLR \leq 1.0$ or $SLR \geq 1.0$. The results showed that, compared with the control group, 1057 genes were differentially expressed in the children from the high fluoride town (those with and without fluorosis). Of these, 148 were robustly up-regulated and 61 were robustly down-regulated. These included transcription factors, genes related to signal transduction, structure proteins, transport proteins, cancer genes, genes related to immunity and genes related to apoptosis. A total of 964 genes were differentially expressed in the dental fluorosis group compared to the control group (71 robustly up-regulated and 60 robustly down-regulated). When the dental fluorosis group was compared to the high fluoride-no fluorosis group, 633 genes were differentially expressed (15 robustly up-regulated and 67 robustly down-regulated); including genes related to immunity, transcription factors, signal transduction and structure proteins.

Several animal studies also indicate that there may be a genetic component to susceptibility to dental fluorosis. Everett et al. (2002) evaluated the development of dental fluorosis in 12 different inbred strains of male weanling (three-week-old) mice (129P3/J, A/J, BALB/cJ, C3H/HeJ, C57BL/10J, CBA/J, DBA1/J, FVB/NJ, SJL/J, and SWR/J). Three treatment groups consisted of 72 mice each, six from each of the 12 strains; one group received distilled water, the second received distilled water with 25 ppm fluoride, and the third distilled water with 50 ppm fluoride. The fluoride ion-specific electrode was used to verify the fluoride concentrations. Once a week each animal was given a complete oral examination and scored for dental fluorosis over the entire upper and lower incisor tooth surfaces using the TF scoring system. Quantitative light-induced fluorescence (QLF) was also used to analyze fluorosis in extracted mandibular central incisors. At day 60 of the treatment period all the test animals were killed, weighed, and examined and selected mineralized tissues were removed. All strains developed various level of fluorosis at 50 ppm, and some strains were also responsive at 25 ppm. A/J mice appeared to be the most susceptible to fluorosis which appeared early, within several weeks, and was seen at both 25 and 50 ppm. In contrast, strain 129P3/J mice were the most resistant and showed only minimal fluorosis at 50 ppm. QLF analysis of control and high-dose mice revealed no significance difference ($p = 0.413$) for the 129P3/J strain, but a statistically significant increase in fluorosis in the A/J mice ($p = 0.006$).

Vieira et al. (2005) examined genetic and environmental factors influencing the development of dental fluorosis in three strains of mice (A/J, 129P3/J and SWR/J) known to have different levels of susceptibility to dental fluorosis. Groups of weanling mice were treated with four different levels of fluoride in their drinking water (0, 25, 50 and 100 ppm) for six weeks, after which their teeth were analyzed for fluoride content using neutron activation analysis. Dental fluorosis was assessed by QLF and tooth quality was determined by enamel and dentin micro-hardness and dentin mineralization. Dental fluorosis increased with increase in fluoride level and was much higher for the A/J mice than the SWR/J mice indicating a greater susceptibility, even though enamel hardness was similar in the two strains. A correlation was seen between fluorosis severity and tooth fluoride concentration, but only 34% of the variance was explained by the concentration of the fluoride in the tooth. The study authors concluded that other factors, such as genetic susceptibility, are likely to be important in fluorosis severity.

3.1.5. Summary

As noted by NRC (2006), the weight of evidence indicates that the threshold for severe dental fluorosis occurs at a water fluoride level of about 2 mg/L. On the basis of a select set of criteria, the study identified as the most appropriate for dose-response modeling for dental fluorosis is that of Dean (1942). This study provides a comprehensive data set on multiple communities using an appropriate fluorosis scoring system that is still in use today.

Other studies such as that of Galagan and Lamson (1953) and Eklund et al. (1987), and a number of non-U.S. studies provide information on the increased prevalence of fluorosis under hot climatic conditions. Increases in dental fluorosis under these conditions have been attributed primarily to increased fluoride intake due to increased drinking water consumption. Current fluoridation guidelines take into account such differences by recommending lower fluoride concentrations in drinking waters available in hot climates (CDC, 1995). Some researchers, however, have suggested that climatic factors may be less important today considering changes

in living conditions (increased use of home and vehicle air conditioning), life style (more time spent indoors), and dietary habits (e.g., increased use of bottled water and filtered tap water which may contain reduced amounts of fluoride). Further research is needed in this area.

Based on studies of non-U.S. populations there is evidence that living at high altitudes may enhance the development of dental fluorosis or produce a condition that cannot be distinguished from dental fluorosis; however, the extent that this is occurring in U.S. populations living at high altitudes cannot be determined at this time.

Various physiological factors, such as calcium deficiency, co-exposure to certain minerals, malnutrition, respiratory or metabolic acidosis or alkalosis, and various pathological conditions affecting urinary output and kidney function, may contribute to increases in the prevalence and severity of dental fluorosis and/or produce dental abnormalities that are indistinguishable from dental fluorosis; conditions which may, in part, account for reports of high levels of fluorosis in some populations exposed to low levels of fluoride. These factors introduce an unquantifiable degree of uncertainty in interpreting dose-response data for fluoride-induced dental fluorosis.

3.2. Relationship between Dental Caries and Dental Fluorosis

Early childhood exposure to fluoride in drinking water has been shown to significantly reduce the occurrence of caries. Although several studies cited by the NRC (2006) suggest that this beneficial effect of fluoride may extend to drinking water concentration as high as 4 mg/L (Englander and DePaola, 1979; Driscoll et al., 1983; Heifetz et al., 1988; Selwitz et al., 1995; Jackson et al., 1995), the NRC (2006) states that the evidence "is not persuasive that caries frequency is appreciably lower at approximately 4 mg/L than at approximately 2 mg/L or 3 mg/L." Of greater concern to the NRC (2006), however, is the possibility that those individuals exposed to fluoride levels above 2 mg/L and suffering from severe fluorosis might be at greater risk of developing caries due to the fluoride-induced pitting of the enamel which would allow food plaque to become entrapped in enamel defects and thereby induce decay. Evidence of an increase in decay rates in this segment of exposed populations would support the supposition that severe fluorosis is not merely an undesirable cosmetic effect, but can also have adverse consequences with the potential to impact health.

Very few studies have specifically investigated the relationship between caries frequency and degree of dental fluorosis. Generally, most studies have documented caries frequency in specific populations exhibiting a range of different levels of dental fluorosis but exposed to a single level of fluoride in drinking water. In cases where drinking water is the major route of fluoride exposure, and the levels of fluoride in the drinking water are high, then the fluoride concentration in the water may be indicative of the expected prevalence of severe fluorosis in the study population. To the extent that the fluoride concentration is directly related to severe fluorosis, data on the relationship between fluoride concentration and caries occurrence (i.e., studies that compare fluoride in drinking water to measures of cavities) can lend support to the severe fluorosis-cavity relationship. Likewise, relationships between the Community Fluorosis Index (CFI, originally referred to by Dean as the Index of Dental Fluorosis) for the study populations and cavities may be useful. The definition of the CFI, as paraphrased from Dean (1942), is as follows:

The Community Fluorosis Index is a weighted average computed by assigning the following numeric weights to the various levels of fluorosis: normal = 0, questionable = 0.5; very mild = 1; mild = 2, moderate = 3, and severe = 4. The Index is equal to the sum of the scores for each fluorosis group (number of individuals times the numeric weight) divided by the total number of individuals in the study population. It is intended only as a relative measure of fluorosis to be used for intra and inter-population comparisons.

In assessing dental caries, most studies have used scores for decayed, missing, and filled teeth (DMFT) or decayed, missing, and filled tooth surfaces (DMFS). These scores reflect the cumulative caries experience of a person or the average caries experience of a population (PHS, 1991). The mean DMFT or DMFS is calculated as the sum of the cases of each of the components across the entire study population divided by the total number of individuals examined; thus the higher the mean value, the worse the dental condition of the population. Mean DMFT and DMFS scores can also be derived for specific segments of a population, such as those that show a specific level of dental fluorosis. This approach allows comparisons between groups with different levels of fluorosis although it only indirectly addresses the issue of whether severely fluorosed teeth are at greater risk of caries. To assess the latter issue a comparison of the DMFS scores between severely fluorosed teeth with those of lesser severity would be needed.

The relationship between caries and fluoride exposure displays the U-shaped dose-response that characterizes many nutrients where there are adverse effects with intakes that are below those that confer a benefit and adverse effects with intakes that are greater than those with benefit. In such cases, comparisons need to be made between the intakes that define the base of the U and those that lie to either side of that base. The base of the U identifies the dose range that defines intakes providing nutritional benefit without risk of adversity for healthy populations. The symmetry of the U is often variable with the slope to the left frequently steeper than that to the right. In the case of fluoride a comparison between caries prevalence for moderate or mild/moderate fluorosis and severe fluorosis would be needed rather than between no or questionable fluorosis and severe fluorosis.

One confounding factor in DMFT or DMFS scoring of severely fluorosed teeth is the degree to which fluorotic pits might be misdiagnosed as pre-carious lesions and filled, thereby resulting in higher DMFT and DMFS scores. Examiner bias is another potential confounding factor which would be difficult to quantify. Few studies used multiple examiners to assess this possibility. During 1999–2002, among children aged 2–11 years, 41% had dental caries in their primary teeth. Forty-two percent of children and adolescents aged 6–19 years and approximately 90% of adults had dental caries in their permanent teeth. Among children aged 6–19 years, 32% had received dental sealants. Adults aged >20 years retained a mean of 24 of 28 natural teeth and 8% were edentulous (Beltrán-Aguilar et al., 2005). The increase of cavity prevalence with age illustrates the importance of comparing caries frequency across similar age groupings.

3.2.1. Dental caries and severe fluorosis

The NRC (2006) identified 14 cases where comparisons were made between dental condition (i.e., DMFS, DMFT, or percent caries) and severity of dental fluorosis. These 14 comparisons are summarized in Table 3-18.

Table 3-18. Studies Evaluating the Relationship between Dental Condition and Severity of Fluorosis

Country (age)	Fluoride (mg/L)	No.	Fluorosis Score	Dental Condition (Endpoint)	Reference
Studies Using Dean's Index of Fluorosis					
U.S. (8–16 yr)	>2 to ≤4	218	VM to Mod	1.58 (mean DMFS)	Driscoll et al., 1986
		54	Severe	2.96	
		218	VM to Mod	4.5% (D or F)	
		54	Severe	19.6%	
U.S. (adults)	3.5	38	M to Mod	43% (DMFT - molars)	Eklund et al., 1987
		125	Severe	40%	
		38	M to Mod.	11% (DMFT - premolars)	
		125	Severe	19%	
		38	M to Mod.	3% (DMFT - anteriors)	
		125	Severe	6%	
Taiwan (6–16 yr)	0.21–4.69	1290	VM to Mod	1.7 (mean DMFT)	Chen, 1989
		10	Severe	2.5	
Sri Lanka (14 yr)	0.14–0.88[a]	44	M	3.4 (mean DMFT)	Warnakulasuriya et al., 1992
		48	Mod to Severe	3.3	
Israel (15–16 yr)	5	83	Mod	4.4 (mean DMFS)	Mann et al., 1987
		46	Severe	10.4	
Israel (6–8 yr)	4.7–5.3	55	Moderate	1.25 (mean DMFS)	Mann et al., 1990
		6	Severe	1.83	
Ethiopia (6–7;13–14)	3.5;12.4[b]		Mod	9% of teeth with cavities	Olsson 1979
			Severe	25% of teeth with cavities	
Studies Using Other Indices of Fluorosis					
Ethiopia (12–15 yr)	0.2–2.2	58	3-4 (TFI)	1.48 ±2.05 (mean DMFT)	Wondwossen et al., 2004
		22	5-7	2.86 ±3.18	
	8.9–14.1	29	3-4	1.58 ±1.91 (mean DMFT)	
		67	5-7	2.31 ±2.23	
Turkey (12–14 yr)	1.42–1.66	24	1-3 (TSIF)	1.7 (mean DMFS)	Ermis et al., 2003
		105	4-7	1.9	
		24	1-3 (TSIF)	1.2 (mean DMFT)	
		105	4-7	1.3	
Brazil (6–12 yr)	2–3	42	3-4 (TFI)	1.1 (1.4 SD)(mean DMFT)	Cortes et al., 1996
		18	≥5	1.3 (1.1 SD)	

Modified from NRC (2006): VM = very mild; M = mild, Mod = moderate; D = decayed; M = missing; F = filled; S = surfaces; T = teeth; TFI= Thylstrup and Fejerskov Index; TSIF = Tooth Surface Index of Fluorosis; SD = standard deviation.

[a]Mean values (total range 0.08 to 8.00 mg/L).

[b]Mean values (ranges were 1.2–7.4 mg/L and 6.0–17 mg/L, respectively).

NRC (2006) concluded that in 11 of the 14 available "contrasts," the measure of caries frequency was higher with severe fluorosis than with mild to moderate fluorosis. The NRC (2006) qualifies this statement by noting that not all the studies evaluated the data statistically, and in some cases the differences were slight. As shown in Table 3-18, ten of the comparisons used Dean's index for scoring the fluorosis, two used the TFI scoring system (Cortes et al., 1996; Wondwossen et al., 2004, and two used the TSIF system (Ermis et al., 2003). Four of the studies involved

different tooth types or different caries units (e.g. DMFT) within the same population (Driscoll et al, 1986; Eklund et al., 1987; Ermis et al., 2003; Wondwossen et al., 2004). Thus, only seven separate studies provide data based on Dean's index of fluorosis (Driscoll et al., 1986; Eklund et al., 1987; Chen, 1989; Warnakulasuriya et al., 1992; Mann et al., 1987; Mann et al., 1990; and Olsson 1979). The studies listed in Table 3-18 are discussed in more detail below.

Driscoll et al. (1983; 1986) evaluated the mean DMFS scores in school children in seven Illinois communities with different levels of fluoride in their drinking water and, correspondingly, different levels of prevalence of severe fluorosis based on Dean's classification of fluorosis (Tables 3-19, see also Table 3-18). Mean DMFS scores decreased and then began to increase as the fluoride level, the Community Fluorosis Index, and the percent prevalence of severe fluorosis increased, although even in the highest exposure group the mean DMFS score did not exceed the value seen at the lowest fluoride level of 1.06 mg/L.

Table 3-19. Severe Fluorosis, Community Fluorosis Index and Mean DMFS Scores for Illinois School Children (8-16 yrs old) Studied by Driscoll et al. (1983)

Fluoride (mg/L)	Population Size	% Severe Fluorosis[a]	Community Fluorosis Index[a]	Mean DMFS
1.06	336	0.6	0.39	3.14
2.08	143	4.9	1.16	1.97
2.84–2.89	192	8.3	1.25	1.41
3.77–4.07	136	22.8	1.88	2.02

[a]Based on Dean's fluorosis scoring system, and calculated from data given in Table 3-4.

In a continuation of these studies, Driscoll et al. (1986) included the prevalence of dental caries in four Iowa communities with very low fluoride in the water supply (<0.3 mg/L). The communities with <0.3 mg F/L exhibited the highest mean DMFS score (Table 3-20). The DMFS values in Tables 3-19 and 3-20 are illustrative of the U-shape for the caries concentration-response.

Table 3-20. Percent Severe Fluorosis and Mean DMFS Scores for Midwestern School Children Studied by Driscoll et al. (1986)

Fluoride Level (mg/L)	No.	% Severe Fluorosis	Mean No. DMFS per child	% Change from 0.3 mg/L Group
<0.3[a]	316	0	5.07	NA
1.06[b]	336	0.6	3.14[c]	38.1
2.08[b]	143	4.9[b]	1.97[d]	61.1
2.84–2.89[b]	192	8.3[b]	1.41[d]	72.2
3.77–4.07[b]	136	22.8[b]	2.02[d]	60.2

[a]Belle Plaine, Durant, Marengo, and Missouri Valley, Iowa.
[b]Illinois towns included in Driscoll et al. (1983) study (optimal fluoride 1 mg/L).
[c]Significantly lower than <0.3 mg/L group.
[d]Significantly lower than <0.3 mg/L group and also 1.06 mg/L group ($p<0.01$).

For the Illinois towns with fluoride concentrations above the ~1 mg/L regarded as optimal (e.g., ≥2 mg/L), Driscoll et al. (1986) compared the mean DMFS score per child directly with the fluorosis score and found that the mean DMFS score for children with a fluorosis score of 4 was significantly higher than those with scores of 0.5 to 3 (Table 3-21). The lowest caries response was that for the questionable fluorosis score. However, had very mild and mild not been combined with moderate, the DMFS for one or both might have been lower than that for questionable based on data from other studies.

Table 3-21. Mean DMFS per Child and Fluorosis Scores for Illinois towns with fluoride water levels above optimal[a] (Driscoll et al., 1986)

Dean's Fluorosis Index	No.	Mean No. DMFS
0	87	1.89
0.5	112	1.40
1–3	218	1.58
4	54	2.96[b]

[a] ≥2 mg F/L.
[b] Significantly higher than children with fluorosis scores of 0.5–3 ($p<0.05$); no other significant differences between groups.

These data suggest that, for this study population, the maximum anti-caries benefit of fluoride occurred in those individuals with questionable to moderate levels of fluorosis (score of 0.5 to 3). Because Driscoll et al. (1986) combined the DMFS data for the groups having fluorosis scores of 1 to 3 it is not possible to determine whether the trend in those intermediate levels increased progressively with increasing severity of fluorosis. [Note: according to Driscoll et al. (1986), data for children in the negligible and optimal fluoride areas were excluded from the analysis because so few of them exhibited fluorosis].

Eklund et al. (1987) evaluated dental caries and fluorosis in adult lifetime residents of Lordsburg (N = 164) and Deming (N = 151), New Mexico. The fluoride concentration in drinking water of Lordsburg was 3.5 mg/L and that in Deming was 0.7 mg/L. All teeth of each subject were examined for dental fluorosis (Dean's Index) and caries (DMFT scores). The overall DMFT score (according to the criteria of Radike, 1972) was 7.0 for Lordsburg and 8.7 for Deming, suggesting that the Lordsburg residents had better protection against dental caries; these differences were statistically significant ($p = 0.0041$) (Table 3-22). Residents of Lordsburg had much higher rates of severe fluorosis (38.4% severe and 37.8% very severe) than residents of Deming (95.4% normal to very mild and 0% severe or very severe) as well as a much higher Community Fluorosis Index (3.74 vs. 0.31 for Deming; $p = 0.0006$). Information was not provided on the mean DMFT scores by category of fluorosis; however, for the Lordsburg subjects, 94.7% of the teeth exhibiting mild to moderate fluorosis were rated as "sound" (138 of 2609), whereas 76.3% of the teeth exhibiting severe fluorosis were rated as "sound." These results are consistent with other observations that the anticaries effects of fluoride are present at concentrations above the 0.7 to 1.2 mg/L range identified as optimal.

Table 3-22. Severe Fluorosis, Community Fluorosis Index, and Mean DMFT Scores for New Mexico Adult Populations Studied by Eklund et al. (1987)					
Town	**Subjects**	**Fluoride (mg/L)**	**% Severe Fluorosis**	**Community Fluorosis Index[a]**	**Mean DMFT**
Deming	151	0.7	0	0.31	8.7
Lordsburg	164	3.5	76.2	3.74[b]	7.0

[a]Calculated from data given in Table 3-4.
[b]Cases of very severe fluorosis are given a score of 4 for calculation of the Index.

The Eklund et al. (1987) publication also evaluated the percent prevalence of decayed, missing or filled teeth by tooth type and level of severity of fluorosis in the Lordsburg study group (Table 3-23). For the anteriors and premolars, the percentage of decayed, missing or filled teeth was higher for severe fluorosis than for mild to moderate fluorosis; however, this trend was not seen in molars which appeared to be more susceptible to caries than premolars or anterior teeth. The data were not analyzed statistically by Eklund et al. (1987). The Eklund et al. (1987) data for Deming and Lordsberg combined illustrate the protective function of fluoride in drinking water even when it leads to fluorosis. However, it is also supportive of the hypothesis that individuals with severe dental fluorosis can experience an increased risk for cavities compared to those with milder cases of dental fluorosis. The Eklund et al. (1987) publication did not report on the presence of fluorosis on the cavity-proned molars compared to that on the anterior teeth and premolars.

Table 3-23. Dental Condition and Level of Fluorosis by Tooth Type in New Mexico Adult Populations Studied by Eklund et al. (1987)				
Tooth Type	**Fluorosis Score[a]**	**No. of teeth examined**	**DMFT**	**Percent**
Molars	Normal to very mild	917	562	61.3
	Mild to moderate	529	230	43.5
	Severe (+ very severe)	483	193	40.0
Premolars	Normal to very mild	1049	262	25.0
	Mild to Moderate	703	73	10.4
	Severe (+ very severe)	489	91	18.6
Anteriors	Normal to very mild	1744	87	5.0
	Mild to Moderate	1474	39	2.7
	Severe (+ very severe)	297	17	5.7

[a]Dean's index.

Iida and Kumar (2009) examined a subset of the 1986–1987 data from the National Survey of Oral Health of U.S. School Children (NIDR, 1992) to determine if there was an association between dental caries and enamel fluorosis. The DMFS data for a total of 16,873 children 7–17 years of age with a continuous residence history were examined and categorized by the degree of fluorosis according to the Dean descriptors. The fluorosis status was assigned according to the two teeth per child with the highest Dean-Index score. The caries prevalence declined with increasing fluorosis level up to the severe fluorosis category where it increased (Table 3-24). The DMFS declined through the mild fluorosis descriptor, increased for the moderate fluorosis and

declined to the lowest level for the severe fluorosis grouping. Standard errors values were higher for the mild and moderate fluorosis categories indicating higher variability among those subjects.

The authors also categorized the data based on the decayed surfaces of the right maxillary first molars as related to the degree of fluorosis. Right maxillary first molars with fluorosis consistently had lower levels of caries experience than did normal molars. For some of the children included in Table 3-24, the right maxillary first molar was not moderately or severely fluorosed, thus reducing the number of children in the moderate group from 190 to 110 and those in the severe fluorosis group from 46 to 31. When limited to the right maxillary molars, the caries prevalence and DMFS were inversely related to the degree of fluorosis. According to the authors, the permanent first molar is one of the teeth most susceptible to both dental caries and fluorosis. Variables associated with DMFS in this later group were age, sex, metropolitan status, school region and sealant use.

Table 3-24. Weighted Child-level Estimates of Caries Prevalence and Mean DMFS of Permanent Teeth According to Degree of Fluorosis as Reported by Iida and Kumar (2009)

Degree of fluorosis	Sample size	Caries prevalence % (SE)	Mean DMFS (SE)
Normal	8261	54.3 (0.7)	3.56 (0.1)
Questionable	5089	56.3 (0.9)	3.24 (0.1)
Very Mild	2685	55.4 (1.2)	2.97 (0.1)
Mild	602	48.1 (2.5)	2.40 (0.2)
Moderate	190	45.9 (4.5)	2.64 (0.5)
Severe	46	52.6 (8.9)	2.24 (0.5)

Chen (1989) evaluated caries prevalence in 5107 children, 6 to 16 yrs old, residing in 14 communities in Shenkang Hsiang Province, Taiwan. The communities were divided into six exposure categories based on water fluoride concentrations in the home. The range of fluoride concentrations in each of the exposure groups was as follows: 0.21–0.25 mg/L (negligible); 0.43–0.48 mg/L (reported as optimal for the region); 0.75–0.98 mg/L (2x optimal); 2.40 mg/L (4x optimal); 2.84–3.24 mg/L (5x optimal); and 4.69 mg/L (7x optimal). Severe fluorosis occurred in 0.2% of the group whose water supply had 0.75–0.98 mg F/L; in 0.3% of the group with 2.84–3.24 mg F/L; and in 1.3% of the group with 4.69 mg F/L. Dental caries were scored with the DMFT index. Compared with the negligible exposure group, DMFT scores were 10.7% lower in the 0.43–0.48 mg F/L group; 14.3% lower in the 0.75–0.98 mg F/L group; and 50.0% lower in the 2.40 mg F/L group. At higher concentrations the presence of fluoride appeared to be less protective against caries. The DMFT scores were only 42.9% lower in both the 2.84–3.24 mg F/L group and the 4.69 mg F/L groups. The maximum reduction in DMFT occurred among the children receiving drinking water with 2.4 mg/L fluoride.

Chen (1989) compared the DMFT scores for different levels of fluorosis severity (Table 3-25). The mean DMFT was 1.7 for very mild to moderate fluorosis and 2.5 for severe fluorosis. Thus, on a total population basis (mean DMFT per child), the higher fluoride level was beneficial, but on a group basis, those with severe fluorosis did not benefit as much. The DMFT score, however, for the severe fluorosis group was not significantly different from the no-fluorosis group, but it was significantly different ($p<0.050$) from those with the milder grades of fluorosis.

Table 3-25. Fluorosis Severity and DMFT in Taiwanese School Children Studied by Chen (1989)		
Fluorosis Score[a]	**No.**	**Mean DMFT/child**
0	3252	2.4
0.5	520	2.2
1–3	1290	1.7
4	10	2.5[b]

[a]Dean's Index of Fluorosis.
[b]Significantly higher than fluorosis scores of 0.5 and 1–3 ($p<0.05$). All other differences not significant.

Warnakulasuriya et al. (1992) recorded the occurrence of dental caries and fluorosis in 380 school children (14 years old) residing in four different geographic areas of Sri Lanka with varying levels of fluoride in the water supplies. The mean fluoride values for each of the four regions were: 0.14 mg/L (range 0.08–0.33); 0.61 mg/L (range 0.09–5.60); 0.62 mg/L (range 0.36–2.80); and 0.88 mg/L (range 0.17–8.00). The areas had similar altitudes with mean annual maximum temperatures of 29–32°C. The study populations were similar in socioeconomic level. Dental fluorosis was assessed using Dean's classification system and the criteria of Russell (1961) to distinguish between fluorosis and non-fluoride enamel opacities. For Dean's Index, the authors used a score of 1 instead of 0.5 for questionable results, thus the most severe category was scored as 5. Each child was examined for dental caries using the DMFT index. Each child supplied a sample of water from his or her domestic source of drinking water and these samples were analyzed for fluoride using a fluoride ion-specific electrode. The children were then grouped into five fluoride categories as shown in Table 3-26. The percent of children that were caries-free in the 0.6–0.79 mg F/L group was significantly larger than that for the lower and higher fluoride groups, and the mean DMFT scores were significantly lower than the other groups. Significant differences were not seen among the other four exposure groups. Local variations in the fluoride concentrations (e.g., 0.17 to 8.00 mg F/L) are likely to have compromised the usefulness of this study in detecting such differences.

Table 3-26. Caries Status in Sri Lanka School Children Studied by Warnakulasuriya et al. (1992)					
Fluoride (mg/L)	**Number examined**	**Percent Caries Free[a]**	**Mean DMFT[b]**	**SD**	**% DMFT Reduction**
<0.4	211	16.6	3.35	2.69	–
0.4–0.59	49	20.4	2.88	2.34	14
0.6–0.79	32	37.5	1.91[b]	2.35	43
0.8–0.99	27	29.6	2.56	2.27	24
>1.0	61	24.5	2.74	2.30	18

[a]$\chi^2_4 = 57.25$; $p<0.001$.
[b]One-way ANOVA – $F^4_{379} = 3.83$; $p<0.01$ (as given in Warnakulasuriya et al., 1992).

The DMFT scores were compared with the level of severity of fluorosis (moderate and severe fluorosis were combined) and the results analyzed statistically (Table 3-27). There were no significant differences between the DMFT scores. However, because the moderate and severe

fluorosis groups were combined, the effects of severe fluorosis on caries may have been masked to some extent. The study authors note that the results may have been influenced by the mobility of children within a given geographic region, and by the consumption of water from schools, because wells within 15 miles of each other sometimes had a 10-fold difference in water fluoride concentrations. Thus, this study does not provide conclusive evidence for or against the supposition that severe fluorosis is associated with an increased occurrence of caries.

Table 3-27. Mean DMFT by Fluorosis Score for Sri Lanka School Children Studied by Warnakulasuriya et al. (1992)

Group	No.	Percent of Population	Mean DMFT[a]	SD
Normal (0)	156	52	3.12	±2.61
Questionable (1)	44		2.82	±2.39
Very Mild (2)	88	35	3.55	±2.54
Mild (3)	44		3.43	±2.76
Moderate (4) + Severe (5)	48	13	3.31	±2.36

[a]One-way ANOVA – $F^4_{379} = 1.96$; $p>0.05$ (as given in Warnakulasuriya et al., 1992).

Mann et al. (1987) reported on the prevalence of dental caries and dental fluorosis in the permanent teeth of 182 school children (15–16 years old) residing in one small village in the Gaza Strip. The concentration of fluoride in the well water was 5 mg/L. Dean's index was used for scoring fluorosis and DMFS for scoring the permanent dentition for caries. The DMFS scores in relation to the severity of fluorosis are shown in Table 3-28. The DMFS scores were significantly higher in the group with severe fluorosis ($p <0.001$, analysis of variance). Boys exhibited a higher prevalence of severe fluorosis (42.2%) than girls (8.7%). The study authors suggested that this might have been due to post-eruptive factors, such as masticatory forces on the defective enamel, higher consumption of tea containing high levels of fluoride, smoking which may have led to staining of the teeth and misdiagnosis of fluorosis severity, and difficulties in differentiating fissure cavities from fluorotic fissure pitting. The data, however, do support the supposition that severe fluorosis is associated with increased caries.

Table 3-28. Mean DMFS by Fluorosis Score for Gaza Strip School Children Studied by Mann et al. (1987)

Group (Fluorosis score)	No.	Percent of Population	Mean DMFS[a]	SD
Mild (2)	53	29	2.81	±4.69
Moderate (3)	83	46	4.42	±5.39
Severe (4)	46	25	10.37	±9.87

[a]Statistically significant association between fluorosis and DMFS, ANOVA, $p<0.001$.

Mann et al. (1990) evaluated caries and fluorosis in the permanent and primary dentition in a population of children (72 boys and 80 girls, 6-8 yrs old) residing in the same village in the Gaza Strip which was the subject of the Mann et al. (1987) study. Fluoride levels in the well water used as drinking water were 4.7–5.3 mg/L. Dean's system was used for scoring fluorosis and DMFS for scoring the primary and permanent dentition for caries (results only for the permanent dentition discussed here). DMFS scores gradually increased in the permanent dentition with increasing

fluorosis severity (Table 3-29), and the score for those individuals exhibiting severe fluorosis was reported to be significantly different from the individuals with no signs of fluorosis. These results generally support those of Mann et al. (1987) as the mean DMFS associated with severe fluorosis is approximately three-fold higher than that for mild fluorosis. Note: the gender differences observed in the Mann et al. (1987) study were not seen in the 1990 study.

Table 3-29. Mean DMFS by Fluorosis Score for Gaza Strip School Children Studied by Mann et al. (1990)

Group	No.	Percent of Population	Mean DMFS	SD
No signs	7	4.6	0.29	±0.76
Mild	84	55.3	0.50	±1.06
Moderate	55	36.2	1.25	±1.54
Severe	6	3.9	1.83[a]	±3.54

[a]Statistically different from group with no signs, ANOVA, $p<0.05$.

Olsson (1979) reported on the occurrence of dental fluorosis and dental caries in 478 Ethiopian school children (ages 6–7 years old and 13–14 years old) residing in either Wonji, a sugar plantation area in the Shoa province; or Awassa, the capital of the Sidamo province. Fluoride levels in Wonji, taken from six wells, ranged from 6.0 mg/L to 17 mg/L (mean of 12.4 mg/L). In Awassa, fluoride levels taken from seven wells ranged from 1.2 mg/L to 7.4 mg/L (mean of 3.5 mg/L). Dental fluorosis was scored according to the criteria of Dean (1934), as modified by Moller (1965). The severe score was reserved for teeth with extensive loss of enamel while teeth with some confluent pits only were scored as moderate. Dental caries were assessed as the percent decayed teeth for primary and permanent teeth (results for the permanent teeth only discussed here). The prevalence of caries for each fluorosis severity level is shown in Table 3-30. The percentage of decayed teeth was significantly higher in the severe fluorosis group ($p<0.05$), and would likely be even higher if the teeth classified as moderate but with pitting were to be reclassified as severe. Olsson (1979) noted that high water consumption during the dry seasons, frequent tea drinking and possible malnutrition may have contributed to the degree of fluorosis seen in the study. Of the 239 children included in the assessment, 100 were not born in the study areas which may have affected the results. The data clearly indicate a higher percentage of teeth with caries in the severely fluorosed group.

