REVIEW OF FLUORIDE

BENEFITS and RISKS

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ABSTRACT

This report is a comprehensive review and evaluation of the public health benefits and risks of fluoride in drinking water and other sources. It was prompted by a study of the National Toxicology Program which found "equivocal evidence" of carcinogenicity based on the occurrence of a small number of malignant bone tumors (osteosarcomas) in male rats.

Extensive studies over the past 50 years have established that individuals whose drinking water is fluoridated have fewer dental caries. Although the comparative degree of measurable benefit has been reduced recently as other fluoride sources have become available, the benefits of water fluoridation are still clearly evident.

Both animal studies and human epidemiology studies are used to identify potential hazards. Taken together, the only two methodologically acceptable animal studies available at this time fail to establish an association between fluoride and cancer. In humans, optimal fluoridation of drinking water does not pose a detectable cancer risk as evidenced by extensive human epidemiological data reported to date, including new epidemiological studies prepared for this report. No trends in cancer risk, including the risk of osteosarcoma, were attributed to the introduction and duration of water fluoridation.

By comparison with the 1940s, the total prevalence of dental fluorosis (mottling of the tooth enamel, ranging from whitish areas to pitting of the enamel) has increased in non-fluoridated areas and may have increased in optimally fluoridated areas. Increases in the prevalence of dental fluorosis in a population signify that total fluoride exposures from a variety of sources are increasing and may be more than necessary to prevent dental caries. Since the addition of fluoride to drinking water beginning in the 1940s, other sources of fluoride have become available, including toothpastes, mouthrinses, and fluoride dietary supplements. Beverages and foods are also sources of fluoride.

There is no indication that chronic low level fluoride exposure of normal individuals presents a problem in other organ systems, such as the gastrointestinal, the genitourinary and the respiratory systems. It is also not associated with birth defects or Down Syndrome. Crippling skeletal fluorosis is not a public health problem in the U.S. Further studies are required on the genotoxicity and reproductive toxicity of fluoride and on whether or not there is an association between various levels of fluoride in drinking water and bone fractures.

This report recommends the continued use of fluoride to prevent dental caries and the continued support for optimal fluoridation of drinking water. It also recommends scientific conferences to determine an optimal level of fluoride exposure from all sources combined, not only from drinking water. In accordance with prudent health practice of using no more than the amount necessary to achieve a desired effect, the report recommends specific ways to avoid excessive and inappropriate fluoride exposure. In addition to many research recommendations, there are a variety of recommendations for Federal, State and local agencies, health professionals, communities, manufacturers and parents.

PREFACE

In the early part of this century, researchers observed that people with mottled teeth, or dental fluorosis, had fewer cavities than people without mottled teeth. Naturally occurring fluoride in the drinking water later was identified as being responsible for these effects on tooth enamel. Later, community studies conducted in the 1940s established that, as the level of natural fluoride in the drinking water increased, the level of dental caries declined. These studies led to the public health practice of adding fluoride to fluoride deficient drinking water in order to bring the total level of fluoride to approximately 1 part per million. This optimal level was designed to gain the benefits of reduced dental caries and to minimize the risk of dental fluorosis. The U.S. Public Health Service has supported and continues to support optimal fluoridation of drinking water.

Water fluoridation has not been without opposition, with those opposed to fluoridation arguing primarily on the grounds of personal choice and the possibility of adverse health effects. The controversy escalated in 1975 when antifluoridationists claimed higher cancer mortality rates in fluoridated than in non-fluoridated areas. Although this claim was refuted subsequently by other investigators, the concern over a possible association between cancer and water fluoridation prompted the National Toxicology Program (NTP) of the U.S. Public Health Service (PHS) to conduct a long term study to determine the toxicity and carcinogenicity of sodium fluoride in rodents.

The NTP conducts a standard rat and mouse carcinogenicity study, similar to studies previously performed by the National Cancer Institute (NCI), that have been useful in evaluating the potential carcinogenicity or toxicity of numerous chemicals. These studies serve to identify potential hazards. The NTP and NCI studies have evaluated nearly 400 chemicals for potential chronic toxicity and carcinogenicity. The conduct and conclusions of these studies are routinely reviewed by an independent panel of experts.

In a review of the results of these studies, Haseman et al. (1987) noted that, of 1,237 sex-species studies (considering each sex/species combination as a separate experiment), 31 percent were considered positive for carcinogenicity, 57 percent were negative, 8 percent showed equivocal results, and 4 percent were judged inadequate for evaluation. The category of "equivocal evidence" was introduced by NTP in 1983 to describe results of studies in which an association between administration of a chemical and a particular tumor response was uncertain.

The NTP study of sodium fluoride found "equivocal evidence" of carcinogenicity based on a small number of rare bone tumors (osteosarcomas) in male rats and no evidence of carcinogenic activity of sodium fluoride in female rats or in mice of either sex (DHHS, 1990). The NTP report of the study on sodium fluoride was peer reviewed in April of 1990.

The Assistant Secretary for Health, Dr. James O. Mason, responded to the findings of the NTP study by requesting a thorough review of the benefits and risks of water fluoridation and other sources of fluorides. In his role as Chairman, Dr. Mason asked the PHS Committee to Coordinate Environmental Health and Related Programs (CCEHRP) to conduct this review (Appendix A). CCEHRP is comprised of all PHS agencies with having responsibility for environmental health programs in research, in technical assistance to State and local health agencies, in health care or in regulation. These agencies include: the Agency for Toxic Substances and Disease Registry; the Alcohol, Drug Abuse and Mental Health Administration; the Centers for Disease Control; the Food and Drug Administration; the Health Resources and Services Administration; the Indian Health Service; and the National Institutes of Health. The Food and Drug Administration (FDA) regulates fluoride in toothpaste, in other dental products such as composite fillings and dental cements, in bottled water, in water used in food processing, in infant formula, and in dietary supplements (among other fluoride uses), but the FDA does not regulate fluoride in drinking water.

The regulation of fluoride in drinking water is the responsibility of the U.S. Environmental Protection Agency. Under the Safe Drinking Water Act of 1974 (Public Law 93-523), the EPA sets primary and secondary maximum contaminant levels for natural levels of fluoride in drinking water. The primary maximum contaminant level of 4 milligrams/liter is the fluoride level that the drinking water is not allowed to exceed and the level that is legally enforceable; whereas, the secondary maximum contaminant level of 2 milligrams/liter is the fluoride level that EPA recommends drinking water not exceed, but this level is not enforceable.

This report of the benefits and risks of fluoride was prepared by a specially created subcommittee of CCEHRP, the Ad Hoc Subcommittee on Fluoride. The Subcommittee performed an extensive examination of the worldwide biomedical literature on fluorides and health. To ensure public input, an announcement was published in the Federal Register on March 1, 1990, soliciting peer reviewed published articles on fluorides. A total of 24 individuals submitted over 100 articles to the Subcommittee. Additionally, the Subcommittee evaluated several new PHS reports, which were prepared at the request of the Assistant Secretary for Health. One of these new reports (Appendix D) is a review of an industry sponsored study of sodium fluoride's long term toxicity and carcinogenicity in rodents. Appendices E and F are new human epidemiological studies of cancer incidence and mortality in relation to patterns of water fluoridation.

The Ad Hoc Subcommittee on Fluoride prepared this report by dividing into three workgroups: one that addressed fluoride's benefits, another that addressed fluoride's risks, and a third workgroup that synthesized the reports of the other two into the final document. The report of the Ad-Hoc Committee was approved by CCEHRP.

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INTRODUCTION

This report is a comprehensive review and evaluation of the public health benefits and risks of fluorides from drinking water and other sources. Fluoride was added first to public drinking water in the 1940s to prevent tooth decay. While controversy at times has surrounded community water fluoridation, over half of the U.S. population now is served by fluoridated drinking water that is naturally occurring or adjusted. Since the 1950s, fluorides have been incorporated into toothpaste, other dental products, and dietary supplements.

The Assistant Secretary for Health, Dr. James O. Mason, requested analysis of the risks and benefits of fluorides upon the receipt of the preliminary results of a study in which animals were given sodium fluoride in the drinking water. The study, conducted by the National Toxicology Program (NTP), a research and testing program within the U.S. Public Health Service (PHS), found a small number of malignant bone tumors (osteosarcomas) in male rats. The NTP study concluded that there was "equivocal" evidence of carcinogenic activity in male rats (DHHS, 1990). "Equivocal" evidence is one of five standard categories that are employed in NTP studies to describe the strength of the evidence of individual experiments. According to NTP's standard definition, "equivocal evidence of carcinogenicity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related." The NTP independent peer review panel which evaluated the study requested the addition of a statement that further defined the term "equivocal evidence" of carcinogenicity as a category "for uncertain findings (emphasis added) demonstrated by studies that are interpreted as showing a marginal increase in neoplasms that may be chemically related" (DHHS, 1990). The NTP study found no evidence of carcinogenic activity of sodium fluoride in female rats, or in mice of either sex.

Animal studies conducted at a range of dose levels, often at doses that are the maximum which can be tolerated, are used to identify potential hazards to human health. The identification of a potential hazard from animal studies, which is one of the first steps in assessing a health risk to humans, does not necessarily mean that there is a risk to humans. Further steps are taken to determine potential human risks. Human epidemiological studies can help to determine if a hazard to humans exists. The results of epidemiological studies are then analyzed in combination with information from animal studies, dose-response relationships, and patterns of human exposure in order to characterize the magnitude of the risk to humans. This process of characterizing human risk--the likely harm to human health--is called risk assessment.

This report qualitatively assesses fluoride's benefits as well as fluoride's risks. The report analyzes the findings of the NTP study of sodium fluoride and the findings from human and animal studies published in the biomedical literature on both the benefits and the risks of fluoride.

The Assistant Secretary for Health directed the Public Health Service agencies to thoroughly examine any possible association between fluoride and cancer. The U.S. Food and Drug Administration (FDA) conducted a thorough review of a methodologically acceptable chronic bioassay in animals, a study sponsored by the Procter and Gamble Company. The National Cancer Institute (NCI) performed new epidemiological studies to update and expand a previous study published in 1976 which did not find a relationship between water fluoridation and human cancer mortality rates.

PROPERTIES, METABOLISM AND SOURCES OF FLUORIDE

PHYSICAL AND CHEMICAL PROPERTIES OF FLUORIDE

Fluoride, the 13th most abundant element is distributed widely throughout the earth (Finger, 1961). At standard temperature and pressure, fluorine is a pale, yellow gas with an atomic number of 9 and an atomic weight of 18.9984. Fluorine is classified as a halogen, one of the elements that makes up Group VII A of the Periodic Table of Elements. The halogens consist of fluorine, chlorine, bromine, iodine, and astatine; all are electromotive elements that normally exist in the free state as diatomic molecules. As electromotive elements, the halogens can react with less electromotive elements or chemical groups to form a wide variety of inorganic and organic halogenated compounds.

Fluoride compounds are formed when the element fluoride combines with other chemical elements. Fluoride does not occur in a free state in nature; it occurs only in combination with other elements.

One might assume that the biochemical properties of fluorides would be similar to those of other halogenated compounds. This assumption, however, is only partially correct. Like other halogens, inorganic fluorides dissociate in aqueous solution to release the monovalent fluoride anion (F-), along with its associated cation. Fluoride, however has many unique chemical properties that impart special biochemical physiological properties to the fluorides and thus affect its metabolism and mechanisms of action within the body.

In addition to the chemical properties, the isotopic nature of fluorine has had an important impact on our understanding of the metabolism, toxicity, and therapeutic effects of fluoride. The only naturally occurring isotope is 19-F, which has an extremely short half-life (Leech, 1956; Sharpe, 1961). The longest lived isotope, prepared by nuclear reaction, is 18-F; it has a half-life of 1.87 hours. Even this isotope, however, is too short-lived to be of any practical use in long-term fluoride studies.

METABOLISM OF FLUORIDE

Kinetic processes are those that describe the absorption, distribution, biotransformation, biochemical interactions, and excretion of chemicals in the body. Dynamic processes are those that describe the parameters of receptor binding and specific biochemical and physiological actions. When these processes relate to therapeutic blood levels, they are characterized generally as pharmacokinetics and pharmacodynamics. When such processes are related to blood levels that produce adverse or detrimental effects, they generally are referred to as toxicokinetics and toxicodynamics. For this review, fluoride "metabolism" is considered to be the sum of the processes by which fluorides are handled in the living body. Such processes include the absorption, distribution, biotransformation, chemical interaction, and excretion of fluoride-containing compounds. Where appropriate, we discuss the biochemical and physiological actions of fluorides.

During the last 60 years, fluorides have been studied widely in biological systems. In many of the earlier studies, deficiencies, combined with the inability to measure fluorides accurately, led to a number of misunderstandings and faulty premises about fluoride metabolism. Today, however, the basic mechanics of fluoride absorption, distribution, biotransformation, and excretion are fairly well understood, and the basic mechanisms underlying many of fluorides' actions in the body are known.

FLUORIDE ABSORPTION

Fluoride absorption is measured according to the various paths of administration relevant to chronic exposure--mucosal and enteral, inhalational, and dermal.

Mucosal and Enteral Absorption

A number of studies both in animals and humans, have shown that ingested fluoride is absorbed rapidly and readily from the gastrointestinal tract. The absorption of soluble fluoride salts in humans has been assayed both by measurement of fluoride disappearance from the gastrointestinal lumen and by measurement of peak fluoride plasma levels after ingestion (McClure et al., 1945; Carlson et al., 1960a; Ekstrand et al., 1977, 1980, 1983; Spencer et al., 1981; Whitford and Pashley, 1984). Results of these studies have shown that fluoride absorption is both rapid and extensive, with peak fluoride plasma levels achieved within 30 to 60 minutes after ingestion and absorption ranging from 90 to almost 100 percent of the administered dose, depending on the form and method of administration.

The currently accepted theory on fluoride absorption is that inorganic fluoride in the form of the undissociated molecule, hydrofluoric acid (HF), is absorbed by rapid passive diffusion along the entire gastrointestinal tract, without any apparent active transport mechanisms being involved (Singer and Ophaug, 1982; Barbakow, 1983; Whitford and Pashley, 1984). Results of numerous *in vitro* and *in situ* animal studies have shown that the undissociated molecule, hydrogen fluoride (HF) is the principal permeating moiety in a number of cellular membranes and epithelia (Barbakow, 1983; Whitford and Pashley, 1984). Ionic fluoride (F-) has been shown to be virtually incapable of such penetration, having a transmembrane permeability coefficient more than 10^6-fold less than that for HF (Gutknecht and Walter, 1981), purportedly because of its ionic charge and large hydrated radius (Whitford and Williams, 1986). These findings have been supported by the results of *in vivo* animal studies that have shown that fluoride absorption is augmented when gastrointestinal pH levels are maintained below the pK_a for HF, 3.45. At the pK_a, 50 percent is nonionized and available for absorption. The nonionized fraction is decreased significantly at much higher pH levels at which the ionized form of fluoride is more prevalent (Whitford and Pashley, 1984).

Since soluble ionic fluorides dissociate to release free F^- in solution and protonate to form HF in the acid milieu of the gastrointestinal tract, all should be absorbed readily in the stomach, since this is the most acidic portion of the gastrointestinal tract. As previously indicated, this accepted theory has been demonstrated in a number of human and animal studies. Moreover, since this uptake is thought to be a purely passive diffusion process, the only factors that should influence it are those that affect either the transmembrane pH or the HF concentration gradients (Whitford et al., 1979; Ekstrand et al., 1980). Alteration of the transmembrane pH gradient has been shown to affect the uptake of fluoride. In addition, the fluoride concentration, the solubility of the fluoride salt, and the stomach contents at the time of fluoride intake, all factors that would affect the transmembrane HF concentration gradient, have also been shown to effect fluoride gastrointestinal absorption (Barbakow, 1983). Several studies have reported inconsistent results regarding the effects of various metal ions on the gastrointestinal absorption of fluoride (Spencer et al.; 1980, 1981); aluminum consistently has been demonstrated to form fluoride complexes and thus drastically reduce its gastrointestinal absorption (Spencer and Lender, 1979; Spencer et al., 1980, 1981; Elsair, 1980).

Oral absorption of fluoride salts is considered to be controlled by the mechanisms discussed previously; the fluoride dentifrice sodium monofluorophosphate (MFP) which is not an ionic form of fluoride, is bonded covalently to phosphorus. MFP is hydrolyzed readily by phosphatases secreted from the gastric acid mucosa (Ericsson, 1983; Whitford et al., 1983). Once hydrolyzed, free F^- is released into the gastric milieu, where it is absorbed as HF by passive diffusion, independent of the parent MFP molecule.

Fluoride absorbed via the oral mucosa makes an insignificant relative contribution to plasma fluoride levels. Oral clearance as a result of salivation and swallowing causes orally retained fluorides to be ingested, and fluoride is ionized at the nearly neutral pH of the oral cavity. Where acidic products are employed (e.g., acidulated phospho-fluoride gel), the potential for direct mucosal absorption increases, but is insignificant relative to fluoride that is swallowed.

Inhalational Absorption

Because of the limited ability of most of the inorganic fluorides to vaporize, investigations of the airborne absorption of inorganic fluorides have been limited primarily to studies involving hydrofluoric acid. In addition, the scientific literature has little information on controlled exposure studies on either humans or animals. The following section describes basic information obtained from these studies.

In a study conducted by Rye (1960), urinary fluoride levels were measured in employees engaged in producing rock phosphate and triple rock phosphate. Airborne exposure levels were reported to be 2.4 parts per million (ppm) fluoride, consisting of 40 percent hydrogen fluoride and 60 percent dust (exact fluoride form unknown). Urinary fluoride levels increased from 0.5 mg/L to 4.0 mg/L within 2 to 3 hours after onset of exposure and peaked at 7.0 to 8.0 mg/L at 10 hours. In a similar experiment in rabbits, Smith and Gardner (1949, 1960) measured blood fluoride levels in rabbits continuously exposed to airborne hydrofluoric acid and found that fluoride levels increased immediately after exposure, reached a plateau within 24 hours, and persisted at elevated levels for 72 hours following cessation of exposure. Morris and Smith (1982) exposed rats to airborne hydrofluoric acid via surgically isolated upper respiratory tracts and found that absorption from the air was nearly 100 percent, with plasma fluoride concentrations correlating closely with the fluoride concentrations in the air. These studies indicate that inhaled fluoride is absorbed to some degree but provide no conclusive information regarding the rate, extent, exact site, or mechanism of the absorption.

Organic fluorides such as methoxyflurane, halothane, and enflurane were developed and used as inhalation anesthetics. Study results reported in the scientific literature show that these compounds are rapidly and extensively absorbed across the pulmonary epithelium after inhalation (Cousins and Mazze, 1973; Dahlgren, 1979).

Dermal Absorption

Study results show only hydrofluoric acid is absorbed through the dermis. Liquid hydrofluoric acid is either a strong or weak acid, depending on its aqueous concentration (Leech, 1956; Rudge, 1962). Dermal exposure to concentration of hydrofluoric acid solutions can result in severe chemical burns (Dale, 1951; Greco et al., 1988).

Numerous unintentional exposures have provided information to indicate that dermal exposure to hydrofluoric acid results in rapid and extensive absorption and the systemic distribution of fluoride (Dale, 1951; Dibbell et al., 1970; Burke et al., 1973; Browne, 1974). Similar results have been shown in controlled dermal absorption studies in mice (Watanabe et al., 1975)

FLUORIDE DISTRIBUTION

Once fluoride is absorbed, either by ingestion, inhalation, or dermal absorption, it passes into the blood for distribution throughout the body and for partial excretion. In plasma, fluoride has been shown to exist in two distinct forms: a nonionic form covalently bonded to carbon-containing, lipid-soluble molecules and in an unbound ionic form (Guy, 1979). Little is known about the source, fate, or biological significance of the nonionic form of fluoride, other than that it does not appear to be exchangeable with ionic fluoride and does not seem to be sequestered in bone (Waterhouse et al., 1980). Ionic or free fluoride is considered to be the biologically important form in regards to fluoride therapeutics and toxicity; numerous studies have shown that this biologically active form of fluoride circulates unbound in plasma (Taves, 1968; Ekstrand et al., 1977). From plasma, fluoride complexes with calcified tissues and is distributed to either the extracellular or intracellular spaces of the soft tissues or is excreted (see Section on Fluoride Excretion).

Most of the ionic fluoride retained in the body enters into the calcified tissues (bone and dentition), either by substituting for the hydroxyl ion (OH^-) or bicarbonate ion (HCO_3-) in hydroxyapatite in bone

or enamel to form fluoroapatite, or as an ionic exchange within the hydration shell of the crystalline surface (McCann and Bullock, 1957; Neuman and Neuman, 1958). Approximately 50 percent of the fluoride absorbed each day will be deposited in the calcified tissues, resulting in more than 99 percent of the fluoride in the body found in association with the calcified tissue (Whitford, 1983). The incorporation of fluoride into the crystalline structure of hard tissues is thought to increase the strength and stability of the crystalline structure of bone and tooth enamel (Posner, 1978; Eanes and Reddi, 1979).

Although fluoride has a high affinity for bone, it is not irreversibly bound in the bone, but forms a reversible, sequestered pool (Armstrong et al., 1970; Whitford, 1990). Fluoride in bone can be remobilized, either rapidly by interstitial ionic exchange or slowly as a result of the ongoing process of bone remodeling. Alterations in the acid-base balance of the body appear to influence fluoride sequestration and mobilization in bone, presumably because acidosis increases the rate of bone resorption, whereas alkalosis increases the rate of bone deposition (Barzel and Jowsey, 1969; Ekstrand et al., 1980; Singer and Ophaug, 1982). The process of bone remodeling is more active in the young where bone is more hydrated with a greater surface area for fluoride exchange compare to mature bone. Thus, it is not surprising that fluoride deposition in bone has been found to be inversely proportional to age (Whitford, 1990). In fact, the elderly often show a net loss of fluoride from bone along with a rise in plasma fluoride levels, most likely because the rate of bone resorption exceeds the rate of bone deposition in older people.

Researchers believe that fluoride in the soft tissues distributes from plasma to the intracellular space by the passive diffusion of hydrofluoric acid (Whitford et al., 1977, 1978, 1979; Armstrong and Singer, 1980; and Eisenberg and Marquis, 1981). Approximately 1 percent of systemically absorbed inorganic fluoride is distributed in the soft tissue compartments of the body because of the high affinity fluoride has for the hard tissues and the relatively rapid renal excretion of free inorganic fluoride (discussed in Section on Fluoride Excretion) (Navia et al., 1988). Since little, if any, soft tissue binding of fluoride has been shown to occur, either to plasma or cellular components, it is not surprising that fluoride distribution has been shown to reach a "steady state" between plasma and soft tissues, with intracellular tissue levels reflecting the continual fluctuation in plasma levels (Whitford et al., 1979), and with tissue levels generally much lower than the corresponding plasma level.

Exceptions to this soft tissue-to-plasma range of fluoride concentrations occur in bone, kidney, fat, and brain (Whitford et al., 1979). Fluoride levels in kidney have been shown to greatly exceed those found in plasma, presumably because fluoride levels are extremely concentrated in renal tubular fluid (Whitford and Taves, 1973; Whitford et al., 1979). In contrast to the higher fluoride levels found in kidney tissue, fluoride levels in brain and fat rarely exceed a tissue-to-plasma ratio of 0.2, presumably because of the low water content of fat tissue and the relative impermeability of the blood-brain barrier to fluoride (Whitford et al., 1979; Whitford, 1990).

FLUORIDE EXCRETION

Renal excretion is the predominant route for removal of inorganic fluoride from the body. Approximately 50 percent of the daily intake of fluoride is cleared by the kidneys (Whitford, 1990). Renal excretion and deposition in calcified tissues account for almost 100 percent of the clearance of inorganic fluoride from plasma (Jamberg et al., 1983). As fluoride deposition in bone decreases with age, fluoride levels in plasma increase (Parkins et al., 1974).

Renal clearance of fluoride is both a pH-dependent and concentration-dependent (passive) diffusion process (Whitford, 1983). Since the pH of renal tubular fluid is normally well above the pK_a of HF (3.45), the majority of fluoride in the tubular fluid will be in the ionized form (F^-) and will be eliminated via urine (Singer and Ophaug, 1982; Whitford, 1983; Whitford, 1990). The remaining HF in the filtrate can diffuse readily across the tubular membrane to the interstitial fluid (where in the relatively neutral pH of the interstitial fluid, it again becomes ionized (F^-) and subsequently re-enters

the circulatory system. This passive absorption process is thought to occur along the entire tubular system (Whitford et al., 1976, 1979; Whitford, 1990).

As a result of the other major factor that affects passive diffusion, the transmembrane concentration gradient, tubular absorption of fluoride has been shown to range from approximately 40 percent to almost 80 percent of the HF in the glomerular filtrate. (Carlson et al., 1960; Schiffl and Binswanger, 1980, 1982). Ekstrand and coworkers (1980) have shown that over the physiological range of urinary pH (4.5 - 8.0), the urine-to-plasma concentration gradient for nonionized HF ranges from more than 10,000 to a lower, but still physiologically meaningful, 15.7. As a result, although most tubular fluoride is in the undissociated form, the unequal urine-to-plasma concentration gradient still results in the tubular absorption of a relatively large proportion of the filtered fluoride.

A number of factors affect the pH and concentration gradients across the renal tubular epithelium and therefore effect the renal excretion of fluoride. Alterations in the acid-base balance of the body, which result in a decrease in the urinary pH (acidosis), have been shown to decrease urinary excretion of fluoride, whereas alterations that increase urinary pH (alkalosis) have been shown to increase urinary fluoride excretion (Whitford et al., 1976; Singer and Ophaug, 1982; Jamberg et al., 1983). In addition, metabolic alterations that affect the extracellular fluid volume have been shown to affect the urinary excretion of fluoride; expansion of the extracellular fluid volume has been shown to increase glomerular filtration of fluoride and thus its overall rate of renal excretion (Carlson et al., 1960; Whitford et al., 1976).

TABLE 1

Renal clearance of fluoride and fractional renal clearance values for healthy human adults from four studies (Whitford, 1990)[a]

Renal Clearance (ml/min)	Range (ml/min)	Fractional Renal Clearance (ml/min)	References
36.4 (±4.2)	28.0 to 52.0	0.35 (±0.03)	Waterhouse et al. (1980)
26.6 (±2.4)	not given	0.23 (±0.03)	Schill and Biswanger (1980)
31.3 (±1.7)	19.5 to 44.0	ca0.26	Cowell and Taylor (1981)
41.8 (±6.0)	12.4 to 71.4	0.38 (±0.02)	Schill and Biswanger (1982)

[a] Data are expressed as mean ± Standard Error of the Mean (SEM).

A few studies have shown that fluoride can also be excreted in other body fluids, including sweat, saliva, breast milk, and digestive juices (Van Rensburg, 1979; Ekstrand et al., 1984a, 1984b). Excretion by all these routes is usually less than the renal clearance, but excretion in sweat may account for 15 to 25 percent of the fluoride eliminated from the body; and, if sweating is excessive, as much as 50 percent (Zipkin, 1972). Researchers found that fluoride concentrations in both breast milk and saliva are below the corresponding plasma concentrations (van Rensburg, 1979; Ekstrand et al., 1984a, 1984b). Since no active scretory mechanisms have been associated with fluoride excretion by body fluids, researchers believe that fluoride excretion through these fluids is a passive diffusion process. Overall, with the exception of excretion in sweat, saliva, breast milk, and digestive juices these other routes are thought to play a relatively insignificant role in the elimination of fluoride from the body.

BIOTRANSFORMATION OF FLUORIDE

Although fluoride is not metabolized, it forms fluoride-phosphate complexes with various metals, for example, aluminum, calcium, beryllium, and magnesium (Holland, 1979; Sternweis and Gilman, 1982;

Whitford et al., 1987). Such reactions in the gastrointestinal tract have been reported to decrease the rate of fluoride absorption.

The organic fluoride inhalation anesthetics, methoxyflurane, enflurane, and halothane, are excreted primarily unchanged in expired air (Chenoweth et al., 1962). They have also been shown to undergo some degree of metabolic biotransformation, primarily in the liver and possibly in other tissues (Dahlgren, 1979; Singer and Ophaug, 1982) to form CO_2, organic acids, and free fluoride ion (F^-). As a result, investigators have found increased plasma levels of inorganic fluoride after exposure, via inhalation, to all three of these anesthetics. Free ionic fluoride is then distributed or excreted from the body just as ionic fluoride from other exposure sources is distributed or excreted. Since methoxyflurane is much more fat soluble than halothane or enflurane, it builds up stores in the body and is released much more slowly to the circulation following cessation of exposure. As a result, methoxyflurane anesthesia produces higher and more prolonged elevations in the plasma fluoride than the other two produce (Jarnberg et al., 1979; Singer and Ophaug, 1982).

MECHANISMS OF ACTION

Although fluoride compounds occur naturally, both in the environment and in most constituents of the body, there is no conclusive evidence that fluorine or any of the fluoride compounds are essential for human homeostasis or growth. (McIvor et al., 1985). Researchers have found that, depending upon the level of exposure and the mechanism involved, fluoride produces beneficial or adverse effects.

Preventive and Therapeutic Mechanisms

Fluoride was first investigated as an anticaries agent because of the inverse relationship noted in many areas of the country between the prevalence of dental caries and the level of fluoride in drinking water (Dean, 1938; as reviewed by Whitford, 1983). At first, scientists believed that the anticaries activity of fluoride was a direct result of its incorporation into the apatite crystal of enamel, thus increasing its stability and reducing its acid solubility (Eanes and Reddi, 1979; Posner, 1978; Whitford, 1983). Investigators have failed to show a consistent correlation between anticaries activity and the specific amounts of fluoride incorporated into enamel. The theory of preeruptive fluoride incorporation as the sole, or principal mechanisms of caries prevention has been largely discounted (Whitford, 1983). Recent studies have shown that the anticaries action of fluoride may be related to the fluoride levels in the saliva and plaque fluids rather than the enamel surface itself; fluoride in those media (which continually bathe the enamel surfaces) purportedly inhibits bacterial metabolism and, at the same time, increases the rate of remineralization of enamel lost as a result of bacterial acid production (Beltran and Burt, 1988). Although these findings provide us with some understanding of the mechanisms of fluoride anti-caries activity, the exact mechanisms are not yet fully understood (Whitford, 1983).

Sternweis and Gilman (1982) reported that fluoride activates the adenylate cyclase (AC) system for transducing signals across the cell membranes by interacting with G-proteins. In an organ culture of human fetal anterior pituitary tissue, the addition of NaF to the medium activated the AC-system, as measured by the level of cyclic adenosine monophosphate released, which resulted in an increased secretion of pituitary hormones (Groom et al., 1971).