Table 3-30. Caries Prevalence and Fluorosis Severity in Ethiopian School Children Studied by Olsson (1979)

Fluorosis Group	No. Teeth Examined	Percent Teeth Decayed
No signs	1723	1
Very Mild	902	2
Mild	1076	4
Moderate	2714	9
Severe	99	25[a]

[a]Significantly higher, ANOVA, $p<0.05$.

Wondwossen et al. (2004) also reported on the occurrence of dental fluorosis and dental caries in Ethiopian children. In this study, 306 children, 12–15 yrs old, from three neighboring villages in the Rift Valley were examined. The children were grouped into two exposure categories based on well-water fluoride concentrations measured in 1982–1997 (average values 0.4–1.4 mg F/L in 1982 to 0.3–2.2 mg F/L in 1997 for the moderate exposure group and 8.9–14.1 mg F/L in 1982 to 10.0–14.0 mg F/L in 1997 for the high exposure group). Dental fluorosis was scored using the Thylstrup-Fejerskov Index (TFI), and dental caries was recorded as DMFS and DMFT. Based on a graphical presentation of the data, the proportion of the population showing severe fluorosis was estimated to have been about 63% in the high fluoride area, and about 13% in the moderate fluoride area. As shown in Table 3-31, mean DMFT scores increased with increasing median TFI score, and the scores for the severe fluorosis group were significantly greater than those for the other groups.

Table 3-31. Mean DMFT Scores and SD by Dental Fluorosis Grouping for Ethiopian School Children Studied by Wondwossen et al. (2004)

Median TFI Score	Moderate Fluoride Area (0.3–2.2 mg F/L)		High Fluoride Area (8.9–14.1 mg F/L)	
	No.	Mean DMFT (SD)	No.	Mean DMFT (SD)
0	16	0.75 ±1.34	-	0 ±0
1-2	98	0.86 ±1.45	16	0.31 ±0.70
3-4	58	1.48 ±2.05	29	1.58 ±1.91
5-7	22	2.86 ±3.18[a]	67	2.31 ±2.23[a]

[a]Significantly different from other groups, $p<0.05$.

Ermis et al. (2003) compared the prevalence of dental caries and dental fluorosis in 278 school children (12–14 yrs old) in three communities in Turkey; one with a fluoride level of 0.30–0.40 mg/L in the water supply; the second with a fluoride level of 1.42–1.54 mg/L; and the third with 1.55–1.66 mg/L F. Fluorosis was assessed using the TSIF method, and caries was scored by both the DMFT and DMFS methods (Table 3-32). Ermis et al. (2003) reported no significant differences in caries prevalence in the three groups of children (ANOVA, $p>0.05$).

Table 3-32. DMFS and DMFT Scores in Turkish School Children Studied by Ermis et al. (2003)

Fluoride (mg/L)	No.	Percent TSIF ≥5	Mean DMFT	SD	Mean DMFS	SD
0.30–0.40	149	0.00	0.84	0.98	1.58	2.24
1.42–1.54	63	0.89	1.30	1.46	1.78	2.52
1.55–1.66	66	5.49	1.26	1.42	1.97	2.60

However, as was observed in other studies, when mean DMFT and mean DMFS scores were compared by the severity of the fluorosis, they increased (Table 3-33). The differences were not significant (Spearman's correlation analysis, $p>0.05$); therefore, these data do not support the supposition that severe fluorosis is associated with an increase in caries. They are somewhat limited as a test for the hypothesis because they combine a TSIF score of 4, at which pitting of the enamel does not occur, with the higher grades of fluorosis (5–7) which do include enamel pitting.

Table 3-33. DMFS and DMFT Scores and Severity of Fluorosis in Turkish School Children Studied by Ermis et al. (2003)					
TSIF Score	**No.**	**Mean DMFT**	**SD**	**Mean DMFS**	**SD**
1–3	24	1.25	1.22	1.67	1.99
4–7	105	1.29	1.48	1.92	2.67

Cortes et al. (1996) conducted a screening study on the occurrence of dental caries and dental fluorosis in 457 school children (6–12 years old) residing in three regions of Brazil: Olho D'Agua with 2–3 mg F/L drinking water, Vitoria with 0.7 mg F/L, and Maceio with less than 0.01 mg F/L. Participating schools were selected for similarities in socioeconomic profiles, although Olho D'Agua was a more rural community while Maceio and Vitoria were more urban (no other information was provided on the study populations). Dental caries were scored with the DMFT/dmft notation for permanent/primary dentition, and the TFI was used to score fluorosis. Table 3-34 shows the mean caries prevalence in six permanent teeth (upper central incisors and first molars) for subjects with different TFI scores (the criteria for selecting these particular tooth types were not given). For the permanent dentition, the DMFT scores for the Vitoria children were significantly less ($p<0.01$) than those for the other two regions. In Olho D'Agua, there was a statistically significant ($p<0.05$) increase in the mean DMFT in those children with TFI scores of 3 or greater when compared to those with TFI scores less than 3.

Cortes et al. (1996) concluded that the overall caries prevalence in these children was lower than expected but felt that it was because of the screening nature of this study. Only six permanent teeth per individual were examined to make comparisons easier, but this likely underestimated the prevalence of caries. The authors also stated that increasing the fluoride levels above 0.7 mg/L was beneficial in reducing caries prevalence for primary dentition but did not appear to be as beneficial for the permanent dentition. Children in the high fluoride areas with a TFI score of 3 or greater had higher levels of dental caries suggesting that if fluoride intake is too high, enamel hypomineralization takes place and increases the risk of caries. Note, however, that the mean DMFT scores (1.1 and 1.2 DMFT) in the Maceio study group with minimal fluorosis were similar to those in Ohlo D'Agua with moderate and severe fluorosis (1.1 and 1.3) illustrating the U-shaped response of DMFT to increasing fluoride concentration in the water.

Table 3-34. DMFT Scores in Brazilian School Children Studied by Cortes et al. (1996)						
TFI Score	**Maceio (0.01 mg F/L)**		**Vitoria (0.7 mg F/L)**		**Olho D'Agua (2-3 mg F/L)**	
	Number examined	**Mean DMFT[a] (SD)**	**Number examined**	**Mean DMFT[a] (SD)**	**Number examined**	**Mean DMFT[a] (SD)**
0	148	1.2 (1.6)	96	0.6 (1.1)[b]	8	0.9 (1.5)
1–2	12	1.1 (1.6)	95	0.3 (0.8)[b]	28	0.6 (0.8)
3–4	–	–	9	0.3 (0.7)[b]	42	1.1 (1.4)[c]
≥5	–	–	1	0.0	18	1.3 (1.1)[c]

[a]Six permanent teeth (upper central incisors and first molars) were scored.
[b]Significantly less than values for the two other study areas.
[c]Significantly greater than mean DMFTs for TFI scores of 0–2 in Olho D'Agua.

3.2.2. Dental caries and fluoride levels in drinking water

A number of studies have evaluated the prevalence of dental caries in various communities with water supplies containing different levels of fluoride, but without correlation to specific levels of dental fluorosis. Nevertheless, the data may be useful in showing trends regarding effects of severe fluorosis on caries prevalence, especially in cases where fluoride in drinking water is the primary factor in fluorosis induction. The most relevant of these studies are discussed below. It should be noted, however, that in these studies – especially the more recent ones – several confounding factors increase the difficulty of determining whether the fluoride levels in drinking water correctly reflect the total fluoride intake of the populations studied. Beginning in the early 1980's, fluoride containing dental products such as dentifrices and mouth rinses became more widely available, and consequently, total fluoride intake may have increased. Use of fluoridated municipal water in the preparation of foods and beverages in the home, in restaurants and in commercial food manufacturing plants could have also increased fluoride intake, especially in communities that originally had non-fluoridated municipal water. Conversely, the increased consumption of bottled water and the use of home water treatment devices beginning in the 1990's may have had the effect of reducing total fluoride intake from tap water. Thus, although these studies may show trends, they cannot be used as conclusive evidence of the association of increased fluoride intake and an increase in dental caries. Because of these factors, the summaries of the studies are arranged chronologically in the paragraphs that follow.

Striffler (1955) examined the caries prevalence in junior high school students in several cities in New Mexico having different levels of fluoride in the drinking water supply and found that the average DMFT scores were inversely proportional to the fluoride level; the lowest average DMFT score was recorded for Lordsburg where the fluoride level was the highest (3.25 mg F/L, see Table 3-35).

Table 3-35. Fluoride Levels and DMFT Scores for New Mexico Junior High School Children Studied by Striffler (1955)

City	Number examined	F (mg/L)	No. of Continuous residents examined	Overall average DMFT score	Average DMFT score for continuous residents
Santa Fe	888	traces	255	5.9	7.3
Lovington	485	0.8	–	2.6	–
Belen	573	0.9	126	2.5	1.9
Lordsburg	263	3.25	92	1.6	1.5

Forsman (1974) studied the prevalence of dental caries (DMFT or DMFS scores) and dental fluorosis (see Section 3.1) in residents (mostly school children) in three communities in southern Sweden (Gadderås, Påskallavik and Billesholm) who were exposed to fluoride in drinking water, and compared the results to that from a control population (school children from the city of Eskilstuna and Kronoberg county). For the purposes of this study, Forsman (1974) considered the fluoride level in Gadderås and Påskallavik to be approximately 10 mg/L and that in Billesholm, to be ~5 mg/L. In the control areas, fluoride levels of 0.9–1.7 mg/L had been recorded, and Forsman (1974) refers to these as the ~1 mg F/L areas.

Forsman (1974) reported that the caries frequency in the permanent teeth in areas with ~10 mg F/L was higher than that in the ~ 1 mg F/L areas. The study author provided age-caries regression plots for groups exposed to different fluoride levels and having different degrees of fluorosis (see Figs 3-3 and 3-4). Information on caries frequency can be visually extracted from these figures. For individuals from the 10 mg F/L areas who had a fluorosis score of ≥ 3, the average DMFS was about 14 (N = 38 subjects), whereas in the control areas the average DMFS score for individuals with a fluorosis score of ≤ 2, was 4 (N = 160 subjects). In the ~5 mg F/L area (Fig. 3-4), average caries frequency (DMFS) in permanent teeth for individuals with a fluorosis score of ≥ 3 was about 8.5 (N = 37 subjects) whereas it was about 4.5 (N = 91 subjects) for individuals with a fluorosis score of ≤ 2. This difference was significant at the 1% level.

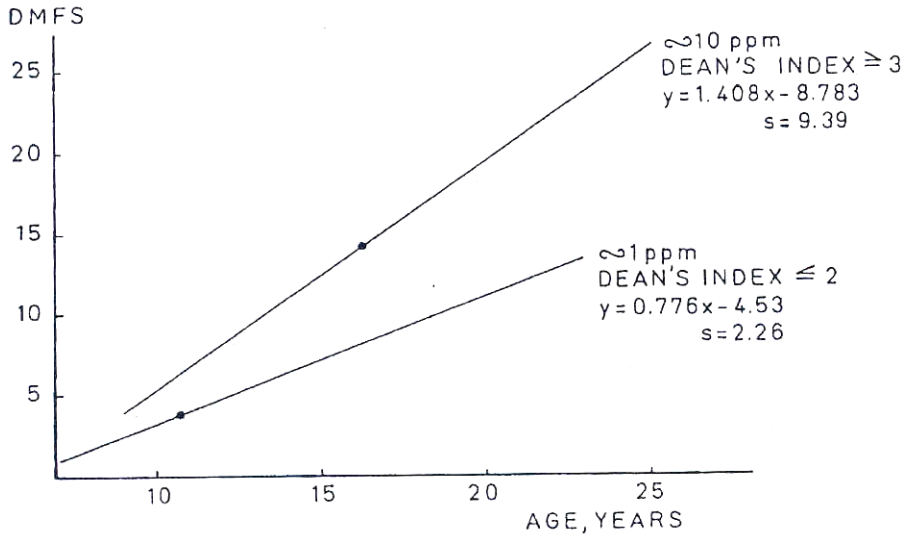

Figure 3-3. Age-caries regression lines for groups with different degrees of enamel fluorosis. The regression lines cover the age ranges and the caries averages are marked by the points on the lines; "s" denotes standard deviations of the lines.

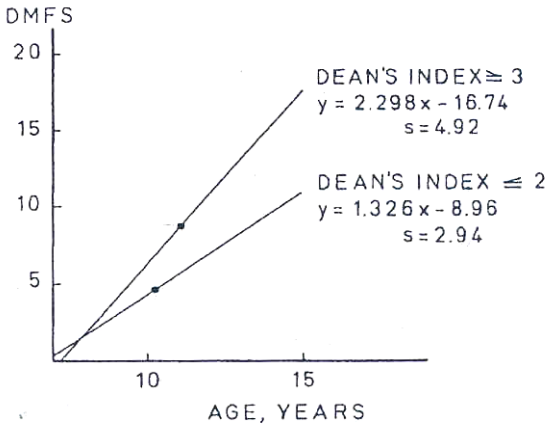

Figure 3-4. Age-caries regression lines for groups with different degrees of enamel fluorosis exposed to water containing approximately 5 mg F/L. The regression lines cover the age ranges and caries averages are marked by the points on the lines; "s" denotes standard deviations of the lines.

The regression lines shown in Figs. 3-3 and 3-4 indicate that DMFS increases with age and that, beginning at about the age of 5, the higher the fluorosis score (Dean score of ≥ 3 vs. ≤ 2) the

greater the age related difference in DMFS. The regression coefficients for the lines shown in Fig. 3-3 were not significantly different from one another. In Figure 3-4 (ingestion of water with 5 ppm F), the higher level of fluorosis was associated with a higher DMFS value, and the difference in DMFS observed at two fluorosis levels was significant at the 1% level.

Englander and DePaola (1979) reported the results of a study in which 1,878 white adolescents (aged 12 to 15 years) residing in seven communities in five U.S. states were examined for caries in their permanent teeth. The seven communities and their fluoride levels were: Boston, MA (<0.1 mg F/L); Danvers, MA (approximately 1 mg F/L); Mecklenburg County, NC (<0.1 mg F/L, from well water); Kalamazoo, MI (approximately 1 mg F/L); Stickley, IL (approximately 1 mg F/L); Charlotte, NC (approximately 1 mg F/L); and Midland, TX (5 to 7 mg F/L). The subjects were all lifelong residents of the communities. Although there were confounding factors which may have influenced the results, the data indicate that DMFS and DMFT scores decreased with increasing fluoride level and were lowest for Midland, TX (approximately 1.8 DMFT and 2.4 DMFS) where the fluoride level was 5–7 mg/L (Table 3-36).

Table 3-36. Dental Caries Experience of 13–15 yr olds in Seven U.S. Communities as Reported by Englander and DePaola (1979)

Town	Number of Subjects	F (mg/L)	Mean DMFT[a]	Mean DMFS (SE)
Boston, MA	302	<0.1	8	13.96 (±0.59)
Danvers, MA	305	1	5	8.60 (±0.43)
Mecklenburg County, NC	120	<0.1	4.5	7.20 (±0.54)
Kalamazoo, MI	315	1	3.7	5.12 (±0.27)
Stickley, IL	312	1	3.2	4.51 (±0.29)
Charlotte, NC	213	1	3.0	4.41 (±0.32)
Midland, TX	311	5–7	1.8	2.40 (±0.21)

Modified from Englander and DePaola (1979).
[a]Estimated from graphical presentation of the data.

Heifetz et al. (1988) conducted a five-year follow-up study of 8–10 yr olds and 13–15 yr olds in seven Illinois communities originally studied by Driscoll et al. (1983 and 1986). For the 13–15 year olds, there was little difference between 1980 and 1985 in the relative differences in the mean DMFS scores between the optimal fluoride area ["optimal" was defined by the study authors as 1 mg F/L for the study region (Midwest USA)] and the above optimal fluoride areas (Table 3-37). For the 8–10 yr olds, the mean DMFS score was substantially higher at 4x optimal fluoride than at 3x optimal (but not reported as statistically significant) in 1980 but not in 1985. In 1985, there was very little difference between the 3x optimal and 4x optimal groups. In all cases, the lowest DMFS scores occurred in the 3x optimal groups, with higher scores in the 4x optimal groups. The study did not compare the DMFS scores based on the severity of fluorosis; however, a greater percentage of the tooth surfaces exhibited TSIF scores > 5 in the 4X optimal groups (Table 3-37).

Table 3-37. Mean DMFS Scores of Illinois School Children by Age Category and Water Fluoride Levels in 1980 and 1985 (Heifetz et al., 1988)					
Group	**No.**	**TSIF Scores ≥5 (% of Surfaces)**	**Mean No. DMFS**	**% Difference from Optimal**	**% Difference from 1980**
8–10 yr-olds – 1980					
Optimal[a]	113	0.1	1.79	–	
2x Optimal	61	0.2	1.20	33.0	
3x Optimal	82	1.4	0.76	57.5	
4x Optimal	59	4.1	1.41	21.2	
8–10 yr-olds – 1985					
Optimal[a]	156	0.1	1.51	–	-15.6
2x Optimal	102	1.3	1.07	29.1	-10.8
3x Optimal	112	1.9	0.82	45.7	+7.9
4x Optimal	62	4.6	0.85	43.7	-39.7
13–15 yr-olds – 1980					
Optimal[a]	111	0.0	4.56	–	
2x Optimal	39	0.1	2.59	43.2	
3x Optimal	50	0.8	1.92	57.9	
4x Optimal	34	1.9	3.38	25.9	
13–15 yr-olds – 1985					
Optimal[a]	94	0.0	5.09	–	+11.6
2x Optimal	23	1.3	2.87	43.6	+10.8
3x Optimal	47	2.2	2.53	50.3	+31.8
4x Optimal	29	5.4	3.86	24.2	+14.2

[a]"Optimal" is defined by the study authors as 1 mg F/L for the study region (Midwest USA).

In a 10-yr follow-up study conducted on children from these same Illinois communities, Selwitz et al. (1995) found the same trend seen in the earlier studies; the DMFS scores for children living in areas with 2x and 3x optimal fluoride in the water supply were lower than the scores for children living in the optimal and 4x optimal fluoride communities (Table 3-38) and the percent of cavity-free children decreased, meaning the percent of those with cavities increased. Comparisons of mean DMFS scores were not made on the basis of the severity of fluorosis. In 1990, the mean DMFS score for communities with 4x optimal water fluoride was similar to that for the communities with optimal water fluoride, even though the mean percent fluorosed surfaces per subject was significantly greater in the 4x optimal fluoride communities. The highest examined fluoride level of about 4 mg/L did not provide any additional anti-caries benefit over that occurring at 1 mg/L, nor did it contribute to a substantial increase in caries. However, in 1990 only a small proportion of the surfaces examined in the 4x optimal group (3%) had TSIF scores of 4 or more; therefore, the prevalence of severe fluorosis was quite low.

Jackson et al. (1995) examined caries prevalence (DMFT and DMFS) and dental fluorosis (Dean's fluorosis score) in 7–14 yr old children (born between 1978 and 1985) residing in three communities in Indiana having different fluoride drinking water levels [0.2 mg/L (non-fluoridated or NF); 1.0 mg/L (optimal fluoride level or OPF); and 4.0 mg/L (4x OPF)]. The mean DMFT score of the OPF group was not significantly different from that of the other two exposure groups, but the mean DMFT score of the 4X OPF group was significantly lower than that of the NF group (Table 3-39). The mean DMFS scores for both the OPF and 4x OPF groups were significantly lower than that of the NF group. In the 4x optimal group, the prevalence of severe fluorosis was

11.3% and the Community Fluorosis Index was 2.06, approximately 13 times greater than that for the NF group.

Table 3-38. Percent Caries-free and Mean DMFS Scores of Illinois Children in 1980, 1985 and 1990 (Selwitz et al., 1995)

Group	No.	MPFS[d]		Caries Data			
		8–10 yr olds	13–15 yr olds	% Caries-free	Mean DMFS (SE)[b]	% Difference from Optimal	p-value
1980							
Optimal	224	18.2	11.1	35.3	2.86 (0.20)	–	
2x Optimal	100	47.3[e]	38.4[e]	52.0	1.71 (0.29)	40.2	0.001[c]
3x Optimal	132	52.4[e]	45.5[e]	57.6	1.21 (0.25)	57.7	<0.001[c]
4x Optimal	93	69.2[e]	63.5[e]	44.1	2.13 (0.30)	25.5	0.043
1985							
Optimal	250	28.9	30.5	44.0	2.81 (0.18)	–	–
2x Optimal	125	52.8[e]	67.2[e]	53.6	1.86 (0.26)	33.8	0.003
3x Optimal	159	50.9[e]	69.1[e]	54.1	1.50 (0.23)	46.6	<0.001[c]
4x Optimal	91	77.1[e]	77.8[e]	48.4	1.91 (0.31)	32.0	0.012
1990							
Optimal	258	17.8	14.9	51.9	1.85 (0.18)	–	–
2x Optimal	105	55.6[e]	48.9[e]	58.1	1.45 (0.28)	21.6	0.235
3x Optimal	117	55.2[e]	45.4[e]	56.4	1.41 (0.27)	23.8	0.176
4x Optimal	77	59.8[e]	67.6[e]	50.7	1.85 (0.33)	0.0	0.989

[a]"Optimal" fluoride in drinking water is defined by the study authors as 1 mg/L for the study region (Midwest USA).
[b]All mean DMFS scores have been age-adjusted.
[c]Significant, $p<0.002$, adjusted α level for multiple comparisons using the Bonferroni procedure.
[d]Mean percent fluorosed surfaces per subject.
[e]Significantly greater than score at optimal fluoride ($p<0.001$).

Table 3-39. DMFS and DMFT Scores in Indiana School Children Studied by Jackson et al. (1995)

Group[a]	Number examined[b]	Percent Severe Fluorosis	Community Fluorosis Index[c]	Mean DMFT (SD)	% Diff. from NF	Mean DMFS (SD)	% Diff. from NF
NF	124; 126	0	0.153	3.68 (2.49)[d]	–	5.54 (4.36)	–
OPF	116; 117	0	0.457	3.34 (2.11)[d,e]	-9.2	4.35 (2.92)[d]	-21.2
4x OPF	97; 101	11.3	2.12	2.95 (1.93)[e]	-19.8	4.26 (3.02)[d]	-23.1

[a]NF = 0.2 mg F/L; OPF = 1.0 mg F/L; 4x OPF = 4 mg F/L.
[b]Number examined for fluorosis; number examined for DMFT.
[c]Calculated from data presented in Table 3-11.
[d,e] Values with same superscripts not significantly different at $p<0.05$.

The mean DMFT and DMFS scores of the study populations were analyzed by Jackson et al. (1995) by age of the subjects (Table 3-40). The data indicated that only the older children (11–14 yr old) showed significant decreases in these scores at the optimal and 4x optimal fluoride levels. The DMFT score for this age group at the 4x optimal fluoride was similar to the scores seen for all

the 7–10 yr olds regardless of fluoride level. These data indicate that, in this study population, fluoride provides maximum anti-caries benefits at the 4x optimal level.

Table 3-40. DMFS and DMFT Scores by Age Group in Indiana Populations Studied by Jackson et al. (1995)

Group	Number examined	Mean DMFT (SD)	% Diff. from NF	Mean DMFS (SD)	% Diff. from NF
7–10 yrs					
NF	77	3.01 (1.48)[a]	-	4.77 (3.08)[d]	-
OPF	69	2.99 (1.58)[a]	-0.7	4.03 (2.45)[d]	-15.5
4x OPF	69	2.96 (1.64)[a]	-1.7	4.30 (2.91)[d]	-9.9
11–14 yrs					
NF	49	4.73 (3.30)[b]	-	6.76 (5.65)	-
OPF	48	3.85 (2.63)[b,c]	-18.6	4.81 (3.44)[e]	-28.8
4x OPF	32	2.94 (2.47)[c]	-37.8	4.16 (3.30)[e]	-38.5

NOTE: Values with same superscripts not significantly different at $p < 0.05$.

Another study that evaluated caries experience and dental fluorosis relative to the drinking water concentration is that of Heller et al. (1997). Data from the 1986–87 National Survey of Oral Health of U.S. School Children were used to assess the relationships between caries experience and dental fluorosis at different fluoride concentrations in drinking water. Fluoride levels of school water were used as an indicator of the children's water fluoride exposure. The use of fluoride drops, tablets, professional fluoride treatments, and school fluoride rinses was also evaluated from caregiver questionnaires. Subjects 4–22 years old with a single continuous residence (n = 18,755) were included in this analysis. Dental caries was assessed using the system of Radike (1972). Fluorosis was assessed on all erupted teeth using Dean's classification. The sharpest declines in dfs and DMFS were associated with increases in water fluoride levels between 0 and 0.7 ppm F, with little additional decline between 0.7 and 1.2 ppm F. Fluorosis prevalence was 13.5%, 21.7%, 29.9%, and 41.4% for children who consumed <0.3, 0.3 to <0.7, 0.7 to 1.2, and >1.2 ppm F water, respectively. In addition to fluoridated water, the use of fluoride supplements was associated with both lower caries and increased fluorosis.

The authors did not examine caries relative to severe fluorosis. However, they did provide the prevalence of severe fluorosis relative to the drinking water concentration. The >1.21mg F/L grouping had a 2% severe fluorosis prevalence (~15 per 772 subjects); there were no cases of severe dental fluorosis in any other concentration grouping.

Grobler et al. (2001) studied the prevalence of fluorosis and dental caries in populations of children, 10–15 yrs old, residing in three South African towns: Leeu Gamka, 3.0 mg F/L; Kuboes, 0.48 mg F/L; and Sanddrif, 0.19 mg F/L. The prevalence of severe fluorosis (Dean's score of 4) was 0% and 0.8% in Sanddrif and Kuboes, respectively, but 30% in Leeu Gamka, the high F area (see Table 3-14). The mean DMFT was slightly higher in Leeu Gamka than in the other two towns (Table 3-41, data were not analyzed statistically). The mean DMFT scores were not analyzed across study populations on the basis of fluorosis severity; however, Leeu Gamka was shown to have a higher Community Fluorosis Index than the other two towns, suggesting that the higher mean DMFT values were associated with greater fluorosis severity.

Table 3-41. DMFT Scores in S. African School Children Studied by Grobler et al. (2001)								
Town	**F (mg/L)**	**No.**	**Mean Age**	**% Caries Free**	**% Fluorosis free**	**% Severe Fluorosis**	**Community Fluorosis Index[a]**	**Mean DMFT (SD)**
Sanddrif	0.19	47	11.77	47	38	0	0.80	1.64 (0.30)
Kuboes	0.48	115	12.01	50	40	0.8	0.82	1.54 (0.24)
Leeu Gamka	3.00	120	11.48	29	1	30	2.67	1.98 (0.22)

[a]Derived from fluorosis scores given in Table 3-11.

Ruan et al. (2005) reported on dental fluorosis (TFI scores) and dental caries (DMFS index) in 12–13 yr-old schoolchildren living in two rural areas in Shaanxi Province, China, a region where the prevalence of both dental and skeletal fluorosis is high. The children were subdivided into five fluoride exposure groups (A, B, C, D, E) based on their well water fluoride concentration (Table 3-42). The number of children (39) receiving well water with the highest fluoride concentration was about one third of those (95–116) for the lower fluoride concentrations. As the mean TFI score increased across the five groups, the percent of the population with caries and the mean DMFT scores decreased. Children exposed to the highest fluoride concentration (group E), who had the highest mean TFI score (4.78), had the lowest prevalence of caries (0.03 DMFT) among all the groups. The highest fluorosis severity group was categorized as having a mean TFI of >4; thus, for this as well as the other groups, the percentage of children that had severe fluorosis (TFI ≥5) is not reported.

According to Ruan et al. (2005), the high prevalence of dental fluorosis and low prevalence of dental caries, even in areas with low fluoride concentrations in drinking water, differs from the situation normally found in industrialized Western nations. Ruan et al. noted that calcium deficiency is common in China, and this may promote fluoride uptake and an increased prevalence of fluorosis. Storage of water in clay pots appeared to increase the percent of children with fluorosis scores ≥3 when compared to other storage vessels (55.6% vs. 20.5%). The authors concluded that this might reflect leaching of fluoride from the clays used to make the pots.

Table 3-42. Fluorosis and Dental Caries in Chinese School Children Studied by Ruan et al. (2005)					
Group	**No.**	**F (mg/L)**	**% with Caries**	**Mean TFI[a] (95% CI)**	**Mean DMFT (95% CI)**
A	95	0.4	22.1	0.30 (0.02–0.57)	0.38 (0.21–0.55)
B	116	1.0	19.8	1.40 (1.15–1.65)	0.28 (0.16–0.41)
C	115	1.8	7.0	3.16 (2.91–3.40	0.09 (0.0—0.15
D	112	3.5	5.4	3.62 (3.32–3.92)	0.06 (0.01–0.11
E	39	5.6	2.6	4.78 (4.36–5.21)	0.03 (0.00–0.08)

[a]Group mean based on the individual median TFI score.

3.2.3. Dental caries as an adverse health effect

Dental caries are caused by bacteria in dental plaque which erode calcium in the tooth enamel and expose the dentin. If a cavity is untreated, bacteria invade the dentin and gain access to the pulp. White cells move to the pulp to combat the bacteria, increasing pressure on nerves and causing a toothache. If untreated, the infected tooth may abscess and die, leading to tooth loss. The infection can also become systemic, leading to bacterial endocarditis (inflammation of the

heart muscle) and death in some cases (AMA, 1982). A cavity that irritates the gum can lead to gingivitis or periodontitis; an infection in the gums leading to damage to the bone supporting the teeth.

About one-half of low income children aged 6–19 have untreated decay (CDC, 2007) which can lead to pain, dysfunction, loss of tooth surface, absence from school, reduced weight, and poor appearance; problems that can greatly reduce a child's capacity to succeed in life (CDC, 2007). Tooth decay occurs in more than 90% of adults over age 40 (CDC, 2007). One fourth of adults over age 60 have lost all of their teeth—primarily because of tooth decay (CDC, 2007). Especially among the elderly, tooth loss leads to eating difficulties and resultant nutrient intake problems. These statistics illustrate the scope of the public health problems associated with tooth decay, even in the face of wide-spread fluoridation of drinking water and use of fluoridated dental products. Therefore, any factor that increases decay is a matter of public health concern.

Cavities and their treatment are associated with several secondary risks. To reduce the risk of bacterial endocarditis, the American Heart Association recommends prophylactic administration of antibiotics during repair of cavities for immune suppressed populations as well as for individuals with congenital heart disease, prior infection, or those who have had valve or heart replacements (Wilson et al., 2007).

It has been proposed that there may be a relationship between focal infections of the oral cavity, resultant inflammation, and other diseases, especially heart disease (Genco et al., 2002; Slots, 1998; Wu et al., 2000). Periodontal disease provides an opportunity for bacteria and bacterial products such as lipopolysaccharide toxins to gain access to systemic circulation, possibly disturbing lipid metabolism and increasing circulating cytokines (Wu et al., 2000). However, increased relative risks are observed in some epidemiology studies but not in others (Genco et al., 2002; DeStefano et al., 1993). An alternative hypothesis is that dental disease is a confounder for socioeconomic and behavioral factors (i.e., smoking and diet), and it is those factors that have the greatest impact on cardiovascular risk (DeStefano et al., 1993; Janket et al., 2004).

In a study by Janket et al. (2004), an asymptomatic dental score (ADS) was used to evaluate the relationship between five dental problems and biomarkers for heart disease in a group of 506 Finnish adults, 256 with heart disease and 250 without heart disease. The best correlation was that between the ADS and C-reactive protein and fibrinogen (biomarkers for inflammation) and high density lipoprotein. Of the five dental problems considered contributors to risk, cavities ranked fourth after infection or inflammation around impacted or erupting wisdom teeth, tooth remnants and gingivitis. Missing teeth ranked last. The data from this study suggest that, although cavities may be a contributor to the association between cardiovascular risk and dental problems, they are a smaller contributor than oral cavity problems generating a strong inflammatory response.

3.2.4. Summary and conclusions

A close examination of the studies cited by NRC (2006) as being relevant for an assessment of the relationship between severe fluorosis and caries (Table 3-43) indicates that, in some studies, caries prevalence in groups with severe fluorosis was significantly greater than that in groups with mild and moderate fluorosis but generally lower than those in groups with no fluorosis.

Groups with no or minimal fluorosis are generally those with minimal fluoride exposure and little fluoride-associated anticariogenic benefit.

Table 3-43. NRC Critical Studies Assessing the Effects of Severe Fluorosis on Caries Scores in Permanent Dentition

Study/ Fluorosis Index	Fluorosis Score[a]	Caries Score (see footnotes)	p Values[b] of Significance		Comment
Chen, 1989 (Taiwan); 0.2–4.7 mg F/L					
Dean's Index	0	2.4[c]	**<0.05**	**NS**	Anti-caries maximal at fluorosis scores of 1–3. Caries at fluorosis score of 4 not significantly different from that for group with fluorosis score of 0 but significantly different from those in the 0.5-3 grouping, indicative of a U-shaped dose-response curve.
	0.5–3	1.7–2.2[c]			
	4	2.5[c]			
Cortes et al., 1996 (Brazil); 0.7 mg F/L					
TFI[h]	0	0.6[c]	**NR**	**NR**	Only one individual with fluorosis score of ≥5. No statistically significant differences. Only 6 teeth per individual scored for caries.
	1–4	0.3[c]			
	≥5	0[c]			
Cortes et al., 1996 (Brazil); 2–3 mg F/L					
TFI[h]	0	0.9[c]	**NR**	**NR**	Statistical analysis of data limited to the following: for TFI scores ≥3, DMFT scores (1.1–1.3) significantly greater ($p<0.05$) than DMFT score of 0.6–0.9 for TFI scores less than 3. Lowest DMFT scores at TFI of 1–2. Only 6 teeth per individual scored for caries.
	1–4	0.6–1.1[c]			
	≥5	1.3[c]			
Driscoll et al., 1986 (USA); 2–4 mg F/L					
Dean's Index	0	1.89[d]	**<0.05**	**NS**	A well conducted study. Anti-caries maximal at fluorosis scores of 0.5-3. Caries at fluorosis score of 4 not significantly different from that for group with no fluorosis but significantly different from the 0.5 to 3 grouping.
	0.5–3	1.40–1.58[d]			
	4	2.96[d]			
Eklund et al., 1987 (US); 3.5 mg F/L					
Dean's Index	0–1	1.2–18.8–69.2[f]	**NR**	**NR**	Percent teeth "not-sound". Caries scores for anteriors─premolars─molars, respectively. Results inconsistent across teeth.
	2–3	2.7–10.2–43.2[f]			
	4–5	5.7–18.6–40[f]			
Ermis et al., 2003 (Turkey); 0.3-0.4 mg F/L for TSIF = 0; 1.42-1.66 mg/L for other groups					
TSIF[h] Index	0	0.84[c]	**NS**	**NR**	No statistical comparison between the fluorosis and non-fluorosis groups.
	1–3	1.25[c]			
	4–7	1.29[c]			
Mann et al., 1987 (Gaza Strip); 5 mg F/L					
Dean's Index	0–1	─	**<0.001**	**ND**	No individuals with fluorosis score of 0-1. Gender differences and confounding factors reported (excessive consumption of tea).
	2–3	2.8–4.4[d]			
	4	10.4[d]			

Table 3-43. NRC Critical Studies Assessing the Effects of Severe Fluorosis on Caries Scores in Permanent Dentition					
Study/ Fluorosis Index	**Fluorosis Score[a]**	**Caries Score (see footnotes)**	**p Values[b] of Significance**		**Comment**
Mann et al., 1990 (Gaza Strip); 5 mg F/L					
Dean's Index	0	0.29[d]	**NR**	**<0.05**	Study was conducted in the same town as the Mann et al. 1987 study, but on younger children (6–8 yr old); therefore, full caries experience was not reached.
	2–3	0.5–1.25[d]			
	4	1.83[d]			
Olsson, 1979 (Ethiopia); 12.4 mg F/L (range 6–17 mg/L) and 3.5 mg F/L (range 1.2–7.4 mg F/L)					
Dean's Index	0	1[e]	**NR**	**NR**	Percent decayed teeth. Statistical significance not indicated. Mean caries values for individuals not reported.
	1–3	15[e]			
	4	25[e]			
Warnakulasuriya et al., 1992 (Sri Lanka); 0.08-8.00 mg F/L F (56% <0.4 mg F/L)					
Dean's Index	0	3.1[c]	**NS**	**NS**	Large variations in fluoride concentrations within study areas. Fluorosis groups 1–3 include questionable to mild; groups 4 and 5 include moderate and severe. Combining the latter two may have masked effects of severe fluorosis; however, no significant differences between score of 3–4 and that of 0, suggestive of a U-shaped dose-response pattern.
	1-3	2.8–3.4[c]			
	4-5	3.3[c]			
Wondwossen et al., 2004 (Ethiopia); 0.4–2.2 mg F/L					
TFI[g]	0	0.8[c]	**NR**	**<0.05**	Median TFI scores. Statistical significance of DMFT differences between TFI = 5–7 and TFI =1–4 not reported.
	1–4	0.9–1.5[c]			
	5-7	2.9[c]			
Wondwossen et al., 2004 (Ethiopia); 8.9–14.1 mg F/L					
TFI[g]	0	—	**NS**	**ND**	Median TFI scores. No individuals with fluorosis score of 0.
	1–4	0.3–1.6[c]			
	5–7	2.3[c]			

[a]Intermediate fluorosis groups combined.

[b]NS = Not significant; ND = No data; NR = Not reported.

[c]DMFT, decayed, missing, filled teeth.

[d]DMFS, decayed, missing, filled surfaces.

[e]Percent decayed teeth based on all teeth examined; severe group includes teeth with pitting, scored by study authors as moderate.

[f]Percent teeth "not sound" for anteriors, premolars and molars, respectively.

[g]Thylstrup and Fejerskov Index.

[h]Tooth Surface Index of Fluorosis.

In the studies conducted in the U.S. and Taiwan, caries frequency in the severe fluorosis group was not significantly greater than that in the group with no or minimal fluorosis. In some cases it

was significantly greater than that for the groups with the lowest DMFT or DMFS scores, a possible reflection of the U-shape of the fluorosis-cavity relationship. In the U.S., severe fluorosis affects a relatively small number of children or adults which limits the statistical power of the few studies that have looked at cavities as a variable.

In many of the non-U.S. studies evaluated by the NRC (i.e., Ethiopia, Gaza Strip, Turkey), caries prevalence was lowest in groups showing no fluorosis, and there was a progressive increase in caries with increasing severity of dental fluorosis. Some of the factors which might account for differences in the fluorosis/caries relationship between the U.S. and other countries include differences in dental care, dental hygiene practices, dietary habits (i.e., consumption of sugars), and nutrient intakes (i.e., calcium balance). Additional studies with controls for variables that are known risk factors for caries would be very helpful in further defining the relationship between severe fluorosis and caries.

Analysis of the data on the relationship between the degree of fluorosis and cavities is complicated by the fact that different studies used different approaches for scoring both the degree of fluorosis and the prevalence of caries, limiting cross-study comparisons. Nevertheless, the weight of evidence does support the conclusion of the NRC (2006) that, under some circumstances, severe fluorosis may be associated with an increased prevalence of caries.

The study of Driscoll et al. (1986) is most applicable to the U.S. population in general. The results of this study showed that the anti-caries protective effect of fluoride in drinking water reached a maximum under conditions in which the resulting fluorosis ranged from questionable to moderate (Table 3-44). Severe fluorosis (fluorosis score of 4) was associated with a mean DMFS score (2.96) significantly higher ($p<0.05$) than that seen at fluorosis scores of 0.5–3. This high DMFS score of 2.96 was not significantly higher than that associated with a fluorosis score of 0 for no fluorosis (e.g., mean DMFS score of 1.89). When analyzed on the basis of the percent decayed or filled teeth, only approximately 5% was associated with very mild to moderate levels of fluorosis, but approximately 20% was associated with the severe level. These data suggest that severe fluorosis diminished the anti-caries protective action of fluoride to some degree. The Driscoll et al. (1986) study included populations of children residing in locations where the fluoride levels in drinking water ranged from 2 to about 4 mg/L, and excluded populations in areas where the fluoride levels were 1 mg/L and less. The mean caries score for the children in areas with <0.3 mg F/L was 5.07, substantially higher than that for the children exhibiting severe fluorosis (data not analyzed statistically).

Table 3-44. Summary of Results of Driscoll et al. (1986) Study of Illinois School Children[a]		
Fluorosis Score[b]	**No.**	**Value**
		Mean DMFS
0	87	1.89
0.5	112	1.40
1–3	218	1.58
4	54	2.96[c]
		Decayed or filled teeth
1–3	218	4.5 %
4	54	19.6 %

[a]Data limited to children exposed to drinking water fluoride levels of ~2–4 mg/L.
[b]Dean's Index.
[c]Significantly higher than that for children with scores of 0.5–3 ($p < 0.05$); no other significant differences between groups.

Most studies evaluating the effects of fluoride on dental caries have not directly compared specific levels of fluorosis severity with measures of caries occurrences (see Section 3.2.2). However, data from these studies can be compared in several ways:

- Fluoride concentration in the drinking water relative to Community Fluorosis Index (CFI) (Table 3-45, Figure 3-5);
- Fluoride concentration in the drinking water relative to DMFT/DMFS (Table 3-45, Figure 3-6);
- Percent severe fluorosis relative to DMFT/DMFS (Table 3-46, Figure 3-7);
- Community Fluorosis Index relative to DMFT/DMFS (Table 3-47, Figure 3-8).

The Community Fluorosis Index is a weighted average derived from Dean's fluorosis scores and the frequency of occurrence of those scores within the study population, and it is intended only as a relative measure of the fluorotic condition of the study populations (Dean, 1942).

The data in Table 3-45 indicate that as the fluoride concentration in the drinking water increases, the Community Fluorosis Index also increases (see Fig. 3-5). The Table 3-45 data also show that the DMFT/DMFS decrease as the fluoride concentration in the drinking water increases to a concentration of about 3 mg/L at which point the DMFT/DMFS either remain level or increase (Figure 3-6).

Table 3-46 and Figure 3-7 compare the percent of the population with severe dental fluorosis with DMFT/DMFS. Here, as in Figure 3-6, there is a suggestion of a U-shape to the relationship. The outline of a U-shaped dose response can also be seen in Table 3-47 and Figure 3-8 which compare Community Fluorosis Index to the DMFT/DMFS scores. The DMFT and DMFS values are lowest at a CFI of about 1 to 1.5 after which they begin to rise.

Table 3-45. Relationship of Fluoride in Drinking Water to CFI and DMFT and DMFS Scores							
Fluoride (mg/L)	**No.**	**Fluorosis Scoring System**	**% Severe Fluorosis**	**Community Fluorosis Index[a]**	**Mean DMFT**	**Mean DMFS**	**Reference**
0.2	126	Dean's	0	0.15[d]	3.68	5.54	Jackson et al., 1995
<0.3	316	Dean's	0	0.06[b]		5.07	Driscoll et al., 1986
~1	336	Dean's	0	0.46[d]	3.34	4.35	Jackson et al., 1995
1.06	117	Dean's	0.6	0.39[c]		3.14	Driscoll et al., 1983
2.08	143	Dean's	4.9	1.16[c]		1.97	Driscoll et al., 1983
2.6	404	Dean's	1.5	1.3	2.46		Dean, 1946
2.84–2.89	192	Dean's	8.3	1.25[c]		1.41	Driscoll et al., 1983
3.77–4.07	136	Dean's	22.8	1.88[c]		2.02	Driscoll et al., 1983
~4	101	Dean's	11.3	2.06[d]	2.95	4.26	Jackson et al., 1995

[a]Weighted average of Dean's fluorosis scores and the frequency of occurrence of those scores within the study population.
[b]Calculated from data given in Table 3-6.
[c]Calculated from data given in Table 3-5.
[d]Calculated from data given in Table 3-11.

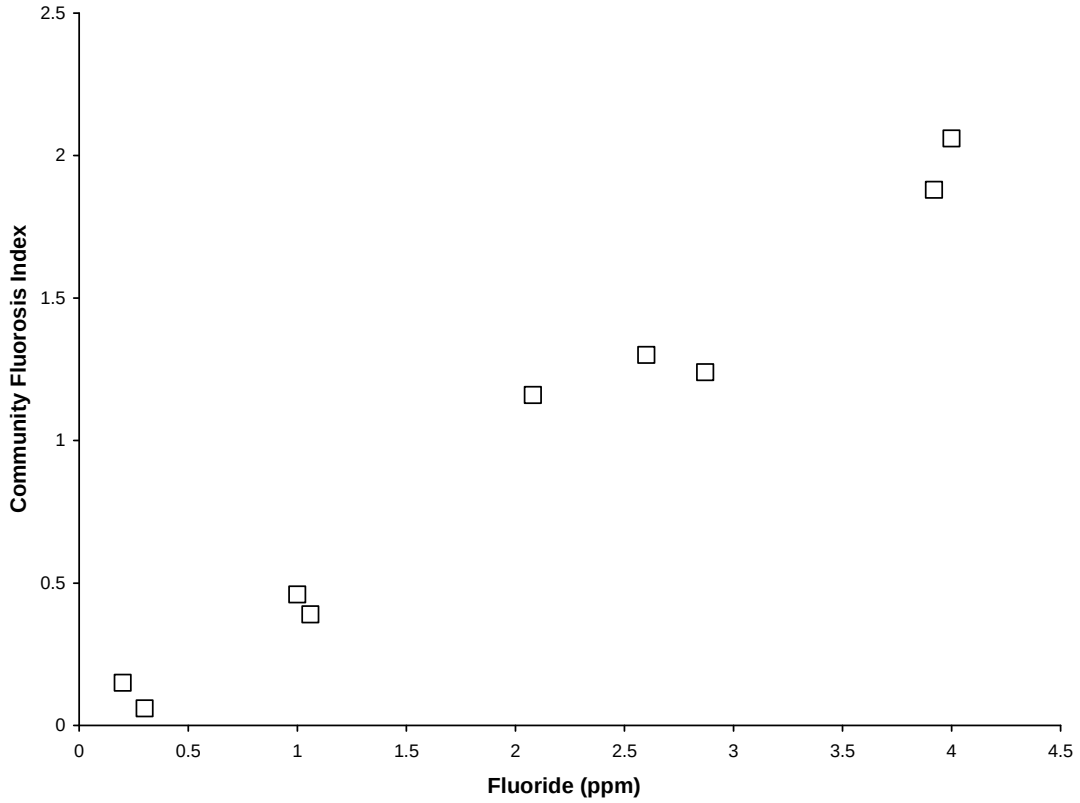

Figure 3-5. Drinking water fluoride concentration vs. Community Fluorosis Index for selected studies.

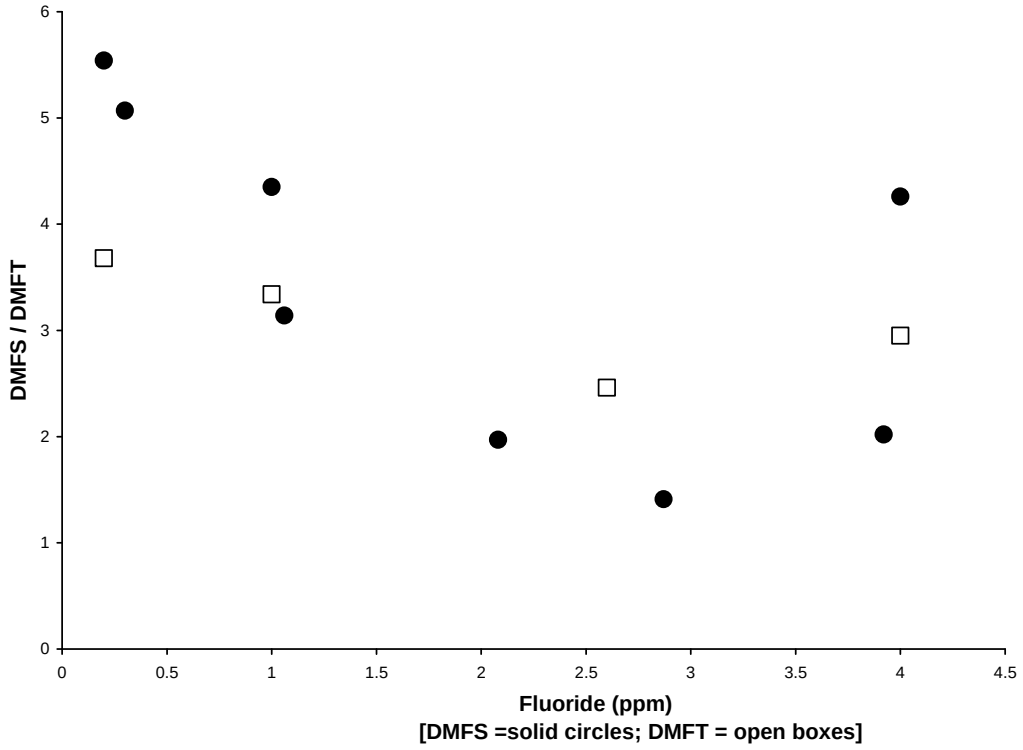

Figure 3-6. Drinking water fluoride concentration vs. DMFT (□) and DMFS (●) scores for selected studies.

Table 3-46. Relationship of Percent Severe Fluorosis to CFI and DMFT and DMFS Scores

% Severe Fluorosis	No.	Fluoride (mg/L)	Fluorosis Scoring System	Community Fluorosis Index[a]	Mean DMFT	Mean DMFS	Reference
0	126	0.2	Dean's	0.15[d]	3.68	5.54	Jackson et al., 1995
0	316	<0.3	Dean's	0.06[b]		5.07	Driscoll et al., 1986
0	336	~1	Dean's	0.46[d]	3.34	4.35	Jackson et al., 1995
0.6	117	1.06	Dean's	0.39[c]		3.14	Driscoll et al., 1983
1.5	404	2.6	Dean's	1.3	2.46		Dean, 1946
4.9	143	2.08	Dean's	1.16[c]		1.97	Driscoll et al., 1983
8.3	192	2.84–2.89	Dean's	1.25[c]		1.41	Driscoll et al., 1983
11.3	101	~4	Dean's	2.06[d]	2.95	4.26	Jackson et al., 1995
22.8	136	3.77–4.07	Dean's	1.88[c]		2.02	Driscoll et al., 1983

[a]Weighted average of Dean's fluorosis scores and the frequency of occurrence of those scores within the study population.
[b]Calculated from data given in Table 3-6.
[c]Calculated from data given in Table 3-5.
[d]Calculated from data given in Table 3-11.

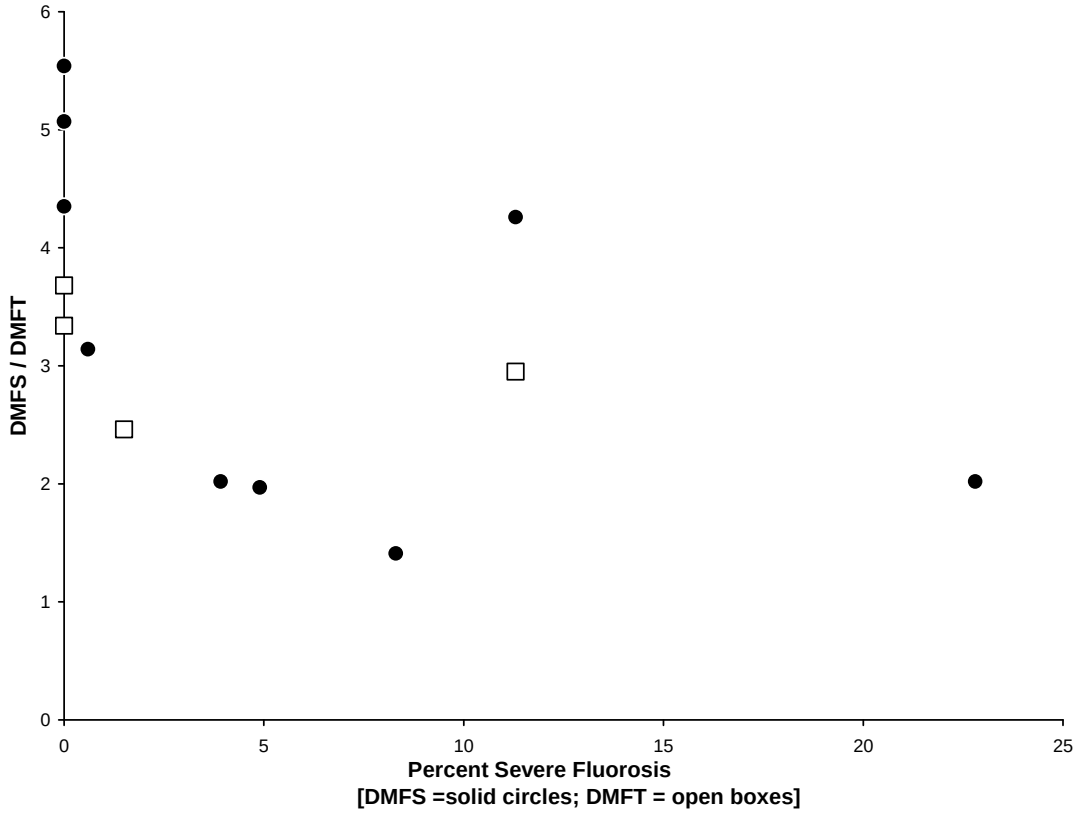

Figure 3-7. Percent severe fluorosis relative to DMFT (□) and DMFS (●) scores for selected studies.

Table 3-47. Relationship of CFI to DMFT and DMFS Scores

Community Fluorosis Index[a]	No.	Fluoride (mg/L)	Fluorosis Scoring System	% Severe Fluorosis	Mean DMFT	Mean DMFS	Reference
0.06[b]	316	<0.3	Dean's	0		5.07	Driscoll et al., 1986
0.15[d]	126	0.2	Dean's	0	3.68	5.54	Jackson et al., 1995
0.39[c]	117	1.06	Dean's	0.6		3.14	Driscoll et al., 1983
0.46[d]	336	~1	Dean's	0	3.34	4.35	Jackson et al., 1995
1.16[c]	143	2.08	Dean's	4.9		1.97	Driscoll et al., 1983
1.25[c]	192	2.84–2.89	Dean's	8.3		1.41	Driscoll et al., 1983
1.3	404	2.6	Dean's	1.5	2.46		Dean, 1946
1.88[c]	136	3.77–4.07	Dean's	22.8		2.02	Driscoll et al., 1983
2.06[d]	101	~4	Dean's	11.3	2.95	4.26	Jackson et al., 1995

[a]Weighted average of Dean's fluorosis scores and the frequency of occurrence of those scores within the study population.
[b]Calculated from data given in Table 3-6.
[c]Calculated from data given in Table 3-5.
[d]Calculated from data given in Table 3-11.

The base of the U- shaped fluoride-caries relationship seems to occur at a drinking water concentration between 2 to 3 mg/L in Figure 3-6, a community severe fluorosis burden of about 5 to 10 percent in Figure 3-7, and at a CFI of about 1 to 1.5. One limitation of the relationships as depicted in Tables 3-45, 3-46, 3-47 and Figures 3-6, 3-7, 3-8 is the fact that the data come from studies conducted at different times periods during which there were changes in exposure to fluoride from sources other than the drinking water. The two data points that indicate an increase in the DMFT and DMFS scores both come from the same study (Jackson et al., 1995). It is the most recent study and the one where intakes of fluoride from sources other than the drinking water were likely to have been the highest.

By limiting the data in Figures 3-6, 3-7, 3-8 to studies conducted in the United States, where 4 mg/L is the enforceable MCL, there is no information from systems with higher drinking water concentrations to more clearly define the upper limit of the beneficial range for fluoride exposures as correlated to the concentration in drinking water.

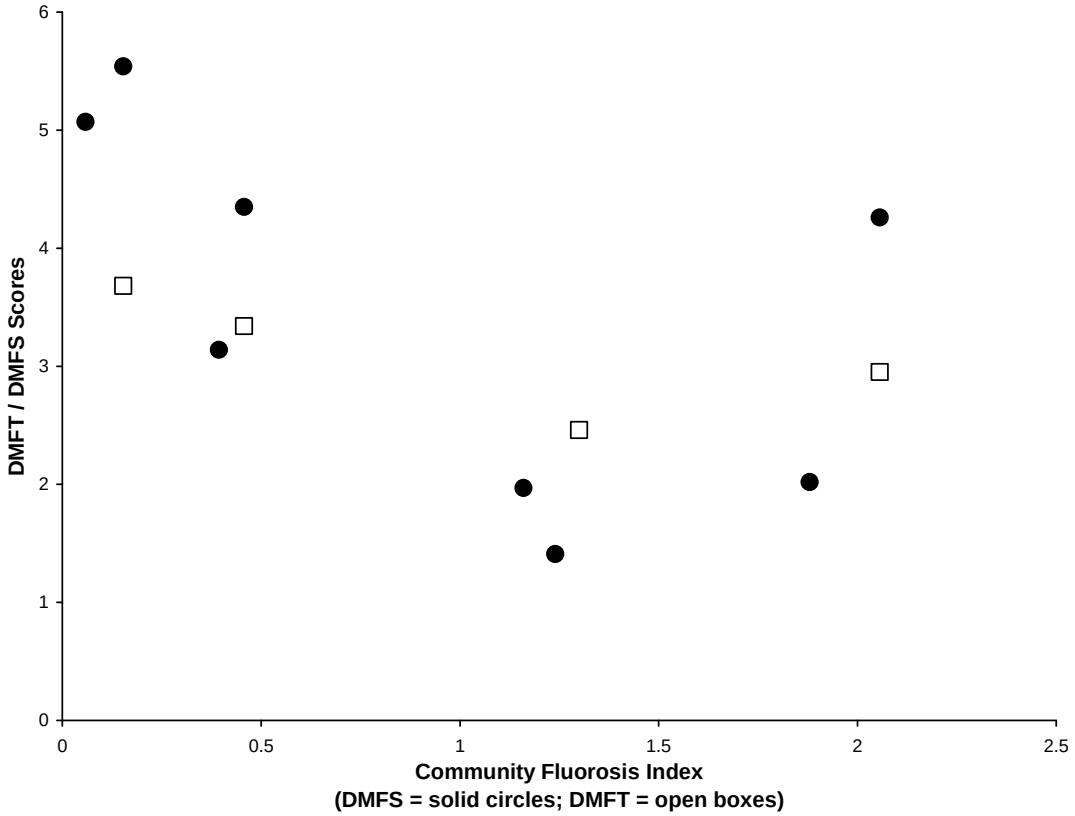

Figure 3-8. Community Fluorosis Index vs. DMFT (□) and DMFS (●) scores for selected studies.

These data appear to support the results of the Driscoll et al. (1986) analysis of DMFS as related to the severity of dental fluorosis. They indicate that there is an increasing anticaries benefit with increasing level of fluorosis up to a certain point, after which the incremental benefit from increased fluoride exposure, as reflected in the drinking water concentration, does not increase

(Fig. 3-6). The data, although limited, are also consistent with the NRC (2006) conclusion that the pitting of enamel that occurs with severe dental fluorosis may increase the cavity risk to levels above that associated with lesser degrees of fluorosis (Fig. 3-7).

Although the data are supportive of the NRC (2006) conclusions regarding enamel pitting they are moderately rather than strongly consistent with the hypothesis that the pitting of the enamel leads to an increased risk for caries. Socioeconomic status, availability of dental care, and personal dental hygiene habits are likely to confound the results from individual studies of the caries relationship. For this reason, the OW selected the pitting of the dental enamel as the critical effect for the dose-response analysis. It is the only endpoint with sufficient data to support dose-response modelling. The EPA finding on the caries association is consistent with NRC (2006) that the"available evidence is mixed but generally supportive".

Pitting of the enamel is a structural defect that weakens the barrier between the oral environment and the dentin of the teeth. It is progressive in that the enamel can flake off from the sides of the pits allowing them to become progressively larger (Fejerskov et al., 1994). Furthermore, the dentin of teeth with severe dental fluorosis is hypomineralized and structurally variant (Rojas-Sanchez et al., 2007; Waidyasekera et al., 2010) increasing the importance of the enamel's protective function. As stated by NRC (2006, page 4), the fact that dentists frequently fill the enamel pits in afflicted patients is an indirect acknowledgement of concern for the defect.

3.3. Skeletal Fluorosis and Bone Fractures

Excessive intake of fluoride can result in skeletal fluorosis, a condition characterized by increasing bone density (preclinical and Stage I); sporadic pain, stiffness of the joints, and osteosclerosis of the pelvis and spine (Stage II); and chronic joint pain, arthritic symptoms, calcification of ligaments, and osteosclerosis of cancellous (porous) bones (Stage III). Alterations in micro-structure of bone tissue (e.g., abnormal crystal structure) resulting from skeletal fluorosis may be manifest as an increase in the likelihood of bone fractures.

Most of the fluoride in the body is found in bones where it exists in both rapidly and slowly exchanging pools. The rapidly exchanging pool involves the hydration shell on the surface of bone crystallites (collagen-mineral structural units). When plasma fluoride levels are high, more fluoride enters the hydration shell with most of it returning to extracellular fluid as plasma levels decline. However, a small portion remains with the crystallite in the newly formed bone as hydroxyfluoroapatite. Fluoride in the mature bone tissue exchanges slowly with the extracellular fluid but is released during bone remodeling and resorption (Stipanuk, 2000). The dose-response evidence for skeletal fluorosis and bone fractures in populations exposed to fluoride is addressed in this section.

3.3.1. Skeletal fluorosis

As mentioned above, skeletal fluorosis is a disorder of the bones and joints which is directly related to the magnitude and duration of fluoride ingestion at levels above those of the general diet in the United States. This disorder is most often manifest in areas of the world with high levels of geological fluoride in the water and sometimes the food supply. It can also occur in occupationally exposed individuals.

Fluoride has an impact on the development and resorption of bone (Krishnamachari, 1986). It is a stimulant for increased activity of ostoblasts, the bone forming cells that lie down the collagen matrix which later becomes mineralized. Excess dietary fluoride, when it becomes incorporated in the hydroxyapatite crystal as hydroxyfluoroapatite, can alter the lattice structure (Mousny et al., 2008) and lead to increases in bone density which may render the bone more prone to fracture. Factors that influence the occurrence of skeletal fluorosis include age, sex, dietary fluoride and calcium intakes, renal function, and parathyroid hormone activity. Bone density measurements, analysis of bone biopsy tissues, and radiography are tools for early diagnosis of skeletal fluorosis. There are no therapeutic treatments for the crippling form of the disease (Stage III) (Krishnamachari, 1986).

Ingestion of fluoride over a long period of time during which calcium intakes are apparently normal can lead to osteosclerosis with immobilization of joints of the axial skeleton and of the major joints of the extremities (Krishnamachari, 1986). Elevated urinary fluoride and increased bone fluoride content are indicators of fluoride toxicity. Hormones associated with bone mineral metabolism may also be affected. Osteomalacia and osteoporosis of varying degrees as well as exostosis formation may also develop (Krishnamachari, 1986). In some cases symptoms can be alleviated when fluoride intake is stopped (Hallanger et al., 2007) but reversal is unlikely for Stage III skeletal fluorosis.

NRC (2006) reviewed the available data on the concentration of fluoride in bone ash of individuals exhibiting various stages of skeletal fluorosis and concluded that the "likelihood and severity of clinical skeletal fluorosis increase with the bone fluoride content, but a given concentration of bone fluoride does not necessarily correspond to certain stage of skeletal fluorosis in all cases". Wide ranges of bone fluoride have been reported for similar degrees of skeletal fluorosis. On the basis of data for the iliac crest or pelvis, fluoride concentrations of 4,300 to 9,200 mg/kg in bone ash have been found in cases of stage II skeletal fluorosis, and concentrations of 4,200 to 12,700 mg/kg in bone ash have been reported in cases of stage III skeletal fluorosis. The overall ranges for other bones (not specifically identified) were 6,300 to 12,900 mg/kg (NRC, 2006). Even so, some studies have reported no evidence of fluorosis with bone fluoride levels falling within these ranges (Zipkin et al., 1958; Erben et al., 1984).

Other factors, such as calcium intake, may alter the severity of fluorosis at a given concentration of bone fluoride. In studies in which skeletal fluorosis was diagnosed at relatively low bone fluoride levels (2650-5850 mg/kg in bone ash), the individuals were reported to be suffering from hypocalcemia or secondary hyperparathyroidism (Teotia and Teotia, 1973, Pettifor et al., 1989). Bone fluoride levels of 10,000-12,000 mg/kg bone ash fall within or exceed the ranges of concentrations that have been associated with stage II or stage III skeletal fluorosis (NRC, 2006), but because of inconsistencies in the entire data set, it is unlikely that bone fluoride concentration can be used in a dose-response analysis of skeletal fluorosis.

3.3.1.1. Exposure to fluoride and changes in bone density

The earliest pre-clinical stages of skeletal fluorosis are associated with increases in bone density. This is generally considered to be the result of the anabolic action of fluoride on osteoblasts (bone forming cells) (NRC, 2006), and is the basis for the use of fluoride compounds in the

therapeutic treatment of osteoporosis. Clinical studies have demonstrated increases in bone density in postmenopausal women suffering from osteoporosis when they were treated with fluoride (see discussion in Reid et al., 2007).