The proposal to use fluoride for treating bone diseases was based on observations of increased bone mass in persons exposed to elevated fluoride levels, either in drinking water or in industrial situations (Roholm, 1937; Leone et al., 1955; Singer and Ophaug, 1982). Researchers believed that fluoride's effect on bone was similar to its effect on enamel, and therefore that fluoride was efficacious in the treatment of certain bone diseases because it increased the stability of the apatite crystal and thereby decreased the rate of bone resorption (Eanes and Reddi, 1979; Navia et al., 1988). In addition, Prince and Navia (1983) found some indication that fluoride may affect the organic matrix of bone and found evidence that fluoride affects the metabolic activity of cells involved in bone deposition and resorption (Punyasingh and Navia, 1984).

Toxic Mechanisms

Exposure to sufficiently high concentrations of HF can cause severe irritation or extensive tissue destruction as a result of the direct caustic action of the acid itself. This is the cause of the dermal burns after exposure to hydrofluoric acid and also for the gastric irritation and tissue destruction after ingestion of large amounts of fluoride compounds.

Although fluoride has beneficial effects on teeth and bone, much higher exposure levels produce correspondingly higher fluoride levels in bone and teeth and can result in structural defects known as dental and skeletal fluorosis (NRC, 1977; IARC, 1982; Navia et al., 1988). Various mechanisms have been proposed as causing or affecting dental and skeletal fluorosis, including fluoride interference with ameloblast activity, osteocyte activity, nucleation, crystalline growth, matrix formation, and calcium homeostasis (Hodge and Smith, 1972; Fejerskov et al., 1977; Prince and Navia, 1983; Punyasingh and Navia, 1984). These studies suggest that several different, and perhaps interrelated, mechanisms may be involved in the development of dental and skeletal fluorosis.

High plasma levels of fluoride (for example, as a result of dermal exposure to hydrofluoric acid) have been reported to complex biologically active calcium, which results in life-threatening hypocalcemia (Burke et al., 1973; Greco et al., 1988). In addition, research (Waldbott, 1962; Holland, 1979; Sternweis and Gilman, 1982; Greco et al., 1988) has shown that fluoride both inhibits and activates a wide variety of enzymes and enzymatic processes (e.g., cholinesterase, adenylate cyclase, glycolysis). Since these enzymatic processes are dependent on a variety of metal ion cofactors, scientists believe that the formation of fluoride-phosphate-metal complexes interferes with these cofactors and thus affects the activity of the corresponding enzymes.

SOURCES OF HUMAN FLUORIDE EXPOSURE

For humans, water, food, dental products, and air generally are the sources of fluoride. Fluoride concentrations from these sources vary considerably, thus human fluoride exposure and intake also vary significantly. Individual exposure differs markedly, depending upon several factors (e.g., lifestyle, dietary practices, age, gender, and health status).

FLUORIDE IN DRINKING WATER AND BEVERAGES

Fluoride is present in both surface water and ground-water. The natural concentration of fluoride in ground-water depends on factors such as the geological, chemical, and physical characteristics of the water-supplying area (Worl et al., 1973). Fluoride concentrations in ground-water fluctuate within wide limits, from less than 0.1 mg/L to more than 25 mg/L. The fluoride concentration in fresh surface water is generally low, ranging from 0.01 mg/L to 0.03 mg/L (WHO, 1984).

On the basis of estimates of the U.S. Environmental Protection Agency (EPA) (1985), about 195.6 million people in the United States are served by public water systems, the remainder by private water systems. About 168.3 million people (more than 86 percent) who are served by public water systems are exposed to fluoride levels of 1.0 mg/L or less (Table 2). An estimated 835,000 people (0.4 percent) are exposed to drinking water with natural fluoride that exceeds 2.0 mg/L, predominately from ground-water sources. More recent estimates, based on data that State health agencies report to the Centers for Disease Control, show that about 132 million people in the United States receive drinking water with fluoride levels higher than 0.7 mg/L. These estimates include water that is naturally fluoridated or to which fluoride has been added (CDC, 1990).

A person's daily intake of fluoride from drinking water is a function of the person's age and the fluoride levels found in the drinking water source. EPA estimated in 1985 that 99.6 percent of the United States population served by public water systems consumed drinking water that ranged from <0.1 to 2.0 mg/L fluoride concentration. The estimated mean daily intake of fluoride ranges from a

high of 0.24 mg/kg/day body weight for formula-fed infants, to a low of 0.029 mg/kg/day for male adults (Table 3). The data indicate that, on a per body-weight-daily basis (mg/kg/day), the fluoride intake from drinking water increases proportionally as the fluoride content of drinking water increases.

TABLE 2

Estimated population exposed to fluoride in public drinking water at various concentration ranges (EPA,1985)

Type of Public Water System	People Served[a] (millions)	Number of people (millions) exposed to fluoride(mg/L) <0.10	0.1-1.0	>1.0-2.0	>2.0-4.0	>4.0-6.0	>6.0-8.0	>8.0
Ground-water	69.239	3.067	61.552	3.872	0.5720	0.1616	0.0113	0.0032
Surface water	126.356	13.548	90.132	22.590	0.0788	0.005	140.0031	0.0001
Total	195.595	16.615	151.684	26.462	0.6508	0.1667	0.0144	0.0033
Percent of Total	100	8.5	77.6	13.5	0.3	<0.1	<0.1	<0.1

[a] Estimated U.S. population served by public water systems.

TABLE 3

Estimated fluoride intake from drinking water (EPA, 1985)

Fluoride Concentration (mg/L)	Estimated Percent U.S. Population[a] served	Estimated Fluoride Intake Per Individual (mg/kg/day) Infants[b] (Formula-Fed)	Children[c] (5-13 years-old)	Adults[d] (Men)
>0.1 to 2.0	99.6	0.24	0.042	0.029
>2.0 to 4.0	0.3	0.73	0.13	0.086
>4.0 to 6.0	<0.1	1.2	0.21	0.15
>6.0 to 8.0	<0.001	1.7	0.30	0.20
>8.0	<0.01	2.4	0.42	0.29

[a] Estimated percent of U.S. Population served by public water systems exposed to fluoride in drinking water at indicated concentration ranges.
[b] Calculation based on infant who weighs 3.5 kg and consumes 0.8 liters of infant formula per day.
[c] Calculation based on 10 year old child who weighs 33 kg and consumes 1.4 liters of water per day.
[d] Calculation based on a man who weighs 70 kg and consumes 2.0 liters of fluid per day.

An indirect result of community water fluoridation is an increase in the fluoride content of foods and beverages that have been prepared commercially or grown domestically using fluoridated water (Clovis and Hargreaves, 1988). Investigators report that the use of fluoridated water (adjusted or naturally occurring levels of 0.7 to 1.2 mg fluoride/L) in the processing of beverages and canned vegetables can add an estimated 0.5 mg/L of fluoride to the product (Marier and Rose, 1966). Researchers also have reported that fluoride concentrations in carbonated beverages prepared with municipal water, fluoridated at 1.0 mg/L, approached the fluoride ion concentrations in the drinking water itself (Shulz et al., 1976; Shannon, 1977). Thus, beverages and food products containing water with measurable fluoride are available and contribute to the total fluoride intake of persons consuming these products, whether or not the drinking water contains fluoride.

When estimating the fluoride intake of an infant during the first 6 months of life, one must consider whether the infant is breast-fed or formula-fed. The concentrations of fluoride in human breast milk are low and range from 5 to 25 micrograms/L (Spak et al., 1983; Krishnamachari, 1987), reflecting different levels of fluoride in the drinking water (0.2 to 1.7 mg/L). Commercially available infant formulas processed from cow's milk contain about 0.1 to 0.38 mg/L of fluoride when prepared with deionized water (Taves, 1983; Johnson and Bawden, 1987; McKnight-Hanes et al., 1988), and soy-based formulas should contain no more than 0.45 mg/L of fluoride (FDA, 1990). When any of the dry or powdered formulas are mixed with fluoride-adjusted water (0.7 to 1.2 mg/L), the fluoride levels to which the infants are exposed approximate 1.45 mg/L, which is within the current recommended daily intake for maximum dental benefit (Johnson and Bawden, 1987; McKnight-Hanes et al., 1988; National Academy of Science, 1989). In the United States, in communities with fluoridated water, the mean daily fluoride intake of formula-fed infants is estimated to range from 0.09 to 0.13 mg/kg body weight and in non-fluoridated areas from 0.01 to 0.02 mg/kg (Singer and Ophaug, 1979). These estimates are considerably lower than the EPA estimates of 0.24 mg/kg/day reported in Table 3, perhaps reflecting the broad range of fluoride concentration in drinking water. In contrast, assuming that human breast milk has a fluoride level of 25 micrograms/L, breast-fed infants may receive 0.003 to 0.004 mg/kg fluoride by body weight (Ericson, 1969).

Where tea drinking is common, tea can contribute substantially to total fluoride intake. Results of the United Kingdom Total Diet Study (Walters et al., 1983) showed that tea was the major source of dietary fluoride for adults in that country (1.3 mg of the total daily intake of 1.8 mg).

FLUORIDE IN FOOD SOURCES

Fresh or unprocessed foods available in the U.S. have fluoride concentrations that generally range from 0.02 to 2.0 ppm (Table 4).

Marine fish that are consumed with bones and bone meal supplements have been shown to be a rich source of fluoride in human food (Muhler, 1970; Kumpulainen and Koivistoinen, 1977). The bones of some land-based animals also contain high levels of fluoride (Sherlock and Corr, 1984).

Food processing influences the fluoride content of foods. The natural fluoride content of most foods is so small that its contribution is insignificant compared with the amount of fluoride produced through cooking and processing food in fluoridated water (Marier and Rose, 1966). In addition, the cooking vessel can influence the level of fluoride (Full and Parkins, 1975).

The dietary fluoride intake of young children (excluding beverages) was reported by Ophaug et al. (1972, 1982) for 6-month-olds and 2-year-olds living in four geographic regions of the United States. Dietary fluoride intake among the 6-month-olds ranged from 0.15 to 0.76 mg/day (Singer and Ophaug, 1979), providing daily intake of about 0.05 mg/kg body weight. By using "market basket" surveys to estimate the fluoride intake (Ophaug et al., 1980), similar levels were established for 2-year-olds (0.41 to 0.61 mg/day in fluoridated areas and 0.15 to 0.26 mg/day in low-fluoride areas).

TABLE 4

Fluoride concentrations in food (Taves, 1983)

	Fluoride Concentration (ppm)		
Foods[a]	Mean	Standard Deviation	Range
Dairy products	0.25	0.38	0.02 - 0.82
Meat, fish, poultry	0.22	0.15	0.04 - 0.51
Grain and cereal products	0.42	0.40	0.08 - 2.01
Potatoes	0.49	0.26	0.21 - 0.84
Leafy vegetables	0.27	0.25	0.08 - 0.70
Legumes	0.53	0.05	0.49 - 0.57
Root vegetables	0.38	0.11	0.27 - 0.48
Fruits	0.06	0.03	0.02 - 0.08
Oils and fats	0.25	0.15	0.02 - 0.44
Sugar and adjuncts	0.28	0.27	0.02 - 0.78
Nonclassifiable foods	0.59	0.19	0.29 - 0.87

[a] The foods were ready to eat or prepared for eating. When preparation required the use of water (e.g., preparing juice from concentrate or boiling vegetables), the local water which contained 1 mg/L fluoride was used. Nonclassifiable foods included certain soups and puddings, among other items.

Several "market basket" estimates have been made of the daily fluoride intake from food in United States adults (exclusive of drinking water and beverages) in both fluoridated adjusted and unadjusted areas (Tables 5 and 6); these estimates generally range from 0.2 to 0.8 mg/day in unadjusted areas and from 0.3 to 2.7 mg/day in fluoride adjusted areas. For an adult weighing 50 kilograms, the daily fluoride intake from food sources range from 0.004 to 0.016 mg/kg/day in low-fluoride areas and from 0.006 to 0.054 mg/kg/day in fluoridated areas.

TABLE 5

Daily fluoride intake of adults in communities with low fluoride levels in drinking water (0.4 mg/L or less)

	Adult Fluoride Intake (mg/day)		
References	Dietary Sources Excluding Beverages and Water	Beverages and Water	Total Fluoride Intake
Armstrong & Knowlton (1942)	0.27 to 0.32	-	-
McClure et al. (1944)	0.3 to 0.5	-	-
Ham and Smith (1954)	0.4 to 0.8	0.0 to 0.3	0.4 to 0.88
Cholak (1959)	0.3 to 0.8	-	-
Kramer et al. (1974)	0.8 to 1.0[a]	-	-
Osis et al. (1974)	0.7 to 0.9[a]	-	-
Singer et al. (1980)	0.3 to 0.4	0.2 to 0.76	0.91

[a] Includes tea and coffee within the dietary source category.

TABLE 6

Daily fluoride intakes of adults in communities with fluoridated drinking water (approximately 1 mg/L)

References		Adult Fluoride Intake (mg/day) Dietary Sources Excluding Beverages and Water	Beverages and Water	Total Fluoride Intake
Marier and Rose	(1966)	1.0 to 2.1	1.0 to 3.2	2.0 to 5.3
Spencer et al.	(1970)	1.2 to 2.7	1.6 to 3.2	2.8 to 5.9
San Filippo and Battistone	(1971)	0.8 to 0.9	1.3 to 1.5	2.1 to 2.4
Kramer et al.	(1974)	1.7 to 3.4[a]	-	-
Osis et al.	(1974)	1.6 to 1.8[a]	-	-
Singer et al.	(1980)	0.3 to 0.6	0.6 to 1.1	1.0 to 1.7
Becker and Bruce	(1981)	0.4	1.6 to 1.9	2.0 to 2.3

[a] Includes tea and coffee within the dietary source category.

DENTAL PRODUCTS CONTAINING FLUORIDE

Fluoride-containing dental products (e.g., dentifrices or toothpastes, mouthrinses, fluoride supplements, and professionally applied topical fluorides) have been identified as sources of systemically available fluoride (Whitford, 1982, 1989; Ophaug and Singer, 1988; Pendrys and Stamm, 1990).

The most commonly used fluoride products are toothpastes, followed by treatment gels and mouthrinses. More than 90 percent of the toothpaste sold in the United States contains fluoride; the concentrations range from 1,000 to 1,500 ppm (Beltran and Szpunar, 1988; Whitford, 1989). The fluoride toothpastes available in the United States contain fluoride from several different compounds: sodium fluoride, 0.188 to 0.254 percent (0.1 percent fluoride with an available fluoride ion concentration of more than 650 ppm; sodium monofluorophosphate, 0.654 to 1.14 percent (0.1 to 0.15 percent fluoride with an available fluorophosphate and fluoride combined of more than 800 ppm); stannous fluoride, 0.351 to 0.474 percent (0.1 percent fluoride with an available fluoride ion concentration of more than 700 ppm); or stannous fluoride products containing the abrasive calcium pyrophosphate, 0.351 to 0.474 percent (0.1 percent fluoride with an available fluoride ion concentration of more than 290 ppm (Whitford, 1989; DHHS/FDA, 1988). Fluoride mouthrinses contain either aqueous solutions of sodium fluoride, 0.05 and 0.2 percent (0.023 and 0.09 percent fluoride yielding fluoride ion concentrations of 230 and 900 ppm respectively); or stannous fluoride, 0.4 percent (0.1 percent fluoride with an effective fluoride ion concentration of 970 ppm) (Whitford, 1989; DHHS/FDA, 1985). Fluoride treatment gels include products containing: either neutral or acidulated phosphate fluoride, 2.72 percent (0.5 to 1.23 percent fluoride with fluoride ion concentrations up to 12,300 ppm); and stannous fluoride in 0.4 and 8.0 percent solutions (0.01 and 1.94 percent fluoride with fluoride ion concentrations of 970 and 19,400 ppm respectively) (Whitford, 1989; DHHS/FDA, 1985).

Children's use of fluoride-containing dental products is associated with an increased daily fluoride intake. Table 7 lists estimates of fluoride ingested during tooth brushing by children of various age groups. The higher amounts ingested by young children have been attributed to their inadequate control of the swallowing reflex.

TABLE 7

Estimated fluoride ingestion during toothbrushing

Reference		Age Group	Dentifrice Used Mean or Range (grams)	Fluoride Ingestion Mean or Range (mg F)
Hargreaves et al.	(1970)	Preschool	-	0 to 0.25
Barnhart et al.	(1974)	2 to 4	0.86	0.30
Ericson et al.	(1969)	4 to 5	0.39 to 0.51	0.13
Barnhart et al.	(1974)	5 to 7	0.94	0.13
Ericson et al.	(1969)	6 to 7	0.41 to 0.48	0.12
Glass et al.	(1975)	8 to 10	0.23 to 2.57	0 to 0.41
Barnhart et al.	(1974)	11 to 13	1.10	0.07
Dowell	(1981)	0 to 2	0.07 to 1.97	-
Brunn et al.	(1988)	3	0.17 to 3.0	-
		7	0.2 to 3.7	-

Children begin brushing their teeth at an early age (Dowell, 1981), and the amount of toothpaste introduced into the mouth varies; it ranges from 0.1 to 2.57 grams per brushing. In a review of the dental literature, Whitford (1989) reported that the amount of toothpaste children use averaged about 1.0 gram per brushing. Based upon the amount of dentifrice used, the amount of fluoride introduced into the mouth with each brushing ranges from 0.1 to more than 2.0 mg and averages 1.0 mg (Hargreaves et al., 1972; Barnhart et al., 1974; Baxter, 1980; Dowell, 1981; Brunn and Thylstrup, 1988); the average is similar for fluoride mouthrinse products (Wei and Kanellis, 1983; Bell et al., 1985). The percentage of fluoride introduced into the mouth, then swallowed or absorbed rather than expectorated, ranges from near zero to 100 percent; (reported fluoride intake per brushing ranges from zero to 0.41 mg or 0.041 mg/kg for a child weighing 10 kilograms). On the basis of these reports, Whitford (1987; 1989) estimated that about 25 percent of the fluoride in toothpastes and mouthrinses is swallowed or absorbed. Thus, a 25 kg or 2-year-old child ingests an average of about 0.25 mg of fluoride (0.01 mg/kg/brushing or rinsing) with each brushing or rinsing.

The practice of giving children daily dietary fluoride supplements was developed as an alternative to fluoridation of drinking water, as a means of preventing caries in children living in low-fluoridated areas. The current recommendations for prescribing dietary fluoride supplements depend on the age of the child and concentration of fluoride in the drinking water (Table 8).

TABLE 8

Dietary fluoride supplement dosage schedule (mg/day)
Council on Dental Therapeutics, American Dental Association, 1978, 1984
and the Committee on Nutrition, American Academy of Pediatrics, 1978

Age of Child (years)	Concentration of Fluoride in drinking water (mg/L) <0.3	0.3 to 0.7	>0.7
Birth to 2	0.25	0.0	0.0
2 to 3	0.50	0.25	0.0
3 to 13	1.0	0.50	0.0

Table 9 shows the estimated ranges of fluoride ingested by the three most important sources of fluoride in young children: diet, dietary fluoride supplements, and dentifrices. If brushing were increased to two or more times per day or more fluoride mouthrinse were used, the contribution from these dental hygiene products would increase proportionally (Pendrys and Stamm, 1990).

TABLE 9

Estimate of maximum fluoride intake from diet, supplements, and dentifrices among 2-year-old children

	Estimated Total Daily Fluoride Intake (mg/day)			
	Drinking water with 1-ppm-fluoride		Drinking water with No detectable fluoride	
Sources	Range	Mean	Range	Mean
Diet[a]	.5 - 0.6	0.6	0.2 - 0.3	0.3
Fluoride Supplements[b]	-	-	0.5	0.5
Fluoride Dentifrice[c]	0.0 - 2.0	0.3	0.0 - 2.0	0.3
Total	0.5 - 2.6	0.9	0.7 - 2.8	1.1

[a] Including water and beverages. Adapted from Ophaug et al., 1980, 1985.
[b] Adapted from Council on Dental Therapeutics of the American Dental Association, 1984.
[c] Adapted from Whitford et al., 1987 and Pendrys & Stamm, 1990.

The use of fluoride supplements and dentifrice in communities that do not have fluoridated drinking water can result in levels of total fluoride equal to that in communities with optimally fluoridated water supplies. Using the mean retention estimate of 25 percent, if teeth are brushed twice each day (1 g of dentifrice or 1 mg of fluoride per brushing), the amount of fluoride ingested would approximately equal the amount ingested from the diet (0.5 mg/day).

AMBIENT AIR LEVELS OF FLUORIDE

Traces of fluoride in the air in rural communities and cities originate from both natural sources and human activities (WHO, 1984). Natural sources of fluoride in the air are the dusts from soil and seawater droplets dispersed into the atmosphere by wind. Most of the ambient levels of fluoride in air found in urbanized areas are generated by industries (Smith and Hodge, 1979). Studies reporting levels of fluoride in air suggest that ambient fluoride contributes little to a person's daily intake (EPA, 1980, 1985). Assuming that an adult male inhales 23 cubic meters of air per day and that he absorbs 100 percent of the inhaled fluoride and assuming that ambient air levels of fluoride are present at the limit of detection (0.05 micrograms/cubic meter) the person will absorb about 1.2 micrograms per day (EPA, 1985). Martin and Jones (1971) estimated that a person living in the highly industrialized city of London inhales 1 to 4 micrograms of fluoride per day.

SUMMARY OF TOTAL HUMAN FLUORIDE INTAKE

The fluoride concentrations in drinking water, beverages, food, and dental hygiene products comprise the major sources of fluorides in humans. Typically, levels of fluoride in air are extremely low, thus the airborne contribution appears negligible. Fluoride levels in water, diet, and fluoride-containing dental products vary considerably, and therefore an individual's exposure to fluoride varies significantly. Exposure is determined by a person's age, weight, diet, drinking-water source, frequency of use of fluoride-containing dental products, and by the climate.

On the basis of information in this section, Tables 10 and 11 summarize the daily intake of fluoride from the major sources of exposure for children and adults. The following observations have been made:

- Although fluoride exposure is greater generally in areas with fluoridated water than in areas with non-fluoridated or low-fluoridated water, populations in both areas are exposed to fluoride from food sources, drinking water, processed beverages, and dental products (Tables 10 and 11).

- If daily fluoride dietary supplements are used appropriately and consistently, the intake levels for children in non-fluoridated areas will be similar to those for children living in optimally fluoridated areas (Table 10). Use of dietary supplements is a voluntary preventive effort, requiring a high degree of parent compliance and continued attention by health care providers who prescribe supplements.

- In optimally fluoridated areas, most of the estimated daily intake of fluoride for children and adults is from drinking water, beverages and food. In low-fluoride or non-fluoridated areas, children can receive their highest proportion of daily intake from fluoride supplements and dentifrice (Table 10). The daily intake of most adults is about equally divided among food, drinking water, beverages, and mouthrinses (Table 11).

- The relative contribution of fluoride dentifrices to a child's fluoride intake varies greatly, but estimates show that, in both fluoridated and non-fluoridated areas, ingestion can constitute a substantial proportion of total intake of fluoride in children.

TABLE 10

Summary--Estimated daily fluoride intake of children[a]

Concentration of Fluoride in Drinking Water mg/L	Intake Estimate[b]	Fluoride Intake				
		Food Sources	Drinking Water and Beverages	Fluoride Dentifrice[c]	Fluoride Supplement[d]	Estimated Total Fluoride Intake
<0.3 mg/L	mg/day	0.15 to 0.3	0.1 to 0.3	0.2 to 1.2	0.50	0.95 to 2.3
	[mg/kg/day]	[0.007 to 0.015]	[0.005 to 0.015]	[0.01 to 0.06]	[0.025]	[0.047 to 0.115]
0.7 to 1.2 mg/L	mg/day	0.4 to 0.6	0.3 to 1.8	0.2 to 1.2	Not Recommended	0.9 to 3.6
	[mg/kg/day]	[0.02 to 0.03]	[0.015 to 0.09]	[0.01 to 0.06]		[0.045 to 0.18]
>2.0 mg/L	mg/day	1.0 to 2.0	0.6 to >3.0	0.2 to 1.2	Not Recommended	1.8 to >6.2
	[mg/kg/day]	[0.05 to 0.10]	[0.03 to 0.15]	[0.01 to 0.06]		[0.09 to 0.31]

[a] Calculation based on the child weighing 20 kg.
[b] Estimated daily fluoride intake of children based upon data extrapolated from various sources reported in the scientific literature; ranges rounded to reflect widest range in listed fluoride sources.
[c] Assumed that fluoride dentifrice used twice per day
[d] Assumed that dental fluoride supplement taken daily.

TABLE 11

Summary--Daily fluoride intake of adults[a]

Concentration of Fluoride in Drinking Water mg/L	Intake Estimate[b]	Fluoride Intake				
		Food Sources	Drinking Water and Beverages	Fluoride Dentifrice[c]	Fluoride Mouthrinse[d]	Estimated Total Fluoride Intake
< 0.3 mg/L	mg/day	0.2 to 0.8	0.1 to 0.7	0.018 to 0.145	0.56	0.88 to 2.2
	[mg/kg/day]	0.004 to 0.016]	[0.002 to 0.014]	[0.0004 to 0.003]	[0.01]	[0.016 to 0.04]
0.7 to 1.2 mg/L	mg/day	0.4 to 2.7	0.6 to 3.2	0.018 to 0.145	0.56	.58 to 6.6
	[mg/kg/day]	[0.008 to 0.54]	[0.012 to 0.064]	[0.0004 to 0.003]	[0.01]	[0.03 to 0.13]
>2.0 mg/L	mg/day	1.2 to 3.4	0.9 to >3.5	0.018 to 0.145	Not Recommended	2.1 to >7.05
	[mg/kg/day]	[0.02 to 0.07]	[0.018 to >0.07]	[0.0004 to 0.003]		[0.04 to >0.14]

[a] Calculation based upon adult weighing 50 kg.

[b] Estimated daily fluoride intake for an adult based upon data extrapolated from various sources reported in the scientific literature; ranges rounded to reflect widest range from listed fluoride sources.

[c] Assumed that fluoride dentifrice and mouthrinse used twice per day.

HEALTH BENEFIT ASSESSMENT OF FLUORIDE

The objective of this chapter is to evaluate (1) the relationship between fluoride and the prevention of dental caries, (2) the contemporary uses of fluoride, and (3) fluoride as a treatment for osteoporosis. Caries and osteoporosis, two common health impairments, have long been studied in relation to fluorides. With respect to dental caries, it is important to determine whether the benefits reported in the past continue today.

DENTAL CARIES

This section, in historical context, concerns the relationship between fluorides and dental caries with respect to the several criteria for establishing causality: detecting an association, seeking a dose-response relationship, replicating the findings under a variety of circumstances and by different investigators, excluding alternative explanations and observer bias, finding biological plausibility for the relationship, and observing the disappearance of the effect when the cause is removed (MacMahon and Pugh, 1970). Not all of these elements are needed to infer causality.

This approach, commonly used in epidemiology -- as in the Surgeon General's first report on smoking and health (U.S. Public Health Service, 1964), is novel in evaluating fluoride exposure in the prevention of dental caries. The literature is too vast to be re-reviewed here. That pertaining to fluoridation of water is well covered through 1960 by McClure in his volumes, Fluoride Drinking Waters (1962) and The Search and the Victory (1970), through 1972 by Murray and Rugg-Gunn (1982), and from 1979 to 1988 by Newbrun (1989). The publications cited in this report, with emphasis on recent work, illustrate the diversity of studies from which one can derive the elements used to evaluate causality.

Studies of various fluoride modalities, such as mouthrinses and toothpastes, although extensive and important (University of Michigan Workshop, 1989), are described here primarily to supplement results pertaining to water fluoridation. The objective of this section was to determine if fluoride prevents caries, and not to evaluate the various fluoride modalities individually or in combination.

HISTORY: "MOTTLED ENAMEL" AND DRINKING WATER

According to McClure (pp 23-29, 1970), study of the effects of fluorides began in 1901 when an association was suspected between markedly discolored teeth and drinking water in two widely separated locations. J. M. Eager, a U.S. Public Health Service officer in Naples, Italy, observed that emigrants bound for the United States often had markedly discolored teeth. He also noted that children of wealthy families, who spent only their summers at the Naples seaside, had thin horizontal dark lines on their teeth, one for each summer. Eager suspected that volcanic fumes from Mt. Vesuvius had affected the drinking water. At the same time, McKay, upon opening his dental practice in Colorado Springs, Colorado, found similarly discolored teeth among his patients who had lived in that location since childhood. In 1930, publication of a report by Kempf and McKay on dental mottling in the aluminum company town of Bauxite, Arkansas, caused Churchill, who headed the laboratory for the company, to have his staff use spectrography to look for chemicals not normally evaluated in water analyses. The staff found a high concentration of fluoride (12 mg/L). In the same year Smith et al. (1931) and Velu and Balozet (1931) independently reported the same laboratory

finding. This observation set the stage for fluoride measurements of drinking water as related to the degree of mottling (a historical term used to describe discolored teeth of any etiology).

ASSOCIATION BETWEEN MOTTLED ENAMEL AND DENTAL CARIES, AND DOSE-RESPONSE EFFECT

Mottling was classified by degree, from barely detectable flecks of white on the normally translucent teeth, through yellow to deepening brown and even corroded-looking teeth (Dean, 1938). In 1916 Black noted that caries were less frequent among residents with mottled teeth in Colorado Springs than among people living in areas where mottling did not occur. Elsewhere in the world others also suggested an association between mottled teeth and reduced caries scores (reviewed by Dean, 1938). Next, Dean and coworkers (1942a) conducted a systematic survey of dental caries in relation to the fluoride content in the water of 21 U.S. cities. These investigators found that the frequency of caries decreased as the content of fluoride in the water supplies increased (Figure 1). This dose-response effect provided evidence that fluoride was related to a reduction in dental caries.