Bone mass is affected by many environmental and physiological factors. Of critical importance in maintaining normal bone density is an adequate intake of calcium throughout life but especially in preteen and teenage years. Adequate dietary intakes of the other bone forming minerals, notably magnesium and phosphorous are also important as is Vitamin D. Conditions that can increase the risk of bone loss include postmenopausal decreases in estrogen production; use of corticosteroids, thyroid hormones, diuretics and blood thinning drugs; tobacco use; alcoholism; and poor nutrition. Each of these conditions needs to be considered when evaluating results of studies assessing the effects of fluoride on bone density.

In a study conducted by Reid et al. (2007) and not available to NRC (2006), 80 postmenopausal women with osteoporosis who had been taking estrogen for one year or more were treated with 20 mg F (as glutamine monofluorophosphate, MFP) or a placebo over 4 years in a double blind trial. Bone density of the total body, lumbar spine, femur, and distal and proximal regions of the forearm was measured with a dual-energy X-ray absorptiometer. At the end of the 4-year trial, bone mass density in the lumbar spine (L2-4 in the anteroposterior projection, AP) of the fluoride-treated group was 22% above baseline and 16% higher than the placebo group which increased 6% above baseline ($p < 0.0001$). In "purely trabecular" bone of the vertebral body (L-3, lateral projection), the increase was 49% above baseline compared to 2.5% for the placebo group ($p < 0.0001$). There was a suggestion of a treatment-related effect in the trabecular-rich region of the ultradistal radius and ulna ($p = 0.1$). In contrast, for the cortical bone of the proximal radius there was no significant change in bone mass density ($p = 0.9$).

The greatest increases in bone density from fluoride treatment have been seen in trabecular bone of the spine, wrist, and hip. [Trabecular bone, characterized as having a sponge-like network of interconnected bony spicules that form a meshwork of spaces filled with bone marrow, can also be found at the ends of the femur, tibia, humerus, and radius; in vertebral bodies, and in the iliac crest]. In contrast, cortical bone (bones, such as the central shafts of the long bones (e.g., mid-radius), which have a thick compact cortex and a relatively small amount of marrow) show little if any increase in density following treatment with fluoride. In fact, some studies have reported cortical bone loss in the "radial shaft" ("cortical bone") or forearm at very high doses (75 mg/day) of sodium fluoride (Riggs et al., 1990; Kleerekoper et al., 1991). Note, however, that in both of these latter studies, the placebo groups also showed loss of bone in the forearm, and the differences from the fluoride groups were not reported to be statistically significant.

In the Riggs et al. (1990) study, a 4% decrease in bone density of the radial shaft (reported to consist primarily of cortical bone and assumed to be the mid-radius), relative to controls, was seen in a group of women 50–75 yr of age with postmenopausal osteoporosis who had been taking 75 mg sodium fluoride and 1500 mg elemental calcium per day for 4 years. Controls received placebo tablets and 1500 mg elemental calcium per day. Riggs et al. (1990) also reported a 35% increase in bone density of the lumbar spine, a 12% increase for the femoral neck and a 10% increase for the femor in the area where it interacts with the hip joint. The differences between the treatment and placebo groups were significantly different from zero at all sites reported.

As noted by Reid et al. (2007), increases in bone density following treatment with fluoride are related not only to the magnitude of the dose administered, but possibly also to the dose rate. Ringe et al., (1999) reported a bigger increase in bone mass density following treatment with 20 mg F/day than with 11 mg F/day, while Rubin et al. (2001) found no significant changes in bone density of individuals with osteoporosis receiving 23 mg F/day in a slow release form with 945 mg calcium.

Hansson and Roos (1987) studied four groups of 25 osteoporotic women over a three-year period in order to evaluate the effects of calcium and two different levels of fluoride on spinal bone mineral content. Group A was given 30 mg of sodium fluoride (13.56 mg/day as F) and 1 g/day calcium as a combination of the bicarbonate, lactate, and gluconate salts. Group B was given 10 mg of sodium fluoride (4.53 mg/day as F) and 1 g/day of calcium bicarbonate, lactate, and gluconate. Group C was given 1 g/day of calcium bicarbonate, lactate, and gluconate combined. Group D was given a starch placebo. The spinal bone (L3, trabecular) mineral content of the women in group A increased from the original 0.272 g/cm to 3.18 g/cm after three years of treatment ($p < 0.01$) while there was no significant change in the bone mineral content of the women receiving the lower fluoride dose, supplemental calcium or the starch placebo.

Epidemiological Studies. Epidemiological studies have evaluated bone density in residents of areas with differing levels of fluoride (high and low) in their drinking water. Based on radiological exams conducted in 1943 and again in 1953 on residents of two towns in Texas, Leone et al. (1955) reported a higher number of cases of increased bone density and coarsened trabeculation (16–17/89 and 12–14/89, respectively) in residents of a town with 8 mg F/L drinking water compared to another town with 0.4 mg F/L (4/101 cases of increased bone density and 2–3/101 cases of coarsened trabeculation). Stevenson and Watson (1957) reported radiological evidence of increased bone density in 23 patients residing in communities where fluoride drinking water concentrations were 4 to 8 mg/L (based on a total of 170,000 radiographic examinations of the spine and pelvis). Extreme bone density (Grade 4 on a scale of 1–4) was found in 11 of these 23 patients.

Sowers et al. (1986) conducted a cross-sectional baseline survey of bone mass in 827 adult women residing in three rural communities in northwest Iowa. The women were divided into groups based on fluoride and calcium levels in drinking water; high fluoride (4.0 ± 0.1 mg F/L) with low calcium (15 ± 3 mg Ca/L); low fluoride (1 mg fluoride/L) with high calcium (375 ± 8 mg Ca/L); or low fluoride (1 mg fluoride/L) with moderate calcium (range 62–71 mg Ca/L for two wells). The community with low fluoride and moderate calcium concentrations in the water supply was considered the referent population. Twenty-four hour dietary recall information for food and beverages was obtained from interviews with each of the participants; however, because of earlier results of a total diet study by Singer and Ophaug (1982) that found drinking water to be the primary source of fluoride, Sowers et al. (1986) did not include dietary fluoride from sources other than drinking water in their assessment. Although more than 80% of the participants reported using fluoridated toothpaste; intake of fluoride through this use was not estimated. Thiazide use and hormone treatment with estrogen were treated as covariates. The study authors did not provide an explanation for including thiazide in their analysis; presumably though, the use of diuretics such as thiazide would increase urinary output and thereby affect fluoride levels in the body.

Sowers et al. (1986) found that mid-radius bone mass (measured distally at a site one-third the distance between the styloid process and the olecranon) did not differ among women 20–35 yrs old in the three communities. Women 55–80 yrs old living in the high fluoride community had significantly less (about 5%) mean mid-radius bone mass than women of the same age range living in the low fluoride and moderate or high calcium communities. When adjusted for estrogen and thiazide use, as well as total calcium intake (including water, food, and supplements), vitamin D intake and muscle area, levels of radial bone mass from women in the high fluoride community were lower than those for the other two groups, but the differences were not statistically significant.

In 1991, Sowers et al. (1991) conducted a follow-up study in these same towns. The populations studied included 81.5–85% of the participants from the earlier study. Women 20–35 yrs old in the higher fluoride community had significantly lower mean mid-radius bone mass values in 1988/89 than did women in the referent and higher-calcium communities (Table 3-48).

Table 3-48. Mean Mid-Radial Bone Mass in 20–35-year-old Iowa Women at Baseline (1983/1984) and at Follow-up (1988/1989) in Studies Conducted by Sowers et al. (1991)

Group	Adjusted[a] Value	p Value for Difference in Means			
Baseline (1983/1984) radial bone mass (g/cm^2)					
Referent[f] (N = 37)	0.75 ± 0.008[b]				**NS[c]**
Higher Ca[e] (N = 33)	0.75 ± 0.008				
Higher F[d] (N = 67)	0.74 ± 0.006				
Follow-up (1988/1989) radial bone mass (g/cm^2)					
Referent	0.73 ± 0.008[b]			}	**0.04**
Higher Ca	0.74 ± 0.009	}	**0.02**		
Higher F	0.71 ± 0.006				
Absolute difference in radial bone mass in 5 yr (g/cm^2)					
Referent	-0.015 ± 0.005[b]			}	**0.08**
Higher Ca	-0.011 ± 0.005	}	**0.03**		
Higher F	-0.027 ± 0.004				
Percent loss of radial bone mass in 5 yr					
Referent	-2.1 ± 0.7			}	**0.08**
Higher Ca	-1.6 ± 0.7	}	**0.03**		
Higher F	-3.6 ± 0.5				

[a]Adjusted for age and Quetelet index [weight in kg/(height in m)^2].
[b]Mean ± standard error.
[c]NS, not significant.
[d]4.0 ± 0.1 mg F/L and 15 ± 3 mg Ca/L.
[e]1 mg F/L and 375 ± 8 mg Ca/L.
[f]1 mg F/L and 67 ± 4 mg Ca/L.

The mean loss of mid-radius bone mass (absolute difference or percentage of loss) over the 5-year period was greater in women of the high-fluoride community than in women of the referent

and higher-calcium communities. The study authors could not identify a factor other than higher fluoride exposure, to explain their observation.

The mean mid-radial bone mass values for women in the 55–80-year age group are shown in Table 3-49. The values are adjusted for age and "Quetelet" index [weight in kg/(height in m)^2]. At both baseline and follow-up, mean mid-radial bone mass was significantly lower in the higher-fluoride community than in the referent and higher-calcium communities. However, the rates of change in radial bone mass were not significantly different among the communities during this 5-year period. The mean bone mass of the femur (measured at three sites, the neck, Ward's triangle, and the trochanter, which the study authors state included cortical and cancellous bone) was consistently lower in the higher fluoride community than in the higher calcium community; however, the mean femoral bone mass measures were not significantly lower than mean values in the referent community.

Table 3-49. Mean Mid-Radial Bone Mass in 55–80-year-old Iowa Women at Baseline (1983/1984) and at Follow-up (1988/1989) in Studies Conducted by Sowers et al. (1991)

Group	Adjusted[a] Value	p Value for Difference in Means			
Baseline (1983/1984) mid-radial bone mass (g/cm^2)					
Control (N = 121)	0.63 ± 0.008[b]				
Higher Ca (N = 148)	0.63 ± 0.007		**0.006**		**0.02**
Higher F (N = 163)	0.60 ± 0.007				
Follow-up (1988/1989) mid-radial bone mass (g/cm^2)					
Control	0.59 ± 0.008				
Higher Ca	0.59 ± 0.007		**0.003**		**0.01**
Higher F	0.56 ± 0.007				
Absolute difference in mid-radial bone mass in 5 yr (g/cm^2)					
Control	-0.039 ± 0.004				
Higher Ca	-0.043 ± 0.003				**NS[c]**
Higher F	-0.046 ± 0.003				
Percent loss of mid-radial bone mass in 5 yr					
Control	-6.4 ± 0.6				
Higher Ca	-6.9 ± 0.5				**NS[c]**
Higher F	-7.4 ± 0.5				

[a]Adjusted for age and Quetelet index [weight in kg/(height in m)^2].
[b]Mean ± standard error.
[c]NS, not significant.

Sowers et al. (2005) measured serum fluoride concentrations and bone mass density (BMD) and evaluated the 4-year incident fracture frequency among adult (20–92 yrs old) women residents of the same three Iowa communities studied by Sowers et al. (1986 and 1991). Bone mass density measurements were made on the femoral neck and lumbar vertebrae using dual X-ray densitometry and on the "distal" radius using single photon densitometry. The study authors reported that the distal radius consisted primarily of cortical bone and the lumbar vertebrae primarily trabecular bone, but they did not characterize the femoral neck (trabecular bone).

BMD of the lumbar spine and femoral neck did not differ among the residents of the three communities; however, the BMD of the distal radius was significantly higher ($p = 0.05$) in the high fluoride community (0.667 ± 0.004 g/cm^2) compared with the mean value in the control community (0.651 ± 0.0053 g/cm^2).

Phipps and Burt (1990) compared cortical bone mass (distal radius with 75% cortical bone) in lifetime female residents of two towns in New Mexico; one town, Lordsburg, had a fluoride level of 3.5 mg/L in its drinking water, and the second town, Deeming, had a fluoride level of only 0.7 mg/L. Bone density was measured by single photon absorptiometry. The study participants consisted of 151 post-menopausal women ranging in age from 39 to 87 years old. Bivariate analysis showed no difference in cortical bone mass between the women of the two communities; however, multiple regression analysis indicated that fluoride exposure was one of several significant predictors of bone mass ($p < 0.05$) (other predictors included weight, years since menopause, current estrogen supplementation, and diabetes). Women living in the high fluoride area had a bone mass ranging from 0.004 to 0.039 g/cm^2 less than those living in the optimal fluoride community, suggesting that high fluoride exposure might result in a reduction in cortical bone mass.

Phipps et al. (2000) conducted a multicentre prospective study on risk factors for osteoporosis and bone fractures. Information was collected for 7129 white women (≥65 yrs old) on exposure to fluoridated drinking water, bone mineral density, and the occurrence of fractures. Exposure to fluoridated drinking water was determined from a questionnaire on residence history. Outcomes for women with 20 years of continuous exposure to fluoridated drinking water (N = 3218) were compared to those for women not exposed to fluoridated drinking water (N = 2563). In women with continuous exposure mean bone mineral density was 2.6% higher at the femoral neck ($p <0.001$), 2.5% higher at the lumbar spine ($p<0.001$) and 1.9% lower at the distal radius ($p=0.002$). The reduction in bone density in the cortical bone of the radius was similar to that reported in other studies.

In a study of 2076 women other than African-Americans living in a rural area of Pennsylvania, Cauley et al. (1995) found no evidence that exposure to residential fluoridated drinking water resulted in increased bone mass. Estimates of fluoride exposure were based on number of years of community water use only (mean of 1.01 mg F/L for those communities that fluoridated their water and 0.15 mg F/L for those that did not fluoridate).

In summary, the available data indicate that changes in bone mass density following exposure to fluoride appear to be dependent on the magnitude of the dose and duration of the exposure and the type of bone evaluated. Trabecular bone appears to be more likely to show a direct response to fluoride with cortical bone showing little-, no-, or in some cases possibly a decrease in density, especially at higher fluoride dose levels. Confounding variables which must be considered in assessing bone mass changes associated with fluoride exposure include calcium and magnesium intake, Vitamin D status, and estrogen therapy.

3.3.1.2. Exposure to fluoride in stage II and stage III skeletal fluorosis

Stage II Skeletal Fluorosis. This stage of skeletal fluorosis is characterized by sporadic pain, stiffness of the joints, and osteosclerosis of the pelvis and spine, calcification of the ligaments,

and, at times osteoporosis of the long bones. NRC (2006) considered Stage II skeletal fluorisis to be an adverse health effect because the described symptoms could affect mobility and are precursors to more serious mobility problems. There is, however, little information on the prevalence of stage II skeletal fluorosis in the U.S.

In a survey conducted in Texas and Oklahoma, Stevenson and Watson (1957) examined the results of 170,000 radiological examinations of the spine and pelvis and diagnosed only 23 cases of fluoride osteosclerosis in individuals living in areas where the drinking water contained 4 to 8 mg F/L. No cases of osteosclerosis were found in individuals living in areas with less than 4 mg F/L in drinking water. The 23 subjects were not classified as to stage of skeletal fluorosis, and NRC (2006) could not determine from the information provided what percentage, if any, of the 23 cases could be classified as stage II fluorosis. It was reported that eleven of the 23 subjects had more than a minimal increase in bone density and 15 had calcification of the pelvic ligaments.

A Public Health Service survey of adult residents living in two towns in Texas, one with 0.4 mg F/L in drinking water (N = 121) and the second with 8 mg F/L (N = 116) found only slight roentgenographic evidence of fluoride-related bone changes (either increased bone density with or without coarsened trabeculation, or coarsened trabeculation with lines of stress and without an increase in bone density) in 10–15% of the population exposed to 8 mg F/L (Leone et al., 1955). The study authors also reported that there was an "equivocal" increased thickening of cortical bone and periosteum with a slight relative narrowing of the bone marrow space. An "occasional" case of ligament calcification was also reported, but it was said to be a "common finding in any older age group." In one 59-yr old individual from the high-fluoride town, the investigators found "definite ossification of the right sacrotuberous ligament", but no other evidence of calcification was reported. Leone et al. (1955) did not refer to any of these cases as osteosclerosis, a histological characteristic of Stage II; therefore, it cannot be determined if any would fall into this category. NRC (2006) points out that the town with 8 mg F/L began a defluoridation program about 18 months before the study was undertaken.

With the exception of the Stevenson and Watson (1957) and Leone et al. (1955) reports, most documentation of fluoride associated skeletal fluorosis in the U.S. is based on case reports. A selected number of these case reports are listed in Table 3-50. The studies reported in Table 3-50 include cases of both Stage II and Stage III skeletal fluorosis.

Only two of the studies listed in Table 3-50 identify cases that would be categorized as Stage II fluorosis. Whyte et al. (2005) reported a case in which a 52 yr old woman developed skeletal fluorosis after consuming excessive amounts of fluoride in instant tea during her entire adult lifetime. The symptoms and radiographic evidence appeared to correspond to stage II skeletal fluorosis although the study authors did not specifically refer to her as having stage II skeletal fluorosis. The subject reported skeletal discomfort including neck and scapular pain and elbow and knee arthralgias. Radiographs documented the appearance of marked osteosclerosis and cortical thickening throughout the spine (especially the lumbar region) and pelvis. The ribs were similarly affected. The subject had reported that she had consumed one to two gallons of instant tea every day throughout her entire adult lifetime. Mean fluoride levels in samples of instant tea products were found to range from 1.0 to 6.5 ppm, and her daily fluoride dose was estimated to be 37–74 mg. Beginning at age 46 yr the subject had estrogen injections followed by oral

estrogen – methyltestosterone therapy for three years. She also took 600 mg calcium twice daily for 4 years and a multivitamin daily for 6 months. Approximately four years after she stopped drinking instant tea, her symptoms abated and her urinary fluoride levels had dropped from a previous high of about 14 mg/g creatinine to a normal level of 2.2 mg/g creatinine, but bone density of the lumbar spine and hip (femoral neck) remained elevated (251% and 131% of control means, respectively, compared to 214% and 124%, respectively, at the time she was first examined). The study authors did not report on any changes in the patient's osteosclerotic condition after she had stopped drinking the instant tea.

Table 3-50. Selected Cases of Severe Skeletal Fluorosis in the U.S.

Estim. F in water (mg/L)	Estim. daily DW intake (L)	Estim. daily dose (mg F/day)	Effect/Comment	Reference
2.8	~4–8	37–74	Signs of stage II fluorosis. Exposure included high levels of fluoride in instant tea mix.	Whyte et al., 2005[a]
7–8	? (high)	?	Signs of stage II fluorosis. Hot climate and excessive intake of water.	Felsenfeld and Roberts, 1991[a]
2.4–3.5	4-8	9.6–28	Crippling skeletal fluorosis (Stage III). Excessive consumption of water.	Sauerbrunn et al., 1965
4–8	?	?	Crippling skeletal fluorosis (Stage III). Excessive intake of water and tea	Goldman, et al., 1971
2–3	3	6–9	Stage III; Renal deficiency	Johnson et al., 1979[a]
8.5	2–4	17–34	Stage III; Renal deficiency	Lantz et al., 1987[a]

[a]As cited in NRC, 2006.

Felsenfeld and Roberts (1991) reported on a case of a 54 yr old woman who was diagnosed with osteosclerosis following a routine chest roentgenogram. The subject had suffered from stiffness of the knees and hips for the two previous years. The only other pertinent physical finding was kyphosis (abnormal curvature of the spine). A metabolic bone survey showed sclerotic bones of the lumbar, thoracic and cervical spines, both clavicles and the pelvis. In addition, a pronounced trabecular meshwork pattern was seen in the pelvis. Certain diagnostic features of skeletal fluorosis, such as interosseous and ligamentous calcifications and bony exostoses were not found. The fluoride concentration of the subject's drinking water, which was obtained from a well, was reported to be 429 µmol/L (8.15 mg/L). The subject had been drinking this well water for the previous 7 years, but the average volume of water she drank was not reported. Her urinary fluoride level was 151 µmol/L (normal range 11–58 µmol/L). Within 6 weeks after she stopped drinking the well water, her urinary fluoride level dropped by 50%.

One case of what appears to be Stage II skeletal fluorosis was reported from the Gaspe Peninsula of Quebec, Canada (Boyle and Chagnon, 1995). A 64-year-old Canadian farmer was admitted to a hospital after complaining of severe pain and stiffness in his joints and difficulty in breathing; his wife reported only mild pain in her hands and wrists. X-rays of the farmer showed increased bone density and urinalysis indicated above normal levels of fluoride. The water supply for the farm had changed six years earlier when a deep well replaced an earlier surface well. Samples from the new well had a fluoride concentration of 25 mg/liter and low calcium and magnesium. The farmer's fluoride intake was estimated to have been approximately 50 mg/day and his wife

about 30 to 40 mg/day for six years. Within a year of discontinuing use of the well for drinking water, the joint pains had decreased and their flexibility increased. The patient was monitored to determine if there was any appearance of excess bone growth. The results suggested that the condition had not progressed to Stage III fluorosis at the time of diagnosis.

Stage III Skeletal Fluorosis. In stage III skeletal fluorosis (crippling skeletal fluorosis), deformities develop in the spine and major joints. There is increased calcification of the joints including the ligaments and, at times neurological effects when the spinal cord becomes compressed by fluorotic bone. For the time period of 1960 to 1997, the Institute of Medicine (IOM, 1997) found only five confirmed cases of stage III skeletal fluorosis in the United States. Two of these case reports are included in Table 3-50; citations were not identified for the others.

Sauerbrunn et al. (1965) documented a case of crippling skeletal fluorosis in Texas. This individual consumed excessive amounts of water (a condition known as polydipsia) and a significant amount of tea for a good portion of his life until his death at age 64. His drinking water was thought to contain from 2.4 to 3.5 mg/L fluoride and his drinking water intake (reported by NRC, 2006) was estimated to range from 4 to 8 L per day, which would correspond to a daily fluoride dose about 10 to 28 mg. A brother who was exposed to these concentrations for the same time period, had the same diet, and lived under similar environmental conditions, but who did not drink excessive amounts of water, only developed mottling of the teeth.

Another case report of an individual with crippling skeletal fluorosis is that of a 55-year old Papago Indian from Gila Bend, Arizona (Goldman, et al., 1971). Similar to the case described by Sauerbrunn et al. (1965), this individual consumed large quantities of water throughout his life and also drank large amounts of hot tea. The fluoride drinking water concentrations were estimated to range between 4 and about 8 mg/L; however, total fluoride intake was not reported.

Endemic stage II and stage III skeletal fluorosis are more frequently reported outside the U.S, most often in Africa, China and India. Cao et al. (2003) evaluated the prevalence of skeletal fluorosis in adults living in the Naqu County area of Tibet. One hundred eleven adults, ≥ 30 years old, selected by a randomized sampling method, were included in the study. The level of fluoride in the water, fuel, soil, food, brick tea, brick tea-water and urine were determined. Total daily fluoride consumption was calculated to be 11.99 mg/day (with 99% coming from the consumption of brick tea which contained 739 mg F/kg). (Note: Brick tea consists of mature leaves, twigs and berries of the tea plant *Camellia sinensis* which is compressed into a brick; the brick is pounded into pieces and then cooked in water to become thick brick tea water which is used in cooking). Physical examinations using standardized activities commonly associated with skeletal fluorosis (e.g., fingers could not touch the shoulder, middle finger could not touch the contralateral ear, etc.) were conducted on all the participants. Individual radiographs were taken for those that presented with more than three physical signs. X-rays were taken of the A-P forearm including the wrist and elbow; the A-P shank including the knee joint; the A-P pelvis; and the A-P and the lateral spine.

Ninety-nine of the 111 subjects presented with more than three physical signs of skeletal fluorosis. The prevalence of the skeletal fluorosis with more than 3 positive signs increased with age and was most prominent in those aged 60–78. Forty-two of these underwent radiographic examination; 3 (7%) were diagnosed with stage I skeletal fluorosis), 13 (31%) with stage II, and 19 (45%) with stage III. The most common radiographic change was an increase in bone matrix

density progressing from a trabecular sand-like granular structure to coarse, dense and rough cloth-like appearance of the trabeculae, extensive fine or coarse dense fusion of trabeculae and marble-like bone sclerosis. In addition, fibrous tissue ossification, tendon attachment calcification and articular degeneration were not uncommon. The study authors concluded that the risk of developing early signs of skeletal fluorosis appeared to be associated with an estimated fluoride intake by adults of 12 mg/day, 67% of which was reported to have come from the ingested tea.

Choubisa (2001) reported on the prevalence of skeletal and dental fluorosis in 21 villages of Banswara, Dungarpur, and Udaipur districts of southern Rajasthan, India, where fluoride (F) concentrations in drinking waters range from 1.5 to 4.0 ppm. At a fluoride concentration of 1.5 ppm, 6.1, 6.8, and 9.5% of adults in villages of Banswara, Udaipur, and Dungarpur districts, respectively, showed evidence of skeletal fluorosis. Deformities such as crippling, kyphosis, and genu varum (Stage III) were observed most frequently in higher age groups (>40 years) at a fluoride concentration of 2.8 ppm or higher. Males were more likely to be affected than females. X-rays of the cervical spine, rib cage, lumbar-dorsal spine with pelvis, forearm, and lower limb of 2–3 subjects from each village showed increased bone mass and density, as well as exostoses, calcification of ligaments and interosseous membranes, and osteosclerosis. A weakness of this study is the fact that there was no assessment of sources of fluoride exposure from non-drinking water sources.

The results of the studies presented in Table 3-50, as well as the epidemiological studies discussed above suggest that a daily fluoride dose in excess of about 10 mg (in the absence of renal deficiency) may produce signs of stage II skeletal fluorosis. The data, however, are too incomplete to be used in a dose-response analysis to estimate a threshold. Although NRC (2006) considered stage II skeletal fluorosis to be an adverse effect, it "could not determine from the existing epidemiologic literature whether stage II skeletal fluorosis is occurring in U.S. residents who drink water with a fluoride concentration of 4 mg/L." Based on data indicating that lifetime exposure to 4 mg F/L drinking water can result in bone fluoride levels (10,000–12,000 mg/kg bone ash) that fall within or exceed the ranges of concentration associated with stage II and stage III skeletal fluorosis, the NRC (2006) concluded that 4 mg F/L in drinking water "has the potential" to induce skeletal fluorosis, but that "more research is needed to clarify the relationship between fluoride ingestion, fluoride concentrations in bone, and stage of skeletal fluorosis before any firm conclusions can be drawn." At a standard intake rate of 2 liters of drinking water per day, a concentration of 4 mg/L would result in a dose of 8 mg/day. This dose is below most of those identified in the studies mentioned above, but does not include the potential for additional intake of fluoride through non-drinking water sources.

3.3.2. Bone fractures

Numerous epidemiological and clinical studies have evaluated the occurrence of bone fractures in populations with differing levels of fluoride exposure. NRC (2006) identified and summarized approximately 30 studies addressing this issue (NRC, 2006, Table 5-1). NRC (2006) focused their review primarily on epidemiological studies of populations exposed to fluoride levels in drinking water of 2 mg/L and above, and also on clinical studies in which fluoride salts were used to treat osteoporosis.

NRC (2006) identified four observational (epidemiological) studies (Sowers et al., 1991; Kurttio et al., 1999; Li et al., 2001; and Sowers et al., 2005) and four randomized clinical trials (Riggs et al., 1990; Kleerekoper et al., 1991; Pak et al., 1995; and Reginster et al., 1998) as key studies in evaluating the potential increased risks of bone fracture following prolonged exposure to fluoride. In evaluating such studies, various confounding factors must be considered that might affect bone loss, including dietary restrictions, nutritional status (i.e., calcium, magnesium, phosphorus, and Vitamin D levels); number of pregnancies; and hormone and drug therapies.

3.3.2.1. Epidemiological studies

The four epidemiological studies identified by the NRC (2006) as being key to evaluating the risk of bone fractures from exposure to fluoride in drinking water (≥1.5 mg/L) are summarized in Table 3-51. All four studies indicate an increased risk of fractures in populations exposed to fluoride levels ranging from >1.5 mg/L to 7.97 mg/L. Only one of the four studies (Sowers et al., 2005) also considered the effects of varying levels of calcium intake. More detailed information on the four studies is provided below.

Table 3-51. Key Observational Studies Identified by NRC (2006) for Evaluating the Effects of Fluoride in Drinking Water (≥ 1.5 mg/L) on the Risk of Bone Fractures

F in Water (mg/L)	Referent Group (mg F/L)	Population Gender/age	Risk Values	Fracture site	Risk	Reference
4[a]	1	F, 55-80 yr	2.1 (1.0, 4.4)[b]	any fracture	Relative risk	Sowers et al., 1991
			2.2 (1.1, 4.7)[b]	hip/wrist/spine		
4[a]	1	F, 20-92 yr	2.55 (0.07)[c]	osteoporotic fractures	Risk ratio	Sowers et al., 2005
2.62–3.56	1	M,F, >20 yr	1.18 (0.35)[c]	all sites	Odds ratio	Li et al., 2001
			1.73 (0.34)[c]	hip		
4.32–7.97	1	M,F, >20 yr	1.47 (0.01)[c]	all sites	Odds ratio	Li et al., 2001
			3.26 (0.02)[c]	hip		
>1.5	≤ 0.1	F, 50-65 yr	2.09 (1.16-3.76)[b]	hip	Adjusted Relative Risk	Kurttio et al. 1999
		M, 50-65 yr	0.87 (0.35-2.16)[b]			

Adapted from NRC (2006, Tables 5-2 and 5-3).
[a]With 15 ± 3 mg Ca/L; referent group exposed to 1 mg F/L and 67 ± 4 mg Ca/L.
[b]95% CI.
[c]p value.

Sowers et al. (1991, see also Sowers et al., 1986) examined skeletal fractures in adult women of three rural communities in northwest Iowa, as part of the same study in which they also evaluated the effects of fluoride in drinking water on bone density (see Section 3.3.1.1). Women aged 20-35 yr in the high fluoride community (4.0 ± 0.1 mg F/L and 15 ± 3 mg Ca/L) had an increased probability of any fracture and of fractures of the spine, hip or wrist as compared with the referent community (1 mg F/L and 67 ± 4 mg Ca/L; Table 3-52). The 95% CI for these values, however, both encompassed 1.0. Women in the 55–80 yr old group in the high-fluoride community had an increased relative risk of 2.11 for any fracture (95% CI = 1.01–4.43), 2.20 for fracture at the spine, hip or wrist (95% CI = 1.07–4.69), and 2.2 for multiple fractures (95% CI = 1.0–4.6) compared with the referent community. The data indicate that almost all of the fractures in the high fluoride (4 mg/L) and high calcium groups (375 mg Ca/L and 1 mg F/L) were multiple site fractures. No significant differences in relative risk of any fracture; fractures of the wrist, spine, or hip; or multiple fractures were found between the high-calcium community and the referent community.

Table 3-52. Risk of Fracture in a Five-Year Period (1983–84 to 1988–89) among Women of Three Rural Iowa Communities Studied by Sowers et al. (1991)

Group	Relative Risk[a] (95% CI)		
	Any Fracture	Fracture of the Hip, Wrist or Spine	Fracture at Multiple Sites
Women aged 20–35 years old at baseline[b]			
Referent (1 mg F/L, 67 mg Ca/L)	—	—	
High Ca (1 mg F/L, 375 mg Ca/L)	0.36 (0.03-3.63)	0.30 (0.04-3.39)	
High F (4 mg F/L, 15 mg Ca/L)	1.81 (0.45-8.22)	2.70 (0.16-8.28)	
Women aged 55–80 years old at baseline			
Referent (1 mg F/L, 67 mg Ca/L)	—	—	—
High Ca (1 mg F/L, 375 mg Ca/L)	1.54 (0.70-3.37	1.60 (0.71-3.40)	1.60 (0.71-3.41)
High F (4 mg F/L, 15 mg Ca/L)	2.11 (1.01-4.43)[c]	2.20 (1.07-4.69)	2.2 (1.04-4.57)

[a]Adjusted for age and Quetelet index [weight in kg/(height in m)^2].
[b]There were no multiple fractures in this age group.
[c]Relative risk adjusted for baseline radial bone mass = 1.99 (95% CI of 0.95–4.20).

Sowers et al. (1991) concluded that fluoride dose (years of residence multiplied by daily intake from beverages) was positively correlated with increased risk of fracture in the higher fluoride community. The relative risk of fracture in postmenopausal women with a fluoride exposure less than the median was 1.9 (95% CI, 0.88–4.0), while those postmenopausal women with an exposure greater than the median had a relative risk of 2.6 (95 % CI, 1.2–6.0) when compared with pre-menopausal women; however, as indicated, the 95% CI of the two groups overlap indicating that the differences were not significant. These relative risks were adjusted for age and Quetelet index [weight in kg/(height in m)^2].

Sowers et al. (2005) measured serum fluoride concentrations and bone mass density (BMD) and evaluated the 4-year fracture frequency among adult (20–92 yrs old) women residents of the

same three Iowa communities studied by Sowers et al. (1986 and 1991). After adjusting for co-variates (including age, body size, thiazide use, hormone use, and menopausal status), no statistically significant association was found between serum fluoride levels and osteoporotic fractures [Risk ratio (RR) = 1.16, p = 0.66). Thus, serum fluoride levels in subjects of the community with 4 mg F/L in the water supply were not statistically associated with bone fractures. NRC (2006) notes that serum fluoride concentrations are not a good indicator of long-term fluoride intake. They vary among individuals based on recent fluoride intakes, returning to baseline within hours of exposure, and therefore, may not be a good indicator of bone fluoride concentrations or long-term exposure.

In the Sowers et al. (2005) study, the RR for osteoporotic fractures in the group from the high fluoride area was elevated (2.55), but not significantly different (p = 0.07) from the referent group (1 mg F/L and 60 ± 4 mg Ca/L). The group from the 1 mg/L F, high calcium area had an RR of 3.01 that was significantly different (p = 0.04) from the referent group. NRC (2006) notes that the latter value suggests that the referent group might have had a low fracture rate because of risk factors not controlled for in the study. The presence of high calcium would also be expected to reduce fluoride absorption which could have influenced the results for this group. Sowers et al. (2005) did not evaluate their results by age class as was done in the Sowers et al. (1991) study.

Li et al. (2001) conducted a retrospective cohort study in six areas of China with fluoride concentrations ranging from 0.25 to 7.97 mg/L (mean total fluoride intake ranged from 0.73 to 14.13 mg/day). The subjects were men and women ≥50 yrs old. Drinking water was considered the major source of fluoride in the study populations. Dietary intakes of bone-forming nutrients for the six study populations were considered adequate based on 3-day dietary surveys which included estimates of calcium, protein and fluoride intake. It was determined that drinking water and diet were the main fluoride exposure sources and there was virtually no exposure from sources such as supplements, dentifrice, mouthwash or infant formula. Data were also collected on degree of physical activity, tea drinking, cigarette smoking and alcohol consumption. Average total daily exposures are reported for each of the exposure groups. However, the publication does not provide any details on how the daily exposures were determined. It is not clear whether they were based on drinking water concentrations and intakes alone or whether the dietary data were included in mean exposure estimates.

When compared to the group exposed to 1 mg F/L, the group exposed to 4.32 to 7.97 mg F/L (average 14.13 mg/day) showed a significant increase in overall fractures since age 20 [Odds ratio (OR) = 1.47, p = 0.01], and in hip fractures since age 20 (OR = 3.26, p = 0.02) (Table 3-53). When the evaluation was based on all fractures occurring since age 50, the group exposed to 4.32 to 7.97 mg F/L had a significantly larger OR of 1.59 (p = 0.02) relative to the 1–1.06 mg/L group. Medical records and X-rays were collected when available. In cases where the records were lacking, an X-ray of the self-reported fracture was taken to verify the event.

NRC (2006) applied the data presented in Li et al. (2001) to a generalized linear model to estimate that the absolute increase in fractures was 1.3% (95% CI = 0.3% to 2.2%, p = 0.01) for the increment from 1.00 to 4.00 mg/L for overall fractures since age 20. When segregated by fracture type and age, the absolute increase was 0.4% (95% CI = 0.0% to 0.8%, p = 0.04) for hip fractures since age 20, and 0.9% (95% CI = 0.2% to 1.7%, p = 0.02) for overall fractures since age 50. NRC, however, also points out that even though a trend for fractures appears to increase

from 1.00 to 4.00 mg/L, the rate for overall fractures at the lowest fluoride exposure level (0.25–0.34 mg/L) was higher than that at 1.0–1.06 mg/L, and in fact, the risk at the lowest level was similar to the risk at the highest exposure level, clearly showing a U-shaped response (Fig. 3-9). Further, the data for fractures of the hip since age 20 suggest that the value at the lowest concentration range may be an outlier, because it is not consistent with the U-shaped dose-response curve observed with the other groupings of the data.

Table 3-53. Relationship between the Prevalence of Bone Fractures and Fluoride in Drinking Water in Chinese Populations Studied by Li et al. (2001)

F in Water (mg/L)	No.	No. with fractures	Prevalence (%)	Odds Ratio (95% CI)[a]	p Value
Since age 20 yr – overall fractures					
0.25–0.34	1363	101	7.41	1.50	0.01
0.58–0.73	1407	90	6.40	1.25	0.17
1.00–1.06	1370	70	5.11	1.00	—
1.45–2.19	1574	95	6.04	1.17	0.33
2.62–3.56	1051	64	6.09	1.18 (0.83–1.67)	0.35
4.32–7.97	1501	111	7.40	1.47 (1.10–1.97)	0.01
Since age 20 – fractures of the hip					
0.25–0.34	1363	5	0.37	0.99	0.99
0.58–0.73	1407	6	0.43	1.12	0.85
1.00–1.06	1370	5	0.37	1.00	—
1.45–2.19	1574	14	0.89	2.13	0.15
2.62–3.56	1051	8	0.76	1.73 (0.56–5.33)	0.34
4.32–7.97	1501	18	1.20	3.26 (1.21–9.81)	0.02
Since age 50 – overall fractures					
0.25–0.34	1363	59	4.33	1.33	0.16
0.58–0.73	1407	45	3.20	0.97	0.87
1.00–1.06	1370	45	3.28	1.00	—
1.45–2.19	1574	52	3.30	0.96	0.85
2.62–3.56	1051	38	3.62	1.04 (0.65–1.66)	0.87
4.32–7.97	1501	72	4.80	1.59 (1.08–2.35)	0.02

[a]95% Confidence Limits were estimated by NRC (2006) using the approach of Greenland (1998).

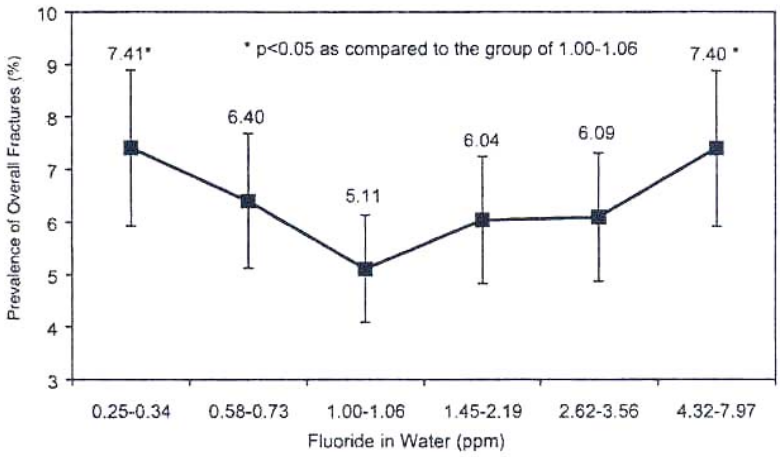

Figure 3-9. Prevalence of overall fractures and fluoride concentration in drinking water in six Chinese populations since the age of 20 yr (Li et al., 2001).

Kurttio et al. (1999) conducted a retrospective cohort study in Finland to determine the effects of water fluoride on the risk of hip fractures. The study cohort consisted of 66,742 men and 77,885 women born in 1900–1930, who lived in the same rural area from at least 1967 to 1980. The study population was divided into exposure groups based on the following fluoride concentrations: ≤0.10; 0.11–0.30; 0.31–0.50; 0.51–1.00; 1.10–1.50; and >1.50 mg F/L. The modeled estimates of fluoride concentrations in well water ranged from below 0.05 mg/L (detection limit) to 2.4 mg/L. When all ages were combined for each gender, there was no correlation (age or area-adjusted) between the rate ratios (RR) of hip fractures and water fluoride concentrations, independent of whether fluoride concentration was treated as a stratified variable or a continuous variable. Age-adjusted and age-area-adjusted RRs for men were 0.97 and 0.90, respectively, and for women 1.07 and 1.10, respectively.

Analysis of the Finnish subjects stratified by age (six 5-year increments), however, found that the crude and adjusted (age, area) RRs for men aged 50–59 were below 1.0, whereas those for women aged 50–64 were above 1.0 (Fig. 3-10). No correlation was found between fluoride concentration and hip fracture in the older subjects (65–80 years old), which the study authors suggested might be due to other more prominent risk factors at higher ages (e.g. age-related changes in calcium absorption, fluoride metabolism, hormonal status, etc.). However, the factors noted are not confounders for the elderly alone and decreased calcium absorption and hormonal status are more often associated with increased fracture risk in the elderly than decreased fracture risk. Accordingly, the increased risk for the 50 to 65 year group compared to the older subjects is counter intuitive.

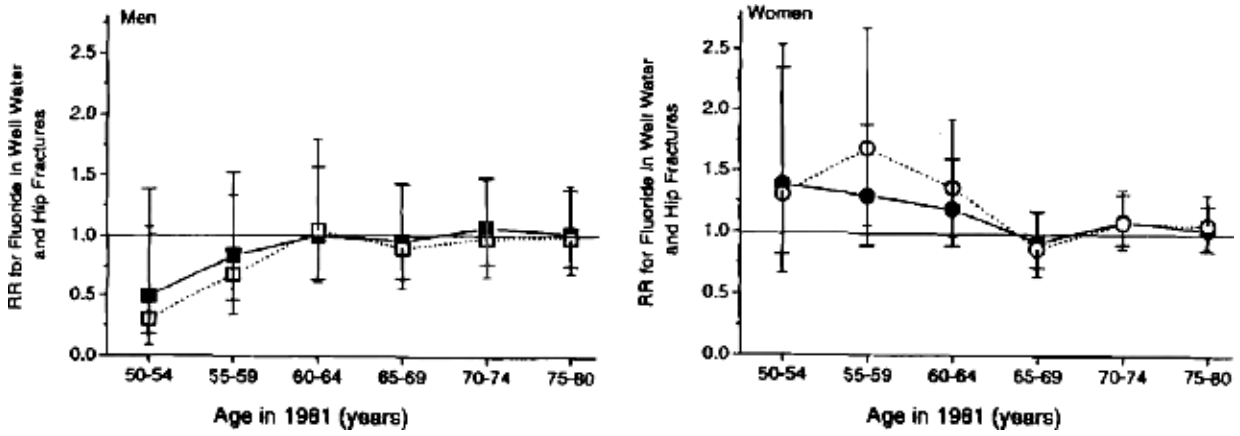

Figure 3-10. Rate ratios and 95 percent confidence intervals for the association of the estimated fluoride concentration in well water (df = 1) and hip fractures in men and women (Cox regression). Age-adjusted (■ and ●) and age- and area-adjusted (□, ○) and the narrower cap of 95 percent confidence intervals) are shown (Kurttio et al., 1999)

Analysis of the data for women aged 50–65 indicated that fluoride was associated with an increased risk of hip fracture this age group (Table 3-54). The adjusted RR was 2.09 (95% CI, 1.16–3.76) for women who were exposed to the greatest fluoride concentrations (>1.5 mg/L) as compared to women exposed to the lowest fluoride concentrations (≤ 0.1 mg/L).

Table 3-54. Effects of Fluoride in Drinking Water on the Risk of Hip Fractures in Finnish Women Aged 50–65 yr (Kurttio et al., 1999)

F in Water (mg/L)	Crude RR	95% CI	Age and Area Adjusted RR	95% CI
≤0.1	1.0		1.0	
0.1–0.3	1.12	0.94–1.35	1.16	0.93–1.43
0.3–0.5	1.18	0.80–1.74	1.31	0.86–1.99
0.5–1.0	1.31	0.99–1.73	1.53	1.08–2.16
1.1–1.5	1.06	0.68–1.65	1.24	0.77–2.01
≥1.5	1.70	0.98–2.96	2.09	1.16–3.76

There are a number of weaknesses in this study, especially the lack of information on the fluoride concentration range represented for the ≥1.5 mg/L grouping and limited identification of possible confounding factors. Fluoride concentrations were modeled. When the modeled values were compared to measured values they were found to be lower by a factor of about 0.7. The grouping of subjects in narrow concentration ranges combined with the uncertainty in the fluoride concentration estimates increases the opportunity for exposure misclassification.

Many earlier studies compared the occurrence of fractures in populations continuously exposed to near optimal levels of fluoride in drinking water with those in populations with shorter exposures and/or to less than optimal levels. Results varied from study to study; some showed a small positive association, others showed a small negative association (see Hillier et al., 1996 for

review of pre-1995 studies, and Demos et al., 2001, for a review of 1991–1998 studies). A select number of studies are briefly summarized here:

A small, statistically significant increase in relative risk of hip fracture in white men and women aged 65 and older was associated with water fluoridation in a national ecologic study conducted by Jacobsen et al. (1992). The relative risk was 1.08 (95% CI = 1.06–1.10) for women and 1.17 (95% CI = 1.13–1.22) for men. The relationship was observed at the county level and the study authors noted that the observation needed to be further assessed at the level of the individual. In a later study Jacobsen et al. (1993) examined hip fracture incidence among men and women aged 50 years and older living in Rochester, MN, before and after fluoridation of the public water supply was instituted (1.1 mg F/L). The study authors did not find a positive association between hip fracture incidence and water fluoridation.

In a study of 2076 women other than African-Americans living in a rural area of Pennsylvania, Cauley et al. (1995) found no evidence that continuous exposure to residential fluoridated drinking water (mean of 1.01mg F/L) was associated with increased risk of wrist or hip fractures when compared to populations with lower fluoride exposures (mean 0.15 mg F/L drinking water).

In a case-control study conducted in the UK, Hillier et al. (2000) evaluated the occurrence of hip fractures in men and women aged 50 years and older living in the English county of Cleveland. The study population consisted of 914 individuals with hip fractures and 1196 controls, of which 514 and 527, respectively, were interviewed. Exposures to fluoride in water were estimated from residential histories and information provided by water suppliers. Estimated lifetime exposure to fluoride ranged from 0.15 to 1.79 mg/L. After adjustment for potential confounders (age, sex, place of residence), the odds ratio associated with lifetime exposure to ≥0.9 mg F/L was 1.0 (95% CI = 0.7–1.5).

Phipps et al. (2000) conducted a multi-city prospective study on risk factors for osteoporosis and bone fractures in 7129 white women (≥65 yrs old) living in four locations in the United States. Women were classified as having been exposed or not exposed (or unknown exposure) to fluoride from 1950 to 1994. Exposure to fluoride was determined from a questionnaire on residence history and information provided by water system maps, the 1992 fluoridation census, or as a result of direct contact with the water supplier. Outcomes were compared in women with 20 years continuous exposure to fluoride (N = 3218) to women with no exposures to fluoridated water (N = 2563) during the same time period. No quantitative information was given on the levels of exposure to fluoride in drinking water. In women with "continuous" exposure the multivariable adjusted risk for hip fractures was slightly reduced (risk ratio 0.69; 95% CI = 0.5–0.96, p = 0.028) as was risk of vertebral fracture (risk ratio 0.73; 95% CI = 0.55–0.97, p = 0.033).

McDonagh et al. (2000) utilized data from 29 published studies to conduct a meta-analysis of bone fracture rates in populations exposed to 1 mg/L compared with those in populations living in non-fluoridated areas. The resulting data were evenly distributed around the "no effect point," but statistical testing showed significant heterogeneity among studies. Some of them showed a positive association whereas an almost equal number showed a negative association. Furthermore, the 95% confidence limits on the measures of effects varied considerably from study to study. McDonagh et al. (2000) stated that, although the results suggest no association between water fluoridation at 1 mg/L and bone fractures, such a conclusion should be interpreted with extreme caution.

3.3.2.2. Clinical trials

The NRC identified four randomized clinical trials involving postmenopausal women which they considered relevant to the evaluation of the effects of fluoride on new non-vertebral bone fractures (Table 3-55). As noted by NRC (2006), the four studies were prospective, double-blinded and placebo controlled, and the subjects received supplemental calcium. In some cases (Riggs et al., 1990), a number of the subjects were being treated for osteoporosis and received vitamin D and/or estrogen supplements as well; in other cases (Kleerekoper et al., 1991) women on estrogen therapy were excluded from the trials. NRC reported that the summary risk estimate for new non-vertebral fractures was 1.85 (95% CI = 1.36–2.50) after four years.

In comparing the epidemiological data to that from the clinical trials, the NRC (2006) noted that, although fluoride dose levels used in the clinical trials were much higher than the doses estimated for the epidemiological studies, the estimated total fluoride exposures were similar, as were the estimated bone fluoride concentrations, which would account for the similarities in the increased risk estimates for bone fractures seen in both types of studies. The study using a slow-release form of fluoride (Pak et al., 1995) actually showed a lower relative risk for non-vertebral fractures (see Table 3-55). Pak et al. (1995) also reported that the group receiving slow-release fluoride had a significantly lower vertebral fracture rate (0.064 ± 0.182 per patient-year compared with 0.205 ± 0.297 per patient-year; $p = 0.002$).

An increase in non-vertebral bone fracture risk was observed in a clinical trial conducted by Reid et al. (2007). In this double blind, 4-yr trial, postmenopausal women with osteoporosis, who had been taking estrogen for one year or more, were treated with 20 mg F (as glutamine monofluorophosphate, MFP) or a placebo. The data indicated that the individuals treated with fluoride had an increased risk of non-vertebral fractures (hazard ratio 3.3, 95% CI = 0.8–12.0), but a reduced risk of vertebral fractures (rate ratio of 0.12, 95% CI = 0.06–0.23, $p < 0.01$; and hazard ratio of 0.20, 95% CI = 0.05–1.30). The hazard ratio takes into account the time to first fracture using a proportional hazard model; the rate ratio compares fracture incidents per 1000 patient-years at risk between groups, assuming a Poisson distribution.

Reid et al. (2007) commented that Riggs et al. (1994), in a reanalysis of the data presented by Riggs et al. (1990), suggested that a rapid increase in bone mass density, brought about by high fluoride doses is associated with increased fracture risk, whereas "modest increments" in bone mass density were protective. Reid et al. (2007) cite a study by Ringe et al. (1999) which showed that fracture rates were lowest in individuals receiving 11 mg F/day compared to those receiving 22 mg F/day, with significantly fewer non-vertebral fractures. A reduction in vertebral

fracture rates (and similar numbers of non-vertebral fractures in both fluoride and placebo groups) had been observed in a study in which 23 mg F/day (together with 945 mg calcium) was administered in a slow release formula (Rubin et al., 2001). Reid et al. (2007) theorized that the bioavailability of the slow-release preparation was lower than that of many other preparations, and the co-administration of the calcium could have further reduced fluoride absorption. Reid et al. (2007) concluded that doses of fluoride less than 20 mg/day are more likely to demonstrate anti-fracture efficacy.

Table 3-55. Clinical Studies Assessing the Risk of Non-Vertebral Fractures in Postmenopausal Women Receiving Therapeutic Doses of Fluoride

Dose (mg F/day)	Avg. Period (yr)	Relative Risk	95% CI	Rate Ratio[a]	95% CI	Reference
20[b]	3.4	1.1	0.5–2.4	1.1	0.5–2.3	Reginster et al., 1998
23[c]	3.1	0.6[d]	0.2–2.5	—	—	Pak et al., 1995
34[e]	2.4	1.5	0.7–3.5	3.0 (hot spots)	2.0–4.6	Kleerekoper et al., 1991
34[e]	3.1	16.8 (incomplete)	3.9–1.7			Riggs et al., 1990
		1.6 (complete)	1.0–2.5	1.9 (complete)	1.1–3.4	
		2.5 (total)[f]	1.7–3.7	3.1 (total)[d]	1.8–5.6	
		2.3 (complete, hip)	0.6–8.8			

SOURCE: Adapted from NRC (2006, Tables 5-3 and 5-4); based on a meta-analysis of Haguenauer et al. (2000).
[a]Rates were computed, presumably by NRC, "by dividing the number of incident fractures (possibly more than one per subject) by participating person-time".
[b]Administered as sodium monofluorophosphate, 4 years.
[c]Administered as 50 mg sodium fluoride/day, slow-release; 12 months on, 2 months off; 4 cycles.
[d]Presumably calculated by Haguenauer et al. (2000); not specifically reported by Pak et al. (1995).
[e]Administered as 75 mg sodium fluoride/day, 4 years.
[f]Total fractures includes complete and "incomplete" stress fractures, the latter observed by roentgenography in participants reporting acute lower extremity pain syndrome.

3.3.3. Summary and conclusions

3.3.3.1. Skeletal fluorosis

Stage II skeletal fluorosis, characterized by sporadic pain, stiffness of the joints, and osteosclerosis of the pelvis and spine, was identified by NRC (2006) as an adverse effect associated with exposure to fluoride. In the United States very few reports of stage II and stage III skeletal fluorosis have been documented. The results of the limited epidemiological studies and cases histories suggest that a daily fluoride dose in excess of 10 mg may be required to produce signs of stage II skeletal fluorosis (except possibly in the case of individuals with renal disease). A daily dose of 10 mg is above the dose level that would result from a fluoride concentration of 4 mg/L and a daily drinking water intake rate of 2 L (excluding fluoride intake through non-drinking water sources). However, based on data indicating that lifetime exposure to 4 mg F/L drinking water can result in bone fluoride levels (10,000–12,000 mg/kg bone ash) that fall within or exceed the ranges of concentration associated with stage II and stage III skeletal fluorosis, the NRC (2006) concluded that 4 mg F/L in drinking water "has the potential" to induce these levels of fluorosis, but that "more research is needed to clarify the relationship

between fluoride ingestion, fluoride concentrations in bone, and stage of skeletal fluorosis before any firm conclusions can be drawn." Consequently, the currently available data are not sufficiently robust to support a dose-response analysis of the effects of fluoride in drinking water on the skeletal fluorosis.

3.3.3.2. Bone fractures

After evaluating the available data, NRC (2006) concluded that there was sufficient consistency among the small set of relevant epidemiological studies "to suggest the potential for increased risk" of bone fracture from exposure to drinking water containing 4 mg F/L or higher, compared to exposure to 1 mg/L. NRC (2006) further stated that data from animal studies and randomized clinical trials are consistent with the observational (epidemiological) evidence in humans, and that biochemical and physiological data indicate a biologically plausible mechanism by which fluoride could weaken bone (i.e., the incorporation of fluoride into the hydroxyapatite of the bone leading to an alteration of the crystalline structure and resulting in lower strength per unit volume). The majority of the NRC (2006) committee concluded that "lifetime exposure to fluoride at drinking water concentrations of 4 mg /L or higher are likely to increase fracture rates in the population, compared with exposure at 1 mg F/L, particularly in some susceptible demographic groups that are more prone to accumulate fluoride in their bone." Indeed, as summarized in Table 3-56, an increased relative risk of bone fracture at the higher fluoride drinking water levels is identified in all the key studies (although not at statistically significant levels in all cases). Since drinking water fluoride intake may be only a fraction of total fluoride intake, risks of bone fracture may be elevated at relatively low fluoride drinking water concentrations.

Additional information on bone fracture rates at fluoride concentrations lower than 2 mg/L, are included in Table 3-56. The results, in general, support the conclusions of the NRC that relative risk of fracture increases with increasing fluoride concentration; however, there are a few studies (Simonen and Laitinen, 1985; Jacobsen et al. 1993; Lehmann et al., 1998) in which the occurrence of fractures and/or relative risk at 1 mg F/L were actually lower than those seen at lower fluoride concentrations. Furthermore, as pointed out by NRC (2006), in the study of Li et al. (2001), the risk of overall fractures in the > 20 yr old group exposed to 0.25–0.34 mg F/L was significantly increased when compared to the 1 mg/L group (odds ratio 1.50; $p = 0.01$) (Fig. 3-9). Fractures of the hip in the >50 yr olds in the lowest exposure group (0.25–0.34 mg F/L) were also increased when compared to the 1 mg/L group, although not statistically significant ($p = 0.16$). According to NRC (2006), this is evidence of a U-shaped dose-response curve and is plausible based on some animal studies indicating a biphasic relationship between bone fluoride concentrations and bone strength. One possible explanation is that fluoride delivered to bone tissue at low sustained doses is more likely to produce a more stable skeletal microstructure than that which occurs following intermittent spikes of exposure which could lead to instabilities in the microstructure. If true, then under certain circumstances and in some populations, fluoride water concentrations in the range of 1 mg/L may, in fact, reduce the risk of fractures compared to lower exposure levels. In three of the key epidemiologic studies (Sowers et al., 1991, 2005, and Li et al., 2001), the referent group was exposed to 1 mg F/L drinking water. Likewise in the clinical trials assessing the occurrence of fractures following therapeutic use of fluoride (Section 3.3.2.2), relative risks were based on comparisons to placebo-dosed individuals who were from

areas with 1 mg F/L in drinking water. Thus, in both types of studies the referent groups were usually exposed to 1 mg F/L in drinking water.

Table 3-56. Epidemiological Studies Evaluating the Effects of Fluoride in Drinking Water on the Risk of Bone Fractures

F in Water (mg/L)	Referent Group (mg F/L)	Gender/age	Risk Values	Fracture site	Type of Risk	Reference
<0.1	1	M, >50 yr F, >50 yr	2.5 (1.6–3.9)[d] (p<0.001) 1.5 (1.2–1.8)[d] (p<0.05)	femoral-neck	Relative risk	Simonen and Laitinen, 1985
Fluoridated	Not fluoridated ≤0.3	F, ≥65 yr M, ≥65 yr	1.08 (1.06–1.10)[c] 1.17 (1.13–1.22)	hip	Relative risk	Jacobsen et al., 1992
1.1 (for 10 yr)	<1.1 (for 10 yr)	M,F; ≥50 yr	a. 0.63 (0.46, 0.86)[d] b. 0.48% (pre-fluoridstion) c. 0.45% (post-fluoridation)	hip	a. Relative risk b. % incidence[f] c. % incidence[f]	Jacobsen et al., 1993
Fluoridated (~ 1)	Not fluoridated (~0.15)	F, ≥65	1.04 (0.84–1.29)[c] 0.99 (0.78–1.24) 1.09 (0.70–1.72) 0.83 (0.44–1.59) 0.94 (0.58–1.54)	non-spine osteoporotic wrist hip vertebral	Relative risk	Cauley et al., 1995
0.08–0.36 0.77–1.20		F, ≥60 yr	0.18% 0.14% (p<0.0001)	hip	Age-adjusted mean annual incidence	Lehmann et al., 1998
1	<0.3	F, ≥65	1.27 (1.08-1.46)[d]	hip	Relative risk	Danielson et al., 1992
0.11–0.25 >0.25	0.05–0.11	M,F, ≥65 yr	3.25 (1.66–6.38)[d] 2.43 (1.11–5.33)[d]	hip	Odds ratio	Jacqmin-Gadda et al., 1998
0.11–1.83	0.05–0.11	M,F, ≥65 yr	1.86 (1.02–3.36)[e]	hip	Odds ratio	Jacqmin-Gadda et al., 1995
4[b]	1	F, 55–80 yr	2.11 (1.01–4.43)[d] 2.20 (1.07–4.69)[d]	any fracture hip/wrist/ spine	Adjusted relative risk	Sowers et al., 1991
4[b]	1	F, 20–92 yr	2.55 (0.07)[c]	osteoporotic fractures	Risk ratio	Sowers et al., 2005
Fluoridated	Not fluoridated	F, ≥65 yr	0.69 (0.50–0.96)[d](0.028)[c] 0.73 (0.55–0.97)[d](0.033)[c] 1.32 (1.00–1.71)[d](0.051)[c] 0.85 (0.58–1.23)[d](0.378)[c]	hip vertebrae wrist humerus	Risk ratio	Phipps et al., 2000
0.25–0.34 2.62–3.56 4.32–7.97	1	M,F, >20 yr	1.50 (0.01)[c] 1.18 (0.35)[c] 1.47 (0.01)[c]	all sites	Odds ratio	Li et al., 2001[a]
0.25–0.34 2.62–3.56 4.32–7.97	1	M,F, >20 yr	0.99 (0.99)[c] 1.73 (0.34)[c] 3.26 (0.02)[c]	hip	Odds ratio	Li et al., 2001[a]
0.25–0.34 2.62–3.56 4.32–7.97	1	M,F, >50 yr	1.33 (0.16)[c] 1.04 (0.87)[c] 1.59 (0.02)[c]	all sites	Odds ratio	Li et al., 2001[a]
>1.5	≤ 0.1	F, 50–65 yr	2.09 (1.16–3.76)[d]	hip	Adjusted rate ratio	Kurttio et al., 1999[a]

[a]Identified by NRC (2006) as providing the most useful data for fluoride concentrations ≥2 mg/L.
[b]With 15 ± 3 mg Ca/L; referent group 1 mg F/L and 60 ± 4 mg Ca/L.
[c]p value; compared with referent group.
[d]95% CI.
[e]90% CI.
[f]Based on person years.

The one key drinking water study that did estimate relative risk by comparison to negligible fluoride exposures was that of Kurttio et al. (1999). Women aged 50–65, exposed to >1.5 mg F/L drinking water were found to have an adjusted relative risk of 2.09 (95% CI = 1.16–3.76) for hip fractures, when compared to the group exposed to <0.01 mg/L. The NRC (2006) considered this study "not sufficient alone to base judgment of fracture risk for people exposed at 2 mg/L" (their proposed point of departure for increases in dental fluorosis). In the Kurttio et al. (1999) study, the reported fluoride concentrations were modeled estimates which showed a strong association with measured values (N = 1411). The study authors reported a 0.71 correlation between analyzed and estimated values. At the highest concentrations, the estimated values tended to be 0.7 times less than the measured values which may have biased the risk estimates towards the null. However, monitoring data at some sites indicated concentrations in excess of 6 mg/L which would have been estimated at less than 2 mg/l based on the model. Thus, it is unclear what levels of exposure were responsible for the hip fractures in the 50–65 yr old women in the >1.5 mg/L group. Specific information on other non-drinking water sources of fluoride for the study population, as well as data on nutritional state, alcohol and tobacco use, estrogen therapy and physical activity was not included in the report. Only 13 hip fractures were recorded in the 50–65 yr old women in the high exposure group (>1.5 mg F/L). There may be a number of confounding factors associated with women in this age group which may not have been accounted for. The women studied were of child-bearing age during the wartime years and nutritional deficiencies and number of pregnancies, may have affected their health status in later years.

Overall, the available data indicates that exposure to concentrations of fluoride in drinking water of 4 mg/L and above is suggestive of and appears to be positively associated with increased the relative risk of bone fractures in susceptible populations when compared to populations exposed to 1 mg F/L. However, there are insufficient data to conclude that this increase in relative risk would also apply if comparisons were made to groups exposed to negligible fluoride concentrations or if comparisons were made based on total fluoride intake rather than on the basis of drinking water concentrations.

4. Approaches to Quantification of Dose-Response

Based on information summarized in Section 3, the critical effect associated with exposure to fluoride (i.e., the adverse effect most likely to occur at the lowest exposure level) is severe dental fluorosis, a condition considered by the NRC (2006) to be an adverse health effect. Severe dental fluorosis has been identified in a small percentage of populations exposed to fluoride levels in drinking water as low as 2 mg/L. In contrast, there is no clear evidence that fluoride will cause other types of adverse health effects such as stage II skeletal fluorosis or bone fractures at levels as low as those associated with severe dental fluorosis. Therefore, the endpoint considered here for a dose-response analysis is severe dental fluorosis.

4.1. Critical Study for Severe Dental Fluorosis

As noted in Section 3, the study that appears to provide the most useful data regarding the effects of fluoride in drinking water on the occurrence of severe dental fluorosis is that conducted by Dean (1942). Dean (1942) surveyed 22 U.S. communities in 10 states where fluoride levels in drinking water ranged from 0.0 to 14.1 mg/L (Table 4-1), and examined a total of 5824 children for dental fluorosis. The strengths of this study are: 1) the dataset is sufficiently large and robust; 2) the range of fluoride concentrations is quite wide; 3) the protocol is sound; 4) there were few alternate sources of commercially available fluoride at the time the study was conducted (e.g., mouthwash, dentifrice, etc.) to confound the data or findings; 5) the concentration-response relationship shows a clear increasing risk of severe fluorosis with increasing fluoride concentration; and 6) the findings are consistent across several different communities. Weaknesses of the study include the following: 1) only white children were included in the survey; 2) potential socio-economic and cultural differences between the samples populations from the different towns were not documented; 3) cultural (e.g., dental hygiene practices and care, and dietary habits) and physiological differences (changes in average body weight, and hormonal changes resulting in decreasing age of menarche) between children in the Dean's study populations and today's children may complicate extrapolation of the Dean data to present day populations.