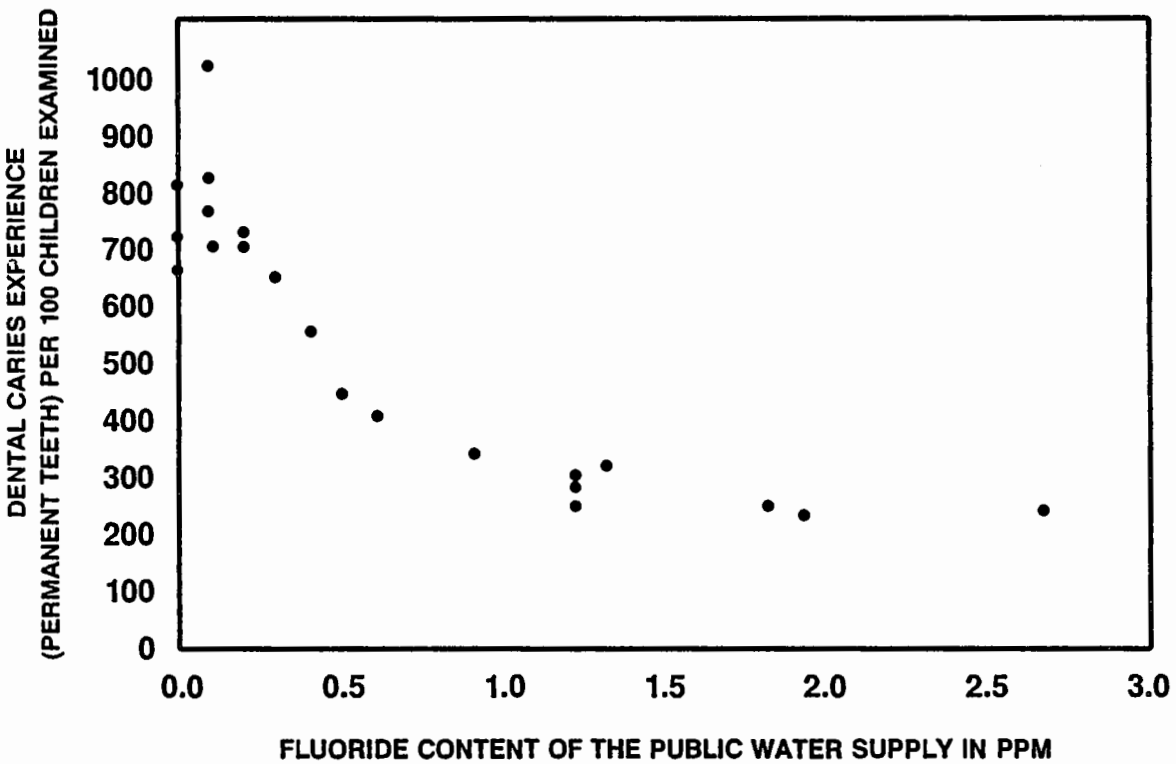

Figure 1. Relationship between the amount of dental caries (permanent teeth) observed in 7,257 selected 12-14 year old white school children of 21 cities of 4 states and the fluoride content of public water supply (Dean et al., 1942)

DISTRIBUTION OF FLUORIDATED WATER

The distribution of naturally fluoridated waters (0.7 ppm or more) is concentrated in certain areas of the United States (Figure 2). The worldwide distribution of naturally or adjusted fluoridated water, as compiled by Collins of the Centers for Disease Control (CDC, 1990) as of June 1, 1989, is shown in Table 12. Countries with the most people receiving fluoridated water were the United States, 132.2 million; Brazil, 42.5 million; and the U.S.S.R., 41.3 million. The increase in fluoridation over time in the United States is shown in Figure 3 (CDC, 1990).

SCORING THE OCCURRENCE OF CARIES

In caries research, reference is generally made to abbreviations in scoring decayed, missing, needing extraction, and filled teeth or surfaces; deft or dmft refers to the primary teeth and DMFT to permanent teeth. These scores reflect the cumulative caries experience of a person or the average caries experience of a population. Counts based on tooth surfaces (dmfs or DMFS) are more informative than those based on the tooth as the unit of measure, because the five surfaces of each tooth are at different risks of caries (Brunelle and Carlos, 1990).

REPLICATION OF THE EARLY FINDINGS

Reduced dental caries following fluoridation of water supplies has been shown many times. In Grand Rapids, Michigan, the site of the first adjusted fluoridated water supply (1 ppm), the caries scores 9 years later showed a marked reduction at each year of age from 6 to 10 years as compared with Muskegon (Figure 4). Soon after, the water supply of Newburgh, New York, was fluoridated, and caries scores were compared with those for nearby non-fluoridated Kingston, New York, with similar results, and a few months later, Evanston, Illinois, followed suit (reviewed by McClure, 1970, pp 122-131). Dean (1942a) identified the optimum level of fluoride in the water supply as 1 ppm for maximal caries reduction with minimal or no risk of dental fluorosis. Galagan (1953) related the optimum level to the environmental temperature, which influences the amount of water consumed (Figure 5).

A cross-sectional study of 5-year-olds, from 1975 to 1978, in four communities in Northeast England showed that the higher the level of fluoride in the drinking water (from 0 to 1 ppm), the lower the scores for dental caries (Figure 6) (Rugg-Gunn et al., 1981).

In four cities of Michigan, with natural fluoride levels of 0.0, 0.8, 1.0 or 1.2 ppm, children 6 to 12 years old were examined in the mid-1980s. These recent results (Table 13), consistent with Dean's (1942a), show a dose-response effect in protection against caries (Szpunar and Burt, 1988).

Heifetz and coworkers (1988) studied dental caries and dental fluorosis in four areas of Illinois in which they found the naturally fluoridated water to be one, two, three and four times the optimal (1 ppm) for 1980 as compared with 1985, and they found a dose-response effect. At fluoride levels higher than optimal, caries scores were lower than optimal (Table 14). Dental fluorosis increased at each level.

Some of the numerous water fluoridation studies that different investigators have conducted in various places are shown in Table 15, from Newbrun (1989). In 11 tables, he summarized the world literature on caries and fluoridation as derived from Medline, 1984 through 1988, and from the relevant

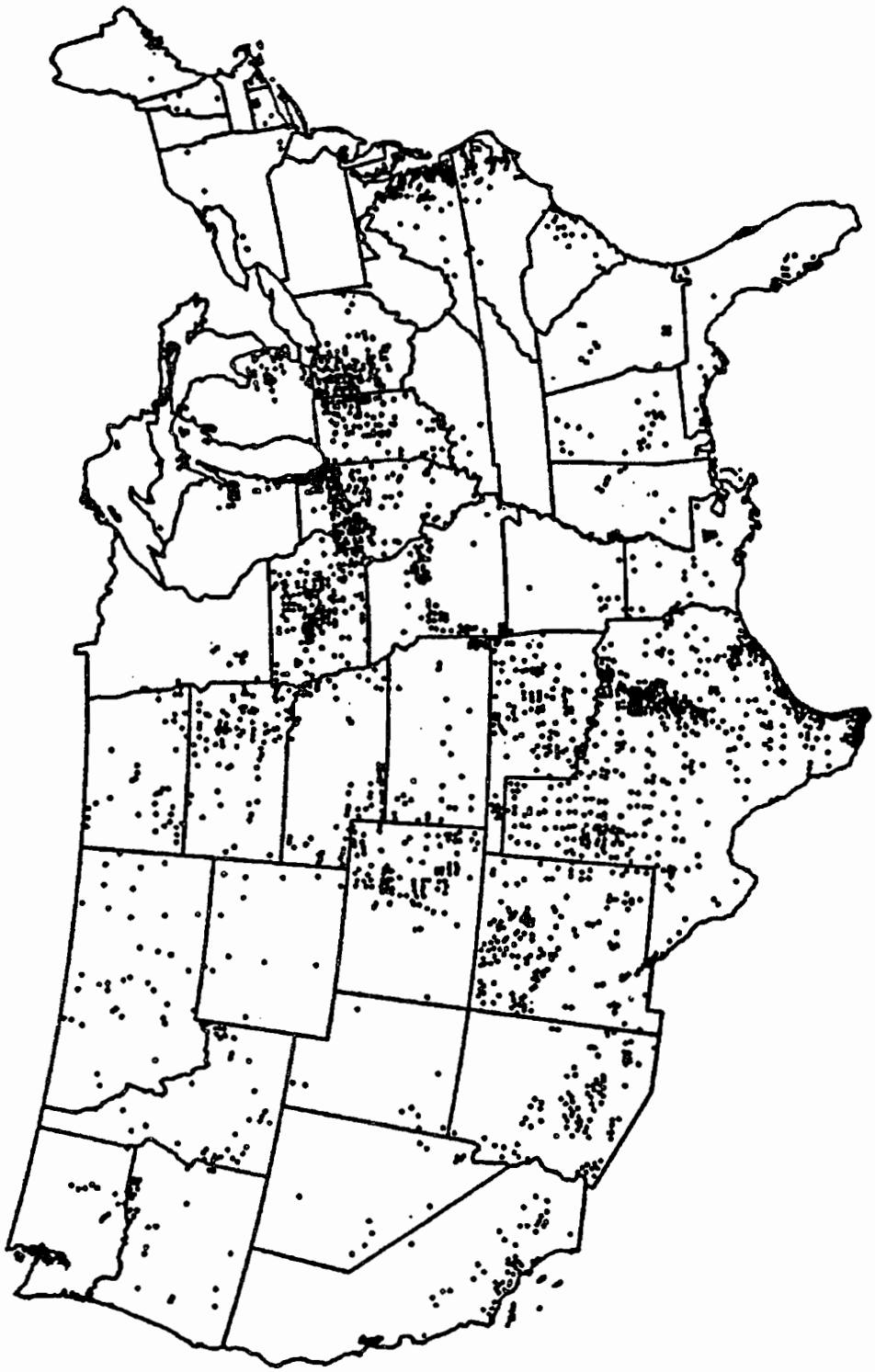

Figure 2. Towns in the United States using naturally fluoridated water (0.7 ppm or more of fluoride) (U.S. PHS, Division of Dental Health, 1969)

TABLE 12

Estimated number of people (by country)
receiving fluoridated water as of June 1, 1989 (millions)

Country	Number of people	Year started	Source[a]
North America			
United States	132.2	1945	1
Canada	9.6	1945	2
Mexico	4.7	1960	3
	146.5		
Central America			
Cuba	0.1	1974	3
Dominican Rep	0.3	-	3
Netherland Antilles	0.2	-	3
Panama	0.6	1950	3
	1.2		
South America			
Argentina	0.1	1969	3
Brazil	42.5	1953	3
Chile	0.4	1953	3
Colombia	8.5	1953	3
Ecuador	1.3	1961	3
Venezuela	4.9	1952	3
	57.7		
Africa			
Libya	0.4	-	2
Europe			
Czechoslovakia	3.5	1956	4
Finland	0.1	-	4
German Dem Rep	3.4	1952	4
Ireland	2.3	1964	4
Poland	1.3	1967	4
Spain	0.1	-	5
Switzerland	0.3	1972	4
United Kingdom	8.0	1955	5
USSR	41.3	1960	4
	60.3		
Asia			
Hong Kong	5.0	1961	2
Israel	1.0	1977	4
Malaysia	7.7	1966	2
Singapore	2.5	1958	2
Taiwan	0.6	-	2
S. Korea	0.3	-	2
Philippines	3.0	-	2
	20.1		
Oceania			
Australia	10.2	1956	
New Zealand	1.8	1954	
Fiji	0.2	-	2

[a] 1. U.S. Fluoridation Census 1988 Summary; 2. FDI-Federal Dentaire Internationale, 1984; 3 PAHO-Pan American Health Organization, 1988; 4 WHO RO-World Health Organization-European Office, 1987; 5 British Fluoridation Society, 1988.

As compiled by James Collins, Dental Disease Prevention Activity, Centers for Disease Control, Atlanta, Georgia.

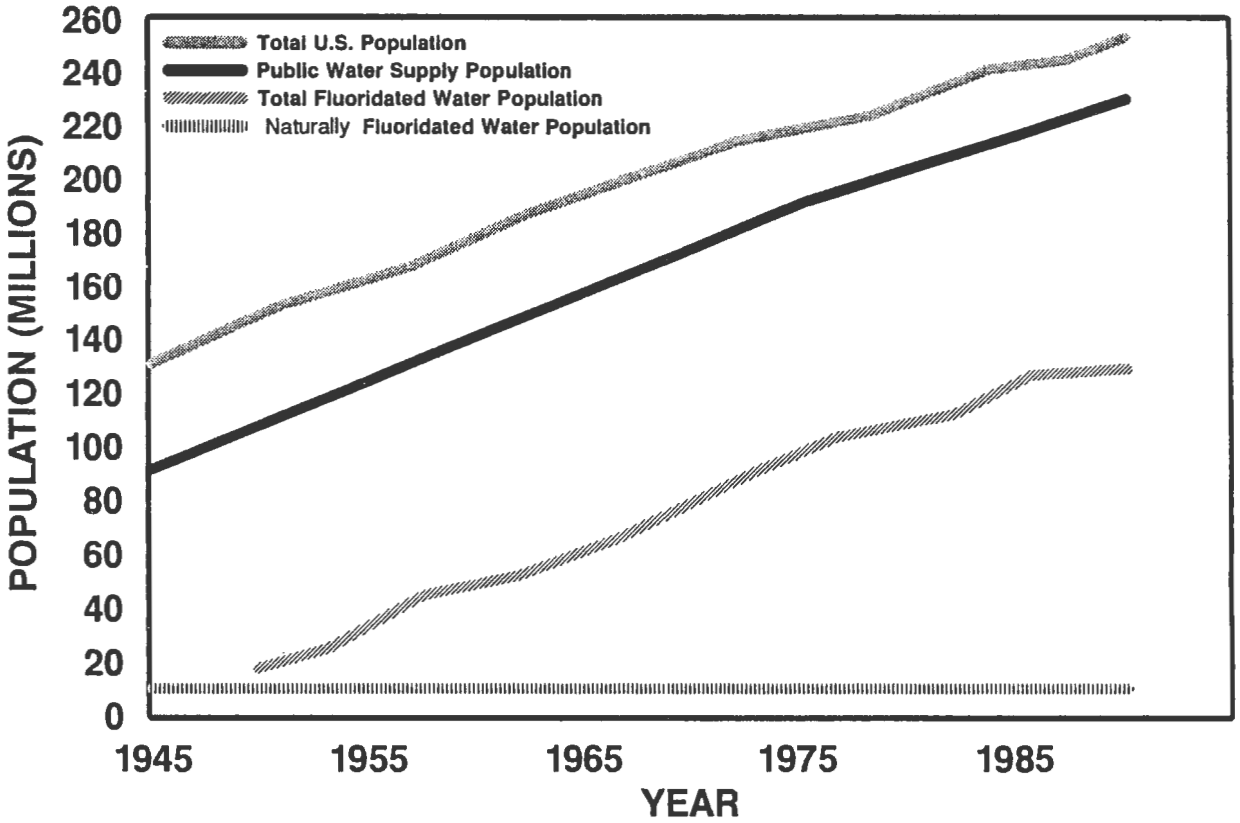

Figure 3. Availability of Fluoridated Water, by Population, United States, 1945 - 1988 (CDC, 1990)

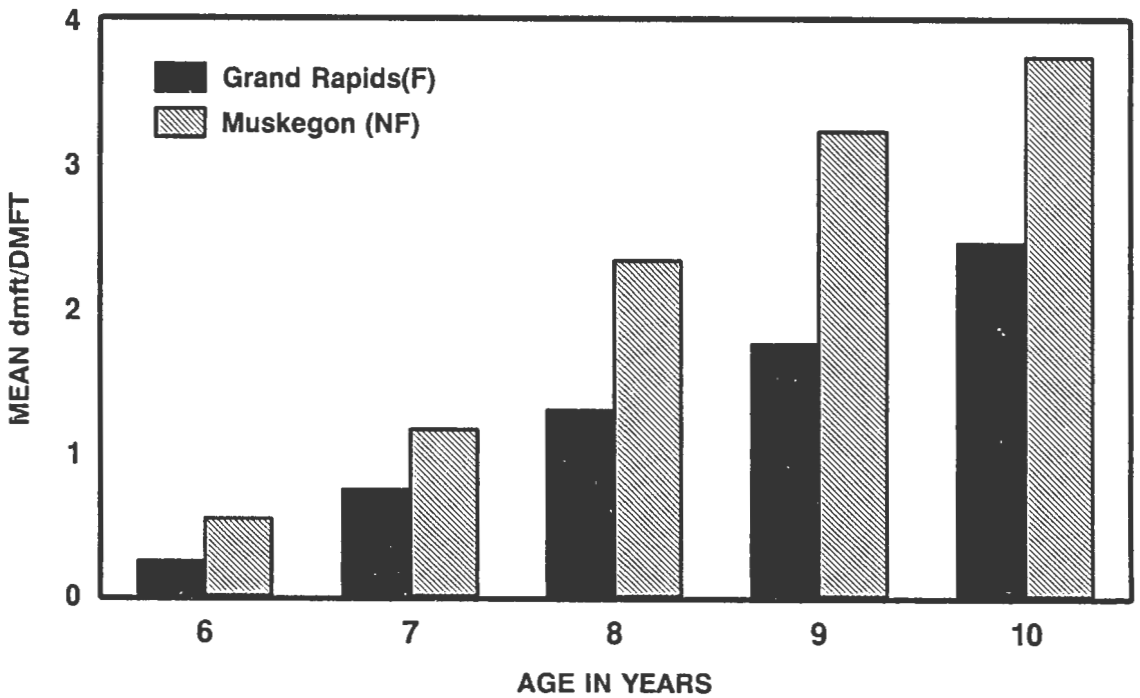

Figure 4. Average number of dmft/DMFT per child by age in Grand Rapids after nine years of fluoridation as compared with no fluoridation in Muskegon, MI, 1955 (Arnold et al., 1956)

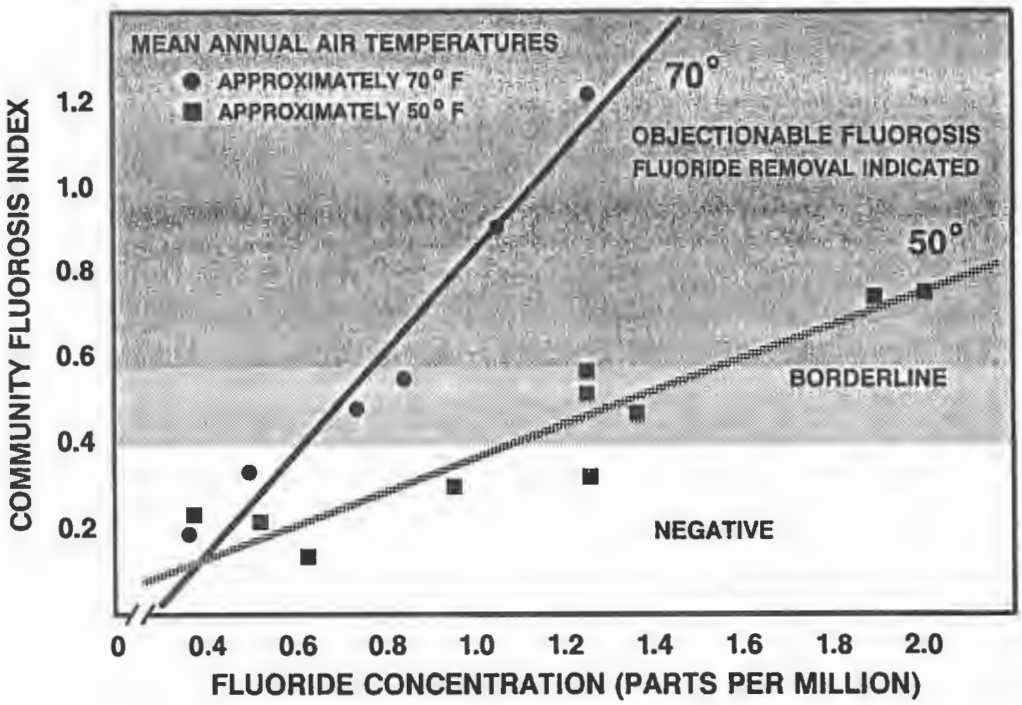

Figure 5. Relation between fluoride concentration of municipal waters and fluoride index for communities with mean annual temperatures of approximately 50°F (Midwest) and 70°F (Arizona) (adapted from Galagan, 1953)

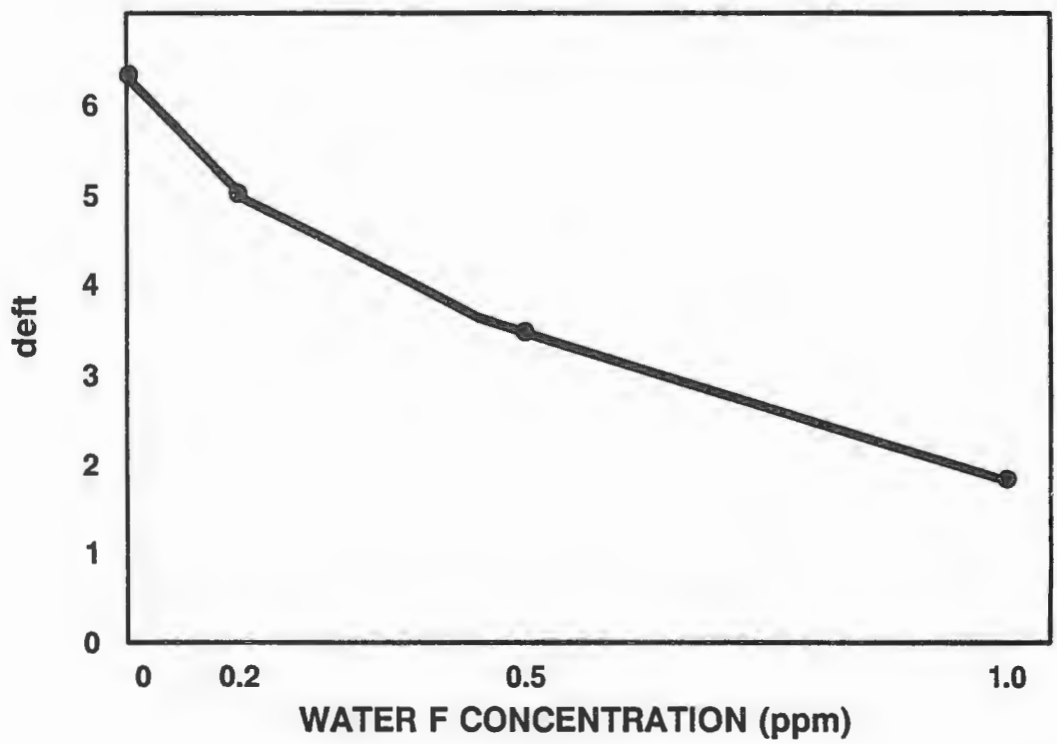

Figure 6. The relationship between caries experience (deft) in 1,038 5 year-old children living in 4 areas of N.E. England and the fluoride concentration in their drinking water (Rugg-Gunn, et al., 1981)

Figure 6. The relationship between caries experience (deft) in 1,038 5 year-old children living in 4 areas of N.E. England and the fluoride concentration in their drinking water (Rugg-Gunn, et al., 1981)

TABLE 13

Percent of children who were caries-free,
mean DMFT and DMFS scores, by community
(Szpunar and Burt, 1988)

Community	Fluoride Level (ppm)	Percent Mean Caries-free[a]	Mean DMFT	DMFS
Cadillac	0.0	55.1	1.32	1.99
Hudson	0.8	58.3	1.04[b]	1.54[b]
Redford	1.0	73.7	0.61[c]	0.87[c]
Richmond	1.2	69.8	0.58[d]	0.74[d]

[a] X^2= 14.783, df=2, $p < 0.002$.
[b] DMFT for Hudson differed for that for Redford, p = 0.0197; DMFS, p = 0.0215.
[c] DMFT and DMFS for Redford differed from those for Cadillac, p = 0.0001.
[d] DMFT for Richmond differed from that for Cadillac, p = 0.0073; DMFS, p = 0.0045.

TABLE 14

Mean DMFS scores of children
by age category and water-fluoride level
in 1980 and 1985 (Heifetz, et al., 1980)

Water Fluoride Level	8 to 10-Year-Olds		13 to 15-Year-Olds	
	1980	1985	1980	1985
Optimal	1.79	1.51	4.56	5.09
2 X optimal	1.20	1.07	2.59	2.87
3 X optimal	0.76	0.82	1.92	2.53
4 X optimal	1.41	0.85	3.38	3.86

literature from 1979 through 1983. In some studies, caries scores were compared over time as fluoride was added to the water supply; other studies were made in parallel, with the caries scores being compared in relation to water supplies with different fluoride levels. Table 15 shows the percentage reduction in DMFS scores in recent years by age-group. The efficacy was greatest for deciduous teeth (ages 3 to 5), with 30 to 60 percent less caries, followed by a 20 to 40 percent reduction for mixed dentition (ages 6 to 12), 15-35 percent for adolescents, and about the same for adults. These results

are in accord with many previous studies, as summarized by Murray and Rugg-Gunn (1982), who reviewed 95 studies in 20 countries between 1945 and 1972 and found reductions in caries scores of 20 to 85 percent.

TABLE 15

Studies published since 1983 of fluoride effectiveness and selected other studies, 1979-1983 (Newbrun, 1989)

Newbrun's Table Numbers	Number of Studies Reported	Number of Groups Compared[a]	Survey Dates	Survey Country	Age of Subjects (Years)	Percent Caries Reduction
1	2	3	1984-87	U.S.A.	3.5 - 5	30
2	8	8	1976-87	U.K.	4 - 54	3 - 60
3	9	17	1977-87	U.S.A.	6 - 16	9 - 57
4	3	3	1976-83	U.K.	9 - 12	42 - 50
5	4	7	1979-87	U.S.A.	14 - 17	8 - 37
6	3	3	1979-87	U.K.	14 - 17	25 - 40
7	1	2	1983-85	Canada	9 - 13	35 - 38
8	1	4	1984	Ireland	5 - 15	21 - 40
9	3	5	1979-85	Aus/NZ	6 - 10	29 - 55
10	5	5	1950-84	U.S./U.K. Canada	20+	20 - 88
11	2	3	1982-85	U.S.A.	60+	17 - 35

[a] For example, Newbrun's Table 1 consists of one publication by Johnson concerning two F vs. NF comparisons and one paper by Brunelle and Carlos concerning another such comparison.

In 1986 and 1987 the National Institute of Dental Research (NIDR) conducted a National Survey of Oral Health to determine the prevalence of oral disease in 39,000 U.S. children 5 to 17 years of age (Brunelle and Carlos, 1990) and to compare the results with those of similar studies conducted in 1979 and 1980. Children who resided continuously in fluoridated areas had 18 percent lower DMFS than did those who resided in non-fluoridated areas, where fluoride was also widely available through sources other than community water supplies. Among children in the non-fluoridated areas, 54.2 percent reportedly had used fluoride tablets or drops at some time in their lives. Further, the use of topical fluorides in school programs was more than twice as common (22 percent) for students in non-fluoridated than in fluoridated areas (9.8 percent). Exclusion of data from children who received tablets, drops or topical applications showed that children in fluoridated communities had a 25 percent reduction in caries scores.

EXCLUSION OF ALTERNATIVE EXPLANATIONS

In studies of dental caries in relation to fluoride exposure the scoring may be unwittingly influenced by the examiner's knowledge of the child's exposure status, particularly with respect to water fluoridation. Efforts have been made to reduce observer bias in studies of fluoridation and dental

Preventive Dentistry Demonstration Program (NPDDP) conducted in 10 areas of the United States (Bell et al., 1982; Bohannan, et al., 1984; Klein et al., 1985; Disney et al., 1990). The purpose of the study was to determine the costs and effectiveness of various school-based preventive procedures so that a foundation could be built for developing the most effective school-based preventive dental programs. In so doing, valuable information was collected on the demography of dental caries in relation to five fluoridated and five non-fluoridated areas. A comparison of combinations of fluoride modalities showed that fluoridated water provided the greatest benefit at the lowest cost (Bell et al., 1982).

In another study, the examiners were unaware by chance that the community water supply was fluoridated. They had been told that fluoride had not been added to the water supply. When the DMFT scores were found to be only half those for non-fluoridated communities, further inquiry showed that the water supply in question was naturally fluoridated (Marchment and Gray, 1986).

Ideally, a controlled clinical trial should be done in which the examiner does not know the exposure status of the person, but such a study is impossible when the entire community's water supply is fluoridated. Clinical trials, which also control for other variables are possible, however, when dental products containing fluoride are tested. Agents that have been so evaluated and proven effective are professionally applied solutions, gels, toothpastes, and mouthrinses. Furthermore, many clinical trials have been made of self-applied products. In a review of clinical trials of fluoride gels, Ripa (1989) found, on average, a 26 percent reduction in caries scores among children residing in communities without fluoridated water supplies. He noted that there is some clinical evidence for a similar percentage reduction in fluoridated communities beyond the protection already afforded by the water supply. Among persons over 18 years of age, no clinical trials have been made of fluoride gels compared with placebos (Ripa, 1989).

Clinical trials of fluoride mouthrinses conducted between 1971 and 1982 showed, on the average, a 25 to 30 percent reduction in caries scores among treated subjects as compared with controls (Carlos, 1985). The clinical importance of this level of benefit among children with low caries experience has been questioned (Leverett, 1989). However, Disney and coworkers (1990) concluded that the consistently positive results of 13 clinical trials show that fluoride mouthrinses are beneficial to caries-prone children.

The groups of persons compared in clinical trials were alike except for exposure to fluoride. Therefore, only fluoride can be credited with the reduction in dental caries. Investigators have reported similar results in clinical trials of fluoride tablets (Driscoll, 1974) and of dentifrices (reviewed by Mellberg, 1990).

Alternative hypotheses have been offered for the reduction of caries scores attributed to fluoridation. There are three principal proponents of alternative explanations. Colquhoun (1985), in studies of children in New Zealand, reported that those in a non-fluoridated lower-class district of Auckland experienced so much improvement in caries scores between 1966 and 1982 that their oral health was better than that of lower class children in fluoridated districts. His studies have been criticized for misclassification of individuals by socioeconomic status and the use of counts of dental fillings, an imperfect measure of dental caries (Burt and Beltran, 1988). Recently Colquhoun (1990) hypothesized that fluoridation may have "impeded decay reductions." Studies by other investigators found a similar reduction in dental caries among New Zealand children as in other countries (Burt and Beltran, 1988).

Diesendorf (1986) claimed that the decline in caries is due not to fluoridation, but to changes in diet, sugar intake in particular, increased use of antibiotics and improved oral hygiene and immunologic defenses. As noted below, there is a lack of evidence for the roles played by these factors.

Yiamouyiannis (1990) analyzed raw data from the NIDR survey reported by Brunelle and Carlos (1990) and, contrary to their findings, reported no differences between the fluoridated and non-fluoridated groups. Among dissimilarities in his analysis and that of NIDR are 1) his use of the tooth (DMFT) as the unit of measure, as contrasted with NIDR's use of a more sensitive measure, the tooth surfaces (DMFS), 2) his classifying fluoridation status according to the area surveyed rather than the place of residence, and 3) his lack of incorporating information on the use of supplemental fluorides. Comparison of his analysis with that of Brunelle and Carlos is impaired by these differences.