The children studied by Dean (1942) were primarily in the age range of 9 to 14 yrs old and/or in school grades 2–12. The dental fluorosis status of each participant in the study was recorded according to Dean's Index of Fluorosis (see Section 2 for description), a categorical scoring system in which 0 represents no evidence of fluorosis; 0.5, questionable; 1, very mild; 2, mild; 3, moderate; and 4, severe fluorosis (including pitting). The frequency of occurrence of each score was computed within each study population (Table 4-1).

The Dean (1942) data and results are summarized by increasing fluoride concentration (mg/L) in drinking water (Table 4-1), rather than fluoride intake from the drinking water. The water intakes (L/day) were not reported, and would have varied across the surveyed population. It is recognized that the levels of dental fluorosis observed in the Dean (1942) study are the result of cumulative fluoride exposure and dose during the most sensitive period of tooth enamel formation and not the fluoride exposure at the time observations were made. It is also understood that, in addition to fluoride exposures from drinking water ingestion, the Dean (1942) populations are likely to have also been exposed to fluoride present in dietary items grown or cooked with fluoride-containing water.

Table 4-1. Percent Distribution of Fluorosis in Populations Studied by Dean (1942), Sorted by Concentration of Fluoride in Community–specific Drinking Water Supplies									
Town	No	Age (yr)	F (mg/L)	Dean's Index					
				0	0.5	1	2	3	4
Waukegan, IL	423	12–14	0.0	97.9	1.9	0.2	0.0	0.0	0.0
Michigan City, IN	236	12–14	0.1	97.5	2.5	0.0	0.0	0.0	0.0
Zanesville, OH	459	12–14	0.2	85.4	13.1	1.5	0.0	0.0	0.0
Lima, OH	454	12–14	0.3	84.1	13.7	2.2	0.0	0.0	0.0
Marion, OH	263	12–14	0.4	57.4	36.5	5.3	0.8	0.0	0.0
Elgin, IL	403	12–14	0.5	60.5	35.3	3.5	0.7	0.0	0.0
Pueblo, CO	614	12–14	0.6	72.3	21.2	6.2	0.3	0.0	0.0
Kewanee, IL	123	12–14	0.9	52.8	35.0	10.6	1.6	0.0	0.0
Aurora, IL	633	12–14	1.2	53.2	31.8	13.9	1.1	0.0	0.0
Joliet, IL	447	12–14	1.3	40.5	34.2	22.2	3.1	0.0	0.0
Elmhurst, IL	170	12–14	1.8	28.2	31.8	30.0	8.8	1.2	0.0
Galesburg, IL	273	12–14	1.9	25.3	27.1	40.3	6.2	1.1	0.0
Clovis, NM	138	9–11	2.2	13.0	16.0	23.9	35.4	11.0	0.7
Colorado Springs, CO	404	12–14	2.6	6.4	19.8	42.1	21.3	8.9	1.5
Plainview, TX	97	9–12	2.9	4.1	8.3	34.0	26.8	23.7	3.1
Amarillo, TX	289	9–12	3.9[a]	3.1	6.6	15.2	28.0	33.9	13.2
Conway, SC	59	9–11	4.0	5.1	6.7	20.4	32.2	23.7	11.9
Lubbock, TX	189	9–12	4.4	1.1	1.1	12.2	21.7	46.0	17.9
Post, TX	38	~8–11[c]	5.7[b]	0.0	0.0	0.0	10.5	50.0	39.5
Chetopa, KS	65	~7–17[d]	7.6[b]	0.0	0.0	9.2	21.5	10.8	58.5
Ankeny, IA	21	~6–17[e]	8.0[b]	0.0	0.0	0.0	9.5	47.6	42.8
Bauxite, AK	26	14–19	14.1[b]	0.0	0.0	3.9	3.9	38.5	53.8

SOURCE: Modified from Dean (1942).

[a] "Subject to a possible correction to 4.2 mg/L during susceptible period of age group examined" (no other explanation given by Dean, 1942).

[b]Single determination, all others are arithmetical means of 12 consecutive monthly samples.

[c]Grades 4–6.

[d]Grades 3–12.

[e]Grades 2–12.

4.2. Categorical Analysis of Dean (1942) Study

Dean's (1942) entire data set was initially analyzed during the present assessment by use of a categorical data analysis procedure (categorical model, or CATMOD) developed by the SAS© Institute of Cary, NC. Categorical analysis is particularly well suited for rank or score data such as are presented in Dean (1942), and for which it is useful to investigate and measure the strength of association between and among data categories. The CATMOD procedure incorporates a maximum likelihood logistics regression model to identify best-fit models (as log-linear modeling, logistic regression, and repeated measurement analysis) to the dataset of response frequency functions.

For the Dean (1942) dataset, the CATMOD procedure (detailed in Appendix A) aggregated the 6 classification responses (e.g., Dean's scores 0, 0.5, 1, 2, 3, 4) and determined not only that the Dean's score data were positively correlated with variable concentration but also that high fluoride concentrations in water could indicate the presence of the more severe dental fluorosis

categories. For the severe fluorosis category (Dean's score of 4), one of the 22 data points examined is that for Bauxite, AR, where the presence of alumina dusts generated by a nearby aluminium smelter may have been a compromising factor. As a consequence, the data from Bauxite were then removed and the resulting modified dataset re-analyzed. Categorical analysis of variance indicates that fluoride concentration in this dataset is significantly and positively associated with severity of effect ($\chi^2 = 1101.86$, $p < 0.0001$).

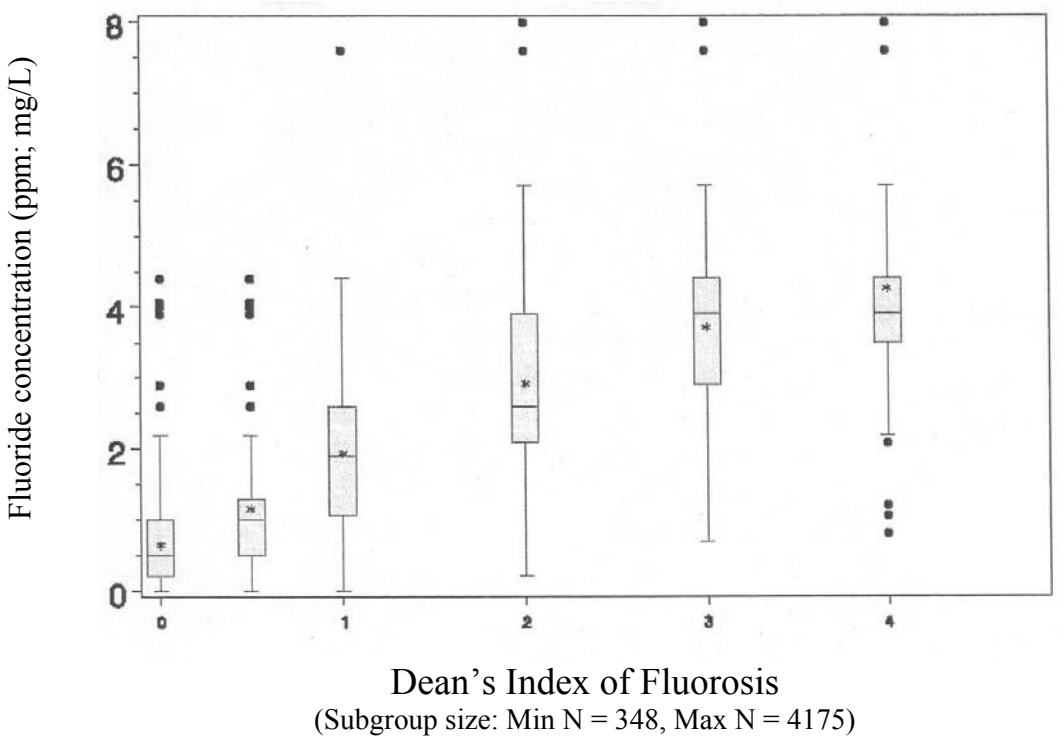

Figure 4-1. Maximum likelihood logistics regression analysis (CATMOD Procedure) of Dean (1942) data. Asterisk (*) indicates mean, horizontal line within box is median, and box boundaries indicate interquartile range surmounted by error bars. Solid circles represent data points that are not bounded by interquartile range or error bars.

4.3. Benchmark Dose Analysis of Dean's Data on Severe Fluorosis

A Benchmark Dose (BMD, USEPA Benchmark Dose Software ver. 2.0) analysis was also conducted using the severe dental fluorosis data of Dean (1942). Because the categorical analysis indicated that the Bauxite, AR, data point was an outlier (a confounding factor for the city of Bauxite was the presence of excessive amounts of alumina in the environment due to proximity of an operational aluminum mine and smelter), this data point was removed from the data set and not used in the BMD analysis. The data set used in the BMD analysis consisted of nine study sites where there was at least one occurrence of severe dental fluorosis, and also Galesbury, IL, the town that had the highest fluoride concentration without any cases of severe fluorosis. A preliminary run was conducted using the BMD logistics, log logistics, probit, log probit, and dichotomous Hill models. The results indicated that the best-fit model was the dichotomous Hill model (Table 4-2).

Table 4-2. Comparison of Regression Models Used to Analyze Dean's (1942) Data				
Model	AIC[a]	Goodness of Fit		
		χ^2	d.f.	p value
log Probit	721.805	5.96	8	0.6518
Probit	742.463	24.42	8	0.0019
log Logistic	728.566	11.64	8	0.1681
Logistic	754.388	34.00	8	0.0000
Dichotomous Hill	721.162	3.284	7	0.8576

[a]Akaike Information Criterion (AIC), a measure of comparison for statistical models (as intercept–only vs. fitted models). The model exhibiting the smallest AIC value is preferred.

This model contains an asymptotic term useful for modeling responses that plateau at less than a 100% response level. The dichotomous Hill model was run to ascertain the BMD and BMDLs for 0.5%, 1% and 5% severe fluorosis. The BMD for 5% severe fluorosis is 3.28 mg/L (BMDL = 3.11 mg/L). The BMD for 1% severe fluorosis is 2.43 mg/L (BMDL = 2.18 mg/L). For 0.5% severe dental fluorosis, the BMD was 2.14 mg/L and the BMDL 1.87 mg/L. Statistical analysis of the data indicated that estimation of 0.1% severe fluorosis was outside the range of probability that the data set can support. The complete BMD output of the dichotomous Hill model for 0.5% severe fluorosis is given in Appendix B, and the resulting plot is shown in Fig. 4-2.

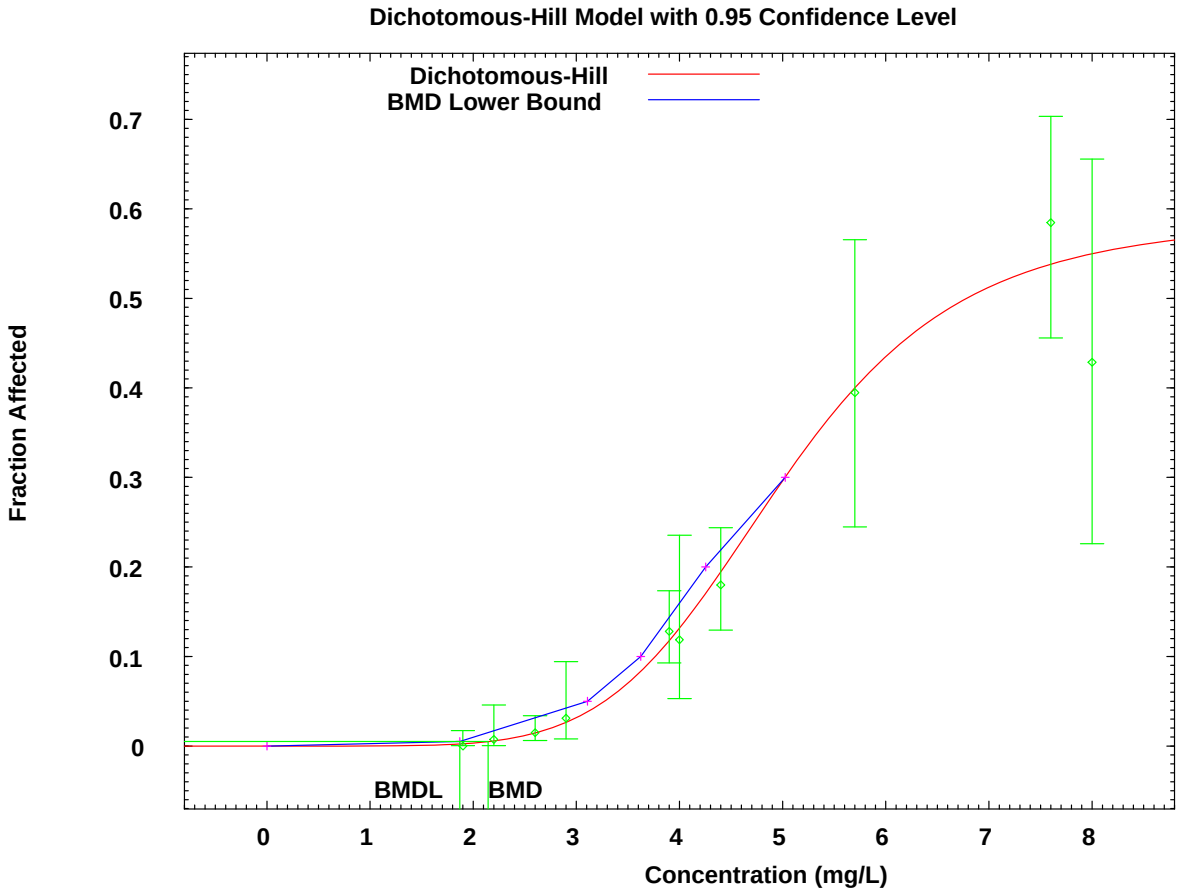

Figure 4-2. Benchmark dose analysis (BMDS vers. 2.0; with 95% CL) for the dichotomous Hill model for 0.5% severe fluorosis (data of Dean, 1942).

The OW also tried to model the Dean (1942) data on moderate fluorosis in order to determine the prevalence of moderate dental fluorosis at the BMD for 0.5% severe dental fluorosis. Unfortunately, none of the models was able to obtain an acceptable fit to the data even with the sequential removal of the locations with the three highest fluoride concentrations.

Sensitivity Analysis of the Benchmark Dose Derivation. To determine to what degree the BMD might be affected by a plateau in the response at the highest fluoride concentrations, the dicotomous Hill model was run with sequential elimination of the data for the two highest concentrations. These two data points differ from the remaining Dean data set in that the fluoride concentrations were based on single measurements rather than 12 consecutive monthly samples, and the study population covered a wider age range (grades 2-12, roughly equivalent to ages 6–17 years), both factors which could introduce a unquantifiable degree of uncertainty in the results.

The p values for goodness of fit were 0.9551 and 0.9216 for running the model without the highest concentration and the two highest concentrations, respectively, and the corresponding BMD values for 0.5% severe fluorosis were 2.12 and 2.16 mg/L (BMDL values 1.84 and 1.85 mg/L, respectively) indicating that removing these values improved the fit, but had very little effect on the BMD (2.14 mg/L) and BMDL (1.87 mg/L) which were obtained when these two data points were included. These results support the use of the Dean data set for deriving a point of departure for the severe fluorosis endpoint using the BMD approach.

Effect of Altitude and Elevated Temperatures on BMD Derivation. Several studies have suggested that high altitudes or elevated ambient temperatures may affect the development of dental fluorosis (see Section 3.1.4). Several of the study sites included in Dean's study fall within these two categories (see Table 4-3). In order to test the effects of temperature and altitude on the resulting BMDL derived from Dean's data, the BMD analysis (dichotomous Hill model) was conducted on data sets which excluded the two sites with the highest maximum temperatures and also the two highest altitude sites. Results are shown in Table 4-4. The best model fit was achieved using all the data points (but excluding that for Bauxite, AR). Therefore, it can be concluded that for this particular data set, the results were not affected by altitude or temperature.

Table 4-3. Temperature and Altitude Data for Selected Dean Study Sites

Site	Year	Annual Mean of Monthly Mean Maximums	Annual Mean of Monthly Means	Altitude
Clovis, NM	1943	78.4°F	61.7°F	4289 ft
Colorado Springs, CO	1942	64.3°F	49.9°F	6104 ft
Amarillo, TX	1947	69.8°F	56.4°F	3608 ft
Plainview, TX	1942	74.3°F	59.5°F	3369 ft
Lubbock, TX	1947	73.0°F	58.7°F	3253 ft
Post, TX	1964*	76.5°F	63.3°F	2619 ft
Conway, SC	1942	77.2°F	65.4°F	20 ft

SOURCE: NOAA; http://cdo.ncdc.noaa.gov/ancsum/ACS
* Incomplete data.

Table 4-4. Comparison of BMD and BMDLs for 0.5% Severe Fluorosis Using Dean's Data Set Adjusted for Warm Climates and High Altitudes			
Data Set	**BMD (mg/L)**	**BMDL (mg/L)**	**p value**
A. Site with highest F level excluded (Bauxite, AK)	2.14	1.87	0.8576
B. As in A, but with two high altitude sites excluded (Clovis, NM and Colorado Springs, CO)	2.19	1.75	0.6543
C. As in A, but with two high temperature sites excluded (Post, TX and Conway, SC)	2.15	1.86	0.6718
D. As in A, but with two high altitude and two high temperature sites excluded	2.20	1.73	0.3617

4.4. NOAEL/LOAEL Approach for Severe Fluorosis

The Dean (1942) data for severe fluorosis (see Table 4-1) can be applied directly to the LOAEL/NOAEL (Lowest-Observed-Adverse-Effect Level/No-Observed-Adverse-Effect Level) approach. In this case, a fluoride drinking water concentration of 2.2 mg/L in Clovis, NM, is the lowest concentration associated with severe fluorosis (0.7%), and is therefore the LOAEL. The NOAEL corresponds to a fluoride concentration of 1.9 mg/L recorded in Galesburg, IL, where no occurrence of severe fluorosis was recorded.

4.5. Summary and Conclusions

The critical study chosen for analysis of the association between dental fluorosis and fluoride concentrations in drinking water is that of Dean (1942) due to its large size and geographic scale (22 U.S. communities in 10 states; 5824 children), range of fluoride concentrations evaluated (from 0.0 to 14.1 mg/L; see Table 4-1), and selection of an appropriate age class (school children primarily between the ages of 9 and 14; an age class in which a very high percentage of permanent teeth have erupted). In addition, every tooth per subject was examined using the same scoring protocol (see Section 2 for description), and the community water supplies were tested for fluoride content by the same chemist (see Table 1, p. 29 of Dean, 1942). This dataset is sufficiently large and robust to support statistical analysis, the protocol is sound, and there were few alternate sources of commercially available fluoride (e.g., mouthwash, dentifrice, etc.) or fluoridated community water supplies to confound the dental fluorosis data collected by Dean (1942) at the time this study was conducted (late 1930's and early 1940's). Study weaknesses include lack of information on dietary fluoride intake, and lack of data on drinking water intakes. Another limitation is the relatively smaller numbers of children examined in high-fluoride communities. Although Dean (1942) notes that water chemistry data collected during the period of dental examination might not reflect the fluoride concentrations present during the years of tooth development, a requisite for inclusion of data in his study was a common water supply within each study location whose history showed no relevant changes in either physical set-up,

source, or composition during the time period that covered the life span of the subjects examined.

Categorical analysis of the Dean (1942) data set comparing Dean scores vs. fluoride water concentrations indicates that these rank data are acceptable for dose-response modeling. Further, results from the SAS© CATMOD (Categorical Model) Procedure indicated that the fluorosis score data of Dean (1942) were positively correlated with the fluoride concentration in water, and that high fluoride water concentrations were predictive of more severe fluorosis levels in teeth.

The benchmark dose model was applied to determine the statistical association between the prevalence of severe fluorosis and the concentration of fluoride in drinking water at the studied locations. The Benchmark Dose for a 0.5% severe fluorosis was determined to be 2.14 mg/L, with a lower 95% CL of 1.87 mg/L. The BMD is very close to the LOAEL of 2.2 mg/L for 0.7% severe fluorosis identified in the Dean (1942) study (see Table 4-1), and the BMDL is only slightly below the NOAEL of 1.9 mg/L identified for the community of Galesburg, IL.

In this report, data sets characterizing the relationship between severe dental fluorosis and dental caries, as well as the relationship between fluoride exposure and skeletal fractures in adults, were evaluated to determine if these associations were candidates for dose-response modeling. Background conditions for the cavity data were highly varied (different geographic locations, variation in accessibility to dental care, and subject age). Some evidence is available supporting the hypothesis that caries prevalence increases at fluoride levels greater than those having an anticariogenic effect. However, those data are not amenable to dose-response modeling and the dose-response varies across the different studies. The available database characterizing the relationship between skeletal fractures relative to fluoride exposure is limited at this time. Additional dose-response research is needed before modeling for association with cariogenic or skeletal endpoints can be undertaken.

5. Reference Dose Derivation

Section 4 of this report establishes severe dental fluorosis as the critical effect for fluoride exposure during the period of pre-eruptive, permanent tooth-enamel formation. Prior evaluations of the effects of excess fluoride (Koop, 1984; NRC, 1993) considered all stages of enamel fluorosis as a cosmetic effect, albeit one to avoid if possible. However, NRC (2006) classified severe dental fluorosis as adverse due to the associated thinning and/or pitting of the enamel that weakens its role in protecting the dentin.

After examining the dose-response data for several aspects of dental fluorosis, EPA determined that data on the relationship between drinking water concentration and the occurrence of severe fluorosis was amenable to modeling. Dean (1942) was selected as the critical study because of the size of the population studied and the number of data points provided. In addition, this study was conducted prior to the introduction of fluoridation of water systems and the introduction of fluoride into dental products. Accordingly, the confounding contribution of non-dietary sources to total fluoride exposure was minimal at that time. In many of the localities evaluated by Dean (1942), the source of fluoride in water was of geochemical origin. Therefore, the Dean (1942) dataset represents a population for which drinking water was the major source of fluoride exposure, with dietary intakes from local produce contributing only a small amount to total exposures.

The Benchmark Dose analysis (Section 4) of the relationship between drinking water concentration and the severe enamel fluorosis identified a BMD of 2.14 mg/L and BMDL (95% confidence bound) of 1.87 mg/L for the prevalence of severe dental fluorosis in 0.5% of the children evaluated.

Utilization of the BMD/BMDL data in derivation of an RfD requires conversion of the exposure associated with a drinking water concentration of 1.87 mg F/L to a dose in mg/kg/day for the sensitive population and knowledge of the vulnerable ages for development of enamel fluorosis. Severe fluorosis of the permanent teeth is a condition that lasts for the lifetime of the tooth, but can only occur during a defined period of tooth development. Determination of the dose associated with severe dental fluorosis is not an easy task because data on drinking water intakes and body weights for the populations studied and the individuals with severe fluorosis were not collected. Accordingly, an indirect approach must be employed. Consideration of the beneficial fluoride doses that increase the resistance of enamel to cavities is also an important consideration in selecting a point of departure for the RfD determination.

5.1. Nutritional Guidelines

Risk assessment for elements such as fluoride with beneficial as well as adverse properties is a challenge, especially when there is a narrow boundary between the doses that are beneficial and those that have adverse effects. The NAS established the first dietary recommendations for fluoride in 1989 (NRC, 1989). At that time, fluoride was not classified as an essential element, but was considered beneficial for humans because of its valuable contributions to dental health (NRC, 1989). The estimated range of safe and adequate dietary intakes (including drinking water) for adults was defined as a daily intake of between 1.5 and 4 mg F/day. The estimated

safe and adequate intake range for the first year of life was identified as 0.1 to 1 mg/day and that for the 1–3 year old age group as 0.5 to 1.5 mg/day.

The dietary guidelines for fluoride were revised by the Institute of Medicine (IOM) in 1997. The 1997 revisions (see Table 5-1) considered fluoride as a nutrient based on its presence and function in bones and tooth enamel. The dietary intake information (including fluoride from drinking water) was used to establish Adequate Intake (AI) guidelines for each age, life-stage (i.e. pregnancy or lactation), and/or gender grouping covered by the Dietary Reference Intakes (DRI). An AI is defined as an estimate of the average nutrient intake by a group or groups of healthy people within a designated age, life-stage, and/or gender grouping and is the recommended dietary guideline when data to determine a more precise Estimated Average Requirement (EAR) are not available. The AI is based on observed or experimentally determined estimates of average intakes by a group or groups of healthy people; in this case, those receiving drinking water optimally fluoridated to achieve optimal anticaries protection. The AI established for fluoride is 0.05 mg/kg/day for all age groups above 6 months, and is based on data from four studies of the dietary fluoride intake of children in the United States or Canada from optimally fluoridated communities (~ 1 mg/L) that were published after 1980. The IOM (1997) converted the 0.05 mg/kg/day AI to mg/day intakes based on the average body weights for the age groups of concern in the OW assessment (Table 5-1).

Table 5-1. Adequate Intake (AI) Reference Values and Tolerable Upper Intake Levels (UL) for Select Age Groups.

Age Range (Body wt. in kg)	Criterion	AI (mg/day)[a]		UL (mg/day)
		Males	Females	
0–6 mon (not reported)	Human milk content	0.01	0.01	0.7
7–12 mon (9 kg)	Caries prevention	0.5	0.5	0.9
1–3 yr (13 kg)	Caries prevention	0.7	0.7	1.3
4–8 yr (22 kg)	Caries prevention	1	1	2.2
9–13 yr (40 kg)	Caries prevention	2	2	10
14–18 yr (boys, 64 kg; girls, 57 kg)	Caries prevention	3	3	10

SOURCE: IOM (1997).
[a]AI is the observed estimate of nutrient intake that reduces the occurrence of dental caries in a group of healthy individuals.

The IOM (1997) also established a Tolerable Upper Intake Level (UL) for fluoride in different age, life-stage, and/or gender groupings. A UL is defined as the maximum level of a total chronic daily intake of a nutrient that is unlikely to pose risks of adverse health effects for almost all individuals in the general population. In the case of fluoride, the UL for infants and children up to age 8 was selected based on prevention of moderate dental fluorosis; for all other age groups, the UL was selected based on the prevention of skeletal fluorosis.

In the derivation of the UL for children, IOM (1997) used the Dean (1942) data and considered that there was a less than a 5% prevalence of moderate dental fluorosis at a 2 mg/L drinking water concentration. At this concentration IOM estimated that fluoride intakes would range from 0.08 to 0.12 mg/kg/day. The body weight and water intakes used for this estimate are not provided. The middle of the range (0.1 mg/kg/day) was identified as a LOAEL for moderate dental fluorosis "the threshold beyond which moderate enamel fluorosis appears in some

children" (IOM, 1997). The LOAEL was divided by an uncertainty factor of 1 to establish a dose of 0.1 mg/kg as the UL for infants and children through eight years of age. Based on reference weights of 7 kg and 9 kg, respectively, the UL for infants in the first six months of life is 0.7 mg/day and that for the second six months is 0.9 mg/day. Children were divided into two age groups, those one to three years old (bw = 13 kg) and those 4 through 8 years old (bw = 22 kg). The UL for the first group of children is 1.3 mg/day and that for the second group is 2.2 mg/day. The UL for all other age groups is 10 mg/day and was based on a NOAEL of 10 mg/day for the development of skeletal fluorosis.

5.2. Period of Developmental Sensitivity to Dental Fluorosis

The U.S. EPA (1985) and the IOM (1997) have used birth to the age of 8 or 9 years as the period of concern for dental fluorosis. These early assessments focused on fluorosis of the anterior teeth because, from a cosmetic perspective, they are the most visible teeth. Data suggesting that severe dental fluorosis may increase the risk for caries throughout the lifetime (NRC, 2006; Forsman, 1974) broadens the concern for severe fluorosis induction to cover the time period of enamel formation of both the anterior and posterior teeth. A study by Groenveld et al. (1990) concluded that about 66% of the anticaries impact of fluoride on pit and fissure cavities of the posterior teeth with high caries susceptibility was due to pre-eruptive fluoride exposure, while the pre-eruptive contribution to protection of the anterior teeth with smooth surfaces was 25%.

A study of fluorosis in 70 children, ages 6.5 to 13, living in a village in Greenland and who had received sodium fluoride tablets (0.5 mg/day) showed that the age at which fluoride administration was initiated increased the risk of developing dental fluorosis and the teeth impacted (Larsen et al., 1985). Exposures during ages 2.5 to 5.5 years were associated with fluorosis of the upper central incisors, from 2.5 to 4.5 years with the first molars, and from 5.5 to 8.5 years with the 2nd molars. The controls were children from the same age range who had not receive the fluoride tablets; all children were lifetime residents of the same village. The local water supply had a concentration of 0.1 mg F/L. Outside of these age periods the risk was not significantly greater than that for the controls. None of the children had TFI scores greater than 3. It is important to note that the third molars would not have erupted and thus would not have been included in the analysis.

The dose-response curve developed from the Dean data is for the secondary teeth since, at the time of examination, approximately 94% of the permanent teeth were present (Dean, 1942). There are no dose-response data for primary teeth comparable to that from Dean (1942) for secondary teeth. The mineralization of the secondary teeth begins at about 6 ± 2 months with the incisors, whereas that for the primary teeth begins in utero (Massler and Schour, 1958). The developing secondary teeth remain rather quiescent until age 2 years ± 6 months when formation of the other permanent teeth begins and the incisors begin to increase in size. Tooth mineralization continues until age 10 years when all teeth except the wisdom teeth appear to be completely calcified. Eruption of all teeth except for the wisdom teeth is complete by about age 13 (ADA, 2005). The wisdom teeth erupt between ages 17 and 21 but are formed by age 15 years ± 6 months. Since enamel formation appears to be complete by age 15 years, EPA has considered the period of greatest sensitivity to severe enamel fluorosis as the time from six months through 14 years of age in this assessment in order to cover the formation of the wisdom teeth.

The data indicate that fluoride exposure and developmental age are the major factors influencing the occurrence of severe dental fluorosis. However, as described in Section 3.1.4, there are other stressors that influence fluorosis development including diet, climate, altitude and possibly genetics. Low intakes of enamel-forming nutrients such as calcium and phosphorous could, when combined with exposure to excess fluoride, increase the tendency for fluorotic defects in the hydroxyapatite crystal lattice of the tooth enamel. However, no data were identified that directly support this hypothesis. Co-exposure to some other minerals (strontium, zinc; see Section 3.1.4.3) can influence the staining of teeth but not the enamel (pitting) defects of severe dental fluorosis.

Some studies (Section 3.1.4.1) show that there is an impact of climate on the prevalence of severe dental fluorosis. Areas with higher ambient air temperatures have a greater prevalence of fluorosis than those with a more temperate climate, hypothetically because of the direct relationship between temperature and drinking water intake (Galagan and Lamson, 1953; Galagan and Vermillion, 1957). Neither climate nor diet is likely to have had a major impact on the fluorosis data in the Dean (1942) study since all of the key cities represented in the concentration-response assessment have fairly comparable latitudes and average ambient air temperatures (See Figure 5-1 and Table 3-15).

Figure 5-1. Sampling sites used in Dean (1942) dental fluorosis study.

There are some data (Section 3.1.4.2) from countries outside of the United States that suggest the prevalence of dental fluorosis may be increased at elevated altitudes (>2000 m). Respiratory water loss may account for the altitude effects because at higher altitudes and low atmospheric pressure there is a greater than normal loss of water vapor from the lungs (IOM, 2005). In areas where ambient air temperatures are high, respiratory water losses may be accompanied by increased water intake. None of the sites in the Dean (1942) data set fell at altitudes over 2000 m although Colorado Springs, CO (one of 21 sites) has an altitude of 1900 m. Dose-response modeling in the presence and absence of the high temperature and high altitude sites, as well as both combined, showed there was little impact on the BMD and BMDL. Other factors discussed in Section 3.1.4 such as acid/base balance would not be influenced by the geographic position of the Dean (1942) observation sites.