SOCIOECONOMIC AND RACIAL DIFFERENCES

The NPDDP study (described above) evaluated the DMFS scores by fluoridation status, age, and socioeconomic status (SES) quartile based on parent's education (the higher of the two), occupation, and family income (Bell et al., 1982). Table 16 shows that the lower the SES quartile, the more tooth surfaces were saved in fluoridated areas. Thus, at 12 years of age, the mean DMFS score in the non-fluoridated areas for the lowest quartile was 9.47 compared with 5.71 in fluoridated areas, a saving of 3.76 tooth surfaces. The corresponding scores for the highest quartile were 6.70 and 4.91, respectively, for a saving of 1.79 tooth surfaces. The study results also showed markedly higher DMFS scores among black children in non-fluoridated areas than in fluoridated areas (Figure 7).

TABLE 16

Mean scores for decayed, missing, or filled surfaces
by age and quartile of socioeconomic status (Bell et al., 1982)

	Ages in Years			
	6	8	10	12
Non-fluoridated				
Lowest 25%	0.68	3.30	5.39	9.47
Next to lowest 25%	0.55	2.67	4.90	8.47
Next to highest 25%	0.52	2.46	4.16	7.23
Highest 25%	0.43	2.09	3.22	6.70
Fluoridated				
Lowest 25%	0.52	2.28	3.44	5.71
Next to lowest 25%	0.49	1.92	3.74	4.97
Next to highest 25%	0.44	1.86	3.27	5.04
Highest 25%	0.39	1.89	2.87	4.91

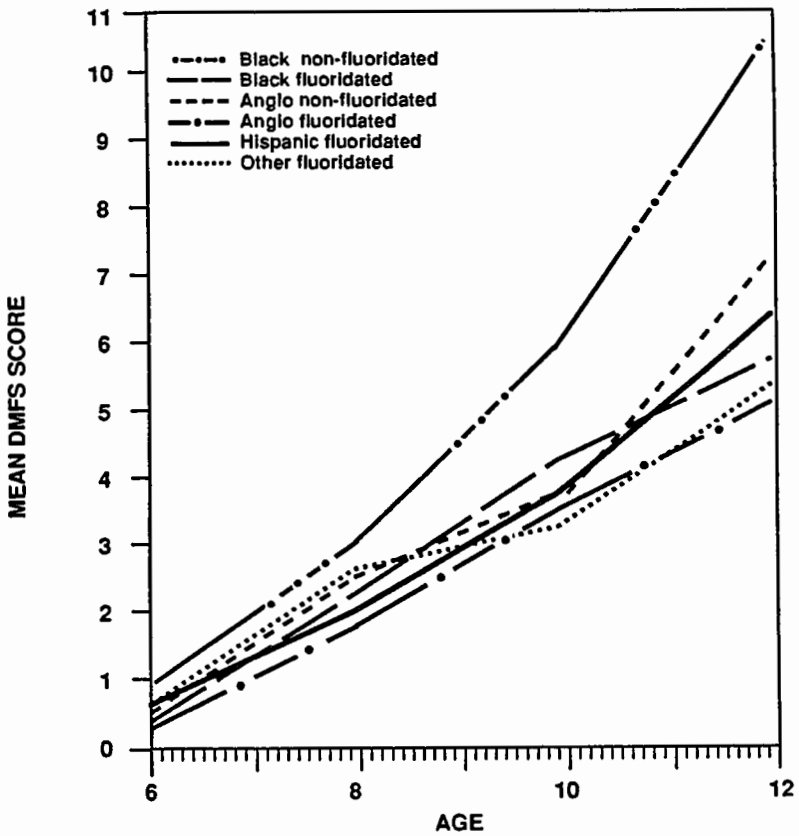

Figure 7. Mean DMFS score, by age, racial/ethnic group, and fluoridation status (Bell et al., 1982)

In England, the mean dmft scores of 5-year-olds in Newcastle, which has had fluoridated water since 1969, were compared with those of children in non-fluoridated Northumberland, according to social class (Carmichael et al, 1989). There, the Registrar General has defined social class from professionals (Class I) to laborers (Class V). The lowest social classes (IV and V) had the highest caries levels, and, in 1976, showed the greatest reduction among those in the city with fluoridated water (Table 17). A decline in caries scores was recorded in both cities in 1981, and the scores remained about the same in 1987, except for social classes IV and V, which had the smallest and most variable samples of children. Similar findings were made in Australia, where the socioeconomic status of the school was used, based on the occupation of the major breadwinner in each family (Brown et al., 1990). The data indicate that a substantial proportion of caries is prevented by fluoridation regardless of social class. Other factors are indicated by the inability of fluoridation alone to reduce the decay to the same low level among all social classes.

BIOLOGICAL PLAUSIBILITY

An important element in establishing a cause-and-effect relationship is its biological plausibility. The link between fluoride and the prevention of caries is strong. In the late 1920s, before it was known that excessive amounts of fluoride could discolor human teeth, investigators noted that it also

discolored teeth of rats given very high doses (reviewed by Sebrell et al, 1933). McClure and Arnold (1941) first described a reduction in caries among rats given 125 ppm of fluoride in their drinking water. Since then, animal experimentation has amply confirmed the observations in humans (McClure, 1962).

TABLE 17

Caries experience (1976-1987) in 5 year-old children in fluoridated (F) and non-fluoridated (NF) areas of Northeast England in three social class group[a] (Carmichael et al., 1989)

	1976				1981				1987			
Social Class	F	NF	Diff	(%)	F	NF	Diff	(%)	F	NF	Diff	(%)
I & II	2.5	3.4	0.9	(26)	1.0	2.2	1.2	(54)	1.1	2.2	1.1	(49)
III	2.4	6.0	3.6	(60)	1.5	3.6	2.1	(57)	1.7	3.7	2.0	(54)
IV & V	2.0	7.0	5.0	(71)	1.5	3.9	2.4	(61)	2.4	5.0	2.6	(51)

[a] Social class I (professional) to class V (laborer or unemployed) as defined by the Registrar General.

Fluoride in ionic form can act on the dental enamel, dentin, and cementum before the tooth erupts -- that is while it is forming, or after it erupts, through the saliva or blood or by direct contact with fluoridated water or dental products. Fluoride preferentially replaces hydroxyl ions in the crystal lattice structure of enamel, forming fluorapatite (Whitford, 1983; Ekstrand et al, 1988; Ten Cate, 1990; Silverstone, 1979 and 1988; Silverstone et al, 1981), and it is also deposited in dental plaque (Geddes and Bowen, 1990). This change in enamel increases its resistance to acid dissolution. When caries start to form, some of the mineral dissolves and releases fluoride, which enhances remineralization. Thylstrup (1990) has noted that the decline in caries attributed to fluoride is probably the result of a reduced rate of enamel dissolution, rather than the total inhibition or delay in the start of caries. Although opinion is divided about the degree to which fluoride is effective before or after the teeth erupt (Beltran and Burt, 1988; Leverett, 1989; Groeneveld et al, 1990), an effect of fluoride available at the tooth surface has gained wide acceptance. Solutions containing low concentrations of fluoride (less than 1.0 ppm) have remineralized early carious lesions *in vitro*, as seen in electron photomicrographs (Featherstone and Ten Cate, 1988, Ten Cate 1990; Silverstone, 1979 and 1988, Silverstone et al., 1981). This remineralization is not simply reverse demineralization; instead, it is a process that makes the teeth more acid resistant through the substitution of fluoride for hydroxyl, as described above (Beltran and Burt, 1988). Topical application may also contribute to remineralization of the roots of the teeth, and this effect would particularly benefit the elderly who are prone to root caries (Beltran and Burt, 1988).

TRENDS OVER TIME

When fluorides were first used in the 1940s to prevent dental caries, large reductions in caries scores (about 50 percent) were observed in fluoridated as compared with non-fluoridated communities

(Figure 4). Since the 1970s, caries scores have been declining in both fluoridated and non-fluoridated communities in Europe, the United States, and elsewhere. Kaminsky and coworkers (1990) list 14 studies of non-fluoridated areas in which the frequency of dental caries declined 17 to 60 percent over time. When fluoridated and non-fluoridated areas were taken together, a marked decline in caries scores was also found in three U.S. national surveys conducted between 1971 and 1987 (Figure 8) (Brunelle and Carlos, 1990).

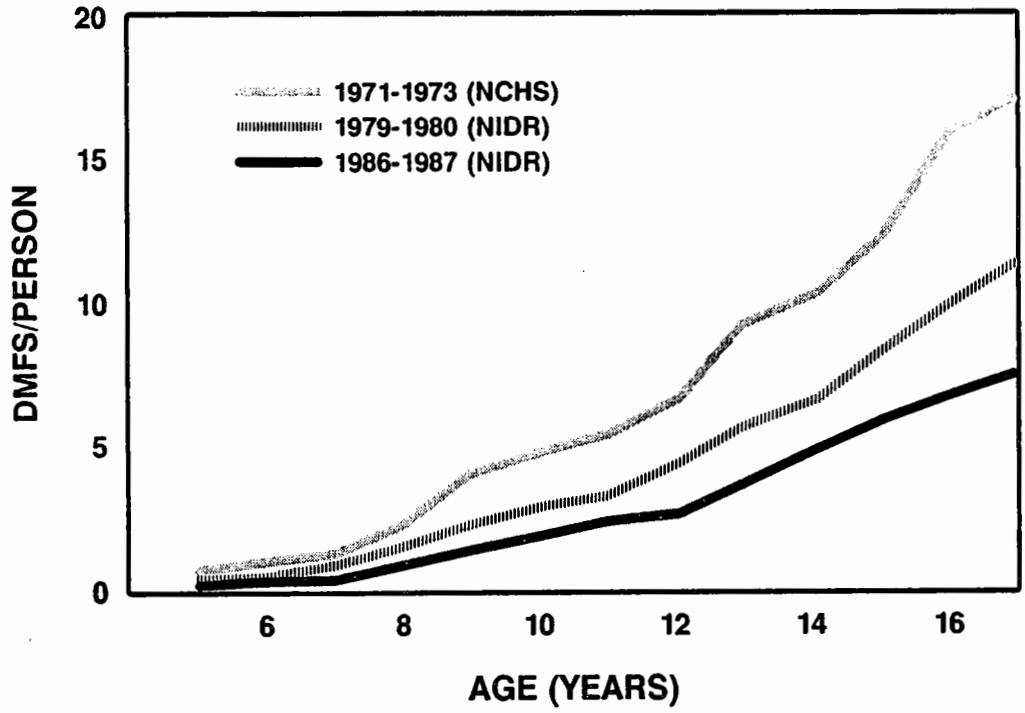

Figure 8. Age specific mean DMFS in three national epidemiological surveys, U.S. (Brunelle and Carlos, 1990)

National decreases have not occurred in all countries, notably Brazil and France where the caries scores have not changed, and Japan, Nigeria, and Thailand where the scores have increased (Renson, 1986). Various reasons for the declines over time have been suggested. Increased immunologic defenses or the wide use of new antibiotics might have contributed to the decline in dental caries, but there are no data to support these theories. Decreased exposure to caries-producing food, sucrose in particular, has been suggested. Renson found that the patterns of dietary consumption were difficult to evaluate because of national differences in reporting dietary as contrasted with commercial use of sucrose. Furthermore, the proportion of sucrose use to that of corn syrup and fructose varies from country to country.

Areas in which the water supplies were not fluoridated may have received fluoride in other ways. From a review of the literature, Renson (1986) found that the decline in childhood caries over time could not be explained by the small, scattered organized programs for a) regular mouthrinsing with fluoride; b) oral prophylaxis, and dental health education; or c) the use of fluoride gels. Fluoride tablets were used more extensively in a few countries than in others and apparently did reduce DMFT

scores. Controlled clinical trials of these products, as stated earlier, implicated fluoride as the only reasonable explanation of the observed reduction in caries scores.

Temporal declines in caries frequencies in other countries followed the increasing market-share for fluoridated dentrifices, which climbed from as little as 1 percent in 1972 to 90 to 98 percent in 1980 and 1982--in Finland, New Zealand, the United Kingdom, Australia, Ireland, Denmark, and Colombia. The market-share in the United States in 1984 was 84 percent, and in the Netherlands it was 82 percent. Countries with lower market shares included France in 1981 with 58 percent and Thailand with 55 percent in 1981, and Japan in 1983 with 15 percent. Only Japan reported rising DMFT scores from 1957 through 1981. Although its dental resources are good and sugar consumption during those years was low, Japan has a strong environmental lobby that opposes the use of fluorides except for topical applications by dentists (Renson, 1986). Fluoridated toothpaste is available there only as an import.

Thus, the global temporal decline in caries scores appears to be due in part to the spread of fluorides through dentrifices into areas with non-fluoridated water supplies. A similar decrease in dental caries occurred in fluoridated areas -- that is, in addition to the effect of fluoridated water supplies. Furthermore, food and beverages may be shipped in from areas where the water is fluoridated. In addition, some people move from non-fluoridated to fluoridated areas, and others, who live in non-fluoridated areas, spend part of the day in areas where they drink from fluoridated water supplies.

DISAPPEARANCE OF THE EFFECT WHEN THE CAUSE IS REMOVED

A valuable criterion in attributing an effect to an exposure is its disappearance when the exposure is removed. For exposure to fluoride, searches have been made for the disappearance of a beneficial effect -- an increase in caries scores when fluoridation of the water supply is discontinued. Results of at least six such studies have been reported.

The water supply of Austin, Minnesota, was fluoridated from 1952 until 1956 (Jordan, 1962). Figure 9 shows an upturn in DMFT scores 2 years after discontinuation among children who were 6, 7, or 8 years old. Among children who were 9 to 12 years old in the same community, this upturn was not observed by 1959, the last year in which the children were examined.

In Galesburg, Illinois, naturally fluoridated well-water was used from 1919 until May 1959, when the city switched to non-fluoridated water from the Mississippi River. The DMFT scores for children 14 years old were 2.01 in 1938 and 2.02 in 1958. Two years after use of the naturally fluoridated water was discontinued, these scores rose to 2.79 (Way, 1964).

In Antigo, Wisconsin, the water supply was fluoridated from June 1949 until November 1960. Dental examinations of children in the second, fourth, and sixth grades showed that less than 4 years after the use of fluoridated water was discontinued the DMFT scores had risen dramatically (Table 18) (Lemke et al, 1970).

In Wick, Scotland, the water supply was fluoridated from 1969 until 1979. Among children 5 and 6 years of age, dmfs scores by clinical examination and radiography went from 7.80 in 1979 to 13.33 in 1984 (Stephen et al, 1987). Fluoridation of the water supply had been discontinued when children in the 1984 group were infants.

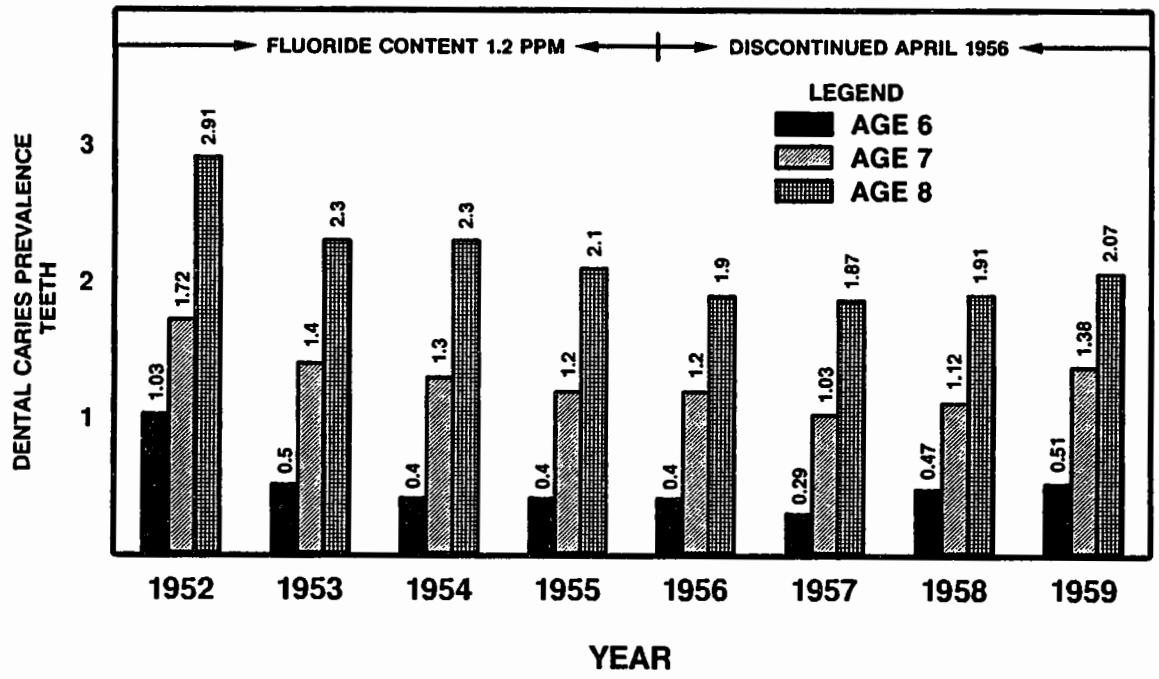

Figure 9. Comparison of DMFT rates of ages six, seven, and eight years according to year, Austin, MN; fluoridation started in 1952 and was discontinued in April, 1956 (Jordan, 1962)

TABLE 18

Scores for decayed, missing or filled teeth of children in Antigo, Wisconsin, by school grade since 1960 when fluoridation was discontinued. (Lempke et al., 1970)

Grade	1960	1964	1966
Second	0.6	1.7	2.0
Fourth	1.7	2.4	2.9
Sixth	2.4	---	4.6

In Karl-Marx-Stadt, East Germany, the water supply was fluoridated in 1959 (1.0 ppm), but from late 1970 until 1972 technical problems reduced the fluoride level during this interval to 0.2 to 0.5 ppm. From 1973 to 1977 the level was 0.65 to 0.9 ppm. In general, at each year of age from 3 to 15 the dmft and DMFT scores declined until 1974, when the caries scores rose, 2 years after the fluoride level was suboptimal (Kunzel, 1980).

The above observations of increased caries scores after fluoridation of water was stopped were made before the recent substantial temporal declines in dental caries were documented. Since then, a study

similar to the earlier ones was made in Scotland. In 1983, fluoridation was stopped in the town of Stranraer. In 1980 DMFT scores among 10-year-olds in Stranraer had been compared with those for the non-fluoridated town of Annan. The two locales were compared again in 1985 and 1988, 2 and 5 years after fluoridation in Stranraer was stopped (but fluoride-containing dentifrices were widely available) (Figure 10). At a time of general temporal decline in caries scores, Stranraer's children experienced an increase in caries after fluoridation was stopped. For non-fluoridated Annan, however, caries scores showed a continued temporal decline that is bringing the scores for the two towns together.

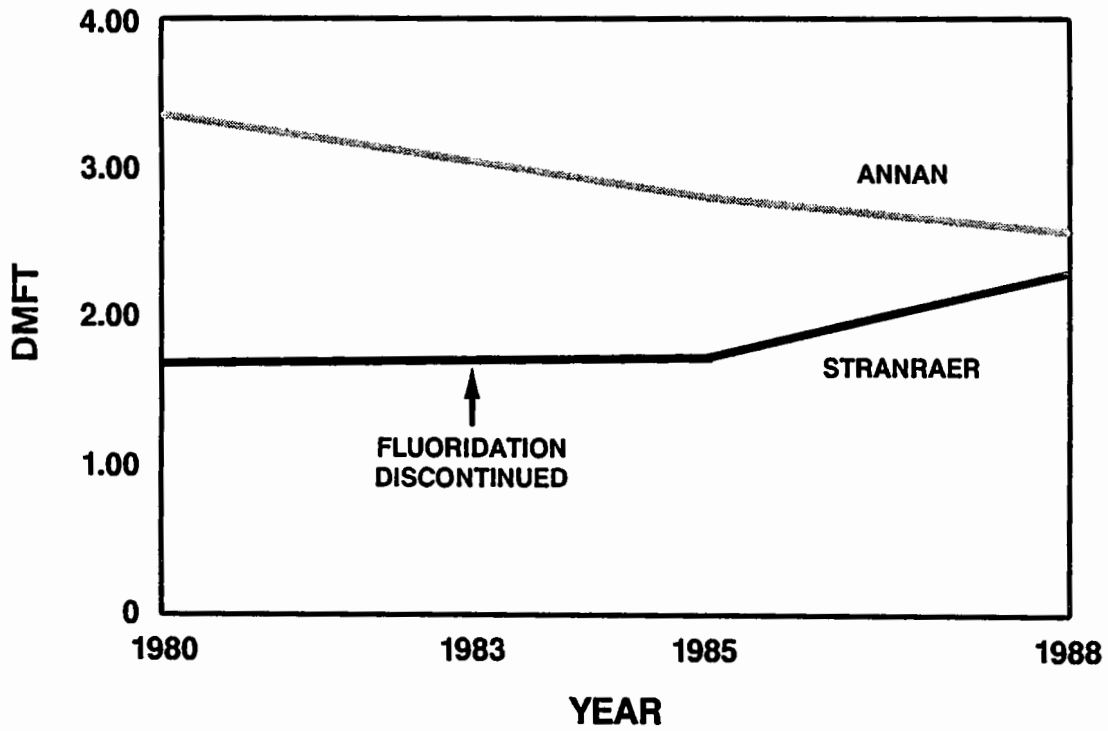

Figure 10. DMFT scores in Annan Scotland (never fluoridated) and Stranraer (Fluoridated until 1983), in 1980, 1985, and 1988 (Attwood and Blinkhorn, 1989 and 1990)

THE COST OF FLUORIDE MODALITIES IN PUBLIC HEALTH PROGRAMS

Water fluoridation has not only a health benefit but also a cost benefit, to which brief reference is made here. At a workshop at the University of Michigan (1989), five subgroups individually estimated the cost-effectiveness of fluoride modalities in preventing dental caries. Table 19 shows the estimated annual cost of providing fluoride regimens per person served. The cost of fluoridation decreased as the size of the community increased, and the savings, as compared with the costs of restorative dentistry, are substantial. As a frame of reference, in the United States the average cost of simple restorations of teeth ranges from $30 to $45.

TABLE 19

Estimated Annual Cost of Fluoride Regimens
Per Person Served in Public Health Programs

Method	Cost
Community Water Fluoridation	
>200,000 Persons	$0.12 - .21
10,000-200,000 Persons	.18 - .75
National Weighted Average	.51
School Water Fluoridation	3.55 - 4.73
Fluoride Supplements	.81 - 5.40
Fluoride Mouthrinse in Schools	.52 - 1.78

SUMMARY

The reduction in dental caries among persons exposed to fluorides fulfills all the criteria for a causal relationship: an association was found with a dose-response effect, the findings were replicated under a great variety of circumstances by different investigators, alternative explanations and observer bias have been excluded, the findings are biologically plausible, and the effect, prevention of dental caries, disappeared when the cause, fluoridation, was removed. The most recent data available, up to 1987, continue to show that the fluoridation of water supplies substantially reduces the scores of dental caries. The decline over time in differences in caries scores between fluoridated and non-fluoridated areas is due in part to the increased availability of fluorides in non-fluoridated areas, as in toothpaste and other vehicles for fluorides.

The need for research into the best regimens for preventing caries continues as circumstances change over time. Refined, more specific protocols must be developed concerning the best ways to provide fluoride to different populations, taking into account their particular age and socioeconomic groups.

The reduction in caries through water fluoridation diminishes the need for restorative dentistry, with its cost in dollars, pain, loss of teeth, time away from school or work, and risks related to anesthesia. A particular advantage of fluoridated water is that it reaches rich and poor alike. It provides the greatest benefit to those who can least afford preventive and restorative dentistry.

THE CONTEMPORARY USE OF FLUORIDES IN CARIES PREVENTION

When H. Trendley Dean first quantified the caries-preventive benefits of fluoride in drinking water, the only persons with substantial exposures to fluoride were those living in areas where drinking water contained levels of naturally occurring fluoride (more than 0.7 ppm) or those few persons who were exposed occupationally. Thus, it was easy to distinguish between those who were exposed to fluoride and those who were not. Moreover, striking differences were evident in caries scores between persons exposed or not exposed to fluoride in drinking water.

Since the advent of fluoridation, new methods of using fluoride have given both children and adults increased opportunities for fluoride exposure. Toothpaste with fluoride was introduced in the 1950s, fluoride supplements and professional fluoride treatments became popular in the 1960s, and mouthrinses containing fluoride became available to public health programs in the early 1970s and to the general public in the 1980s. This proliferation of products was stimulated by the caries preventive benefits that had been demonstrated for fluoride and by the desire of health officials to make these benefits available to those without fluoridated drinking water or to those who needed additional levels of protection from dental caries.

Common practice historically has dictated that the more fluoride protection one received, the better. Even where optimal levels of systemic fluoride have been available through drinking water or supplements, dental professionals have generally recommended the additional protection of dentifrices containing fluoride, professional fluoride treatments, and fluoride mouthrinses.

Recently, however, some have questioned these traditional approaches to the use of fluorides in clinical and public health practice. With average caries scores in the permanent teeth of children declining, is it still useful to recommend combined forms of fluoride for persons, especially those served by public health programs? Emerging evidence about increases in dental fluorosis among children in some communities makes it prudent to reconsider current recommendations for the use of fluorides (Pendrys and Stamm, 1990). Furthermore, those who live in non-fluoridated communities have partial access to the benefit of fluoridated drinking water through consumption of commercial beverages or foods prepared with fluoridated water, or through working or going to school in areas where the drinking water is fluoridated (Clovis and Hargreaves, 1988).

The need to balance the caries-preventive benefits of fluoride against the potential cosmetic concerns of the more advanced forms of dental fluorosis is just as important today as in Dean's time. Still, the sources of fluoride are more varied today, and fluoride's control more complicated. In Dean's time, if the fluoride content of the water supply could be established at the appropriate level, no conscious choices about the use of fluoride were required by individuals or professionals. By contrast, there are now many participants in decisions about fluoride use: dentists decide whether topical fluoride treatments are offered; dentists and physicians decide whether to prescribe fluoride supplements or recommend other fluoride treatments; adults decide whether to use a toothpaste or mouthrinse containing fluoride and whether children will or will not be supervised in their use of available fluoride products; and industry and government officials decide what fluoride-containing products will be available, what their constituents will be, and what instructions will be provided for their use.

Without doubt, the variety of available fluorides has contributed greatly to reducing children's average dental caries scores (DMFS) from more than 10 in the 1940s down to slightly more than 2 in the 1980s (Carlos and Wolfe, 1988). The results of hundreds of clinical or community trials have shown that various fluoride regimens reduce dental caries (University of Michigan, 1989). These include: community and school water fluoridation; professionally and self-applied fluoride pastes, rinses, gels, and solutions; fluoride tablets and drops; and toothpastes and mouthrinses containing fluoride. Although the results from all studies are not constant, a number of clinical trials and community demonstrations have established that people can further reduce dental decay by using multiple forms of fluoride (Horowitz, 1989; Ripa, 1989; Leverett, 1989). These additional benefits are not simply additive, however. At some point, additional percentage reductions in decay may offer insignificant health benefits on a population basis.

The important challenge for public health officials and clinicians, given multiple options for fluoride exposure, is to employ the most appropriate uses of fluorides, both individually and in combination. In doing so they should: set goals for caries reductions; attempt to maximize the efficiency of fluoride use; and attempt to minimize the risks of dental fluorosis. Work in these areas has already started:

- Many individuals, health professionals, and health organizations and programs (e.g. health departments, professional organizations) are working to prevent decay. The adoption of local water fluoridation and other fluoride-based caries preventive programs continues, as does the promotion of personal oral health care practices and the professional use of fluoride-based preventive techniques. At the local, State, and national level, targets are being set for reduced dental caries scores in populations, in the proportion of individuals who never experience caries and/or the average level of caries experienced by individuals (Healthy People 2000, Model Standards).

- In May 1989, at a conference at the University of Michigan, maximizing efficiency in the prevention of dental caries was addressed (Burt, 1989). Although several fluoride modalities were found to be effective in reducing caries in children and adults, the relative costs of these benefits diverged widely. The actual risk of dental caries in particular groups (for example children, adults, the elderly, and those in institutions) was an important factor in the benefit-cost relationship. Conference participants concluded that water fluoridation provided by far the most protection for the resources expended, at a national average cost of only 51 cents per person annually.

- In March of 1989, at a conference in Pine Mountain, Georgia, minimizing the risk of dental fluorosis associated with fluoride use was addressed. Dental researchers agreed that the milder forms of dental fluorosis have increased among children in some U.S. communities (Pendrys and Stamm, 1990) (See pages 103-115). Although this increase has not created concern for the overall health or well-being of individuals, it points to the need for clear reassessment of the current uses of fluoride in caries prevention.

- In Spring, 1991, the University of North Carolina will convene a conference to address the multiple systemic fluoride exposures possible, towards making recommendations for optimizing caries-preventive benefits and minimizing risks of dental fluorosis.

Increased levels of fluorosis, although generally associated with lower levels of dental caries, are not necessary sequelae. By eliminating inappropriate uses of fluoride, people can reduce the risk of fluorosis without significantly increasing the risk of dental caries. The following are examples of such inappropriate uses of fluorides:

- The use of fluoride supplements by people in fluoridated communities (Brunelle and Carlos, 1989; Kumar et al, 1989; Williams and Zwemer, 1990).

- The use of inappropriate dosages of dietary fluoride supplements[1] in communities without optimal fluoridation (Levy et al., 1987; Kuthy and McTigue, 1987).

- The unsupervised use of dentifrice containing fluoride by children younger than 6 years of age, who cannot determine the proper amount of toothpaste to use, and who may swallow toothpaste while brushing rather than spitting it out (Smith and Ekstrand, 1988; Drumond et al., 1990).

- The unsupervised use of a fluoride mouthrinse at school or home by children younger than 6 years of age, which has resulted in some children swallowing fluoride mouthrinse (Carlos, 1985).

Although not necessarily inappropriate, some current uses of fluoride may be questioned:

- Many people do not need to use additional forms of fluoride (that is, rinses or professional fluoride applications) beyond the combination of water fluoridation or dietary supplements and fluoride toothpaste, when their risk of caries is believed to be low.

- Are adequate instructions available from manufacturers, health professionals, and public programs on the proper use of individual and combined fluoride modalities? Clear instructions on products intended for use by children are especially critical.

- Are fluoride products for children available in concentrations and amounts that would confer caries protection while minimizing the chances for dental fluorosis?

The development of toothpastes with a lowered fluoride concentration and the availability of dose limiting dispensers could be of value.

- On the basis of the observed prevalence of dental fluorosis, Pendrys and Stamm have suggested that the supplementation schedules for children older than 2 years of age may be too high (Pendrys and Stamm, 1990). Practitioners should also consider whether bottled water is being used and what its fluoride content is when deciding whether to prescribe fluoride supplements and at what dosage (Stannard et al., 1990).

SUMMARY

Those who use or recommend disease prevention techniques to individual patients or populations need to take into account: the risk of the disease to an individual or group, the benefits to be achieved by using a particular technique, the alternative techniques that may be available to prevent or control the

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[1]The American Academy of Pediatrics and the American Dental Association have developed fluoride supplement schedules. Different dosages up to 1.0 mg daily are recommended on the basis of two factors: 1) the degree of fluoride deficiency in the community water supply, and 2) the age of exposed children (Table 8, page 30). A low dose could result in denying the full caries-preventive benefits, whereas a high dose could result in an increased risk of dental fluorosis.

disease, the possible adverse effects, the costs of various techniques, and the practicality of usage. How and when to use fluorides are difficult decisions for health professionals and the public to make, given the number of factors involved.

Establishing consensus guidelines for the optimal use of fluorides in the prevention of dental caries would be a significant aid to individual health professionals in determining the most appropriate uses of fluorides for their patients. Such guidelines could assist dental product manufacturers, professional organizations, government agencies and individual practitioners in communicating with the public about the effective and safe use of fluoride-containing products.

Information is available to the public about the fluoride content of drinking water and dental products as shown in Table 20.

TABLE 20

Sources of information on fluoride content of water and dental products

Fluoride Source	Source of Information on Fluoride
Community drinking water supply	• Community water authority (local) • State health department
Private drinking water supply (well or cistern)	• State health department • University laboratory • Private laboratory
Bottled water	• Bottled water producer
Topically applied fluoride dental products: • Toothpastes • Mouthrinses • Professionally or personally applied gels and solutions	• Dentists • Product manufacturers (labels and direct inquiry) • Food and Drug Administration (FDA)
Systemic Fluoride supplements: • Tablets • Drops • Lozenges	• Dentists • Physicians • Manufacturers (labels and direct inquiry) • Physicians' Desk Reference • FDA

OSTEOPOROSIS

For nearly 30 years fluoride (primarily in the form of sodium fluoride) has been used experimentally in the United States and therapeutically in other countries to treat osteoporosis. One benefit of fluoride treatment has been observed by all investigators using modern bone densitometry. The mineral density of the spine (primarily trabecular bone) increases by as much as 35 percent over four years (Riggs et al., 1990) as a group median. The increase is continuous and linear over the treatment period. Some studies (Riggs et al., 1990) have shown small decreases in cortical bone (e.g., at the mid-shaft radius), causing some investigators to express concern that the bone mass increase is effectively "stolen" from other portions of the skeleton. It has become clear that an adequate calcium and vitamin D intake are necessary to avoid inducing osteomalacia and "stealing calcium" from other skeletal sites. Because of concern regarding the quality of the newly formed fluoride-induced bone, fracture incidence is recognized as the most important outcome measure in studies of fluoride treatment for osteoporosis.

Until the last few years, most of the clinical studies of fluoride were open in design and lacked adequate controls and random design. The minimum dose with a demonstrable effect on bone mass is about 40 mg NaF daily (Heaney et al., 1989). Based on the success of these uncontrolled studies, fluoride has been accepted as a treatment for osteoporosis by the licensing bodies of eight European nations and used widely throughout most other countries. Until recently there have been few clinical trials with a random, controlled design to evaluate the safety and efficacy of fluoride treatment of postmenopausal osteoporosis, that may lead to vertebral crush fractures of the spine.

Dambacher et al., (1986) reported a small clinical trial (15 patients treated with 80 mg per day slow-release NaF and 14 patients treated with placebo). After the first year, the treated group had significantly more vertebral fractures than the placebo group. The difference in the fracture rate remained constant and significantly different in years two and three. The vertebral bone density increased, while the total bone density remained constant, during the study. In the treated group 47 percent experienced osteoarticular pain, which was attributed to stress fractures. No controls experienced these symptoms. No gastric distress was observed. Based on prior studies by these authors, no calcium or vitamin D supplements were added to the treatment program.

Mamelle (1988) described a large and complex trial that compared NaF to a variety of other treatments that included combinations of calcium, vitamin D and calcitonin. NaF (50 mg daily) was used for 257 patients and the other therapies for 209 patients. Treatment was administered according to a common protocol by 94 physicians in France. In the first year the mean number of vertebral fractures was not significantly different between groups. In the second year fewer fractures occurred in the NaF treated group. The composite data for both years shows a trend favoring the NaF group, but no significant difference. Analysis of individuals experiencing one or more new fractures demonstrated a significant reduction in the NaF group. There were no differences between the groups in the incidence of non-vertebral fractures and gastric distress. Ankle and foot pain were significantly worse in the NaF group; however, appropriate tests for stress fractures were not conducted.

In response to a Request for Applications by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, two clinical trials on the fracture efficacy of NaF in osteoporosis were conducted at the Mayo Clinic (Riggs et al, 1990) and the Henry Ford Hospital (Kleerekoper et al, 1989). The trial was designed as a double blind, randomized, placebo study of 75 mg per day NaF (37 mg fluoride ion). Both groups of patients received 1500 mg of calcium per day. Rigorous inclusion and exclusion

criteria limited the study to well-defined osteoporosis with no confounding metabolic conditions. The Mayo Clinic study group totaled 202 enrolled patients, while the Henry Ford study had 84 patients. The protocols were the same, but recruitment and retention problems led to much less statistical power in the Henry Ford study.

In the Mayo Clinic study (Riggs et al, 1990), which measured bone mass as well as vertebral fractures, the bone mineral density increased by 35 percent ($P < 0.0001$) in the lumbar spine, increased by 12 percent in the femoral neck ($P < 0.0001$), but decreased by 4 percent ($P < 0.02$) in the radius (predominately cortical bone). There was no significant difference in the rate of new vertebral fractures between the NaF and placebo groups (446 and 525 per 1000 person-years, respectively). In the NaF group the fracture rate was much higher in the first year than in subsequent years. In the Henry Ford study, (Kleerekoper et al, 1989) the occurrence of new vertebral fractures was not significantly different between the NaF and placebo groups (733 and 529 per 1000 person-years, respectively). Again, vertebral fracture rates were higher in the NaF group during the first year of the study.

In both studies gastrointestinal side effects were significantly greater in the NaF group than the placebo group. Hip fractures did not occur at different rates between groups in either study. The Henry Ford study demonstrated on bone biopsy that 17 percent of the NaF group had mineralization defects, while none was present in the controls. Episodes of lower extremity pain were significantly more common in the NaF group compared to the placebo group. Both studies attributed this finding to the presence of stress (or incomplete) fractures. In the Mayo Clinic study, all nonvertebral fractures (both complete and incomplete) were shown to be significantly higher in the NaF group.

In 1988, an International Workshop on Fluoride and Bone (Heaney 1990, personal communication) met to discuss the state-of-knowledge and develop a consensus on the use of fluoride. Fluoride is the only agent known to produce a continuous gain in vertebral bone mass. Most of the studies on treatment of osteoporosis with fluoride considered at the workshop were either uncontrolled or did not include fracture end-points. Where fracture incidence was measured, most showed effectiveness in reducing vertebral fractures. The Mamelle study, while equivocal, did indicate a benefit especially in the second year. After considering the generally positive experience with NaF in Europe, where the drug is approved, the Workshop Consensus approved NaF for osteoporosis therapy at moderate levels (15 to 25 mg per day fluoride ion, plus calcium and vitamin D) with careful patient management to monitor and control for side effects (Kraenzlin et al. 1990). After the workshop was completed the results of the studies at the Mayo Clinic and Henry Ford Hospital became available. These studies were clearly unable to demonstrate a vertebral fracture reduction with NaF. Side effects were statistically significant and, in the case of total non-vertebral fractures, of considerable concern to the investigators. While these recent studies were well designed, critics claim that the higher NaF dosage may have caused the negative results. It is likely that gastric side effects could be reduced with lower dosages or other delivery forms (slow-release or coated). There is no evidence that fluoride efficacy in reducing vertebral fractures would be different with a lower dosage.

Based on these recent data FDA convened an Advisory Committee in the fall of 1989. The Committee deliberated on a number of questions posed by FDA. The final position of the panel was that NaF has not been shown to be effective in decreasing the incidence of vertebral fractures resulting from accompanying osteoporosis at this time. Because of the value of NaF in increasing vertebral bone mass, the panel encouraged further research.

In subsequent epidemiologic studies investigators sought to correlate the presence of fluoride in drinking water with protection against fractures. One study showed fewer hip fractures in a fluoridated community (1 mg/L) compared to a similar community with only trace levels of fluoride in the water (Simonen and Laitinen, 1985).

Recently, an evaluation was made of the incidence of hip fracture rate in two communities, one with 4 mg/L of fluoride in the water, the other with 1 mg/L. The relative risk of fractures of the wrist, spine and hip in the community with the high fluoride was 2.1 when compared with the other community (Sowers et al., 1990, Am J of Epidemiol, in press). Refer to page 94 for further discussion of this paper.

SUMMARY

For nearly 30 years, the administration of fluoride has been used as an experimental therapy to treat osteoporosis, but until recently there were no carefully controlled studies to evaluate fluoride as a treatment. Two recent clinical trials did not show significant reductions in bone fracture rates when fluoride was used as a treatment. An FDA advisory panel has concluded that fluoride therapy has not been shown to be effective in decreasing the incidence of vertebral fractures. In several countries the therapeutic administration of fluoride is approved for osteoporosis.

HEALTH RISK ASSESSMENT OF FLUORIDE

INTRODUCTION

In the following sections, the effects of fluoride toxicity in animals and in humans are reviewed. In these studies, different forms of fluoride have been administered, and the effective dose to the individual may vary. Table 21 provides a conversion between the various forms of fluoride (sodium fluoride or fluoride) and the units of dosage (parts per million, micromole per liter, and milligram per liter). The fluoride dose is sometimes administered in terms of body weight (milligrams per kilograms of body weight). In all cases, we will report the administered dose as described by the author. In this report fluoridated areas refer to those communities where the drinking water supplies is at the optimal level 0.7 to 1.2 ppm either naturally or adjusted, whereas, non-fluoridated areas are those below the optimal level usually less than 0.3 ppm. The amount of fluoride present in those communities designated non-fluoridated will depend on the specific details in each study described by each author. The reader can use Table 21 to convert the dose to the appropriate units (Dawes and Ten Cate, 1990).

TABLE 21

Conversion table for form of fluoride

SOLUTION CONCENTRATION

Fluoride (F) (ppm) or (mg/L)	Fluoride (umol/L)	Sodium Fluoride (NaF) (ppm) or (mg/L)
0.019	1.0	0.042
0.038	2.0	0.084
0.5	26.3	1.10
1.0	52.6	2.21
10.0	526.3	22.1
100.0	5250.0	220.9
250.0	13160.0	552.7
1000.0	52630.0	2209.2

This review focuses on the risks associated with chronic exposure to fluoride, because risks from these exposures are more relevant to the exposures encountered with fluoride in drinking water, food, and dental preparations. Thus, this document will not review the literature on acute high-dose toxicity from intentional and unintentional administration of fluoride products and from occupational exposures, which especially affects the gastrointestinal and respiratory tracts.

STUDIES INVESTIGATING BONE AND OTHER SKELETAL EFFECTS

ANIMAL STUDIES

The principal sources of fluoride exposure to animals are: 1) pollution from industrial processes and the contamination of forage; 2) rock phosphate or bone meal containing fluoride in the diet; and 3) drinking water (Shupe et al., 1982).

Excessive levels of fluoride can produce abnormalities in both teeth and bone. Skeletal fluorosis observed in animals is chronic and progressive; it is governed by several factors, including: 1) the amount of fluoride ingested; 2) the duration of ingestion or exposure; 3) the type and solubility of the

fluoride ingested; 4) age at the time of the first exposure; 5) the level of nutrition; 6) stress; and 7) the individual's response.

Skeletal fluorosis in animals is often associated with loss of appetite, decreased lactation, and shifting leg lameness. Others have reported diarrhea, reproductive failures, anemia, and stunted growth. (Krook and Maylin, 1979). Most of the scientific reports on skeletal fluorosis in animals focus on experimental studies in laboratory rodents and on natural and experimental studies in cattle, sheep, mink, rabbits, chickens, and horses. The sensitivity of these animals to fluoride toxicity based upon normal acceptable dietary intakes is highly variable, as shown in Table 22. Consequently, data from interspecies comparisons (including humans) must be interpreted with this in mind.

TABLE 22

Normal dietary levels of fluoride by selected animal species

Species	Normal (F) Intake
Dairy Cattle	up to 30 ppm
Beef Cattle	up to 40 ppm
Sheep	up to 50 ppm
Swine	up to 70 ppm
Turkeys	up to 100 ppm
Chickens	up to 150 ppm

(Adapted from Buck and Osweiler, 1980)

Skeletal fluorosis has been observed in various animals including: swine (Burnell et al., 1986; Wenzel et al., 1984); cattle, deer, and mink (Shupe and Olson, 1982a, 1982b; Suttie et al., 1958, 1960, 1985; Greenwood et al., 1964; Dale and Crampton, 1955; Krook and Maylin, 1979); chickens, (Lundy et al., 1986); and horses (Shupe and Olson, 1971). Skeletal fluorosis within rodents, dogs, and cattle deserves more detailed discussion because these species have been studied more extensively.

Rodents: Experimentally, skeletal fluorosis has been produced in a variety of laboratory species, but most frequently in the rat and mouse (Baylink et al., 1970; Qiu et al., 1987; Ream, 1981; Weber and Reid, 1969; Stookey et al., 1963; Suttie and Phillips, 1959; Zipkin and McClure, 1952). Studies in rats given excessive amounts (50 or 80 mg/L) of fluoride in the drinking water for 8 months showed inhibition of bone formation and osteomalacia (Qiu et al., 1987). Other investigators have reported defective collagen synthesis (Usla, 1983) and decreased bone growth (Harrison et al., 1984) in rats given 14 and 41 mg/kg/day for 30 days and 5 weeks, respectively. Diets high in fat have been reported to increase deposition of fluoride in bone and, thus, to enhance toxicity (Miller and Phillips, 1955).

Canines: Studies in dogs given 0.7 mg/kg/day sodium fluoride for 6 months resulted in histological bone lesions of skeletal fluorosis, with toxic effects on osteoblasts and osteoclasts (Snow and Anderson, 1986).

Cattle: In studies of bovine skeletal fluorosis, various investigators describe bone changes ranging from exostotic and osteopetrotic lesions to osteomalacia lesions of the bone. Lesions were described in metatarsals and metacarpals, femurs, patellas, ribs, vertebrae, and mandibles. In the metatarsal and metacarpal bones, changes were more pronounced and occurred earlier, according to the investigators, because of increased mobility and physical stress. Joints may be involved in long-term chronic fluorosis, with bridging, fusion, and ankylosing spondylitis and with degenerative arthrosis and inflammatory arthritis. Ligament ossification has also been observed in long-term cases. Microscopic

and morphologic changes include loss of collagen birefrigency, failure of bone resorption by osteolytic osteolysis, numerous osteoid seams, and atrophy of osteoblasts and osteonecrosis. (Shupe et al., 1963a, 1963b, 1964; Shupe and Alther, 1966; Shupe, 1980; Suttie et al., 1960; Greenwood et al., 1964; Dale and Crampton, 1955; Krook and Maylin, 1979).

HUMAN STUDIES

Fluoride has a complex dose-related action on bone. Both bone metabolism and mineralization are affected by fluoride ions. Fluoride increases bone formation mainly through a stimulation or recruitment of proliferative cells to increase the number of osteoblasts. Fluoride may decrease resorption indirectly by enhancing production of mineral tissue that is less soluble (Grynpas, 1990). Direct effects are also possible. Fluoride is incorporated more readily into mineralizing new bone rather than existing bone. This factor may explain fluoride's seemingly larger effect on trabecular bone that has more rapid turnover.

Fluoride increases the stability of the crystal lattice in bone, by substitution of fluoride for hydroxyl ion in the crystal lattice, but makes bone more brittle. In one case report (Carter and Beaupre, 1990) based on a 45-year-old man with endemic fluorosis, compressive strength of bone was increased by 25 percent, while tensile strength decreased by 40 percent. Mechanical failure and fracture propagation are influenced strongly by tensile strength. Long bones and individual struts of trabecular bone are subjected to tensile loads. At the structural level, it is a complex and difficult problem to determine the optimal balance between the increase in bone mass and the decrease in tensile strength. In a recent unpublished study, the effect of fluoride on bone strength was found to be biphasic with an optimum strength at 10 to 20 percent substitution (Heaney, 1990 personal communication) of fluoride in the crystal lattice.

Human Skeletal Fluorosis

The preclinical and three clinical stages of skeletal fluorosis (Smith and Hodge, 1979) are described in Table 23, along with reported correlations of accumulated fluoride in bone ash to the osteosclerotic phase (Franke et al., 1975; Schlegal, 1974). The earliest bone changes associated with skeletal fluorosis are radiographic enlargements of trabeculae in the lumbar spine. These preclinical findings have been associated with bone ash fluoride concentrations of 3,500 to 4,500 ppm. Singh and Jolly (1970) reported that osteosclerosis in the pelvis and vertebral column, coarse trabeculae, and diffuse increased bone density of clinical phase I are seen in industrial cases but rarely are reported in areas where fluorosis is endemic. Most of the latter cases show the more severe changes of phases II or the crippling fluorosis of phase III. Bone changes observed in human skeletal fluorosis are structural and functional, with a combination of: 1) osteosclerosis, the predominant lesion in fluorosis patients who have an adequate dietary intake of calcium; 2) osteomalacia, which predominates in patients who have a marginal or suboptimal dietary intake of calcium; 3) osteoporosis and exostosis formation of varying degrees; and 4) secondary hyperparathyroidism in a proportion of patients (Krishnamachari, 1986).

Boivin and coworkers (1988) reported measuring bone fluoride content from iliac crest bone to determine the degree of fluoride retained in bone and, over time, the amount of fluoride eliminated. Subjects with skeletal fluorosis primarily of an industrial etiology, had bone fluoride values over 0.50 percent of bone ash by weight, and the values were always statistically higher than the highest control value, 0.10 percent ($p < 0.001$). Fluoride retention in bone appeared to be higher in cases of greater fluoride exposure, even if that exposure was difficult to define precisely.

The total quantity of fluoride ingested is the single most important factor in determining the clinical course of skeletal fluorosis (Krishnamachari, 1986); the severity of symptoms correlates directly with the level and duration of exposure (Fisher et al., 1989). As most commonly reported for a person to develop crippling skeletal fluorosis, he or she must ingest 20 to 80 mg/day of fluoride (the equivalent to 10 ppm fluoridated water) for 10 to 20 years (Hodge and Smith, 1965; Hodge, 1979; WHO, 1984;

TABLE 23

Preclinical and clinical stages of human skeletal fluorosis
and correlation of bone ash fluoride concentration

OSTEOSCLEROTIC PHASE	ASH CONCENTRATION (mg F/kg)
Normal Bone	500 - 1,000
Preclinical Phase asymptomatic; slight radiographically-detectable increases in bone mass	3,500 - 5,500
Clinical Phase I sporadic pain; stiffness of joints; osteosclerosis of pelvis & vertebral column	6,000 - 7,000
Clinical Phase II chronic joint pain; arthritic symptoms; slight calcification of ligaments; increased osteosclerosis/cancellous bones; with/without osteoporosis of long bones	7,500 - 9,000
Phase III: Crippling Fluorosis limitation of joint movement; calcification of ligaments/neck,vert. column; crippling deformities/spine & major joints; muscle wasting; neurological defects/compression of spinal cord	>8,400

(Adapted from: Smith and Hodge, 1979; Franke et al., 1975; Schlegal, 1974)

National Academy of Science, 1980). For endemic, tropical areas, the level of clinical effect for skeletal fluorosis is less certain (National Academy of Science, 1980); Singh and Jolly (1970) stated that it may not be possible to determine the average minimal dose of fluoride needed to produce skeletal fluorosis, because of individual variations and the crude level of water analysis in many of the endemic areas.

For almost 40 years, investigators in the United States have searched for evidence of skeletal fluorosis. Radiographic changes in bone indicative of skeletal fluorosis, changes in bone mass, and effects on skeletal maturation were not observed at water fluoride concentrations of 1.2 mg/L for 10 years and from 3.3 to 6.2 mg/L for a lifetime (Hodge and Smith, 1981; Sowers et al., 1986; Schlesinger et al., 1956; McCauley and McClure, 1954). In a survey of 170,000 radiographs of patients living in Texas and Oklahoma with water fluoride levels between 4 and 8 mg/L, Stevenson and Watson (1957) found 23 cases of radiographic osteosclerosis, but no evidence of skeletal fluorosis.

Skeletal fluorosis is highly variable in its clinical severity among individuals living in the same environment and exposed to the same risk of fluoride ingestion (Krishnamachari, 1986). In the past 30 years, only five cases of crippling skeletal fluorosis have been reported in the literature in the

United States (Sauerbrunn et al., 1965; Goldman et al., 1971; Fisher et al., 1981 & 1989; Bruns and Tytle, 1988). Yet, over several generations many individuals in U.S. have consumed water containing high natural levels of fluoride, without demonstrating signs or symptoms of skeletal fluorosis. The unequal world-wide distribution of this disorder generally has been ascribed to unidentified dietary factors that render the skeleton more or less susceptible. Whitford (1989) suggests that differences among populations with respect to fluoride metabolism and fluoride balance are responsible. Acid-base status and the concomitant changes in urinary pH (Whitford and Reynolds, 1979) are the most important contributors to population variation.

In the five cases of crippling skeletal fluorosis in the United States, retrospectively assessed, exposure to natural levels of fluoride in drinking water ranged from 3.9 to 8.0 mg/L. All possible confounding factors were not addressed. Two of these cases were associated with daily consumption of up to 6 liters of water containing fluoride levels of 2.4 to 3.5 ppm in one case and 4.0 to 7.8 ppm in the other (Sauerbrunn et al., 1965; Goldman et al., 1971). Large quantities of tea, itself high in fluoride, were consumed daily as well. The total fluoride intake was estimated to be 15 to 20 mg/day for 20 years. The third case involved a 65 year old Mexican immigrant, who had resided in the United States only a few months; the patient sought medical care because of symptoms of spinal cord compression (Fisher et al., 1989). Radiographic skeletal findings for this individual were consistent with osteosclerotic changes suggestive of skeletal fluorosis. The urine fluoride content was 3.39 mg/L, with an ashed vertebral bone fluoride content of 1,900 ug/g of bone. Analysis of the drinking water from a village in northern Mexico where the patient lived showed a fluoride level of 3.91 mg/L, and results from additional samples from the geographic area ranged from 0.1 to 5.5 mg/L. For the other two diagnosed U.S. cases of skeletal fluorosis, the level of fluoride exposure and duration are unknown (Fisher et al., 1989; Bruns and Tytle, 1988).

In India, Tanzania, and South Africa, crippling forms of skeletal fluorosis have been reported in pediatric age groups (Moudgil et al., 1986; Krishnamachari, 1986). Endemic bent knee is observed primarily in young and adolescent boys and is characterized by: simultaneous exostoses and compression fractures; low calcium intake, and simultaneous osteomalacia; osteosclerosis; and osteoporosis with secondary hyperparathyroidism. Moudgil and coworkers (1986) described 10 children less than 5 years old, and 31 additional children from a rural village in India who had consumed water containing between 7.3 and 29 mg/L of fluoride. These children were reported to have clinical and radiographic evidence of crippling skeletal deformities. A spectrum of radiological changes were observed: osteoporosis; osteosclerosis; findings suggestive of rickets and hyperparathyroidism; and renal osteodystrophy.

Severe crippling fluorosis is not seen in all residents of endemic areas; age of exposure as well as dose and duration of fluoride intake are critical in predicting the clinical signs and symptoms of skeletal fluorosis. Other factors reported to influence the incidence of skeletal fluorosis include: nutritional and calcium deficiencies; renal insufficiency (Singh and Jolly, 1970); the level of bone turnover (Boivin et al., 1988); and diets containing high levels of fluoride (Sauerbrunn et al.,1965; Goldman et al., 1971). Also, in certain occupational settings, the duration and exposure from the inhalation of products of manufacturing, e.g., aluminum, steel, iron, pesticides, fertilizers, and smelting of precious metals (Hodge and Smith, 1972). Other factors influencing skeletal fluorosis include soil type or areas of volcanic rock, geophasia, syndromes of polydipsia, excessive water consumption (Fisher et al., 1989), and the type of physical activity (Singh and Jolly, 1970). Finally, the following factors have been associated with increased incidence of skeletal fluorosis: pre-existing inflammation; increased serum haptoglobin levels; cortisol levels (Susheela et al., 1988); the use of fluoride in the treatment of multiple myeloma and osteopenic conditions; and the use of niflumic acid in the treatment of inflammatory conditions (Bruns and Tytle, 1988).

Osteogenic Effects and Bone Fractures

The issue of the role of fluoride in the etiology of bone fractures initially appeared as a consequence of the findings of clinical trials using fluoride as a treatment for osteoporosis (see page 76). However, several community studies have further investigated this relationship. In one study, 39 countries with varying levels of calcium and concentrations of fluoride ranging from 0.005 mg/L in Sandwell to 0.93 mg/L in Birmingham, United Kingdom were compared. No significant correlations were found between the level of water fluoridation or calcium and the prevalence of hip fractures (Cooper, 1990).

Another geographical correlational study of hip fracture in the United States observed a small but positive correlation between the incidence of hip fracture and diet, calcium content, and fluoride levels (Jacobson et al., 1990). In the most comprehensive study to date, Sowers and coworkers (in press) studied women in three demographically similar rural communities. The communities were identified on the basis of both fluoride and calcium levels in the drinking water supplies. One community (high fluoride, low calcium) had natural levels of fluoride of 4 mg/L and calcium levels of 15 mg/L; the second community had water calcium levels of 375 mg/L, and 1 mg/L fluoride while the control community had fluoride levels of 1 mg/L and calcium levels of 67 mg/L. There were no significant differences in the five-year risk of fractures occurring at the wrist, spine, or hip in the high calcium versus the control community, there was a two-fold increased risk of fractures of all sites in among women 55-80 years of age in the higher fluoride community when compared with the control community. Possible confounding factors such as hormone use, body size and weight, age, and dietary intake of calcium were examined and were not found to be exerting any differential effects in the study communities.

SUMMARY

In summary, human crippling skeletal fluorosis is endemic in several countries of the world, but is extremely rare in the United States. A number of factors govern the amount of fluoride deposited in the skeleton. Important factors include: 1) age at exposure; 2) the duration of exposure; 3) the dose of fluoride (as reflected in the blood concentration); 4) nutritional status; 5) renal status; and 6) individual biological variation. Currently, the body of data on the role of fluoride in the etiology of fractures is not resolved. Further analytical studies are warranted to investigate various aspects of this relationship.

STUDIES INVESTIGATING THE EFFECTS OF FLUORIDE ON TEETH

CLASSIFICATION AND DIAGNOSTIC CRITERIA

Some confusion surrounds the use of the terms "dental fluorosis" and "mottled enamel." "Dental fluorosis" describes fluoride-induced lesions of the dental enamel only, while "mottled" or "discolored enamel" covers a broader clinical spectrum of lesions which include both fluoride- and nonfluoride-induced opacities (Fejerskov et al., 1990; Small and Murray, 1978). Thus, enamel opacities may be classified into three categories: fluoride-induced opacities, i.e., dental fluorosis; nonfluoride-induced opacities of known etiology; and idiopathic opacities (Cutress and Suckling, 1990).

Nonfluoride-induced opacities include all categories of opacities with a known etiology, but are not defined as fluorosis. They are discrete, demarcated, and, in general, distributed asymmetrically. These opacities often affect a single tooth or rarely, multiple teeth, and are a result of systemic or local causes. For example, non-fluoride-induced opacities that may appear distributed symmetrically can be attributed to: trauma, infection, irradiation, environmental, drug, or genetic factors. Idiopathic opacities may exhibit characteristics of dental fluorosis, but are without an apparent history of significant fluoride ingestion, and are attributed to trace elements, for example, strontium, selenium, or "idiopathic" diseases (Cutress et al., 1985; Curzon and Spector, 1977).

Dental fluorosis is a chronic, fluoride-induced condition, in which enamel development is disrupted and the enamel hypomineralized (Dean, 1934; Dean and Elvove, 1936; Murray, 1986; Cutress and Suckling, 1990). It is a clinical diagnosis which always should be supported by an adequate history of exposure to fluoride. Dean (1934, 1942b) described the original clinical classification system for measuring dental fluorosis, and his 1942 version is used today. Dental fluorosis, as described by this index, ranges from normal (no evidence of fluorosis); to very mild or mild (white opacities involving less than 50 percent of the tooth surface); to moderate (opacities involving more than 50 percent of the tooth surface); to severe (the entire tooth surface is involved, accompanied by varying degrees of pitting of the enamel which in the most advanced forms may alter the shape of the tooth). Extrinsic brown stains frequently are observed with moderate and severe dental fluorosis, but are not a criterion for classifying fluorosis. (See Appendix B for further description of these categories.)

Morphologically, the findings support the research model that fluoride affects the forming enamel by making it porous. The degree and extent of the porosity depend on the concentration of fluoride in tissue fluids when the teeth are developing (Fejerskov et al., 1990). Regardless of the degree of enamel porosity, the teeth are not discolored at the time of eruption. The discoloration occurs after eruption because of the uptake of exogenous stains mainly from the diet. Porosity and discoloration can vary in degree among different areas of the same tooth. Histologically, the chalky white areas of fluorosed teeth show hypomineralization of the enamel beneath a relatively well-mineralized surface. The distribution of enamel defects associated with dental fluorosis is described as nondiscrete and bilaterally symmetrical in the dentition (Cutress and Suckling, 1990).

The clinical criteria of these indices appear to identify most cases of dental fluorosis (Cutress et al., 1985; Cutress and Suckling, 1990). Many investigators in this field of study express confidence in their diagnoses of dental fluorosis, stating that dental fluorosis in humans has a characteristic appearance, in terms of distribution within the dentition of an individual and the distinguishing enamel features (Dean, 1934, 1942b; Thylstrup and Fejerskov, 1978; Fejerskov et al., 1988; Horowitz, 1986). Others (Small and Murray, 1978; Moller, 1982) report greater difficulty in a differential diagnosis, indicating "the less severe the observed defects, the greater the diagnostic problem." Confidence in the diagnosis of fluorosis increases with the higher prevalence and increased severity of defects; problems of diagnosis increase with lower prevalence and severity (Cutress and Suckling, 1990). In the United States, where the majority of cases are of the very mild and mild categories, such diagnostic problems might contribute to the variability of dental fluorosis prevalences reported.