5.3. Dose Determination for Severe Dental Fluorosis

As mentioned above, an indirect approach was required in order to estimate the dose associated with severe dental fluorosis in the affected segment of the populations studied by Dean (1942) because data on drinking water intakes were not collected. In the absence of drinking water intake data from the time of the Dean (1942) study, EPA used data collected during the 1977/1978 Nationwide Food Consumption Survey (Ershow and Cantor, 1989) on drinking water intakes and body weights of children during the susceptible age period to estimate their fluoride doses in mg/kg/day for the mean, 75^{th}, 90^{th}, and 95^{th} percentile tap water consumer groupings. These data were selected because they provide the drinking water intake information that lie closest to the time of the Dean study and the body weight data are consistent with the growth curves for children from 1923 (Proudfit, 1923) and 1958 (Cooper et al., 1958); these dates bracket the time of the Dean (1942) studies. The water intake and body weight data were converted to estimated fluoride doses using the following equation:

$$\textbf{Estimated F dose} = \frac{\textbf{F concentration at the BMDL x L tap water consumed}}{\textbf{body weight}}$$

The assumption that drinking water was the primary source of exposure to fluoride in the communities studied is justified by the fact that, at the time of the Dean (1942) study, there was no fluoride in toothpaste or other dental products and no use of fluoride supplements. In addition, there was no intentional fluoridation of community water supplies thus limiting the introduction of fluoride from drinking water into commercial foods and beverages processed in the many areas of the country with low natural levels of fluoride.

This calculation provides a range of doses for different age grouping at mean and each percentile of drinking water intake evaluated. Any drinking water intakes (mean or percentile) that resulted in doses that were less than or equal to the 0.05 mg/kg/day IOM AI value associated with optimal, anticaries protection were eliminated from consideration as doses causing severe dental fluorosis. At the time of the Dean (1942) study there were no data to suggest that severe dental fluorosis was present in situations where the drinking water fluoride concentration fell between 0.7 and 1.1 mg/L, the drinking water concentrations from the seven studies that were used as the basis for the IOM (1997) AI recommendation of 0.05 mg/kg/day.

The BMDL is a lower bound estimate of the tap water fluoride concentration associated with 0.5% severe dental fluorosis in a large population (5,824) of children as determined by the drinking water concentration-response observed in 20 locations studied. The sensitivity analysis presented in Section 4.3 shows that the BMDL is not affected appreciably by differences in the modeling approach. Because it is the deposition of fluoride in the crystal lattice of the tooth enamel that causes dental fluorosis, it is assumed that the small number of children who displayed severe dental fluorosis in the Dean (1942) publication were either sensitive to its effects or those that received excess exposure to fluoride during the period when the affected enamel was being formed. Where exposure was the main contributor to the effects it was assumed that tap water was the source of almost all of the fluoride exposure. Nutritional and/or genetic factors may have played a role in the development of severe dental fluorosis for some affected individuals, however, these factors were assumed to have a minimal impact on the concentration-response noted in communities studied by Dean (1942) with drinking water concentrations near the BMDL. Doses generated from drinking water intakes (mean or percentile) that were greater than 0.05 mg/kg/day AI were considered as points of departure for the drinking water component of the RfD analysis.

5.3.1. Body Weight and Drinking Water Intakes

As mentioned in Section 5.3, EPA was not able to identify data that provide a detailed analysis of average body weights and water intakes for the sensitive population during the time the Dean (1942) data were collected. Comprehensive body weight and drinking water intake data were identified in two important sources covering later time periods. The first source (Ershow and Cantor, 1989), provided body weight and drinking water intake information (direct and indirect) from the 1977–1978 U.S. Department of Agriculture (USDA) Nationwide Food Consumption survey. The other source is the U.S. EPA analysis of the data from the USDA 1994–1998 Continuing Survey of Food Intake by Individuals (CSFII) as presented in U.S. EPA, 2004.

There are some differences in the methodologies used to generate the 1989 and 2004 reports, but the general approach to data analysis and the framework for the analysis are the same. The data are reported by Ershow and Cantor (1989) as gram intakes of tap water per day rather than the milliliters per day (mL/day) used in the EPA reports. Thus, the gram intakes were converted to milliliters using a density for water of 1 g/ml.

Ershow and Cantor (1989) reported mean body weights and tap water intakes; tap water included direct and indirect uses. Tap water intake was defined as the sum of drinking water intake and water added in final home or restaurant preparation of beverages and food. U.S. EPA (2004) reported mean body weights and direct and indirect drinking (tap) water intakes Direct drinking water refers to ingestion of plain drinking water and indirect water was defined as water used in the final preparation of foods and beverages at home or by food service establishments such as school cafeterias and restaurants (U.S. EPA, 2004). The combination of direct and indirect water reported in the U.S. EPA (2004) report is equivalent to the total tap water consumption in the Ershow and Cantor (1989) report. Both groups reported the mean body weights and water intakes using the same age groupings. The Ershow and Cantor data (1989) were derived from survey data contributed by about 26,000 participants (8621 children in the age range of interest) and collected during the 1970's. The data from the 1994–1998 CSFII (U.S. EPA, 2004) were contributed by about 21,000 participants (9687 children in the age range of interest).

Table 5.2 summarizes the body weight and tap water intake data (direct and indirect) from the Ershow and Cantor (1989) report for the age groups of interest; Table 5-3 summarizes comparable data (consumers only for the children) from the EPA (2004) report. Ershow and Cantor (1989) did not provide consumer only data in their report but did use three-days of dietary recall information rather than the two-days used for the EPA (2004) analysis. The consumer-only analysis from (EPA, 2004) was based only on recall data where water intake was provided for both days. It was the judgment of Ershow and Cantor (1989) that their estimates of average intakes were fully representative of population intakes for all age groups other than the infants. They felt that intakes reported for formula-fed infants could be underestimations of actual exposures because the recall information did not distinguish between powdered, concentrate and ready-to-feed formula.

Table 5-2. Body Weight and Tap Water Intake in the United States (Ershow and Cantor, 1989)

Age Range (years)	Mean Body Wt. (kg)	Tapwater Intake			
		Mean (ml)	75th Percentile (ml)	90th Percentile (ml)	95th Percentile (ml)
0.5–0.9	9.2	328	480	688	764
1–3	14.1	646	820	1162	1419
4–6	20.3	742	972	1302	1520
7–10	30.6	787	1016	1338	1556
11–14	47.7	925	1196	1621	1924

As is apparent from a comparison of Table 5-2 and 5-3, tap water intake seems to have been greater in the 1970's than in the 1990's, with the exception of the 0.5 to 0.9 year-old infants. This is consistent with dietary data indicating that there has been an increase in the intake of bottled water and commercial beverages in place of tap water over the last decade (EPA, 2004; IOM, 1997). Measures of bottled water intakes and commercial bottled beverages are not included in the Ershow and Cantor (1989) report because at the time of the Nationwide Food Consumption survey in 1977/1978, bottled water was not as important a commercial product as it was at the time of the 1993–1998 survey. Based on the EPA (2004) report, bottled water accounts for 13 % of mean total water intake. Mean body weights have also increased slightly for the older age groups. The Ershow and Cantor (1989) data were used in the dose analysis that follows because they were collected during a time period closer to the Dean (1942) study.

Table 5-3. Body Weight and Drinking Water Intake Data (Consumers Only) from Estimated Body Weight and Per Capita Water Ingestion in the United States – An Update (U.S. EPA 2004)

Age Range (years)	Mean Body Wt. (kg)	Tapwater Intake		
		Mean (ml)	90^{th} Percentile (ml)	95^{th} Percentile (ml)
0.5–0.9	9	467	971	1,147
1–3	14	349	723	946
4–6	21	442	943	1,176
7–10	32	487	993	1,241
11–14	51	641	1415	1,742

The children studied by Dean (1942) were largely 9 to 14 years old; however, their severe fluorosis developed during the pre-eruptive earlier period of enamel formation. The age ranges

used in this U.S. EPA assessment range from six-months (the beginning of enamel formation on the secondary teeth (Massler and Schour, 1958) through age 14 in order to cover late enamel development of the wisdom teeth. Since wisdom teeth were not likely to have erupted in the children evaluated by the Dean study, they would not be reflected in the severe fluorosis values observed by Dean (1942).

5.3.2. Dose Estimates

The dose estimates generated using the drinking water intake values and mean body weights in Table 5-2 are summarized in Table 5-4. The values in Table 5-4 represent the doses associated with drinking water intakes and body weights for each of the age groups evaluated.

Table 5-4. Estimates of Fluoride Doses at Specific Tap Water Intakes for Age Groupings During the Sensitive Window for Development of Severe Enamel Fluorosis (at 1.87 mg F/L)

Age Range (Years)	Fluoride Exposure (mg/kg/day)			
	Mean	75th Percentile	90th Percentile	95th Percentile
Ershow and Cantor, 1989				
0.5 – 0.9[a]	0.07	0.10	0.14	0.16
1–3	0.09	0.10	0.15	0.19
4–6	0.07	0.09	0.12	0.14
7–10	0.05	0.06	0.08	0.10
11–14	0.04	0.05	0.06	0.08

[a]Dose estimates for infants may underestimate the actual doses because of the lack of reliable information on the type of formula used for bottle-fed infants.

As children grow, their body weights, eating, and drinking water consumption patterns change. It is therefore important to evaluate each combination of water intake and body weight variables to determine the appropriate point of departure for the RfD determination. Consideration of more than one age grouping with associated estimates of drinking water intake provides a fuller picture of the impact of age, water intake and body weight on fluoride dose from ingestion of drinking water containing 1.87 mg F/L, the derived BMDL for 0.5% severe fluorosis (see Section 4.3).

Examination of the dose estimates for individuals based on mean water intakes in Table 5-4 demonstrates that two of the doses, 0.04 and 0.05 mg/kg/day, are not appropriate as the point of departure for the RfD because they fall at or below the recommended fluoride intake level of 0.05 mg/kg/day (IOM, 1997). The same is true of the 0.04 and 0.06 mg/kg/day dose estimates at the 75th and 90th percentile drinking water intakes. The OW selected 0.07 mg/kg/day as the contribution of the drinking water to the RfD because it provided a reasonable difference (0.02 mg/kg/day) between it and the IOM (1997) intake (0.05 mg/kg/day) that was the basis for the AI considering day-to-day dietary variability. Although the lower 0.06 mg/kg/day dose estimate also exceeded the IOM (1997) estimate of need, OW felt that a 0.01 mg/kg/day difference between the IOM estimate and a dose from drinking water that caused severe dental fluorosis was too small given the range of dose estimates in Table 5-4 and the uncertainties surrounding both the AI and the drinking water component of the RfD. The range of estimates for the mean water intakes is 0.04 to 0.09 mg/kg/day and that for the full range of water intakes is 0.04 to 0.19 mg/kg/day. The Dean (1942) report provided only drinking water concentration information; it included no data on diet or drinking water intakes for the children studied. It is thus unclear whether high water intakes, individual sensitivity, or a combination of both factors predisposed

some children to severe rather than mild or moderate fluorosis in the populations studied by Dean (1942).

Support for the EPA fluoride dose estimates, as derived from drinking water intake estimates and the calculated BMLD for severe dental fluorosis, is provided by the data from the Iowa Fluoride Study (Hong et al., 2006a, b). As part of this study, 579 children were evaluated for dental fluorosis of the eight permanent incisors and four first molars at 8–10 years of age (mean 9.2 years). The fluoride intake of these same children had been followed from birth through 48 months by means of questionnaires their parents completed every 3–4 months (Hong et al., 2006b). Daily fluoride intake in mg/kg/day was estimated from water, beverages, and selected foods, fluoride supplements and dentifrice. Fluorosis was evaluated using the Fluorosis Risk Index (FRI). Severe Fluorosis cases were defined as having FRI definitive staining and/or pitting on both maxillary central incisors. [This characterization of severe fluorosis differs from that of the Dean Index in that it includes staining without pitting.] Individuals with FRI questionable fluorosis were excluded. The importance of fluoride intake during different time periods was assessed using t-tests and logistic regression.

One hundred and thirty-nine (24%) subjects had fluorosis (mostly mild) on both maxillary central incisors. Mean age-specific fluoride intake per unit body weight (bw) ranged from 0.040 to 0.057 mg/kg bw, with higher intake during earlier time periods and relative stability after 16 months (Hong et al., 2006a). In bivariate categorical analyses, fluoride intakes during each of the first 4 years were individually significantly related to fluorosis on maxillary central incisors, with the first year most important ($P < 0.01$), followed by the second ($P < 0.01$), third ($P < 0.01$), and fourth year ($P < 0.03$). Multivariable logistic regression analyses showed that, after controlling only for the first year, the later years individually were still statistically significant. When all four time periods were in the model, the first ($P < 0.01$) and second years ($P = 0.04$) were still significant, but the third ($P = 0.32$) and fourth ($P = 0.82$) were not. The lack of severe fluorosis in this population provides some support for considering intakes of 0.04 and 0.05 mg/kg day as below the threshold for severe fluorosis.

In a second publication, (Hong et al., 2006a) reported estimated fluoride intakes from birth to 36 months based on the questionnaire mentioned above. Relative risks for fluorosis were significantly elevated for intakes of 0.04 to 0.06 mg/kg/day and >0.06 mg/kg/day, compared with intakes <0.04 mg/kg/day. The highest relative risk 4.76 (2.39–9.41; 95% CI) was found for the average 24 to 36 month period. The few subjects (8) classified as having severe dental fluorosis all had fluoride intakes >0.06 mg/kg/day. Severe fluorosis was defined by the FRI as including staining and/or pitting of the central incisors or first molars. In that respect, this categorization differed from that of Dean where "discrete or confluent pitting" was necessary in order to categorize the fluorosis as severe.

EPA contacted Dr. Steven Levy, director of the Iowa Study and asked if he would be able to determine if any of the eight cases identified as severe by the FRI demonstrated pitting of the enamel. Dr. Levy (2010) reported back to EPA that only one of the eight cases (0.2% of the subjects with dietary records) had pitting according to photographs of the children's teeth. The pictures for a second child could not be located. Dr. Levy also provided EPA with the fluoride intake estimates (mg/kg bw) from water, selected foods, supplements and dentifrice for each of eight severe fluorosis cases.

The child that had the pitting of the enamel was apparently breast fed for at least the first 6 months. Starting at about 6 months the baby received substantial amounts of infant formula reconstituted with tap water. At 8 months, the tap water source was changed from one with 0.05 mg/L F to one with 1 mg/L F. The 9-month exposure record had the highest estimated daily fluoride intake (0.118 mg/kg bw) reported over the three-year period. The average daily intake from 16 through 36 months was 0.079 mg/kg bw. Between 20 months and 36 months, the fluoride exposure estimate exceeded 0.08 mg/kg/day in 4 of 5 reports. The affected child's average fluoride intake for 3 to 9 months was the lowest of the 8 children in the data set shared by Dr Levy likely reflecting breast feeding for the first six to nine months.

For the 16 month to 36 month period, 2 of seven children had higher estimated average intakes than the child with pitted enamel. Their teeth developed staining, but no pitting. Exposure records for the 8th child were deficient after the first year, with data for only one of seven reports. The child with the missing pictorial dental record had only two exposure reports of the five expected over the first year and both were > 0.1 mg/kg bw. That child's average intake for the 16 month to 36 month period was 0.056 mg/kg bw.

There are limitations to the exposure records from this study as discussed in Hong et al. (2006a). There was no direct verification of the data reported by the parents in the questionnaires. Also the questionnaire was administered at 3 to 4 months intervals and could not capture day to day variations in the children's exposures. The questionnaire did not ask for information on the use of fluoride mouth washes or gels. In some cases, records were incomplete because parents did not submit questionnaires for some of the time periods. Given these limitations, the data suggest that both cumulative and episodic exposures during critical windows of enamel formation could have an impact on staining and pitting of the central incisors and first molars in children when they are exposed during the period 0.5 to 3 or 4 years of age. This is the approximate time these teeth are forming (Massler and Schour, 1958). They are also supportive of the EPA fluoride dose estimates in Table 5-4 for children in this age range with severe dental fluorosis as defined by Dean when they are average consumers of drinking water at the BMDL (1.87 mg/L fluoride) for 0.5% severe fluorosis.

5.4. RfD Determination

The point of departure for the drinking-water RfD is a dose of 0.07 mg/kg/day as identified in Section 5.3.2. This dose is greater than the beneficial dose of 0.05 mg/kg/day and allows a 0.02 mg/kg/day difference between the AI and RfD. It is less than the IOM UL estimate of 0.1 mg/kg/day, which was based on a <5 % increase in moderate dental fluorosis. The OW drinking water RfD estimate is based on the lower bound confidence limit for the fluoride concentration associated with a 0.5% prevalence of severe dental fluorosis. Thus, the two estimates are not necessarily in conflict.

$$\text{RfD} = \frac{0.07 \text{ mg/kg/day}}{1} = 0.07 \text{ mg/kg/day}$$

where:

0.07 mg/kg/day	=	Lower Limit on Benchmark Dose estimates (in mg/kg bw/day) associated with severe fluorosis in the population studied by Dean (1942).
1	=	A composite Uncertainty Factor following EPA guidelines (see Section 5.4.2)

It is unfortunate that the Dean (1942) publication does not provide any data on which teeth were the two most severely fluorotic teeth that became the basis of the fluorosis score. Had those teeth been identified, it might have been possible to more precisely identify the age period of greatest sensitivity. Without that data, it is necessary to consider the entire age period of enamel formation as the time of vulnerability to fluorosis. Dean (1942) does mention that, in one community with a drinking water concentration of 1.2 mg/L, 11.1 % of the teeth positive for fluorosis were incisors or first molars and 88.9 percent were cuspids, bicuspids and second molars; however, none of the children in this population had moderate or severe fluorosis.

When conducting risk assessments involving exposures through drinking water, the BMDL concentration of 1.87 mg/L can be used in place of the RfD as the appropriate point of departure for determination of the MCLG because it does not include the uncertainty associated with assumptions used to calculate the drinking-water RfD. A relative source contribution (RSC) factor would be applied to the BMDL concentration to account for exposure to fluoride through media such as dental products that were not available at the time the Dean (1942) data were collected.

The drinking water-based RfD was adjusted to account for the additional fluoride intake from foods at the time of the Dean (1942) study. OW determined from the data presented by McClure (1943) that an intake of fluoride from a diet where solid foods had an average concentration of 0.50 ppm fluoride appeared to provide a reasonable basis for the contribution of solid foods to total fluoride exposure in the 1930 to 1940 time frame (see Appendix D). The dietary fluoride intakes estimated by McClure (1943) for the 1–3, 4–6, 7–9 and 10–12 year old age groups consuming foods with an average of 0.5 ppm fluoride were divided by the midpoint of the ranges of body weights provided by McClure (1943) to derive the dose estimate for the contribution from solid foods. The result of this calculation was an estimated dietary intake of 0.01 mg F/kg/day when the individual values for each age grouping were rounded to two decimal places (see Appendix D).

The final OW estimated oral RfD for fluoride was therefore:

Oral RfD	=	Intake from DW + Intake from food
Oral RfD	**=**	**0.07 mg/kg/day + 0.01 mg/kg/day = 0.08 mg/kg/day**

5.4.1. Application of Estimated Oral RfD to Adult Populations

The estimated oral RfD (0.08 mg/kg/day) is protective against severe dental fluorosis in children during the critical period of enamel formation. This value is likely also protective against fluoride-related adverse effects in adults, including skeletal fluorosis and an increased risk of bone fractures. The oral RfD includes a drinking water component of 4.9 mg/day [equivalent to a drinking water concentration of 2.45 mg F/L (DWEL) for a 70 kg adult drinking 2 liters per day], and a food component of 0.7 mg/day (for a 70 kg man), and resulting in a total daily intake of 5.6 mg F/day.

In evaluating the data available for skeletal effects of fluoride (Section 3.3), EPA did not identify data that were good candidates for dose- or concentration-response modeling. Unlike severe enamel fluorosis which showed a linear concentration-response, the skeletal effects display a biphasic relationship of fluoride exposure and its impact on bone strength which cannot be accommodated by currently available models. Although the bone effects could not be reliably modeled for dose-response, the data examined in this current analysis indicated that the skeletal effects are unlikely to occur at the 1.87 mg/L BMDL for severe dental fluorosis.

The NRC (2006) qualitatively suggested that adults could be at risk for bone fractures at a fluoride drinking water concentration approaching 4 ppm (8 mg/day assuming a 2 L/day drinking water intake). The World Health Organization (2002) concluded that there was an increased risk of bone fractures at total fluoride intakes of ≥ 14 mg/day in some countries and an increased risk of bone effects at total intakes above about 6 mg/day based in part on a study of bone fractures by Li et al. (2001) conducted in China. The oral RfD of 5.6 mg/day, including a drinking water component of 4.9 mg/day (for a 70 kg person), is protective compared to each of these benchmarks.

5.4.2. Uncertainty factors

In establishing an estimated oral RfD for fluoride, data on nutritional benefit were assessed in combination with the data on severe dental fluorosis to define a level that provides anticaries protection without causing severe dental fluorosis when consumed daily for a lifetime. Conventional application of uncertainty factors is not always appropriate when carrying out a risk assessment for nutrients and other beneficial substances, especially when there is a relatively small difference between the levels that satisfy need and those that cause adverse effects. For this reason the total uncertainty factor applied was 1. The widely recognized variability in epidemiological data on the prevalence of severe dental fluorosis combined with the data demonstrating the anticaries benefit of exposures to fluoride at concentrations at or below the BMDL do not support any other approach. The margin of difference between the AI and RfD is 0.03 mg/kg/day.

The point of departure for the oral RfD analysis is the lower bound for 0.5 % severe dental fluorosis in children. The sample size was large (138 to 404 individuals per data point in the critical area around the BMD (1.9–2.6 mg/L) and the participants were randomly selected. Geographic and climate differences related to the places of residence of the children examined were unlikely to contribute to sensitivity. The population studied is the group vulnerable to

dental fluorosis of the secondary teeth (children ages 6 months to 14 years) eliminating the need for an intraspecies UF. The duration of exposure covered the full period of sensitivity to severe dental fluorosis of the secondary teeth. An oral RfD of 0.08 mg/kg/day appears to be protective for possible impacts on bone fractures and skeletal fluorosis in adults, and should be protective of severe dental fluorosis of the primary teeth as well. Accordingly, an uncertainty factor of other than 1 is not needed for intrahuman variability (UF_H) and for extrapolation from a subchronic to chronic exposure (UF_S). In addition, human data provide the basis of the estimated oral RfD. Therefore, an adjustment for the use of animal data (UF_A) is not necessary. The use of a BMDL for 0.5% severe fluorosis as the POD eliminated the need for a LOAEL to NOAEL extrapolation (UF_L)

The standard toxicity database for fluoride is complete negating the need for a database uncertainty factor (UF_D). It includes chronic, reproductive, and developmental studies in animals as well as a variety of epidemiology studies in humans (NRC, 2006). Although NRC (2006) did identify research needs for the endocrine, neurological and other effects of fluoride, they generally concluded that available studies on other effects were not sufficient to assess public health relevance to the U.S. population. To date, the best documented and established public health consequences of fluoride exposure are severe dental fluorosis, skeletal fluorosis and increased risk of bone fractures.

As a consequence, 1 is the chosen value for each of the following uncertainty factors used in this estimate of the fluoride oral RfD: UF_H, UF_A, UF_S, UF_L. The composite UF is also equal to 1.

5.4.3. Confidence in the Estimated Oral RfD

Confidence in the BMDL for fluoride exposure from drinking water is high because of the large number of children evaluated in the critical study and the fact that the data were collected before drinking water fluoridation, fluoridated supplements, and dental products were introduced. There remains some uncertainty that concentrations in water, especially in those communities with high naturally occurring fluoride levels, adequately capture total fluoride exposure. However, other exposures in those communities would increase, rather than decrease the BMDL.

Confidence in the estimated oral RfD may be impacted by uncertainties concerning the accuracy and sensitivity of the method used to measure fluoride in the water sources for the municipalities included in the Dean (1942) study (see Section 3.1.1). The method, modified zirconium-alizarin reagent with visual color comparison to standard solutions, is no longer used; however, the regent has been reported to be sensitive to small increments of fluoride over a range of 0.0 to 3.0 ppm, the critical range for assessing the threshold for severe fluorosis, and within this range it approximates Beer's law (Megregian and Maier, 1952). In addition, Dean's data appear to be:

- Internally consistent as evidenced by the BMD stability when end points at the high and low end of the curve were removed,

- Supported by later studies on some of the same water sources showing similar concentrations,

- Used average concentration values from 12 consecutive months for all but the three systems with the highest prevalence of severe dental fluorosis, thereby compensating for potential individual and seasonal variation,

- Based on water quality data from the same time period, and not likely to have been compromised by high levels of interfering substances.

Confidence in the estimated oral RfD is medium because of the difficulties encountered in converting the concentration-response data to dose estimates for the RfD derivation.

5.4.4. Impact of nutritional requirements on dentition and other uncertainties

There are a few studies that have identified severe dental fluorosis in individuals from the United States exposed to fluoride in drinking water at a concentration lower than 1.87 mg F/L (Driscoll et al., 1983; Galagan and Lamson, 1953). However, both studies were completed after the beginning of fluoridation and the introduction of fluoride from fluoridated water into the food supply. The Driscoll et al. (1983) study was conducted after fluoride was introduced into dental products. Accordingly, they do not contribute to uncertainty regarding the Dean (1942) results.

The prevalence of fluorosis can be affected by factors which alter rates of intake and excretion. Of particular importance are water consumption rates (which may be affected by climate and altitude), the potential for increased fluoride intake through sources other than drinking water (foods or food additives containing high levels of fluoride and cooking of foods in fluoridated water), the use of fluoridated dental products; inadequate intake of essential vitamins and minerals (e.g., Vitamin D and calcium); and physiological and pathological conditions which may alter excretion rates (acid-base balance and kidney diseases).

As discussed in Section 3.1.4, there are a number of additional factors that may produce alterations in dental enamel that resemble those caused by fluoride. These include dental changes caused by living at high altitudes, genetic abnormalities, malnutrition, or exposure to minerals such as strontium or aluminum or medications, which may complicate the diagnosis of dental fluorosis.

Because of fluoride-related and nonfluoride-related variables, the prevalence and severity of fluorosis in a given population may be impacted by factors other than the levels of fluoride in drinking water. However, for the data set from which the BMDL is derived, the only confounding factor that was identified was the co-exposure in one of the study populations to high levels of aluminum. This data point was excluded from the calculation.

5.5. Summary and Conclusions

The estimated oral RfD for fluoride, based on the endpoint of enamel pitting as manifest in severe dental fluorosis is 0.08 mg F/kg/day for children during the period from 6 months to 14 years of age. Beyond the period when the enamel forms on pre-eruptive teeth, the ingestion of fluoride does not cause pitting of enamel. However, the RfD is applicable to the entire population since it is also protective for the endpoints of severe fluorosis of primary teeth, skeletal fluorosis and increased risk of bone fractures in adults.

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APPENDIX A

CATEGORICAL DATA ANALYSIS OF FLUOROSIS DATA SET OF DEAN (1942)

(Categorical Model, or CATMOD, developed by The SAS© Institute of Cary, NC).

Population Profiles		
Sample	CONCENTRATION	Sample Size
1	0	423
2	0.1	236
3	0.2	583
4	0.3	770
5	0.4	345
6	0.5	516
7	0.6	614
8	0.7	467
9	0.8	95
10	0.9	123
11	1	166
12	1.06	336
13	1.2	703
14	1.3	447
15	1.8	170
16	1.9	273
17	2.08	143
18	2.2	138
19	2.6	404
20	2.89	192
21	2.9	97
22	3.5	164
23	3.9	289
24	4	156
25	4.07	136
26	4.4	189

Population Profiles		
Sample	**CONCENTRATION**	**Sample Size**
27	5.7	38
28	7.6	65
29	8	21

Response Profiles	
Response	**condition**
1	Mild
2	Moderate
3	Normal
4	Questionable
5	Severe
6	Very Mild

Response Frequencies						
	Response Number					
Sample	1	2	3	4	5	6
1	0	0	414	8	0	1
2	0	0	230	6	0	0
3	1	0	498	60	0	24
4	3	0	676	75	0	16
5	3	0	204	122	0	16
6	4	0	303	185	0	24
7	2	0	444	130	0	38
8	12	10	224	166	0	55
9	6	2	40	37	1	9
10	2	0	65	43	0	13
11	17	0	78	19	0	52
12	16	6	188	99	2	25
13	17	9	354	215	2	106
14	14	0	181	153	0	99
15	15	2	48	54	0	51
16	17	3	69	74	0	110
17	24	12	26	41	7	33
18	49	15	18	22	1	33
19	86	36	26	80	6	170
20	38	15	44	50	16	29
21	26	23	4	8	3	33
22	1	37	0	0	125	1
23	81	98	9	19	38	44
24	37	45	13	5	18	38
25	34	10	17	21	31	23

Response Frequencies						
	Response Number					
Sample	1	2	3	4	5	6
26	41	87	2	2	34	23
27	4	19	0	0	15	0
28	14	7	0	0	38	6
29	2	10	0	0	9	0

Response Functions and Design Matrix			
		Design Matrix	
Sample	Response Function	1	2
1	1.02600	1	0
2	1.02542	1	0.1
3	1.22470	1	0.2
4	1.15584	1	0.3
5	1.48406	1	0.4
6	1.49031	1	0.5
7	1.39414	1	0.6
8	1.67238	1	0.7
9	1.62105	1	0.8
10	1.65041	1	0.9
11	1.95181	1	1
12	1.47321	1	1.06
13	1.73329	1	1.2
14	1.97539	1	1.3
15	2.12353	1	1.8
16	2.41209	1	1.9
17	1.86713	1	2.08
18	1.48188	1	2.2
19	2.23267	1	2.6
20	1.64323	1	2.89
21	1.77835	1	2.9
22	2.42378	1	3.5
23	1.33564	1	3.9

Response Functions and Design Matrix			
		Design Matrix	
Sample	Response Function	1	2
24	1.61218	1	4
25	1.83088	1	4.07
26	1.28836	1	4.4
27	1.43421	1	5.7
28	2.17692	1	7.6
29	1.52381	1	8

Analysis of Variance			
Source	DF	Chi-Square	Pr > ChiSq
Intercept	1	29644.50	<.0001
CONCENTRATION	1	1101.86	<.0001
Residual	27	1030.63	<.0001

Analysis of Weighted Least Squares Estimates				
Parameter	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept	1.0803	0.00627	29644.50	<.0001
CONCENTRATION	0.2712	0.00817	1101.86	<.0001

Covariance Matrix of the Parameter Estimates			
Row	Parameter	Col1	Col2
1	Intercept	0.00003937	-.00002081
2	CONCENTRATION	-.00002081	0.00006674

Correlation Matrix of the Parameter Estimates			
Row	Parameter	Col1	Col2
1	Intercept	1.00000	-0.40601
2	CONCENTRATION	-0.40601	1.00000

Dean's Index of Fluorosis=0 Condition=Normal

Analysis Variable : CONCENTRATION Fluoride Concentration (ppm)							
N	Lower 95% CL for Mean	Mean	Upper 95% CL for Mean	Median	Std Dev	Minimum	Maximum
4175	0.6251690	0.6448407	0.6645124	0.5000000	0.6483295	0	4.4000000

Dean's Index of Fluorosis=0.5 Condition=Questionable

Analysis Variable : CONCENTRATION Fluoride Concentration (ppm)							
N	Lower 95% CL for Mean	Mean	Upper 95% CL for Mean	Median	Std Dev	Minimum	Maximum
1694	1.1139510	1.1542444	1.1945377	1.0000000	0.8455345	0	4.4000000

Dean's Index of Fluorosis=1 Condition=Very Mild

Analysis Variable : CONCENTRATION Fluoride Concentration (ppm)							
N	Lower 95% CL for Mean	Mean	Upper 95% CL for Mean	Median	Std Dev	Minimum	Maximum
1072	1.8645862	1.9343843	2.0041825	1.9000000	1.1646679	0	7.6000000

Dean's Index of Fluorosis=2 Condition=Mild

Analysis Variable : CONCENTRATION Fluoride Concentration (ppm)							
N	Lower 95% CL for Mean	Mean	Upper 95% CL for Mean	Median	Std Dev	Minimum	Maximum
566	2.7997891	2.9156890	3.0315890	2.6000000	1.4038218	0.2000000	8.0000000

Dean's Index of Fluorosis=3 Condition=Moderate

Analysis Variable : CONCENTRATION Fluoride Concentration (ppm)							
N	Lower 95% CL for Mean	Mean	Upper 95% CL for Mean	Median	Std Dev	Minimum	Maximum
446	3.5842192	3.7071076	3.8299961	3.9000000	1.3205280	0.7000000	8.0000000

Dean's Index of Fluorosis=4 Condition=Severe

Analysis Variable : CONCENTRATION Fluoride Concentration (ppm)							
N	Lower 95% CL for Mean	Mean	Upper 95% CL for Mean	Median	Std Dev	Minimum	Maximum
346	4.0942617	4.2554624	4.4166632	3.9000000	1.5245120	0.8000000	8.0000000