Enamel biopsies have been tested as a diagnostic measure of enamel fluoride concentration in dental fluorosis; however, inconsistencies in the thickness of the enamel biopsy have hindered their usefulness. In a study of serial biopsies of surface enamel (Richards et al., 1989), investigators demonstrated a consistent positive association between enamel fluoride concentration and moderate to severe enamel fluorosis, but no consistent association was found for milder forms of fluorosis. Experimental use of proton microprobe scans *in vivo* (sheep) has demonstrated promising results that correlate positive surface enamel and dentin fluoride concentrations with clinical diagnoses of dental fluorosis (Nelson et al., 1989).

Little research on the psychological effects of dental fluorosis on children and adults has been conducted, perhaps because the majority of those who have the milder forms of dental fluorosis are unaware of this condition. Similarly, there are few reports on frequency of dental treatment by stage of dental fluorosis (e.g., cosmetic treatment of dental fluorosis and the restorative treatment needed because unsupported enamel in severely fluoresced teeth has fractured). Although definitive studies have not been conducted, improved clinical methods for correcting aesthetically objectionable fluorosis have been introduced in recent years. Vital bleaching, abrasion, and bonded veneers are being used by many clinicians. These methods are relatively simple to perform, and can correct the cosmetic effects of dental fluorosis.

DENTAL FLUOROSIS INDICES USED IN ORAL EPIDEMIOLOGIC SURVEYS

Several indices have been developed to measure dental fluorosis in epidemiologic studies. The most common indices are: Dean's Fluorosis Index (1934, 1942b); the Thylstrup-Fejerskov Index, or TF Index (Thylstrup and Fejerskov, 1978); the Tooth Surface Index of Fluorosis, or TSIF (Horowitz et al., 1984); and the Developmental Defects of Dental Enamel Index or DDE (FDI, 1982). Recently, the Fluorosis Risk Index (Pendrys, 1990) has been introduced.

Differences exist among the various indices. Dean's Fluorosis Index scores all teeth, but uses only the two most severely affected teeth to classify an individual person. The TSIF and TF indices score fluorosis of all tooth surfaces, and can be used to report the percentage of tooth surfaces affected as well as the percentage of individuals affected. When the TF index is used, the teeth are dried before they are scored, leading to a measurable elevation of the frequency of opaque tooth enamel areas compared to other measures. The Fluorosis Risk Index relates the risk of dental fluorosis to the varying time of development of the dentition and the exposure to fluoride sources. This index divides the occlusal and buccal enamel surfaces into four zones, assigning each zone one of two classifications based on the age at which the zones begin to form. Choice of an index may depend on the purpose of the study.

DENTAL FLUOROSIS AND DENTAL CARIES

Dean and Elvove (1936a) found that with increasing concentrations of fluoride in water, the prevalence of dental fluorosis increased. Dean (1938, 1942c) also reported that with increasing dental fluorosis, the proportion of caries-free individuals increased. Dean and coworkers (1936a, 1936b) observed individuals in two cities with natural water fluoride concentrations of 0.6 and 0.9 mg/L and found prevalences of very mild dental fluorosis of 2.9 and 10.6 percent, respectively. From these data comes the often quoted statement of Dean (1936b), "from the continuous use of water containing about 1 part per million, it is probable that the very mildest forms of mottled enamel may develop in about 10 percent of the group." Later, in his 21 U.S. city survey, Dean (1942a, 1942c) reported dental fluorosis prevalences of approximately 12 percent (in one community with a natural water fluoride concentration of 0.9 mg/L).

The "optimal" level of fluoride in drinking water was determined empirically by Dean (1942b). Dean plotted two curves--dental caries experience versus natural water fluoride concentration and dental fluorosis prevalence versus natural water fluoride concentration (Figure 11). With respect to fluoride concentration in drinking water, the intersection of the two curves at 1 mg/L of fluoride represented the minimal levels of both dental caries and dental fluorosis. Thus, the recommendation of an "optimum" fluoride concentration of 1 mg/L fluoride attempted to maximize the caries preventive benefits of fluoride, and at the same time attempted to minimize the likelihood of dental fluorosis.

On the basis of studies in which geographic region, climate, and mean water consumption were assessed, the "optimal" level of 1.0 mg/L fluoride was modified to represent a range from 0.7 to 1.2 mg/L, depending on the annual mean of maximum daily air temperature of the geographic location (Galagan et al., 1957a, 1957b). More recently, under the provisions of the U.S. Environmental Protection Agency's (EPA) Safe Drinking Water Act of 1974, the EPA set, on April 2, 1986, drinking water regulations for fluoride as follows: 1) "a Primary Maximum Contaminant Level of 4 mg F/L to protect against crippling skeletal fluorosis," and 2) "a Secondary Maximum Contaminant Level of 2 mg F/L to protect against moderate to severe dental fluorosis" (EPA, 1986).

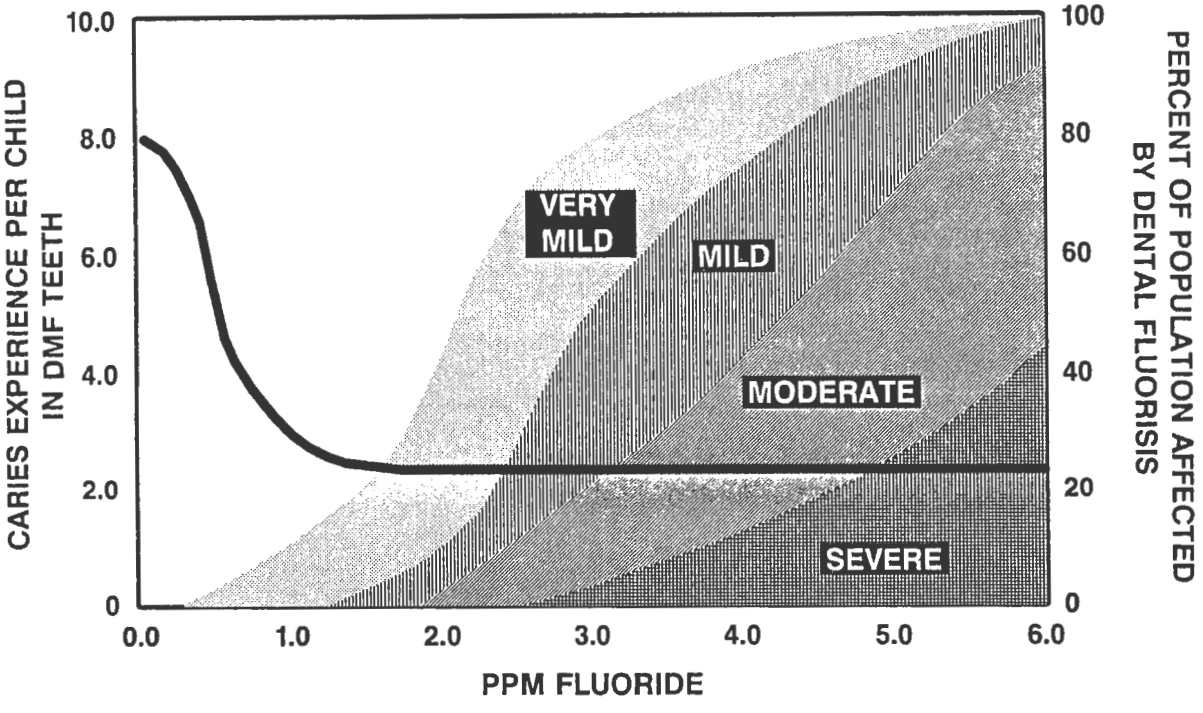

Figure 11. Dental caries and dental fluorosis in relation to flouride in public water supplies.

DENTAL FLUOROSIS PREVALENCE

In Appendix C, the major studies of dental fluorosis prevalence in the United States and Canada from 1939 to 1987 are listed. Studies of dental fluorosis in Africa, India, Europe, and Australia exist, but for purposes of this report, data were restricted to North America. The studies are categorized according to the survey time period and the index used to measure dental fluorosis: (Table C-1) Dean's 21-city survey, 1940s; (Table C-2) studies of the 1980s in which Dean's Index is used; (Table C-3) studies of the 1980s in which the TSIF Index is used; (Table C-4) studies in which the T-F Index is used; and (Table C-5) studies in which the Index was not reported.

In 1939-1940, Dean and co-investigators (1941, 1942a) surveyed approximately 7,000 children in 21 cities in Illinois, Indiana, Ohio, and Colorado where the natural fluoride content of drinking water ranged from 0.0 to 2.6 mg/L. During the 1980s, approximately 5,800 children in 28 communities with water fluoride concentrations ranging from 0.0 to 4.3 mg/L were examined, using Dean's Index, to estimate the prevalence and severity of dental fluorosis. In addition, at least 13 communities in the 1980s have been surveyed using the TSIF index.

The only national data on dental fluorosis were reported by the National Institute of Dental Research (NIDR) from a 1986-1987 survey of 32,241 U.S. schoolchildren. The representative sample included areas that were non-fluoridated, optimally fluoridated, and highly fluoridated; however, the prevalence figures were not reported by water fluoride concentrations (Brunelle, 1989). Total prevalence of dental fluorosis, according to Dean's index, was reported as 22.3 percent (17.0 percent very mild, 4.0 percent mild, 1.0 percent moderate, and 0.3 percent severe thus, of the fluorosis that did occur, less than 6 percent was the moderate or severe category). These data can serve as the national baseline for future dental fluorosis studies in the United States.

TRENDS IN PREVALENCE OF DENTAL FLUOROSIS

With the available epidemiological studies on dental fluorosis, the prevalence of dental fluorosis over time can be assessed by using three types of comparisons:

(1) Comparison, 5 years apart (1980 and 1985), of dental fluorosis in the same cities, and in many of the same children, using the same index (TSIF) and the same examiners.

(2) Comparison, 30 to 40 years apart (1940 to 1980 and 1955 to 1985), of dental fluorosis in the same cities, using the same index (Dean's Fluorosis Index), but different examiners.

(3) Comparison, 40 or more years apart (1940s to 1980s), in different cities, but with similar water fluoride concentrations, using the same index (Dean's Fluorosis Index) and different examiners.

A description of each comparison follows. For all comparisons, fluoride intake from sources other than water is not controlled in these observations.

1980 and 1985 Comparison With the TSIF Index

In 1980 and 1985, epidemiologists from the National Institute of Dental Research assessed cross-sectionally the prevalence of dental caries and dental fluorosis among lifelong resident children, ages 8 to 10 and 13 to 15, in four areas of Illinois with water fluoride concentrations of one, two, three, and four times the recommended

optimal level for the geographic area (Heifetz et al., 1988). Both surveys used the TSIF index which scores dental fluorosis by tooth surface rather than by individuals.

The study was longitudinal for children 8 to 10 years of age in 1980 who were still available in the 13 to 15 year age group in 1985 (Tables 24 and 25). Follow-up examinations of dental fluorosis (1990) are in progress in these same communities.

Findings from the cross-sectional and longitudinal data, as reported by the investigators, indicated that:

- In 1985, in optimally fluoridated areas, approximately 71 percent of all tooth surfaces were reported to be "unaffected" (TSIF category 0) by dental fluorosis.

- Investigators examined changes in the severity of fluorosis from 1980 to 1985. They suggest that both milder (TSIF categories 1, 2, 3) and moderate-to-severe (TSIF categories 4, 5, 6, 7) forms of dental fluorosis varied, by water fluoride level and age (Table 24, and Appendix C, Table C-3). At the optimal fluoride level (1 mg/L), the percentage of surfaces affected by milder forms of fluorosis increased from 1980 to 1985, but the percentage for moderate-to-severe forms remained virtually unchanged during that period. At fluoride levels above optimum, particularly at two times optimal, notable increases in the severity of fluorosis occurred for 13- to 15-year-olds, but not for 8- to 10-year-olds.

- In the 1985 survey, the two and three times optimal water fluoride communities had a similar prevalence of dental fluorosis. For 8- to 10-year-olds (Table 24), the total prevalence of affected tooth surfaces at two and three times optimal levels was identical, 52 percent; for 13- to 15-year-olds, the difference was less than 3 percentage points (66.5 to 69.2 percent).

Using data reported by Heifetz and coworkers (1988), the Ad Hoc Subcommittee assessed differences in dental fluorosis between the age cohorts by the period of fluorosis susceptibility (birth to age 5 years) for each cohort. Table 25, though not part of the Heifetz et al. (1988) paper, describes the

TABLE 24

Percent distribution of dental fluorosis for all tooth surfaces;
by age and water fluoride level, 1980 and 1985

Water Fluoride Level	Percent distribution of TSIF[a] scores for all tooth surfaces							
	1980				1985			
Children 8- to 10-Years-old	0	1 to 3	4 to 7	1 to 7	0	1 to 3	4 to 7	1 to 7
Optimal (1X)	81.2	18.7	0.1	18.8	72.0	28.0	0.1	28.1
(2X) Optimal	52.9	46.7	0.4	47.1	48.0	50.7	1.3	52.0
(3X) Optimal	48.5	49.6	1.9	51.5	48.0	49.9	2.1	52.0
(4X) Optimal	30.3	65.3	4.4	69.7	24.2	70.6	5.2	75.8
Children 13- to 15-Years-old								
Optimal (1X)	88.6	11.4	0.0	11.4	70.7	29.3	<0.1	29.3
(2X) Optimal	61.7	38.2	0.1	38.3	33.5	64.9	1.6	66.5
(3X) Optimal	54.0	44.9	1.0	45.9	30.8	66.7	2.5	69.2
(4X) Optimal	36.9	60.9	2.2	63.1	22.5	71.7	5.9	77.5

(Adapted from: Heifetz et al., 1988)

[a] TSIF scores:

Numerical Scores	Descriptive Criteria
0	Enamel shows no evidence of fluorosis.
1	Enamel shows definite evidence of fluorosis, namely areas with parchment-white color that total less then 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 of the visible surface, but less than two-thirds.
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 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. Larger areas of enamel may be missing and the anatomy of the tooth may be altered. Dark-brown stain is usually present.

TABLE 25
Year of birth, fluoride intake period, year of examination, age cohort, and number of individuals examined -- by water fluoride level.

Year of Birth	Birth Year to Age 5 or Exam Group (Yrs) (Fluoride Intake Period)	Dental Fluorosis Examination Year	Age of Children at Exam Year (Yrs)	Number of Individuals Examined Water Fluoride Levels (X Opt.) (1X)	(2X)	(3X)	(4X)
1965 to 1967	1965 to 1972	1980	13 to 15	111	39	50	34
1970 to 1972	1970 to 1977	1980	8 to 10	113	61	82	59
1970 to 1972	1970 to 1977	1985	13 to 15	94	23	47	29
1975 to 1977	1975 to 1982	1985	8 to 10	156	102	112	62

(Adapted from: Heifetz et al., 1988)

various age cohorts by period of susceptibility to dental fluorosis and water fluoride level. From the investigators' discussion of the relationship of observed scores to temporal ingestion of fluoride during cohort periods of tooth development and fluorosis susceptibility, one might infer that in these communities and at all levels of water fluoride intake was lowest from 1965 to 1970.

The data concerning the nature and extent of fluoride intake and its relationship also suggest that:

- Fluoride intake increased about 1970, after which it has remained relatively constant through 1982.

- From 1980 to 1985, the prevalence of dental fluorosis in 13- to 15-year-olds, at each water fluoride level, increased substantially (Table 24). Such cross-sectional increases in dental fluorosis likely reflect notable increases in fluoride intake during the period of tooth development. For the cohort examined in 1985, those years were 1970 to 1977; for 1980 examinees, the developmental period was 1965 to 1972 (see Table 25). These dates suggest that increases in fluoride intake occurred as early as 1970.
- Further evidence for this likely increase in fluoride intake comes from the 1980 cross-sectional survey (Table 24). At each water fluoride level, 8- to 10-year-olds exhibited more dental fluorosis than did 13- to 15-year-olds. Correlating this finding with the respective periods of tooth development for each cohort, reinforces the likelihood that fluoride intake was greater in 1970 to 1977 than in 1965 to 1972.

  To examine whether the observed cross-sectional differences were spurious, the investigators made longitudinal comparisons, using the cohort born in 1970 to 1972, and examined both in 1980 (as 8 -to 10-year-olds) and in 1985 (as 13- to 15-year-olds). Surface-specific comparisons of teeth present at both examinations (first permanent molars) indicated little or no change in the intensity of fluorosis over the 5 years (data are not presented in this paper). For this reason, the investigators concluded that posteruptive mineralization or abrasion over time could not account for the observed differences in dental fluorosis. Findings by Aasenden and Peebles (1974, 1978), however, suggest that changes (fading) in observed dental fluorosis (particularly very mild and mild) over time may occur in the incisors, if not in the molars, and the use of first molars only to look for possible changes in fluorosis may be questionable.

- From 1980 to 1985, changes in percentages of tooth surfaces affected by dental fluorosis in 8- to 10-year-old children were far less pronounced than changes in prevalence among 13- to 15-year-olds (Table 24). In light of the critical period of tooth development for each cohort, the data reflected little to no change in fluoride intake for the time periods 1970-77 and 1975-82, with the possible exception of the optimally fluoridated (1X Opt.) communities.

Comparison Between Dental Fluorosis Levels Found During the 1940s and 1980s as Measured by Dean's Fluorosis Index

During the 1980s, investigators surveyed three cities that had been surveyed previously between 1939 and 1955: Kewanee, Illinois, was surveyed by Dean et al. (1942a) in 1939 and by Driscoll et al. (1983) in 1980; Kingston and Newburgh, New York, were surveyed in 1955 by Ast and coworkers (1956) and by Kumar and coworkers (1989) in 1985. Direct comparisons of dental fluorosis as measured by Dean's Index, are shown in Table 26.

TABLE 26
Percent prevalence of dental fluorosis in cities
surveyed 30 to 40 years apart, as measured by Dean's Index

				Dental Fluorosis Category						
City	Year	Fluoride N	Fluoride Conc.	Very Mild	Mild	Mod.	Severe	Total Prev-alance	Author	Date
Kingston, NY	1955	612	0.0	0.0	0.0	0.0	0.0	0.0	Ast	1956
	1985	425	0.0	4.4	2.2	0.7	0.0	7.3	Kumar	1989
Kewanee, IL	1939	123	0.9[a]	10.6	1.6	0.0	0.0	12.2	Dean	1942
	1980	336	1.0[a]	7.4	4.8	1.8	0.6	14.6	Driscoll	1983
Newburgh, NY	1955	438	1.0[b]	5.9	1.4	0.0	0.0	7.3	Ast	1956
	1985	459	1.0[b]	4.7	2.1	0.9	0.0	7.7	Kumar	1989

[a] Natural fluoride concentration
[b] Adjusted fluoride concentration

Inferences made from historical comparisons of this type may be interpreted with caution, due to examiner variability and other potential errors. Observations made from these studies are as follows:

- The prevalence of dental fluorosis increased markedly in non-fluoridated Kingston (from 0.0 percent in 1955 to 7.3 percent in 1985) and remained virtually unchanged in the optimally fluoridated communities of Newburgh (7.3 percent in 1955 to 7.7 percent in 1985) and naturally fluoridated Kewanee (12.2 percent in 1939 to 14.6 percent in 1980).

- Kumar and coworkers (1989) reported that most of the dental fluorosis observed in non-fluoridated Kingston in 1985 was of the very mild (4.4 percent) and mild (2.2 percent) categories. Driscoll and coworkers (1983) observed a shift within the categories of dental fluorosis in naturally fluoridated Kewanee and reported a decline in the very mild dental fluorosis (10.6 to 7.4 percent) and an increase in mild dental fluorosis (1.6 to 4.8 percent). Similarly, a comparison of data from the 1955 and 1985 surveys in fluoridated Newburgh showed a decrease in very mild fluorosis (from 5.9 to 4.7 percent) and an increase in mild dental fluorosis (from 1.4 to 2.1 percent).

- Moderate dental fluorosis increased very slightly (less than 2 percent in all three communities) when fluorosis levels of the 1980s' studies are compared with those from the original studies. In 1980, in naturally fluoridated Kewanee, 1.8 percent of the children were reported to have moderate levels of dental fluorosis. In 1985, in fluoridated Newburgh and non-fluoridated Kingston, New York, investigators reported similar percentages of moderate fluorosis, 0.9 and 0.7 percent respectively.

- Although six cases of moderate and two cases of severe dental fluorosis were reported in Kewanee (1980), no history of unusual fluoride intake could be established. No cases of severe fluorosis were reported in any other community.

Comparison Between Dental Fluorosis Levels Found During the 1940s and 1980s in Cities With Similar Drinking Water Fluoride Content--Measured by Dean's Fluorosis Index

In the 1980s, most investigators did not attempt to survey the same cities as Dean; instead, they surveyed cities with similar concentrations of fluoride in the water. Table 27 summarizes the prevalence of dental fluorosis according to clinical classification and the concentration of water fluoride from Dean's 1940 studies and from prevalence studies in the 1980s in which Dean's Index was used. In this summary, the 1986-87 NIDR school children study was not included because water fluoride concentrations were not specified (Brunelle, 1989).

As would be expected over a 40-year period, differences among studies are likely, for example: 1) examiners; 2) interpretations of diagnostic criteria; 3) subject recruitment (Dean studied Caucasians only); 4) residency requirements (lifetime resident or not); 5) sources of fluoride history (parent versus child); and 6) examination conditions (Dean used natural light, but in recent surveys, investigators used artificial light). The value of specific numbers, characterizing the prevalence of dental fluorosis 40 years apart, can be debated; however, such data can be useful in suggesting trends or in considering the findings from other investigations. Given that purpose, the following observations were made concerning the prevalence of dental fluorosis between 1939-1940 and the early 1980s. Fluoride intake from sources other than water is not controlled for these observations. Furthermore, most of these surveys were conducted in the period from 1978 to 1982 among children 8 to 15 years of age. These surveys reflect fluoride intake during the approximate years 1963 (15-year-olds in 1978 were born in 1963) to 1980 (risk periods for 8-year-olds surveyed in 1982 ended at age 6 in 1980). In these surveys, changes in fluoride intake since 1980 would not be reflected as dental fluorosis.

Observations from the comparisons are as follows; however, given the nature of the data available, all comments should be accepted cautiously:

- The prevalence and severity of dental fluorosis increased as the concentration of water fluoride increased. In the 1940s and 1980s, the prevalence of dental fluorosis was greater in fluoridated areas (natural and adjusted) compared with non-fluoridated or low-fluoride areas.

Changes in the percentages of individuals affected (total prevalence) and/or severity (shifts between categories or degrees of dental fluorosis) were observed:

- In areas naturally fluoridated at less than 0.4 mg/L, the total prevalence of dental fluorosis has increased over the period from less than 1 percent to approximately 6 percent (0.9 to 6.4 percent). From 1939 to approximately 1985, nearly all of this increase occurred in the very mild (0.9 to 4.4. percent) and mild (0 to 2.2 percent) categories.

- In communities with optimally fluoridated (0.7 to 1.2 mg/L) water supplies, total prevalence and severity of dental fluorosis apparently has increased from 1939 to approximately 1985. Total prevalence of dental fluorosis increased from approximately 13 to 22 percent (13.6 to 22.2 percent). It should be emphasized that the increase has been limited almost entirely to the milder forms of dental fluorosis (very mild category, 12.3 to 17.7 percent; mild category, 1.4 to 4.4 percent; and combined moderate and severe categories, 0.0 to 0.9 percent).

- In communities with higher than optimal water fluoride concentrations, dental fluorosis prevalence may also have increased slightly: at 1.8 to 2.2 mg/L (44 to 53 percent); and 2.3 to 2.7 mg/L (73.8 to 78.5 percent). Communities with natural fluoride levels between 1.3 and 2.7 mg/L decreased in the very mild fluorosis category, increased in mild fluorosis, and exhibited inconsistent changes in moderate and severe fluorosis. If moderate dental fluorosis has increased since the 1940s, it most likely increased at fluoride water

TABLE 27

Percent Prevalence of Dental Fluorosis by Clinical Classification and Concentration of Water Fluoride from Dean's 1940 21-City Survey and 1980's Surveys Using Dean's Index.

Water Fluoride Level	Total No. Cities/ Studies		Mean N per City/Study		Percent Very Mild Prevalence		Percent Mild Prevalence		Percent Moderate Prevalence		Percent Severe Prevalence		Percent Total Prevalence	
	1940s	1980s	1940s mean	1980s mean	1940s mean±sd	1980s mean±sd	1940s mean±sd	1980s mean±sd	1940s mean±sd	1980s mean±sd	1940s mean	1980s mean±sd	1940s mean±sd	1980s mean±sd
< 0.4	10	5	360	326	0.9±0.7	4.4±3.0	0	2.2±2.8	0	0.1±0.3	0	0	0.9±0.7	6.4±2.6
0.4-0.6	3	1	427	126	5.0±1.4	2.4	0.6±0.3	0	0	0	0	0	5.6±1.2	2.4
0.7-1.2	4	4	270	471	12.3±2.3	17.7±16	1.4±0.4	4.4±2.0	0	0.8±0.8	0	0.1±0.3	13.6±2.0	22.2±14
1.3-1.7*	1	3	477	175	27.0±4.2	19.5±4.1	3.1±1.1	5.6±4.7	0	0.7±0.6	0	0	30.2±4.3	25.7±9
1.8-2.2*	2	1	222	143	35.1±7	23.1	7.5±2	16.8	1.1±0.1	8.4	0	4.9	44.0±6	53.2
2.3-2.7	1	5	404	174	42.1	40.5±7	21.3	29.5±5	8.9	8.4±5	1.5	0	73.8	78.5±9
2.8-3.2	0	3	NA	124	NA	26.2±16	NA	30.0±11	NA	15.0±16	NA	2.8±5	NA	74.0±22
3.3-3.7	0	0												
> 3.7	0	2	NA	163	NA	24.8±11	NA	27.8±4	NA	19.3±17	NA	13.9±12.	NA	83.4±16
	21	24												

Note: Table constructed as follows: the 21 cities represented in the 1940s column are those cities surveyed by Dean in the 1940s and are listed in Appendix C: Section A. The 24 cities represented in the 1980s column are those cities surveyed by different investigators in the 1980s using Dean's Index and are listed in Appendix C: Section B-1. The means and standard deviations are derived from the cities classified by respective water fluoride levels.

* Fluoride concentration for E. Moline and Maywood, Il were reported originally as 1.2 mg/l. Subsequent reports by Dean and Arnold indicate that there is presumptive and direct evidence that the fluoride concentrations for these two cities were 1.4 to 1.6 mg/l during the period of the survey (Dean 1942c; Arnold, 1948).

concentration levels between 1.8 to 2.2 mg/L (1.1 to 8.4 percent). These observations are limited because of the sparse number of studies in communities with natural water fluoride concentrations greater than 1.3 mg/L.

RISK FACTORS ASSOCIATED WITH HUMAN DENTAL FLUOROSIS

Clearly dental fluorosis is associated with ingested fluoride during the tooth-forming years, but the exact mechanism by which dental fluorosis occurs is not fully understood, nor is it clear at which stage of development the primary or permanent teeth are most at risk (Robinson and Kirkham, 1990). The most important risk factor in determining whether dental fluorosis will occur and how severe it will be, is the total amount of fluoride consumed from all sources during the period of tooth calcification (Pendrys and Stamm, 1990).

The permanent teeth begin to mineralize at birth, and this process is completed by about age 5, third molars excluded (Horowitz, 1986); however, Pendrys and Stamm (1990) have reported that the risk period may extend to age 6 or 7. Teeth which develop and mineralize later in life appear to be more severely affected by dental fluorosis. This finding is probably due to the fluoride intake being greater for older children (Thylstrup and Fejerskov, 1978; Larsen, 1988). In areas of high exposure to fluoride, fluorosis can be seen in the primary teeth; in the permanent teeth, the result of long-lasting exposure to "excessive" fluoride can be seen when the first permanent molars and incisors erupt at 6 to 7 years of age. Precise estimates of the effect on the teeth of excessive fluoride intake can be observed only when the premolars and second molars erupt at ages 10 to 12 (Fejerskov et al., 1990).

Dental fluorosis may be influenced directly or indirectly by metabolic factors as well as factors related to sources of available fluoride. These factors include dietary fluoride supplements, toothpaste with fluoride, dietary sources of fluoride, professionally and self-applied topical fluoride products, and multiple risk factors. These potential influences on the prevalence of fluorosis are discussed below.

Metabolic factors

Susceptibility to dental fluorosis can be influenced by chronic systemic acid-base disturbances (acidosis), which affect the kidneys' ability to remove fluoride from the body. Examples include the composition of the diet--normally the major determinant of acid-base status and urinary pH; certain drugs; and a variety of metabolic and respiratory disorders. Manji and coworkers (1986) reported that at any level of fluoride intake, high altitude or hypobaric hypoxia, led to an apparent increased prevalence of dental fluorosis. Whitford (1990) has questioned the classification of such enamel defects, on the basis that hypobaric hypoxia would lead to increased urinary pH causing an increased excretion of fluoride and a hypothesized decreased likelihood of dental fluorosis. The hematocrit level also influences the level of fluoride in plasma, independent of the fluoride level in whole blood (Whitford, 1989; Angmar-Mansson and Whitford, 1990).

Use of Fluoride-Containing Products or Simultaneous Use of Multiple Fluoride Products

The differential effects of exposure to fluoride from water-related sources (i.e., drinking water, reconstituted infant formula, and soft drinks) and exposure to fluoride-containing dental products (i.e., supplements, toothpaste, and mouthrinse) are not equal. The former is a slow, constant exposure of about 1.0 mg/L fluoride; the latter is an exposure to much higher fluoride concentrations producing intermittent and higher plasma fluoride peaks. Risk factors reportedly investigated with respect to changes in the prevalence of dental fluorosis are listed below.

Dietary fluoride supplements: Several investigators have reported an association between the use of dietary fluoride supplements in non-fluoridated communities and increases in the prevalence of very mild and mild forms of dental fluorosis (Pendrys and Katz, 1989; Kumar et al., 1989; Woolfolk et al.,

1989; Ismail et al., 1990; Pendrys and Morse, 1990). European studies have reported a similar association as well (Aasenden and Peebles, 1974; Holm and Andersson, 1982; Granath et al., 1985).

In a case-control study of seventh- to ninth-grade schoolchildren who lived the first 6 years of their lives in areas with water low in fluoride, Pendrys and Katz (1989) reported that early and late exposure to fluoride supplements during the first 6 years conferred a fourfold increase in the relative risk of developing mild-to-moderate enamel fluorosis. The investigators also suggested that the risk associated with mild-to-moderate fluorosis was greater during the third through the sixth years of life than during the first year.

Ismail and co-investigators (1990) reported fluoride tablets to be a strong risk factor associated with dental fluorosis in 11- to 17-year-old students born and living the first 6 years of life in two Quebec cities where fluoride tablets are sold over-the-counter. Logistic regression analysis assessing the non-fluoridated community (fluorosis prevalence 31 percent; tablet consumption 67 percent) and the fluoridated community (fluorosis prevalence 55 percent; tablet consumption 13 percent) showed children whose parents provided a history of fluoride tablet use were 1.7 times more likely to have dental fluorosis than children who had not taken tablets.

In non-fluoridated Kingston, New York, Kumar and coworkers (1989) reported a prevalence of dental fluorosis of 7.7 percent, primarily in the very mild and mild forms. On the basis of a fluoride history of the first 8 years of life, the investigators suggested that the relative risk of fluorosis increased when fluoride tablets or drops were taken daily.

Other investigators have reported that some physicians and dentists may prescribe dietary fluoride supplements inappropriately, since they prescribe the supplements without first determining the water fluoride content (Levy et al., 1987; Kuthy and McTigue, 1987). The potential exists for an increased risk of dental fluorosis if fluoride supplements are prescribed for any child living in areas where water is fluoridated at a level above 0.7 mg/L or for children from birth to 2 years of age living in areas where the water is fluoridated at a level above 0.3 mg/L.

Toothpaste with fluoride: Fluoride-containing dentifrice, or toothpaste, has been implicated as a risk factor contributing to the development of dental fluorosis (Osuji et al., 1988). The risk is attributed to young children (prior to age 5 or 6) swallowing rather than expectorating fluoride-containing dentifrice. Nearly all of the ingested dentifrice is absorbed completely if absorption is not interfered with by the presence of other metal ions (Trautner and Einwag, 1988). The potential increase in a child's intake of fluoride, on average, is estimated to be 0.25 mg per brushing (Whitford et al., 1987). In a community with fluoridated water (above 0.7 mg/L), early use of fluoridated dentifrice (for children from ages 6 to 24 months) has been reported to increase the risk of very mild fluorosis when these young children are compared with children who begin brushing with toothpaste at a later age (Osuji et al., 1988). In addition, Pendrys and Katz (1989) reported a possible association (odds ratio of 2.8) between mild and moderate fluorosis and a history of fluoride dentifrice use during the first 6 years of life. Less than 10 percent of all toothpastes do not contain fluoride (Graves & Stamm, 1985). The inability to find sufficient numbers of users of nonfluoride toothpastes makes it difficult to obtain sufficient power in these studies to detect differences in the prevalence of dental fluorosis among users of fluoridated and non-fluoridated dentifrices.

Dietary sources of fluoride: Results of market-basket surveys of food (excluding water and beverages) indicate that, during the past 40 years, fluoride ingestion from dietary sources has remained relatively constant (McClure, 1943; Ophaug et al., 1980; Singer et al., 1980; Taves, 1983; Pendrys and Stamm, 1990). A possible exception may be in the use of infant formula and beverages. Use of liquid and powder infant formula concentrates, reconstituted with fluoridated water, and of soy-based infant formulas have been reported to present an increased risk for dental fluorosis (Johnson and Bawden, 1987; McKnight-Hanes et al., 1988). Using a case-control design in an optimally fluoridated community, Osuji and coworkers (1988) reported that the use of formula for infants beyond the age of

13 to 24 months was associated strongly with very mild dental fluorosis (odds ratio of 10.5) when compared with breast-fed infants. In 1978, manufacturers of infant formula voluntarily lowered the concentrations of fluoride in the formulas; however, the reported results of the fluorosis studies cited in this document would not reflect these lower fluoride concentrations in infant formulas.

Another contemporary source of dietary fluoride is beverages and "soft drinks," processed using fluoridated water. Drinking large quantities of beverages produced in areas with fluoridated water has been reported to raise the dietary fluoride intake of some children living in non-fluoridated or low-fluoride areas to within the range of persons living in optimally fluoridated areas (Clovis and Hargreaves, 1988). Dental fluorosis was not evaluated in these areas, however.

Professionally and self-applied topical fluoride products: Elevated levels of fluoride in plasma have been reported as a result of the ingestion of professionally applied topical fluoride solutions (Larsen et al., 1985); however, the epidemiological evidence relating dental fluorosis to these fluoride agents is limited. Larsen and coworkers (1985b) found no association between professionally applied topical fluoride treatments and dental fluorosis in 14 year-old Danes. Szpunar and Burt (1988) reported 57 percent more dental fluorosis among children (6 to 12 years of age) who reported "ever used" fluoride rinses compared with those who reported "never used;" most of the children in the survey had not used rinses until age 4 or older. The investigators suggested that the association could be spurious, with the rinsers' parents more dentally aware in general and the children being exposed to other fluoride products as well.

Multiple risk factors: Only two studies, both in Canada, have reported the relative risk of dental fluorosis associated with the presence of multiple risk factors. In a case-control study conducted in a fluoridated community (Toronto, Canada), Osuji and coworkers (1988) compared 67 children with very mild fluorosis to 74 children without dental fluorosis. Preliminary evidence suggested that, when the mother's education was controlled the relative risk associated with multiple risk factors (infant formula use beyond 13 months of age and toothpaste use before 24 months) may be synergistic. That is, the effects of fluoride from both risk factors acting together are greater than the separate effects of each factor added together.

The second study (Ismail et al., 1990) evaluated dental caries and dental fluorosis in 936 randomly chosen, 11 to 17-year-old students (born and having lived the first six years in the same community) from a private and a public school in both a fluoridated (1.0 mg/L) and non-fluoridated (less than 0.1 mg/L) community in Quebec. An analysis of odds ratios (OR) and their 95-percent confidence intervals (CI) revealed a significantly higher prevalence of fluorosis among students from the fluoridated, compared to those from the non-fluoridated community (OR 3.43; CI 1.28, 2.27), and among students from the private rather than the public school (OR 1.19; CI 1.03, 1.39).

Several risk factors identified could contribute to dental fluorosis and may be responsible for the apparent increase in some forms of dental fluorosis documented during the past decade. Theoretically, however, other factors could contribute to an alteration and possible decrease in fluorosis since 1985.

The most recent surveys of dental fluorosis used for trend comparisons in this review were completed in 1985 (Heifetz et al., 1988 and Kumar et al. 1989) among children 8 to 15 years old. Assuming the risk period for dental fluorosis is from birth to about 6 years of age, these surveys reflect the fluoride intake period from about 1970 to 1983. Changes in fluoride intake since 1979 would not be reflected in most of the survey results reported. Examples of possible changes in fluoride availability include the following: 1) In 1979, the American Academy of Pediatrics, conforming with the American Dental Association's guideline schedule, adopted a new schedule that specified lower fluoride supplement dosages for children younger than 2 years (Committee on Nutrition, 1979). 2) In late 1978, manufacturers of infant formulas, baby cereals, and juices began processing products voluntarily with water containing negligible levels of fluoride (Feigel, 1983); 3) Recently, cautionary advice has

been given with respect to the unsupervised use of fluoride toothpaste for young children, and an educational campaign on fluoride supplements has been directed towards physicians (Horowitz, 1985).

Projections of the future prevalence and severity of dental fluorosis may be impractical, considering the possible changes in fluoride intake that have occurred. Current findings indicate that the prevalence and severity of dental fluorosis must continue to be monitored.

SUMMARY

Human dental fluorosis is associated with high tissue fluoride concentration during tooth formation. The greater the fluoride exposure during tooth development, the greater the likelihood of dental fluorosis. The actual concentration of fluoride that correlated with an observed clinical presentation, in a given individual, is difficult to quantify. The prevalence and severity of fluorosis depend on: 1) the amount, concentration, and duration of exposure to fluoride; 2) the stage of tooth development; 3) individual variations in susceptibility; and 4) certain environmental variables.

Overall, dental fluorosis remains more prevalent in fluoridated than non-fluoridated areas. Dental fluorosis appears to have increased in both non-fluoridated and fluoridated communities, but has increased much more in non-fluoridated or low-fluoride areas. Apparently, in non-fluoridated areas over the period 1939 to about 1980, increases in very mild and mild forms of dental fluorosis have occurred. Total prevalence and intensity of dental fluorosis may have increased in optimally fluoridated areas over this same time period. Virtually all of the increase observed in optimally fluoridated areas since Dean's time has occurred in the very mild and mild categories. If moderate dental fluorosis has increased, the increase is minimal and has been most pronounced between the water fluoride range of 1.8 to 2.2 mg/L. During this period of time the prevalence of severe forms of dental fluorosis continues to be very low in optimally fluoridated areas.

In comparison of cross-sectional studies from 1980 and 1985 in the same Midwestern communities, investigators reported an increased prevalence of dental fluorosis by tooth surface (rather than by individual) which may be due in part to increased fluoride ingestion among children. Apparently, the increased ingestion began in the early 1970s, and since then the total fluoride intake has changed very little. Evidence from 1980 and 1985 surveys identified increases in percentages of tooth surfaces with dental fluorosis at optimum, 2X, 3X, and 4X optimal levels of water fluoride in children 13 to 15 years of age and at optimal levels in children 8 to 10 years. There may have been a slight increase in moderate-to-severe forms of dental fluorosis in some children 13 to 15 years old in communities fluoridated at the 2X, 3X, and 4X optimal levels. The study was geographically restricted to four areas of Illinois, so the general applicability of the study is unknown.

Factors found to be associated with an increase in the reported prevalence of dental fluorosis include the daily--and possible inappropriate--use of dietary fluoride supplements, the use of fluoride-containing toothpaste before a child is 24 months of age, and the use by children beyond 13 months of age of powdered and concentrated forms of infant formula reconstituted with fluoridated water. Because of changes in manufacturing practices of infant foods since 1978, the risks associated with these products may no longer be operative.

In most studies in which the risk of developing dental fluorosis has been assessed, investigators have focused almost exclusively on a single risk factor, that is on a single source of fluoride and have not controlled for multiple sources. The effect on dental fluorosis of multiple risk factors or of the simultaneous use of multiple fluoride modalities, remains largely unknown.

STUDIES INVESTIGATING THE EFFECTS OF FLUORIDE ON THE RENAL SYSTEM

The kidney is a potential target of acute fluoride toxicity probably because kidney cells are exposed to relatively high fluoride concentrations. Fluoride shows an increasing concentration from the cortex to

the medulla. Thus, those portions of the nephron responsible for concentrating urine and conserving water are exposed to the highest fluoride concentrations within the kidney.

Fluoride exists in serum as two fractions, nonexchangeable and exchangeable (Taves, 1968). Ingested inorganic fluoride adds to the exchangeable fraction, the portion that has toxicological consequences. Here we discuss only the exchangeable fraction.

Fluoride, after being administered by mouth, is rapidly absorbed into the blood. The increase of fluoride concentration in the plasma begins within minutes after ingestion, and the peak concentration reportedly occurs between about 30 to 90 minutes, with a rapid decline thereafter (Cowell and Taylor, 1981; Whitford, 1990). The fluoride ion is filtered from plasma in the glomerular capillaries into the urinary space of Bowman's capsule, followed by a variable degree of tubular reabsorption. The reabsorption mechanisms may involve diffusion of the hydrogen fluoride (HF) molecule (Whitford, 1990). The epithelium is essentially impermeable to the fluoride ion but not to the nonionized HF. Therefore, reabsorption should be dependent upon the pH of the urine in the tubules, as was found to be the case in rats (Whitford et al., 1976).

ANIMAL STUDIES

Postnatal (29-day-old) rats administered a single high dose intraperitoneally of either 30 or 48 mg/kg of fluoride developed polyuria, decreased urine osmolality, increased urinary pH, changes in urinary chloride excretion, mild glucosuria, and morphological evidence of proximal tubular cell necrosis, 24 to 48 hours after dosing. The excretory abnormalities and histological effects were transient and disappeared 120 hours after dosing. The urinary pH of suckling rats is much lower than that of weanling rats and, therefore, suckling and weanling rats handle fluoride in very different ways. The decrease in urinary pH probably favors the reabsorption of fluoride and would increase the likelihood of renal damage; however, these effects have not been observed in suckling rats (Daston et al., 1985). An alternative explanation is that the redistribution to bone and hard tissues in the immature rat accounts for increased reabsorption from the nephron and the increased clearance from the blood. Another explanation for the relatively refractive nature of the immature rat kidney to toxic insult is that the kidney does not mature until the third week of life.

Some investigators have reported swelling and necrosis of the proximal and distal renal tubules in weanling rats consuming water with extremely high levels of sodium fluoride (380 ppm NaF for 6 weeks) (Lim et al., 1975). Taylor (1961) reported interstitial nephritis and dilation of tubules at the corticomedullary junction in rats maintained for 6 months on water containing 100 ppm fluoride compared with the optimal level of 1 to 1.2 ppm sodium fluoride. Other investigators have reported structural changes in the kidneys of animals following chronic exposure to fluoride (Hodge and Smith, 1977; Manocha et al., 1975; Greenberg, 1986).

HUMAN STUDIES

Understanding the renal kinetics of fluoride is of particular importance because of the potential toxicity when fluoride is present in plasma at high concentrations. The healthy kidney removes fluoride from the blood much more efficiently than it removes other halogens. The renal clearance values for chloride and bromide are typically in the range of 0.5 to 2.0 mL/min (Whitford 1990). Cowell and Taylor (1981) reported that values for clearing fluoride ion from plasma ranged from 19.5 to 44.4 mL/min and tubular reabsorption of the fluoride ion from 61.5 to 86.5 percent for individuals with normal renal and parathyroid function. Whitford (1990) cited a wider fluoride clearance range of 12.4 to 71.4 mL/min; Jarnberg and coworkers, (1983) reported values from 52.3 to 89.1 mL/min.

Whitford (1990) cites a wide range of average fluoride values (0.7 to 2.4 umol/L) for human plasma even when the populations studied appeared to have similar fluoride intake. These differences could

be due to unrecognized population differences in fluoride intake or in fluoride metabolism, but Whitford believed that they were more likely to be associated with the analytical techniques used.

Jarnberg and coworkers (1983) showed a relation between urinary flow and fluoride renal clearance for urine flow rates from about 1 to 21 mL/min. Other investigators have found a positive relationship between urine flow rate and fluoride clearance (Cowell and Taylor 1981; Ekstrand et al., 1978), with correlation coefficients of 0.576 and 0.90, respectively.

Some surgical patients who received methoxyflurane anesthetics have suffered renal insufficiency beginning within a few hours of surgery. Metabolism of the methoxyflurane produced fluoride concentrations in serum that were high enough to produce the effect (Mazze, 1984). Whitford and coworkers (1987) state that exposures which produce serum fluoride concentrations greater than about 30 umol/L in rats, dogs, and humans cause a renal-concentrating defect, a transient phenomenon in which normal renal function returns once the serum fluoride concentration decreases. However, the concentration must fall well below the threshold level (30 umol/L) before normal kidney function resumes.

Hanhijavi (1975) studied inorganic fluoride plasma concentrations in 2,200 patients in Finland, including 501 persons who lived in an area with low fluoride (less than <0.2 ppm F) drinking water and 1,083 persons who lived in a community with adjusted fluoride levels in the drinking water (1 ppm). Plasma inorganic fluoride concentration increased throughout the age groups studied (ranging from 2 to 87 years). Both the fluoride concentration and the percent increase with age were greater in those persons residing in the community with adjusted fluoride in the drinking water, but not in proportion to the levels of fluoride in the drinking water. In both groups, renal clearance of fluoride increased with age, with a peak at about 50 years. Mean renal clearance was about twice as high for those patients from the fluorinated area as those from the low fluoride area. Decreased renal clearance occurred in persons with renal insufficiency and in patients with diabetes mellitus. Kono and coworkers (1984) and Spak and coworkers (1985) have also shown decreased fluoride clearance among both adults and children with impaired renal function. These findings indicate that persons who have impaired kidney function may have a reduced margin of safety for developing skeletal and dental fluorosis compared with persons who have normal kidney function. Although no cases of either skeletal or dental fluorosis have been reported among these individuals, continued follow-up of these populations is needed especially for children with impaired renal function.

Juncos and Donadio (1972) noted fluorosis of both teeth and bones in two teen-age patients who suffered renal failure. Both individuals had regularly ingested large quantities of water with 1.7 to 2.6 ppm of fluoride, concentrations that are below that considered to be the threshold for the development of fluorosis in persons with normal consumption habits and healthy renal function.

Lantz and coworkers (1987) also reported a case of renal failure and features of chronic fluoride intoxication in a person who ingested 2 to 4 liters a day of mineral water containing 8.5 mg/L of fluoride for 20 years. The patient stopped drinking this water 4 years before "presenting" to the investigators with chronic renal insufficiency. In several epidemiologic investigations, however, researchers have found no human kidney disease from long-term nonoccupational exposure to fluoride at drinking water concentrations up to 8 mg/L (EPA 1985). No differences in urinary excretion of albumin, sugar, red blood cells, and formed elements were found among children from a community with drinking water containing 1.2 mg/L fluoride compared with those from a community with water containing essentially no fluoride (Schlessinger et al., 1956). No unusual incidence of either kidney pathology or disease was seen as a cause of death in a population whose water supply contained 2.5 mg/L fluoride (Geever et al., 1958), and the renal status of people in two communities, one with a drinking water concentration of 8 mg/L and the other with a concentration of 0.4 mg/L, was similar (Leone et al., 1954).

SUMMARY

The kidney is a potential target organ for chronic fluoride toxicity because the healthy kidney removes fluoride from the blood much more efficiently than it removes other halogens. The fluoride ion is filtered from plasma in the glomerular capillaries into the urinary space of Bowman's capsule; with a variable degree of tubular reabsorption by diffusion of the hydrogen fluoride molecule. Renal fluoride clearance increases with age, with a peak at about 50 years, but the mean renal clearance was about twice as high for persons from an area with fluoridated drinking water as for those from an area with water low in fluoride. Serum fluoride concentrations above about 30 umol/L cause renal concentrating defects in rats, dogs, and humans. Decreased fluoride clearance may occur among persons with impaired renal function, but the overall health significance of reduced fluoride clearance is uncertain, with no cases of symptomatic skeletal fluorosis being reported among persons with impaired renal function. Several epidemiological investigations have found no human kidney disease from long-term nonoccupational exposure to fluoride concentrations in drinking water up to 8 mg/L.

STUDIES INVESTIGATING THE EFFECTS OF FLUORIDE ON THE GASTROINTESTINAL SYSTEM

All the soluble, fluoride-releasing compounds except monofluorophosphate form hydrogen fluoride when mixed with hydrochloric acid in the stomach. In the acid environment of the stomach (pH = 1), 99.7 percent of the fluoride and hydrogen ions associate to form the nonionized molecule hydrogen fluoride ($pK_a = 3.45$). The semipermeable cell membrane that is essentially impermeable to the fluoride ion also protects stomach cells from the completely ionized hydrochloric acid. The cell membrane, however, does not prevent nonionized molecules from entering the cell membranes by diffusion. This passive diffusion depends on a concentration gradient across the membrane.

Hydrogen fluoride is irritating to the stomach mucosa. At concentrations as low as 10 mmol/L, fluoride has shown both structural and functional adverse effects on the gastric mucosa of the rat and dog. The structural changes range from patchy loss of the mucous layer and scattered desquamation of mucous cells to widespread and full-thickness erosions of the gastric mucosa, with exposure of the underlying lamina propria. The functional changes include dose-related disruption of the physiological gastric mucosal barrier, as indicated by increased fluxes of sodium, potassium, chloride, protons, and water (Whitford 1990).

Once inside the cell the hydrogen fluoride dissociates, releasing hydrogen and fluoride ions in neutral pH. The released hydrogen ion can disrupt the normal intracellular pH. The liberated fluoride ion can interfere with normal intracellular enzyme activity. Thus, hydrogen fluoride can act in two ways to produce effects at the cellular level. The quickest is likely to be that caused by disruption of intracellular pH. An appreciable amount of fluoride is absorbed from the stomach to the blood, as demonstrated by the rapid rise in the concentration of fluoride in serum within minutes of ingestion. This absorption is also a pH-dependent, diffusion-controlled process (Whitford and Pashley 1984).

ANIMAL STUDIES

Studies conducted in intubated rats given extremely high levels of sodium fluoride (100 mmol) demonstrated erosive and hemorrhagic injury to the gastric mucosa and disruption of the structure and integrity of the gastric glands, with progressive healing over 7 days (Easmann et al., 1984, 1985; Pashley et al., 1984). These changes in the stomach were similar to those described in humans after they have suffered acute fluoride toxicity (deLopez, 1976; Basman et al., 1985). In rats, chronic exposure to 4, 10, and 25 mg/kg sodium fluoride in the diet produced dose-dependent chronic gastritis and glandular stomach acanthosis (FDA, 1990). Gross lesions were observed in the mucosa of the glandular stomach of most male rats treated for 6 months with 300 ppm sodium fluoride (DHHS, 1990). Histopathologic lesions in male and female rats included subtle diffuse hyperplasia of the mucosal epithelium of the glandular stomach, along with individual cell necrosis.

Four groups of investigators have produced varied reports about the toxicity of fluoride in the gastrointestinal tract, primarily in cattle, sheep, and dogs (Schmidt and Rand, 1952; Hibbs and Thilsted, 1983; O'Hara et al., 1982; Nichols et al., 1949).

HUMAN STUDIES

In two studies of workers occupationally exposed to varying levels of fluoride, investigators reported various gastrointestinal effects. They include chronic gastritis with and without accompanying skeletal fluorosis, duodenal ulcers, and erosion of the gastric mucosa (Medvedeva, 1983; Desai, 1986). Neither of these investigators reported air measurement of fluoride, so the distribution of exposure type, gaseous versus particulate, for the workers is unknown but can be presumed to be higher than in the general population. In these studies, the major route of entry was probably inhalation because, for most adults, hand-to-mouth activity is not great enough to provide for the necessary doses by ingestion. Although inhalation was the main route of entry, clearance of dust particles from the respiratory tract into the gastrointestinal tract probably provided the exposure reflected in the symptoms.

SUMMARY

All the soluble, fluoride-releasing compounds except monofluorophosphate form hydrogen fluoride when mixed with hydrochloric acid in the stomach. At optimal levels of fluoride in water, however, gastrointestinal effects are not a problem. Hydrogen fluoride at high concentrations is very irritating to, and has shown both structural and functional adverse effects on, gastric mucosa in both animals and humans. Both cellular and serum absorption of fluoride from the stomach is pH-dependent and occurs by passive diffusion. Industrial workers with exposure to high concentrations of fluoride dust reported chronic gastritis, sometimes accompanied by duodenal ulcers. Even though the workers' exposure was primarily that of dust inhalation, the principal symptoms reported were gastrointestinal, thus showing the significance of respiratory clearance in some inhalation exposures. There are no reports of gastrointestinal problems in populations with non-occupational fluoride exposure.

STUDIES INVESTIGATING THE EFFECTS OF FLUORIDE ON THE REPRODUCTIVE SYSTEM

IMPAIRMENT OF FERTILITY

Animal Studies

Adverse effects on reproduction were reported by Messer et al. (1973) who found reduced fertility in female Swiss Webster mice during 25-week studies with drinking water containing concentrations of fluoride (as sodium fluoride) that were lethal (200 ppm) or caused significant decreased maternal weight gain (100 ppm). No effects on fertility of two generations of mice were seen with dams given water containing 50 ppm fluoride. Conflicting reports exist regarding spermatozoal abnormalities in mice resulting from fluoride treatment. Pati and Bhunya (1987) reported abnormal spermatozoa in Swiss mice given intraperitoneal injections of 8 mg sodium fluoride/kg on 5 successive days. However, Li et al. (1987) reported no increases in sperm abnormalities in B6C3F1 mice given 5 daily oral doses of sodium fluoride of 70 mg/kg, and Dunipace et al. (1989) found no increase in abnormal sperm morphology in B6C3F1 mice maintained for 21 weeks on drinking water containing up to 75 ppm fluoride (as sodium fluoride). Mice given sodium fluoride in drinking water at concentrations of 500 or 1000 ppm for two or three months showed clear evidence of damage to spermatogenesis (Kour and Singh, 1980).

Male rats fed diets with added fluoride sufficient to give doses of 25 to 30 mg fluoride/kg body weight did not show adverse effects on reproductive organs or their function; female rats given equivalent doses of fluoride (0.043 percent of the diet) showed reduced diet consumption and

suppression of estrus (Phillips et al., 1933). Sodium fluoride administered to male rats at dietary concentrations of 100 or 200 ppm for 60 days caused a dose-related decrease in reproductive performance; a decrease in serum testosterone levels was observed with the higher dose (Araibi, et al., 1989).

Aulerich et al. (1987) found no effects on reproduction of mink maintained for 7 months on diets containing as high as 350 ppm fluoride (as sodium fluoride), although postnatal mortality of kits maintained on the high-dose fluoride diet was increased. In contrast, decreased calving rates have been reported in young cows given drinking water containing 5 mg/L fluoride for 4 years (Van Rensburg and De Vos, 1966), and poor growth, abnormal skeletal morphology and dental fluorosis have been observed in the offspring of cows receiving feed contaminated with fluoride (Maylin et al., 1987).

Early studies provided somewhat conflicting evidence for the possibility that fluoride is an essential element for normal fertility. It has been suggested that fluoride promotes a more efficient dietary utilization of iron and other trace minerals (Messer et al., 1973; Tao and Suttie, 1976). Further reports of avian and bovine studies have provided evidence consistent with the concept that fertility impairment occurs when animals are exposed to levels either higher or lower than normally occur in the diet (Guenter and Han, 1986; Carriere et al., 1987; Hoffman, 1985; Patee, 1988; Van Rensburg and De Vos, 1966). Inhibition of lactation in cows (Maylin and Krook, 1982) and silver foxes (Eckerlin et al, 1986) was observed after exposure to high levels of fluoride.

Human Studies

While results of epidemiological studies seeking an association between fluoride exposure and changes in reproductive function were not found in the literature, two Russian studies have related chronic occupational exposure to fluoride-contaminated compounds, with effects which may impact on reproductive function. Males demonstrating clinical skeletal fluorosis who had worked in the cryolite industry for 10 to 25 years, showed decreases in circulating testosterone and compensatory increases in follicle stimulating hormone when compared to controls (Tokar and Savchenko, 1976). Of the exposed males, those with 16-25 years exposure to cryolite had increased luteinizing hormone levels compared to men with 10-15 years service. Females exposed occupationally to air heavily laden with superphosphates demonstrated increases in menstrual irregularities and genital irritations when compared to unexposed controls (Kuznetzova, 1968). Occupational exposure to many other compounds in the cryolite and superphosphate industries makes it difficult to implicate any one element, such as fluoride, in producing these effects.

Summary

In summary, it is not yet clear whether fluoride is essential for reproductive performance. Several species are sensitive to fluoride levels higher than those normally encountered, such that their fertility and reproductive performance is impaired. The association of adverse reproductive effects of fluoride exposure in humans has not been adequately evaluated.

TERATOLOGIC AND DEVELOPMENTAL EFFECTS

Animal Studies

Fluoride passes the placenta of laboratory rats in limited amounts and is incorporated into tissues at levels less than 0.01X the amount administered (Theuer et al., 1971). Recent studies of conventional design to evaluate developmental toxicity of sodium fluoride have not been reported, but defects of the teeth following fluoride exposure *in utero* have been reported in several species including the dog (Knouff et al., 1936) and the mouse (Fleming and Greenfield, 1954). Studies in mice and rats

(Cavagna et al., 1969) did not result in any adverse effects on offspring after direct subcutaneous injections of 0.2 mg fluorine neonates.

A study by Shellenberg, et al., (1990) of 20 Shetland sheepdogs randomized to four treatment groups, two with high fluoride (460 ppm) dog chow, and two with low fluoride (55 ppm) dog chow, observed no significant differences in the rates of major congenital anomalies between the four study groups.

Human Studies

Intrauterine effects: Transfer of fluoride across the human placenta has been reported (Armstrong, et al., 1970; Ron, et al., 1986; Teotia, et al., 1979). The birth-defects registry of the metropolitan Atlanta, Georgia area has been evaluated for evidence of a possible adverse effect of fluoridation. Erickson and coworkers (1976) studied data from hospital records for 120,000 live births in the five counties of the metropolitan Atlanta area, 1967 to 1973, and birth-certificate diagnoses for more than 1.25 million live births in the 29 states and two cities covered by the National Cleft Lip and Palate Intelligence service. The incidence of congenital malformations among live born infants in fluoridated areas did not differ from that in non-fluoridated areas.

Another approach is to look for an excess of birth defects among offspring of women heavily exposed to fluoride in geographic areas with high levels of naturally fluoridated water. In reports from South Africa (Jackson, 1962) and Pradesh, India (Teotia et al., 1971) investigators described skeletal fluorosis among 11 to 14-year olds but not among 6 to 10-year olds. The water fluoride levels were 10.35 to 13.5 ppm. Fluorosis was documented at the earliest age in the Arusha region of northern Tanzania (Christie, 1980) where knock-knees, bowlegs, and saber shins were observed among children 2 years of age or older. The fluoride level in the bore-hole well water used by these children was 21 ppm. Presumably, the first exposure occurred *in utero*, but effects were not seen at or soon after birth. Thus, very heavy exposure beginning *in utero* and continuing postnatally did not cause detectable skeletal fluorosis until the third year of life. Malnutrition and calcium deficiency may have been contributing factors. Birth defects have not been reported among children born in these areas.

The same investigators (Teotia et al, 1979) compared the metabolism of fluoride in 15 pregnant women from an area in India with high natural fluoridation (10 ppm) with that of 18 nonpregnant women. Another comparison was made of 10 pregnant and 10 nonpregnant women in an optimally fluoridated area (1 ppm). The results from the highly fluoridated area showed a dramatic fall in the pregnant mother's fluoride level in plasma and urine, beginning between the 12th and 24th weeks of pregnancy and a small decline in the comparison group of pregnant women whose water fluoride level was 1 ppm. These observations may be related to the accumulation of fluoride in fetal bones. The investigators did not mention the health of the infants at birth.