APPENDIX B

BENCHMARK DOSE ANALYSIS OF SEVERE FLUOROSIS DATA SET OF DEAN (1942)

(USEPA Benchmark Dose Software ver. 2.0)

DICHOTOMOUS HILL MODEL FOR 0.5% SEVERE FLUOROSIS

```
 ================================================================
        Dichotomous Hill Model. (Version: 1.0; Date: 09/24/2006)
        Input Data File: C:\USEPA\BMDS2\Data\DicFluSet.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS2\Data\DicFluSet.plt
                                          Wed Aug 13 13:21:23 2008
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

        where: 0 <= g < 1, 0 < v <= 1

               v is the maximum probability of response predicted by the model,

               and v*g is the background estimate of that probability.


   Dependent variable = Severe
   Independent variable = DOSE
   Slope parameter is not restricted

   Total number of observations = 10
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                              v =        -9999
                              g =        -9999
                      intercept =     -8.26097
                          slope =      4.28252


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -g
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                       v    intercept        slope

         v             1         0.36        -0.53

 intercept          0.36            1        -0.97

     slope         -0.53        -0.97            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
```

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
v	0.587691	0.073515	0.443604	0.731778
g	0	NA		
intercept	-9.0565	0.888417	-10.7978	-7.31524
slope	5.63552	0.70711	4.24961	7.02143

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test d.f.	P-value
Full model	-355.599			
Fitted model	-357.581	3.96504	7	0.7838
Reduced model	-495.125	279.053	9	<.0001

AIC: 721.162

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
1.9000	0.0025	0.694	0	273	-0.8339
2.2000	0.0058	0.797	1	138	0.2285
2.6000	0.0146	5.889	6	404	0.04617
2.9000	0.0264	2.562	3	97	0.2772
3.9000	0.1175	33.958	37	289	0.5557
4.0000	0.1315	7.758	7	59	-0.292
4.4000	0.1941	36.686	34	189	-0.494
5.7000	0.3994	15.177	15	38	-0.05862
7.6000	0.5376	34.944	38	65	0.7603
8.0000	0.5494	11.536	9	21	-1.112

Chi^2 = 3.283691 d.f. = 7 P-value = 0.8576

Benchmark Dose Computation

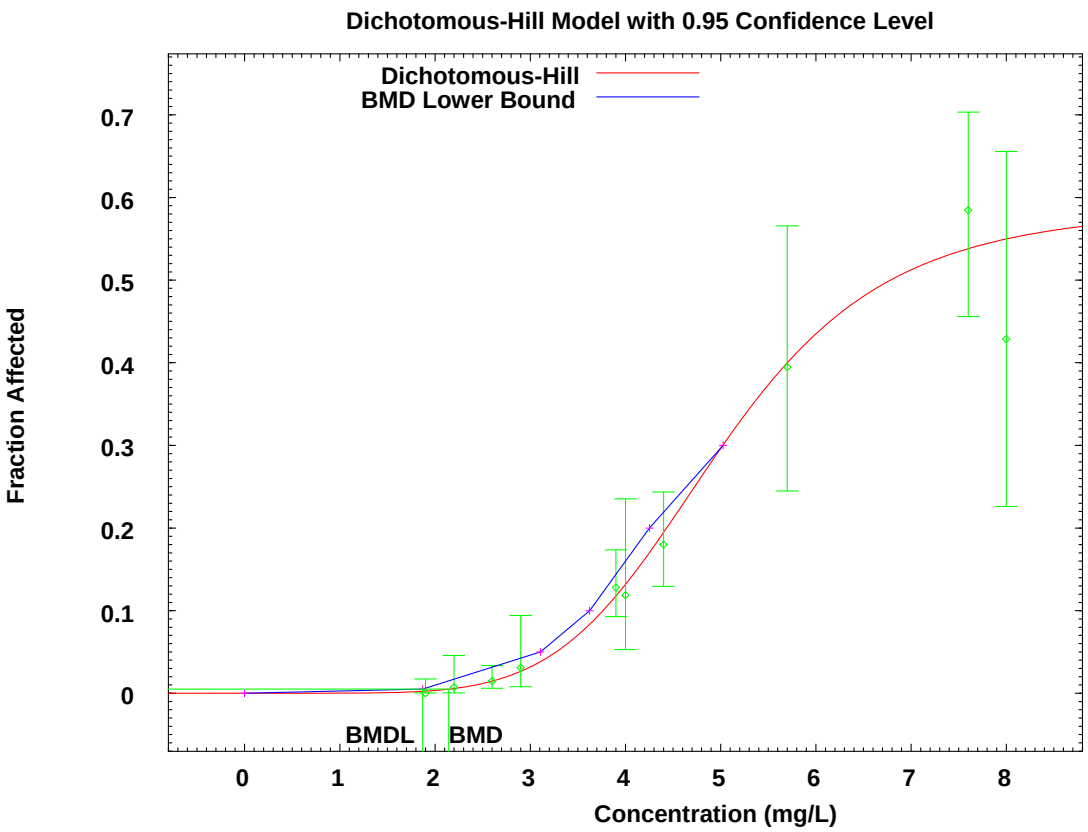
Specified effect = 0.005

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.14408

BMDL = 1.86945


Dichotomous-Hill Model with 0.95 Confidence Level
Dichotomous-Hill
BMD Lower Bound
Fraction Affected
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
BMDL
BMD
0
1
2
3
4
5
6
7
8
Concentration (mg/L)

APPENDIX C

EPIDEMIOLOGY STUDY EVALUATIONS

EPIDEMIOLOGY STUDY EVALUATIONS

The key epidemiological studies used in the dose-response analysis are evaluated in Table C-1. Notes on the categories, and brief insight into scoring logic, are given below.

1. **Author** – Self explanatory.
2. **Study design** – The higher values were given to studies for factors such as: including the population of concern (children); pertinent selection criteria, appropriate monitoring endpoints; multiple fluoride concentrations in the drinking water; appropriate analytical and statistical methods.
3. **Population size** – Was there enough participants overall and were there enough at each exposure level?
4. **Endpoint definition & indices** – How was dental fluorosis defined? Was severe dental fluorosis well-defined? Were consistent/well-accepted indices used for scoring?
5. **Ability to estimate exposure from fluoride through drinking water** – Higher ratings were given to those who had resided in their communities continually during most susceptible periods. Also, higher ratings were given if samples or concentration information was taken during that time period.
6. **Concentration appropriate for U-shaped dose-response** – If we only have fluoride concentration information at the low and/or high end, valuable information is missing in the middle that tell the data story. Do we have a distribution of data between none/negligible and the MCL (4mg/L)?
7. **Statistical significance and confidence intervals** – Was statistical analysis done? Were p-values and/ or confidence intervals included? Were complex statistical text conducted when possible?

Table C-1. Evaluation of Epidemiology Studies[a]							
Study Author	**Study Design[a]**	**Population Size**	**Endpoint definitions and indices**	**Inter/ Intra-examiner reliability**	**Ability to estimate exposure in drinking water**	**Concentrations appropriate for U- shaped Dose Response**	**Statistical significance, Confidence bounds**
Dean, 1942	High	High	High	NS[b]	High	NA[c]	Low
Driscoll et al., 1983	Medium	Medium	Medium	Medium	High	High	Medium
Driscoll et al., 1986	Medium	Medium	High	NS[b]	High	High	High
Eklund, et al., 1987	Low	Low	High	Medium	Medium	Low	High
Heifitz et al., 1988	Medium	Low	Medium	High	High	High	NS[b]
Selwitz et al., 1995	High	Medium	High	Medium	High	High	High
Jackson et al., 1995	Medium	Low-Medium	High	Medium	Medium	Low	Medium
Iida & Kumar, 2009	Medium-High	High	High	Medium	Medium-High	NS[b]	High

[a]Confidence in each portion can be scored numerically or categorically (1-Low, 2-Medium, and 3-High).

[b]Not specified in the published report.

[c]Not applicable; the Dean (1942) study did not include an evaluation of cavities.

APPENDIX D

FLUORIDE DOSE FROM SOLID FOODS AT THE TIME OF THE DEAN (1942) STUDY

Introduction

The Dean (1942) paper that provides data for the dose-response analysis covers a duration of exposure vulnerability from about 1930 to 1940 for the children evaluated. That was an era when many sources of fluoride exposure that are present today did not exist. The availability and distribution of foods was also far different. Imported foods were rare and much of the food supply (i.e. dairy, produce) was of local origin. A much larger portion of the population, especially those outside of large cities, grew at least some of their fruits and vegetables and preserved them through canning, or drying techniques.

The historic information on fluoride in the food supply is important because, other than drinking water, diet was the major fluoride source for the children from whom the dose-response data were collected. The 1930–1940-era food data are needed to adjust any dose-response estimate derived from concentrations in drinking water so as to appropriately incorporate food as an additional exposure source when estimating the RfD for inorganic fluoride.

Fluoride in Solid Foods (1930–1940)

McClure (1949; see also McClure, 1939, 1943), summarized information published between 1933 and 1948 on the fluoride content of various foodstuffs. Many of these early studies used the Williard and Winter (1933) distillation method to recover fluoride from the food coupled with a colorimetric titration-based quantification approach (see McClure, 1939, 1949). [See U.S. EPA, 2010) for a discussions of analytical methods and their changes over time.] With the exception of items grown in an area characterized by high levels of fluoride in soils, or sprayed with a fluoride-containing pesticide, McClure (1949) reported typical concentrations below 10 ppm, and frequently below 1 ppm (see Table D-1). However, levels of detection appear to have been better with some food matrices than others.

When available, McClure (1949) reported fluoride content based on fresh weight and dry weight. Table D-1 presents only the fresh-weight or "as consumed" values. The data reviewed for his publication included non-U.S. studies; studies evaluating the effects of fluoride-containing pesticides on the fluoride content of produce; and studies evaluating the effects of high soil fluoride levels on fluoride content of plants, or high fluoride dietary intake on the fluoride content of animal products (i.e., milk, eggs and meat). Consequently, some of the values listed by McClure (1949) may not be representative of the fluoride content of a typical U.S. diet for that time period, not withstanding difficulties presented by the analytical methods used at the time. However, McClure (1949) did report that soil fluoride had little impact on the fluoride content of plants other than edible roots and tubers. Fluoride in irrigation water also appeared to have little impact on plant content (McClure, 1949).

Table D-1. Fluoride Contents of Selected Foods as Reported by McClure (1943, 1949)			
<0.2 ppm	**0.2 to <0.5 ppm**	**0.5 to <1 ppm**	**≥1 ppm**
Milk (0.07-0.0.17)	Milk (0.22-0.38)	Milk (0.55)	Butter (1.50) Cheese (1.62)
Egg White (0-0.14) Eggs (0.12-0.42)	Egg white (0.20-0.47) Egg yolk (0.42)	Egg yolk (0.59-0.90)	Egg white (1.48) Egg yolk (1.20)
Beef (<0.20-0.29) Pork (<0.20) Mutton (<0.20) Calf liver 0.19	Pork (0.34)	Chicken, boned and canned , (0.63) Pork chop (0.98) Veal (0.90) Beef liver (0.99)	Beef (2.00) Chicken (1.40) Chicken liver (1.43-1.59) Pork shoulder (1.20) Frankfurter (1.67-1.70) Roundsteak (1.28) Lamb (1.20)
Mackerel, boned (<0.20) Tuna (0.1)		Shrimp (edible portion (0.93) Oysters (0.65)	Fish (1.49-1.63) Codfish (5.0-7.0) Mackerel, canned (12.10) Herring, smoked(3.5) Sardines, canned (7.3-12.5) Salmon, canned (4.16-9.0) Salmon, fresh (5.77) Crab meat (2.00) Shrimp, canned (4.4) Oysters (1.50-1.58)
Beans (0.11-0.15) Cabbage (0.12-0.15) Cauliflower (0.08-0.12) Carrots (<0.20) Celery (0.10-0.14) Chick peas (0.14) Potatoes (0.07-0.16) Potatoes, sweet (<0.2) Turnips (<0.20)	Beets (0.20-0.38) Blackeye peas (0.23) Carrots (0.4) Cabbage (0.3-0.38) Celery (0.20-0.24) Lettuce (0.30) Potatoes (0.2) Spinach (0.21-0.44) Tomatoes (0.24)	Cabbage (0.8) Cauliflower (1.0) Celery (0.7) Peas (0.6) Tomatoes (0.6-0.9)	Potatoes, whole (6.4) Spinach (1.0-1.8)
Citrus Fruits (0.04-0.18 Noncitrus fruit (0.02-0.19) Apples (0.035) Strawberry (0.18)	Citrus Fruits (0.25-0.36) Orange (0.22) Noncitrus fruit (0.22-0.34) Pears (0.21) Peaches (0.21) Banana (0.23)	Noncitrus fruit (0.52-0.92) Apples (0.8)	Noncitrus fruit (1.05-1.32) Apples (1.32)
Corn, canned (<0.20) Wheat, bran (<0.20) Rice (<0.1-0.19 Oats, crushed (<0.2) Peanuts (0.20) Coffee (0.20)	Corn meal (0.22) Wheat, bran (0.29) Flour (0.27-0.45) Oats, fresh (0.25) Hazelnuts (0.30)	Wheat germ (0.88) Rice (0.67) Almonds (0.90) Spaghetti (0.8) Coffee (0.7) Cocoa (0.5) Milk chocolate (0.5)	Wheat germ (1.7-4.0) Soy beans (1.33) Honey (1.00) White bread (1.0) Coffee (1.1-1.6) Cocoa (2.00) Milk chocolate (1.0-2.00) Tea (4.1-398) Tea infusion (1.19)

SOURCE: Multiple sources as reported in McClure (1943, 1949).

[a]Concentration based on fresh weight or "as consumed." The same food may appear in more than one concentration grouping because of the range of results reported.

McClure (1949) also reported on an earlier study by Smith et al. (1945) that found that cooking vegetables in fluoridated water resulted in higher levels of fluoride in the cooked foods. When cooked in water containing 5 ppm fluoride, the fluoride content of beets, cabbage, and cauliflower increased from 0 to 1.0, 3.6, and 4.2 ppm, respectively; that of carrots increased from 2.3 to 3.2 ppm; spinach from 2.0 to 4.0 ppm; and Italian squash and Brussels sprouts from 0.2 to 3.8 and 2.9 ppm, respectively. Pinto beans, potatoes and oatmeal did not show an increase in fluoride content when cooked in water containing 5 ppm fluoride, but did increase in F when cooked in water containing 24 ppm F. Differences among individual foods may be related to the presence or absence of cations in the food matrix that form poorly soluble fluoride salts.

In a different study, the fluoride content of vegetables cooked in non-fluoridated water was compared with that of vegetables cooked in water with a fluoride concentration of 1, 2 or 5 mg F/L (Martin, 1951). As with McClure (1949), the Willard and Winter (1933) method was used to determine the fluoride concentration with the modification that magnesium acetate was used as the fixative. The fluoride content of the raw vegetables ranged from 0.14 to 0.84 mg/kg (Table D-2). Vegetables absorbed fluoride in proportion to the fluoride content of the water, and vegetables cooked in a saucepan absorbed more fluoride than those cooked in a pressure cooker. The study authors did not suggest an explanation for this, but it may have been due to the increased concentration of fluoride in the open saucepan following evaporation of the water. Higher concentrations of fluoride in the cooking water increased the absorption of fluoride into the vegetables, regardless of the mode of cooking. The fluoride level in vegetables cooked in a saucepan in water with 1 mg F/L ranged from 0.55 mg/kg (corn) to 2.02 mg/kg (spinach) while those cooked in fluoride-free water ranged from 0.17 mg/kg (carrots) to 1.00 mg/kg (spinach).

Table D-2. Average Fluoride Content (mg/kg) of Vegetables Cooked in Water with Varying Fluoride Levels

Vegetable	Raw	Boiled in Saucepan				Boiled in Pressure Cooker			
		Fluoride Content of Water (ppm)				Fluoride Content of Water (ppm)			
		0.0	1.0	2.0	5.0	0.0	1.0	2.0	5.0
Carrots	0.14	0.17	0.81	1.75	3.61	0.18	0.51	0.69	1.02
Beans	0.20	0.21	0.96	1.72	4.32	0.19	0.52	0.67	1.23
Cauliflower	0.27	0.27	1.24	2.10	5.03	0.27	0.69	1.09	2.24
Peas	0.22	0.28	1.22	2.02	3.88	0.25	0.84	1.08	1.52
Spinach	0.84	1.00	2.02	2.85	4.99	0.76	1.13	1.63	2.81
Cabbage	0.23	0.29	1.13	1.88	4.92	0.23	0.55	0.79	1.03
Beets	0.21	0.26	0.60	1.16	1.88	0.28	0.44	0.57	0.78
Tomatoes	0.17	0.23	0.61	–	–	0.13	0.26	–	–
Corn (cob)	0.24	0.29	0.55	–	–	0.17	0.42	–	–

SOURCE: Martin (1951).

Cholak (1960) reviewed pre-1951 data on concentrations of fluoride ion in various food products. Cholak (1960) did not give the method of analysis used in each case; however, he noted that the preferred method for fluoride determinations at that time was ashing followed by a distillation step and conversion to soluble hydrofluorosilicic acid and a colorimetric or

photometric analysis. Concentrations were highest in meats and fish and lowest in fruits and vegetables (Table D-3).

Table D-3. Fluoride Concentrations in Food Products

Category	Fluoride Concentration (mg/kg food)	
	Mean	Range
Meat	1.06	0.20–3.33
Beef	0.94	0.20–2.0
Pork	1.19	0.20–3.33
Chicken	–	1.40
Fish	9.40	0.10–84.47
Mackerel	25.51	0.02–84.47
Salmon	8.55	4.16–19.34
Oysters	1.24	0.65–1.58
Eggs	0.44	0.00–1.48
Citrus fruit	0.17	0.028–0.360
Noncitrus fruit	0.34	0.00–1.32
Cereals, and cereal products	0.57	0.10–4.00
Cotton seed meal	–	20.0–31.0
Vegetables and tubers	0.58	0.10–6.40
Beans	0.13	0.11–0.15
Cabbage	0.31	0.12–0.80
Potatoes	1.19	0.07–6.40
Cow's milk	0.17	0.07–0.55

SOURCE: Cholak (1960).

Most of the studies discussed in the previous paragraphs were conducted using ashing, followed by extraction of the fluoride and a colorimetric technique for quantification. These methods are subject to interferences from other ions found in the food matrix and co-eluting with the fluoride. The early methods have largely been replaced by nonashing techniques and quantification via a fluoride ion-specific electrode. Singer et al. (1980) evaluated fluoride concentrations in 117 food items placed in 12 composite food groups. Fluoride in the food groups was determined by ashed and unashed techniques combined with either an ion-specific electrode or colorimetric analysis (eriochromecyanine R procedure, Singer and Armstrong, 1959). The results from the ion-specific electrode were found to be more accurate than the colorimetric method, especially for unashed samples. Ashed samples gave different results from unashed samples for some food groups but not for others. In a different study, Singer and Ophaug (1979) found that the use of a colorimetric method with eriochromecyanin R could result in erroneously high fluoride values for some foods.

In order to determine if the analytical method introduced a substantial bias towards erroneously high fluoride concentrations in the foods reported by McClure (1939, 1943, 1949), EPA compared values for a subset of foods from the McClure (1943, 1949) reports with those in the USDA (2005) fluoride database (Table D-4). The USDA database summarizes published and unpublished information on the fluoride content of selected foods and beverages from a variety of sources after critical evaluation of the data. It also includes the results of USDA sampling of food and beverage products at 144 locations across the U.S. The USDA samples were analyzed using a fluoride ion-specific electrode with direct readout for clear liquids, and a microdiffusion method for other foods. Representative foods from the dairy, meat/poultry/fish, grains, fruits, and

vegetable food groups that constitute a substantial portion of the daily solid food intake of children were selected for Table D-4.

Table D-4. Comparison of Fluoride Data (ppm) from McClure (1943, 1949) to that from USDA (2005)

Food	McClure (1943)	McClure (1949)	USDA (2005)	Description from USDA (2005)
Milk	0.07–0.22	0.07–0.55	0.03	1%, 2% and skim
Cheese	1.6	1.62	0.35	Cheddar
Egg	ND	1.18	0.05	cooked
Chicken	1.4	0.63–1.40	0.15	Includes fried and roasted
Beef	<0.2	2.00	0.22	
Frankfurter	1.7	1.67	0.48	Beef hotdog
Tuna	ND	0.1	0.19	Tuna canned
Fish	1.6–7.0	<0.2–12.5	0.18	Includes broiled and fried
Rice	1	0.19–0.67	0.41	Rice cooked
White bread	1.0	dry wt. only	0.49	Includes white and whole wheat bread
Spaghetti	ND	0.8	0.18	uncooked
Apples	0.8	0.035–1.32	0.03	Raw with peel
Pears	ND	0.21	0.08	Raw
Peaches	ND	0.21	0.04	Raw
Banana	ND	0.23	0.01	Raw
Orange	0.22	0.07-0.17	ND	Fruit
Strawberry	ND	0.18	0.04	Raw
Spinach	1.0	0.21–1.8	0.38	Cooked
potatoes	<0.2	0.20	0.45	White, boiled
Beans	ND	0.13	0.18	Green cooked, canned frozen
Peas	ND	0.6	0.29	cooked, canned frozen
Tomatoes	0.6–0.9	0.24	0.02	Raw
Carrots	<0.2	<0.22–0.4	0.03	USDA raw

ND = no matching data

A comparison of the McClure (1943, 1949) data to that in the USDA, 2005 database indicates that the current measurements of the concentrations of fluoride in foods are almost uniformly the same or lower than those made with earlier analytical methods. Differences are often as high as tenfold or greater. These differences cast doubt on the fluoride quantification used in exposure assessments derived from the early food concentration information. A portion of the difference probably results from interferences of other ions with the colorimetric analysis used by the early researchers.

The USDA (2005) database provides more descriptive information than McClure (1943; 1949) regarding the analyzed product. Where possible, the descriptions of food material were matched. However, the lack of detail in the McClure publications may contribute to the difference in results in cases where the form of the food analyzed for the McClure publication (i.e. raw or cooked) is not identified. Cooking and preparing foods with water that contains fluoride increases the fluoride content of the food as served (McClure, 1949; Martin, 1951; Marier and Rose, 1966). This is true for home-prepared and commercial foods. However the uptake of fluoride from the process water varies with the food product. As mentioned earlier, this may

relate to the presence of cations in the water and/or the food. Cations in the water can form poorly soluble fluoride salts (such as calcium fluoride), which can reduce fluoride uptake into the finished product). Fluoride in cooking water can also react with these same cations when present in the food and, in cases where the salt formed is only weakly soluble, may increase the fluoride from water retained in the cooked product.

Dietary Dose Estimation

McClure (1943), used information obtained in the 1930's and early 1940's on the fluoride content of various foods to estimate fluoride intake for four different age groups (1–3; 4–6; 7–9; and 10–12 year olds) using different estimates of water consumption and fluoride concentrations in dry foods. Depending on scenario, plain drinking water consumption was estimated to provide 25-33 % of the total daily water intake requirement. In both scenarios, it was estimated that 10% or 25% of total water content of food was of drinking water origin. McClure (1943) used four estimates of possible fluoride levels in dry food: 0.1 ppm, 0.2 ppm, 0.5 ppm and 1 ppm. The resulting estimates of fluoride intakes from drinking water, food, and drinking water and food combined are shown in Table D-5.

Table D-5. Estimated Fluoride Intakes from Drinking Water with 1 ppm Fluoride and Food with 0.1–1 ppm Fluoride (McClure, 1943)

Parameters	Age Group			
	1–3 yr	4–6 yr	7–9 yr	10–12 yr
Daily Energy allowance (calories)	1,200	1,500	2,000	2,500
Daily Water requirement (cc)	1,200	1,500	2,000	2,500
Drinking water consumption (cc):				
a) Direct: 25% of total daily requirement Indirect: 10% from foods (cc)	390	520	650	812
b) Direct = 25% of total daily requirement Indirect: 20% from foods	480	640	800	1,000
c) Direct = 33% of total daily requirement Indirect = 10% from foods	480	640	800	1,000
d) Direct = 35% of total daily requirement Indirect = 20% from foods	580	746	933	1,165
Total daily fluoride intake (mg) with1 mg F /L in DW:				
Under conditions of (a)	0.390	0.520	0.650	0.810
Under conditions of (b) and (c)	0.480	0.640	0.800	1.0
Under conditions of (d)	0.560	0.745	0.930	1.165
Food Consumption (g):				
Food consumption (g); total daily intake of dry foods when 1 g equals 4.5 calories	265	355	445	555
Fluoride ingested daily (mg) in food when dry food contains the following concentrations of F:				
e) 0.10 ppm	0.027	0.036	0.045	0.056
f) 0.20 ppm	0.053	0.071	0.089	0.111
g) 0.50 ppm	0.133	0.178	0.223	0.278
h) 1.0 ppm	0.265	0.360	0.450	0.560
Estimated total fluoride intake (mg) from water and food:				
Water (a) and Food (a)	0.417	0.556	0.659	0.866
Water (a) and Food (b)	0.443	0.591	0.739	0.921
Water (a) and Food (c)	0.523	0.698	0.872	0.278
Water (a) and Food (d)	0.653	0.880	1.10	0.560

Table D-5. Estimated Fluoride Intakes from Drinking Water with 1 ppm Fluoride and Food with 0.1–1 ppm Fluoride (McClure, 1943)				
Parameters	**Age Group**			
	1–3 yr	**4–6 yr**	**7–9 yr**	**10–12 yr**
Water (b or c) and Food (a)	0.507	0.676	0.845	1.056
Water (b or c) and Food (b)	0.533	0.711	0.889	1.111
Water (b or c) and Food (c)	0.613	0.818	1.023	1.278
Water (b or c) and Food (d)	0.745	1.00	1.250	1.560
Water (d) and Food (a)	0.587	0.781	0.975	1.221
Water (d) and Food (b)	0.613	0.816	1.019	1.276
Water (d) and Food (c)	0.693	0.923	1.153	1.443
Water (d) and Food (d)	0.825	1.105	1.380	1.725

SOURCE: McClure, 1943.

The estimates of dried food intake were derived from caloric intake recommendations for each age group from NRC (1941) and a caloric density estimate of 4.5 cal/g dried food. Total fluoride intake ranged from 0.417 to 0.825 mg for children 1–3 years old; 0.556 to 1.105 mg for children 4–6 years old; 0.659 to 1.380 mg for children 7–9 years old; and 0.866 to 1.725 mg for children 10–12 years old (Tables D-5 and D-6).

McClure (1943) used estimates of average body weights for children of the four age groups to calculate fluoride intakes per unit body weight (Table D-6). According to McClure (1943), the body weight data for children, ages one through six years came from Woodbury (1921), while those for children six to twelve years old were published by the American Child Health Association.

Table D-6. Daily Fluoride Intakes Estimated by McClure, 1943					
Age Interval	**Body Wt.[a] (kg)**	**Daily Fluoride Intake**			
		From DW (mg)	**From Food (mg)**	**Total (mg/day)**	**Total mg/kg/day**
Birth to 3 years	8–16	0.390–0.560	0.027–0.265	0.417–0.825	0.026–0.103
4 to 6 years	13–24	0.520–0.745	0.036–0.360	0.556–1.105	0.023–0.085
7 to 9 years	16–35	0.650–0.930	0.045–0.450	0.659–1.380	0.020–0.086
10 to 12 years	25–54	0.810–1.165	0.056–0.560	0.866–1.725	0.016–0.069

SOURCE: McClure (1943).

[a]Body weights of children 1-6 years old from Woodbury (1921); body weights of 6-12 year olds taken from Baldwin-Wood weight-height-age tables for boys and girls of school age, published by the American Child Health Association.

Earlier studies of fluoride in unprocessed foods found that the range was from 0.2–0.3 ppm F (McClure, 1949, Martin, 1951), with levels in the meat/fish and poultry food group about 1 ppm or higher. In the McClure (1943) analysis above, the food contributed 0.03 to 0.6 mg/day to the diet of children while Armstrong and Knowlton (1942) estimated a daily intake of 0.27–0.32 mg/day. A study by Marier and Rose (1966) found that foods processed with 1 mg F/L water contained 0.6–1.0 ppm fluoride instead of 0.2–0.3 ppm. The authors used a micro-distillation method coupled with colorimetric/spectrophotometric detection which may have inflated

fluoride concentration determinations. Nevertheless, the results indicate an increase in fluoride when foods are prepared in fluoridated water (Table D-7) as in the study by Martin (1951).

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Table D-7. Fluoride Content of Canned Vegetables				
Food	Average Fluoride Content (mg/kg)[a]			
	Non-fluoridated Process Water		Fluoridated Process Water (1 mg F/L)	
	Liquid	Solid	Liquid	Solid
Mixed vegetables	0.30	0.37	1.03	1.05
Green beans	0.14	0.20	0.71	0.89
Whole potatoes	0.13	0.38	0.87	0.76
Diced carrots	0.30	0.19	0.55	0.61
Kernel corn	0.10	0.20	0.48	0.56
Green peas	0.15	0.10	–	–
Wax beans	–	–	0.49	0.60

SOURCE: Marier and Rose (1966).
[a]Results are averages of single determinations for duplicate samples.

The OW used the integrated exposure estimates from McClure (1943) and the USDA (2005) concentrations of fluoride in foods to estimate the fluoride contribution from solid foods (including beverages such as milk and fruit juices) to the diet during the 1930-1940 time period. The OW analysis assumes that the estimated fluoride concentrations measured in food during the 1940 era were most likely higher than would have been determined using the improved analytical methods and instrumentation available today. Taking into consideration the USDA (2005) data on concentrations in foods, the OW selected the 0.5 ppm average dietary fluoride concentration in dry food as the best one for estimating dietary exposure for children in the Dean (1942) study, excluding the contribution that came from the drinking water. The OW selection was based on the following considerations:

- Even a ten-fold concentration of the USDA value for fluoride in foods with high water content (mostly fruits and succulent vegetables) when they were dried would not increase the concentration to above 0.5 ppm for most food items.
- The fluoride in lower moisture-content foods such as bread, uncooked pasta, meats, and eggs is 0.5 ppm or below in the USDA (2005) fluoride database.
- Breakfast cereal products popular with children today but poorly represented in the McClure (1949) publication have fluoride concentrations of about 0.5 ppm in the USDA (2005) database (range of means 0.17 to 0.72 ppm).
- An average concentration of 0.2 ppm seems to underestimate exposure given the USDA (2005) food values for items common in the diets of children when considering the variability in their food consumption habits across the age range covered by the estimates (infancy to age 14 years). An average concentration of 1 ppm appears to be too high to be representative of a mixed diet.

Accordingly, the 0.5 ppm average fluoride concentration is a conservative, albeit uncertain, estimate of the fluoride in diet at the time of the Dean (1942) study.

The McClure (1943) fluoride intakes from dry foods estimated for the 1–3, 4–6, 7–9 and 10–12 year old age groups consuming foods with an average of 0.5 ppm fluoride are given in Table D-8. The intake estimates were divided by the midpoint of the range of body weights McClure (1943) provided (Table D-6) for each age range to derive an estimate for the contribution from dry solid foods at the time of tooth development for the participants in the Dean (1942) study:

Dose from foods = Fluoride from dry food (mg/day) ÷ BW

where:

Fluoride from dry foods = Value from Table D-5 for age group (average F concentration of 0.5 ppm in diet)

BW = Median body weight for each age range (Table D-8)

Table D-8. Estimated Fluoride Intake from Solid Foods with an Average 0.5 ppm F as Derived from McClure (1943)

Age Range (years)	Body Weight Estimated Range (midpoint) (kg)	Fluoride Intake from Solid Foods (mg/day)	Fluoride Intake from Solid Foods (mg/kg/day)[a]
1–3	8–16 (12)	0.133	0.011
4–6	13–24 (18.5)	0.178	0.010
7–9	16–35 (25.5)	0.223	0.009
10–12	25–54 (39.5)	0.278	0.007

[a]Calculated using the midpoint body weights.

The product of this calculation is an estimated intake of 0.01 mg/kg/day when the individual values (Table D-8) were rounded to two decimal places. This dose is added to the 0.07 mg F/kg/day (Section 5.4) for the drinking water contribution from total daily fluoride exposure. Accordingly, the total daily dose is thus 0.08 mg F/kg/day for children receiving drinking water with a concentration of 1.87 mg F/L during the time period between about 1930 and 1940. This concentration is considered the point of departure from the dose-response assessment for a prevalence of 0.5% severe dental fluorosis among the children evaluated by Dean (1942).

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