Down syndrome: In 1959 Lejeune and coworkers found that Down Syndrome (DS) was caused by three copies of chromosome 21 in the child instead of the normal two copies. Usually the extra chromosome comes from the mother, and the risk increases greatly with maternal age at the birth of the child (Lilienfeld, 1969). The rate among live births rises from a low of about 0.4 per 1,000 when the mother is 20 to 24 years old to 10 per 1,000 when she is 40 to 44. An error in the division of chromosome 21 in the ovum or sperm (i.e., before the child is conceived) creates the three copies found in children with DS. If an environmental exposure causes this misdivision, it could occur at any time, even a decade or more before the child is conceived. Thus, DS is a genetic disorder rather than a teratogenic effect.

In two studies, Rapaport looked for a relationship between DS and fluoridation. The first study in 1956, concerned institutionalized children, and was flawed because it did not have a population base from which rates could be estimated. The second, in 1963, involved ascertainment of cases from birth- and death-certificate diagnoses and "registries of specialized medical schools in the state." Ascertainment varied in part because of the limited areas served by the medical schools involved. In

the four areas compared, only 17 to 48 percent of the cases were ascertained, as estimated from hospital data or other investigators (Lillenfeld, 1969). Therefore, inadvertent ascertainment bias may account for the seeming rise in rates for the syndrome as fluoridation levels increased from 0.0 ppm to between 1.0 to 2.6 ppm. Since then, three studies have failed to confirm Rapaport's finding (Berry, 1958; Needleman et al., 1974; Erickson et al., 1976). Rapaport suggested that the excess of DS was more pronounced in the children of young women. Erickson (1980), using birth-certificate data from 44 cities with populations of 250,000 or more, classified as to fluoridation status, could not replicate Rapaport's finding.

Needleman and coworkers studied the prevalence rates of DS in Massachusetts from 1950 to 1966, and found them to be the same in both fluoridated and non-fluoridated areas: 1.5 cases of DS per thousand births. This value attests to the completeness of ascertainment, because it is the same as that found on the basis of hospital registries (Lilienfeld, 1969). The authors concluded that the data provide strong evidence against an effect of fluoride during the years covered by the study.

Summary

Fluoride crosses the placenta and is incorporated in tissues of the developing conceptus. Limited animal data report defects of the teeth in offspring of mothers exposed to high dose levels of fluoride. In humans, studies in areas of India and Africa with high levels of naturally fluoridated water showed no increase in birth defects but signs of skeletal fluorosis became evident during childhood. No association was observed between birth defects and fluoridation of community water supplies based on the birth defects registry of the greater metropolitan area of Atlanta, Georgia.

About 30 years ago, an investigator linked an excess of Down Syndrome, a genetic disorder, to fluoridation, but the results of three later studies conducted by other investigators with fuller ascertainment of cases did not confirm that finding.

STUDIES INVESTIGATING THE HYPERSENSITIVITY AND IMMUNOLOGIC EFFECTS OF FLUORIDE

The literature contains minimal animal and human data on sodium fluoride-related hypersensitivity reactions. In animal studies, investigators often used excessively high doses, inappropriate routes of administration, or both (Lewis and Wilson, 1985; Jain and Susheela, 1987). Consequently, the predictive value of these data, as they relate to human exposures at accepted exposure levels, is questionable. Reports of human hypersensitivity reactions resulting from exposure to sodium fluoride are scattered and largely anecdotal (Razak and Latifah, 1988; Modly and Burnett, 1987; Richmond, 1985; Arnold et al., 1960;). The most common responses observed included dermatitis, urticaria, inflammation of the oral mucosa, and gastrointestinal disturbances. Hypersensitivity reactions associated with dental preparations were mild to moderate in severity and appeared to resolve completely with discontinuation of the product (Adair, 1989). It was reported that these reactions were caused by sodium fluoride or by alcohol, dyes, or flavoring agents in the products.

Waldbott (1962) reported that the ingestion of 1 mg/L fluoride in water produced numerous symptoms, which included gastrointestinal distress and joint pains. These symptoms were also reported in a few patients when a daily dose of 20 mg or more was administered to patients as treatment for bone conditions (Shambaugh and Sundar, 1969; Rich et al., 1964). These symptoms are not believed to be caused by chronic intake of fluoride at any dose level, let alone at the low fluoride exposure levels cited by Waldbott. These findings have been dismissed for the following reasons: 1) insufficient clinical and laboratory evidence of allergy or intolerance to fluorides used in the fluoridation of community water; and 2) no evidence of immunologically mediated reactions in a review of the reported allergic reactions (Austen et al., 1971).

Waldbott (1978) proposed that a specific skin manifestation called Chizzola maculae could be caused by airborne fluorides. Waldbott and Steinegger (1973) claimed that this skin lesion was caused by drinking fluoridated water, but could not offer evidence to support this hypothesis. Additional claims (Waldbott and Cecilioni, 1969) attributed the development of these discrete skin lesions to fluoride exposure in 10 of 32 persons living near fertilizer plants in Ontario, Canada, and Iowa, and near an iron foundry in Michigan. The evidence for Chizzola maculae as a result of exposure to fluoride has been reviewed extensively by several investigators (Hodge and Smith, 1977), who concluded that the evidence was circumstantial and unsupported by field surveys.

The literature pertaining to immunologic and immunomodulation effects of fluoride is limited. Although direct exposure to high concentrations of sodium fluoride *in vitro* affects a variety of enzymatic activities (Okada and Brown, 1988; Salesse and Garnier, 1984; Takanaka and O'Brien, 1985; Alm, 1983; Mizuguchi et al., 1989; Mircevova et al., 1984; Carr et al., 1985; O'Shea et al., 1987), the *in vivo* relevance of these observations is unclear. Standardized immunotoxicity testing of sodium fluoride at relevant concentrations and routes of administration has not been conducted.

STUDIES INVESTIGATING THE GENOTOXIC EFFECTS OF FLUORIDE

Genotoxicity studies are highly dependent on the methods used. A number of conclusions can be drawn concerning the genetic toxicity of fluoride *in vitro* and *in vivo*, and a fuller discussion of the genotoxicity of fluoride and the methodological limitations is contained in Appendix H. Despite the apparently contradictory reports appearing in the published literature, fluoride has not been shown to be mutagenic in bacteria (Ames test). In some studies fluoride has been reported to induce gene mutations in both cultured rodent and human cells. Fluoride has also been reported to transform rodent cells *in vitro*. Although there is disagreement in the literature concerning the ability of fluoride to be a clastogen (induce chromosome aberrations) in cultured cells, it has been suggested that fluoride can cause chromosome aberrations in cultured rodent and human cells. Fluoride induced primarily chromatid gaps and chromatid breaks, indicating that the cells are most responsive in the G stage of the cell cycle, i.e., after chromosome duplication in preparation for cell division. Negative results reported in some cytogenetic studies are likely the effect of inadequate test protocols.

The disagreements among the *in vivo* tests for chromosome damage in rodents can not yet be reconciled. There are a few reports of positive results for chromosome aberrations in rodent bone marrow and testes, but other studies, using similar protocols and dose ranges, have reported no induced chromosome damage. In the majority of studies reported positive, the background frequencies of chromosome damage were significantly higher than the commonly-accepted levels, or the investigators reported categories of chromosome damage that may be associated with procedural artifacts. Other reports have presented insufficient information to allow adequate evaluations. Therefore, at this time, the *in vivo* clastogenicity of fluoride should be considered unresolved.

PROPOSED MECHANISMS

Although the mechanism(s) by which these cellular effects result from exposure to fluoride is not known, a number of possible mechanisms have been proposed to explain the genetic activity observed. These mechanisms have been based on the observed reactions of fluoride in solution with divalent cations or nucleotides, or the physiological and biochemical responses of cells treated with fluoride. Sodium fluoride inhibits both protein and DNA synthesis in cultured mammalian cells. The inhibition of DNA synthesis may be a secondary effect of the inhibition protein synthesis, or a result of the direct inhibition of DNA polymerase. Fluoride can react with divalent cations in the cell so as to affect enzyme activities that are necessary for DNA or RNA synthesis, or chromosome metabolism or maintenance; it may react directly with DNA as part of a complex; or it can disrupt other cellular processes such as cell differentiation or energy metabolism (Edwards and Parry 1986; Harper, et al., 1974; Hellung-Larsen and Klenow, 1969; Holland, 1979a, b; Imai, et al., 1983; Srivastava, et al., 1981).

A hypothesis of indirect or 'secondary' effects of fluoride on DNA or chromosomes is attractive in light of the fact that there is no apparent mechanism by which many of the genotoxic effects observed can be induced by direct interaction of fluoride with DNA.

SUMMARY

Genotoxicity studies of fluoride which are highly dependent on the methods used, often show contradictory findings. The most consistent finding is that fluoride has not been shown to be mutagenic in standard tests in bacteria (Ames test). In some studies with varying methodologies, fluoride has been reported to induce mutations and chromosome aberrations in cultured rodent and human cells. The genotoxicity of fluoride in humans and animals is unresolved despite numerous studies.

STUDIES INVESTIGATING THE CARCINOGENICITY OF FLUORIDE

ANIMAL STUDIES

Five carcinogenicity studies in animals have been reported in the biomedical literature. Three studies, conducted before 1970 and interpreted as negative, had significant methodological limitations, as judged by current standards of experimental design. They were inadequate due to incomplete documentation of methods and results, incomplete selection of fluoride doses and inadequate observation periods (Tannenbaum and Silverstone, 1949; Taylor, 1954; Kanisawa and Schroeder, 1969). IARC in 1987 reaffirmed its 1982 statement that data on animals were not sufficient for the agency to evaluate the carcinogenicity of fluoride in animals (IARC, 1982; IARC 1987). Two subsequent studies were conducted using current standards to evaluate the carcinogenicity of sodium fluoride in experimental animals.

NTP Study

Since the IARC reviews, the National Toxicology Program (NTP) has reported results of a carcinogenicity study. Earlier, NTP undertook a study to evaluate the carcinogenicity of fluoride, but because of serious deficiencies in the methodology, the study was considered inadequate for evaluation of carcinogenicity. In the more recent study, F344/N rats and B6C3F1 mice were treated with 25 ppm, 100 ppm, and 175 ppm sodium fluoride (see Table 28 for conversion). A detailed description of the NTP study protocol, and results is available (DHHS, NIH Report No. 90-2848, 1990).

A panel of experts reviewed the recent study. Osteosarcomas of bone were observed in one mid-dose and three high-dose male rats. No osteosarcomas were observed in control or low dose groups of male rats. An extraskeletal osteosarcoma was also identified in a high-dose male rat. Because this tumor did not originate in bone, it was not grouped with osteosarcomas of bone origin. Four of the five osteosarcomas could be detected with either gross or radiographic examinations. Radiographs were also used to identify osteotic changes (osteosclerosis).

Fluoride concentrations were determined in bone ash (see Table 29).

TABLE 28

Approximate dose received by male and female rats and mice during the NTP study[a]

Sodium Fluoride ppm	Fluoride ppm	Daily Dose of Sodium Fluoride in Water (Mg/kg Body Weight)	
		Male Rats	Female Rats
25	11	1.3	1.3
100	45	5.2	5.2
175	79	8.6	9.5
		Male Mice	Female Mice
25	11	2.4	2.8
100	45	9.6	11.2
175	79	16.7	18.8

[a] ppm sodium fluoride in drinking water (column 1) converted to ppm fluoride in drinking water (column 2) and to mg/kg body weight (BW) sodium fluoride ingested by experimental animals (columns 3 and 4).

TABLE 29

Fluoride Concentrations in Bone Ash (ug F/mg ash)

	RATS		MICE	
Dose ppm	Male	Female	Male	Female
Control	0.445	0.554	0.719	0.917
25	0.978	1.348	1.606	1.523
100	3.648	3.726	3.585	4.370
175	5.263	5.554	5.690	6.241

A few squamous cell neoplasms were identified in the oral cavity of dosed and control rats, but the incidences in the dosed animals were not statistically significantly increased compared to controls. Thyroid follicular cell neoplasms were marginally increased in high dose male rats, but were not considered chemically-related because of the absence of a supporting pattern of progression of increase in hyperplasia, adenoma, and carcinoma. Keratoacanthomas were observed only in high dose female rats, but other benign neoplasms arising from stratified squamous epithelium were observed in control females. Uterine stromal polyps were observed in fewer treated than control rats. Malignant lymphoma and histiocytic sarcoma were marginally increased in high dose female mice, but the rate in the top dose group was similar to that typically seen in historical control groups and this was not considered a chemically-related effect. The incidences of liver neoplasms in all groups of dosed and control male and female mice were higher than incidences previously seen in NTP studies. Several of the hepatocellular carcinomas had cell types and morphologic patterns consistent with hepatoblastomas or hepatocholangiocarcinomas, and were classified as such. However, there was no overall increase in hepatocellular neoplasms in dosed groups. The NTP peer review panel agreed with the conclusions proposed by the NTP that stated there was no evidence of carcinogenic activity in female rats, or in

male or female mice, and that the evidence concerning the association of the osteosarcomas in male rats with chemical treatment was equivocal.

NTP stated:

> The four osteosarcomas of bone (one in the mid-dose group and three in the high-dose groups) in the current studies occurred with a statistically significant dose-response trend by the logistic regression test ($p = 0.027$); the pairwise comparison of the incidence in the high-dose group versus that in controls was not statistically significant ($p = 0.099$). The statistical significance of the trend test is increased when the subcutaneous osteosarcoma in the fourth high-dose rat is included in the incidence, but the pairwise comparison remains not significant ($p = 0.057$) As previously mentioned, because this tumor did not originate in bone, it was not grouped with osteosarcomas of bone origin. The incidence of bone osteosarcomas of 3/80 and the incidence of all osteosarcomas of 4/80 in the high-dose male rats are both significantly greater than the rate of 0.6 percent for osteosarcomas and osteomas at all sites in control male rats in the historical database. (NTP report, page 90).

The appropriateness of using the NTP historical data base in evaluating the significance of bone tumors has been questioned for two reasons. First, the extensive procedures for the gross and histologic examination of bones and teeth used in the NTP fluoride study differed significantly from the procedures used in the studies that make up the NTP historical database. The limited number of bones historically evaluated represented a hindrance to considering the historical data base in evaluating the bone lesions from the extensive sampling done in this experiment. Second, animals in this study were fed a low-fluoride basal diet (to approximately 8 ppm fluoride) as opposed to historical controls that received a standard diet containing levels of fluoride of at least 20 ppm fluoride.

Because of differences in the standard methodology needed to conduct this experiment, investigators deemed that concurrent controls were the most appropriate for a comparison of potential fluoride-induced lesions. Some have questioned the validity of not using historical controls, and some have pointed out the difficulty inherent in assessing the validity of grouping the extraosseous osteosarcoma with the osteosarcomas originating in bone. The NTP Peer Review Panel and the authors of this report discussed these matters at length, and decided that concurrent controls were the most appropriate comparators and that the osteosarcoma tumor was appropriately considered separately.

The Peer Review Panel for the NTP studies concluded:

> Under the conditions of these 2-year dose water studies, there was equivocal evidence of carcinogenic activity of sodium fluoride in male F344/N rats, based on the occurrence of a small number of osteosarcomas in dosed animals. There was no evidence of carcinogenic activity in female F344/N rats receiving sodium fluoride at concentrations of 0, 25, 100, or 175 ppm (0, 11, 45, 79 ppm fluoride) in drinking water for 2 years. There was no evidence of carcinogenic activity of sodium fluoride in male or female mice receiving sodium fluoride at concentrations of 25, 100, or 175 ppm in drinking water for 2 years.

The Peer Review Panel went on to approve the addition of a statement that further defined the term "equivocal evidence" of carcinogenicity. The Panel said that:

> Equivocal evidence is a category for uncertain findings demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

Procter and Gamble Study

From 1981 to 1983, Procter and Gamble sponsored a carcinogenicity study which the Carcinogenicity Assessment Committee, Center for Drug Evaluation and Research, Food and Drug Administration (CAC/CDER/FDA) reviewed under an investigational new drug application (IND). This committee advises the Center on the appropriateness of all carcinogenicity studies submitted with respect to study design and validity, performance of the study, and interpretation of the results. The following comments reflect that review. The Procter and Gamble study is described in more detail in the CAC,CDER,FDA report (Appendix D).

The study was conducted with Cr:CD (Sprague Dawley) rats and Cr_j:CD-1 (ICR) mice treated with 0 (C_1), 4 low (L), 10 medium (M), or 25 high (H) mg/kg/day per body weight sodium fluoride added to a low-fluoride basal (Teklad semisynthetic) diet. The diet was mixed by adding the high dose to Teklad low-fluoride basal diet analyzed as less than .01 ppm fluoride and diluting with diet for the two lower doses. The diets were prepared on the basis of the estimated daily intake and animal weight. Although the Procter and Gamble doses administered can be roughly compared with the NTP doses administered (Table 28), measurements of absorption and the ultimate exposure of the rodents in the NTP and Procter and Gamble studies have not been directly evaluated. A second control group (C_2) that received powdered Purina rodent chow was also studied for comparison of the dietary effects of low-fluoride administered at a rate of about 1 mg/kg/day.

Bone radiographs of the female rats at terminal sacrifice were negative. Fluoride concentrations were determined in bone ash (see Table 30). Because different bones were measured in the two studies and the methods used to determine bone ash were dissimilar, no definitive comparisons can be made between the NTP and the Procter and Gamble Studies.

TABLE 30

Fluoride Concentrations in Bone Ash (ug F/mg ash)

	RATS		MICE	
Dose	Male	Female	Male	Female
Control	0.467	0.505	1.582	0.970
Control	0.691	0.781	1.676	1.294
4 mg/kg	5.014	4.541	4.405	3.380
10 mg/kg	8.849	8.254	7.241	6.189
25 mg/kg	16.761	14.428	13.177	10.572

Two osteosarcomas (malignant bone tumors) were identified in low-dose female rats that "died on study" (did not survive until terminal sacrifice); also identified were one osteosarcoma in a high dose male rat and one fibroblastic sarcoma (possibly with osteoid formation) in a mid dose male rat, respectively (CAC report, Appendix D). Osteomas (benign bone tumors) increased in both male and female mice.

By using the method of Peto et al., (1980) for analyzing statistically significant trends, tumor incidence in the 4, 10, and 25-mg/kg BW groups (L, M, H, respectively) were compared with Teklad control (C_1) and Purina control (C2) and also to the combined control groups ($C_1 + C_2$). Compared with control animals male rats had no statistically significant occurrence of osteosarcoma, fibroblastic sarcoma, or combined osteosarcoma and fibroblastic sarcoma ($p < 0.2$); female rats had no statistically significant occurrence of osteosarcoma ($p < 0.6$).

Using the method of Peto et al. (1980) for analyzing statistically significant trends, researchers compared tumor incidence in the groups of 4, 10, and 25-mg/kg BW mice (L, M, H, respectively) with Teklad control (C_1) and Purina control (C_2) and also with the combined control groups ($C_1 + C_2$). A statistically significant trend was observed when treated animals with osteomas were compared with C_1, C_2, or $C_1 + C_2$ (for male mice, $p < 0.0001$; for female mice, $p < 0.003$. A pairwise comparison for high-dose (25 mg/kg BW) mice with C_1, C_2, or $C_1 + C_2$ also showed a statistically significant increase in male mouse osteomas ($p < 0.0001$) and female mouse osteomas ($p < 0.007$).

The Procter and Gamble studies had a number of limitations, with uncertain effects on the observations reported.

1) Only preliminary data from the draft reports were available for evaluation of the mouse study because the researchers are rereading the slides (CAC report, page 3).

2) The Teklad diet may not have allowed the animals to grow and develop normally because diet-related lesions (pale livers and gastric hairballs) were observed in all rats and mice except those fed the Purina Rodent Chow.

3) The diet and water were often outside specifications having high levels of minerals, ions, and vitamins.

4) FDA concluded that an inappropriate dose-determination study to assess the adequacy of the dose was used. A dentifrice slurry was administered by gavage, which was not an appropriate preparation for this carcinogenicity study of fluoride in the diet.

5) The survival rate for experimental animals, including Teklad control animals, was poor, and studies had to be terminated prior to 2 years. As provided for prospectively in the protocol, the experiment was terminated when any group's size dropped to 10 survivors; this occurred in the surveys of high-dose male mice, mid-dose female mice, low-dose male rats, and control female rats. Thus the male and female mouse and the male and female rat studies lasted 95, 97, 95, and 99 weeks, respectively.

6) In rats, researchers confirmed a virus during the pretest and suspected that it occurred (on the basis of antemortem evidence) during the study. A Type C retrovirus was present in the mice used in the study, and a Sendai viral outbreak was identified during weeks 32 to 36 of the study. The fact that the Type C virus was associated with osteoma suggests that the virus may have caused the osteoma or that the virus was activated by rapid bone growth (Finkel, 1973; Wilson, 1985). The causal relationship between the virus and the lesions is not clear in this case because increased osteomas were observed at the high-fluoride dose. The relationship between retrovirus and osteomas in humans is equally unclear. The CAC also noted the difficulty in assessing the dose-related aspect of the osteomas in mice since 1) all bones from animals sacrificed at termination were not evaluated histopathologically, 2) demarcation between hyperostoses and osteoma was highly subjective, and 3) diagnostic drift was apparent throughout the day. (see Appendix D for further discussion).

Under the conditions of the study, the CAC observed that malignant tumors were not related to dietary fluoride exposure in rats and mice. A highly significant increased frequency of benign tumors (osteomas) was observed in high-dose mice (FDA, 1990).

It is important, in evaluating the adequacy of the data for extrapolation to humans, to understand the unique timing of rat skeletal development. The rat is slower than most other mammals in bone maturation. Ossification is not complete until after the first year of life (Strong, 1929, Baker et al., 1979). This means that for half the rat two-year life span, the formation of bone or conversion of

fibrous tissue or of cartilage into bone, is incomplete. In humans, ossification is generally completed by 18 years of age.

Spontaneous neoplasma of bone and cartilage are rare in the Fisher rat. Osteosarcoma is the more common of the two, occurs more frequently in males than females, and occurs more frequently in the flat bones of the skull and the vertebrae than the long bones (Borman and Eustis, 1990). The International Agency for Research on Cancer reports that ostomas (benign) occur in the long and flat bones in the mouse, but they are vague on the distribution of malignant and benign tumors.

Osteomas and osteosarcomas are so rare in rodents that the histogenetic relationship between benign and malignant bone tumors cannot be accurately stated.

Summary

The mouse data from the Procter and Gamble study can be compared and contrasted with the NTP mouse study data. The NTP study conducted in B6C3F1 mice at lower doses of fluoride administered in the drinking water showed no evidence of carcinogenicity. The Procter and Gamble study conducted in CD1 mice at higher does of fluoride administered via the diet showed osteomas, but was confounded by the Type C retrovirus in mice. The NTP mice had few histopathologic effects on bone but definite discoloration of teeth compared with the Procter and Gamble mice; the latter showed histopathologic bone and teeth effects associated with chronic fluoride toxicity. This difference is consistent with the lower levels of bone fluoride detected in the NTP study (contrast Tables 29 and 30), although many different bones were evaluated. No malignant bone tumors associated with fluoride exposure were seen in mice in either study.

In the Procter and Gamble rat study, two osteosarcomas occurred in the 4-mg/kg-BW females and one osteosarcoma in the 25-mg/kg-BW males. The incidence in either sex was not statistically significant. One osteosarcoma was identified by Proctor and Gamble in the premaxilla of a low-dose female rat (Maurer, 1990). The osteosarcoma in the high-dose male was identified as such by pathologists at the Armed Forces Institute of Pathology (AFIP) and this diagnosis is the subject of divided expert opinion (FDA, 1990). No agreement has been reached regarding this discrepancy; however, CAC and this Subcommittee opted to use the "worst case scenario" in interpreting the data and therefore considers the results to encompass two osteosarcomas in 4-mg/kg-BW female rats, one osteosarcoma in a 25-mg/kg-BW male rat, and one fibroblastic sarcoma in the bone of a 10 mg/kg BW male rat. In the NTP study researchers found three osteosarcomas in the 175-ppm sodium fluoride male rats (8.6 mg/kg BW sodium fluoride) and one osteosarcoma in the 100-ppm male rats (5.2 mg/kg BW sodium fluoride).

When the NTP and the Procter and Gamble studies are combined, there is a total of eight individual sex/species groups examined. Seven of these groups showed no significant evidence of malignant tumor formation. One of these groups, male rats from the NTP study, showed "equivocal" evidence of carcinogenicity, which is defined by NTP as a marginal increase in neoplasms--i.e., osteosarcomas--that may be chemically related. Taken together, the two animal studies available at this time fail to establish an association between fluoride and cancer.

EPIDEMIOLOGIC STUDIES

After water fluoridation programs began in several major American cities between 1952 and 1956, a few studies of general mortality with no particular emphasis on cancer were conducted to determine whether fluoridation was having any adverse health effects (Perkins, 1952; Knutson, 1954; Leone et al., 1955; Hagen et al., 1954; Geever et al., 1958). Analysis of specific causes of death in these reports showed no excess risk of cancer due to water fluoridation. In the United Kingdom, Kinlen (1974) and Nixon Carpenter (1974) reported no significant association between cancer rates and fluoridation.

In 1975, the issue of water fluoridation and risk of cancer resurfaced, after a series of public statements by Yiamouyiannis and Burk. Their claims were based on the analyses of cancer mortality in cities with adjusted water fluoridation. (Yiamouyiannis, 1975a; 1975b; 1975c; 1975d; Burk and Yiamouyiannis, 1975). They analyzed age-standardized cancer mortality rates in various combinations of counties with fluoridated and non-fluoridated water, and concluded that fluoridation accounted for up to 22,500 excess cancer deaths per year (Burk and Yiamouyiannis, 1975). These analyses were criticized for a number of methodological reasons, including the dissimilarity of the study populations regarding a number of socioeconomic, racial, and other demographic factors that may affect cancer rates (Oldham and Newell, 1977). Cancer rates vary widely across different age and sex groups. Failure to account for these differences in data analyses (through "adjustments" for age and sex) can lead to improper interpretation of data. The general consensus was that these study results did not show that fluoridation causes cancer.

In response to various criticisms of their original analyses, Yiamouyiannis and Burk further analyzed time trends in cancer mortality among fluoridated (adjusted) and non-fluoridated cities (Burk, 1976; Yiamouyiannis, 1977; Yiamouyiannis and Burk, 1977; Yiamouyiannis, 1980; Graham and Burk, 1984). They suggested that after 10 U.S. cities implemented adjusted water fluoridation programs, cancer mortality rates increased rapidly when compared with rates in 10 non-fluoridated U.S. cities during the same period. The greatest change in the crude cancer mortality rate occurred between 1953 and 1957, and their estimate of trend was based on the change in rates that occurred during this period. These rates were not adjusted, however, for the age and sex of the various populations studied and, as a result, were confounded (Ericson, 1978). Furthermore, the divergence in the crude cancer mortality rates between the fluoridated and non-fluoridated cities was not a consequence of increased cancer rates in the fluoridated cities, but was the result of a diminished rate in the non-fluoridated cities. An evaluation of the city-specific cancer mortality trends during this time confirmed this finding (Knox, 1985).

Yiamouyiannis and Burk conducted additional analyses of the same set of data, looking at cancer mortality rates within broad age groups directly standardizing the data for the effects of age. These analyses were criticized as having errors and inconsistencies in data analyses, inappropriate methods of analyses, and a lack of statistical tests of significance (Taves, 1979; Kinlen and Doll, 1981). Moreover, investigators have reanalyzed the cancer mortality data in the "20-cities studies" of Yiamouyiannis and Burk (1977) and have not found any association between cancer mortality and water fluoridation (Hoover et al., 1977; Doll and Kinlen, 1977; Oldham and Newell, 1977; Kinlen and Doll, 1981; Chilvers, 1983).

Additional studies of the possible link between water fluoridation and cancer morbidity and mortality have been conducted in several countries, including the United States (Hoover et al., 1976; Taves, 1977; Erickson, 1978; Rogot et al., 1978; and Chilvers, 1982), Great Britain (Kinlen, 1975; Cook-Mozaffari et al., 1981; Griffeth, 1985; and Chilvers, 1985), Canada (Glattre and Wiese, 1978), Austria (Binder, 1977), Australia (Richards and Ford, 1979), and New Zealand (Goodall and Foster, 1980). These studies failed to provide evidence for an effect of water fluoridation on cancer morbidity or mortality.

Studies have been conducted of workers exposed to high levels of fluoride through the mining of fluorospar or cryolite or in the production of aluminum or superphosphate. The route of exposure to fluoride is usually through the inhalation of dust containing fluoride and is marked by relatively high exposure levels. Skeletal fluorosis (clinical stage not defined) has been diagnosed in workers exposed to high concentrations of fluoride dust in air for up to 45 years. Although increased risks of lung cancer have been reported in several studies, presumably as a consequence of exposure to particulates and other workplace carcinogens, no increased risk of bone cancers has been observed (de Villiers et al., 1964; Gibbs and Horowitz, 1979; Milham 1979; Anderson et al., 1982; Grandjean et al., 1985). Rockette and Arena (1983) observed an increased risk of cancers of the pancreas, genital-urinary tract, and lymphohematopoietic system, but not of the respiratory tract in 20,000 workers who had been

employed for 5 or more years in an aluminum reduction plant. The increased risk of these types of cancers was believed to have been caused by exposure to a variety of chemicals in the workplace.

Because of the continuing importance of the question of fluoride in drinking water and human cancer, several independent expert panels of epidemiologists have reviewed the relevant scientific literature. The British Working Party on the Fluoridation of Water and Cancer (Knox, 1985) and an international panel of epidemiologists, convened by the Monographs Programme of the International Agency for Research on Cancer (IARC, 1982), conducted the two most comprehensive evaluations. In addition, a subcommittee of the Drinking Water Committee of the U.S. National Academy of Sciences (NAS, 1977) reviewed the literature in the field of epidemiology.

Both the Knox and IARC panels considered in detail all of the available evidence relating to fluoride in drinking water and cancer in human populations. With the exception of one study (Stocks, 1958) in which individual fluoride intake from tea was considered, all of the available studies included in the Knox (1985) and IARC (1982) evaluations are descriptive studies in which both exposure and disease were measured at the community level. Correlations of exposure and disease (or mortality) rates among population aggregations made in such studies are usually considered less satisfactory in determining risk (or the lack thereof) than investigations that ascertain individual exposures and health outcomes. However, the exposure measure used in correlational studies of fluoridated water and cancer is unusual in that it applies to most or all of the populations within the study areas. This trend may be changing because of other sources of fluoride in dental products and the diet, which will probably result in disproportionate distributions of fluoride among individuals in a community. Nevertheless, such studies may be better indicators of risk than other correlational studies such as those of occupation or ethnicity, where usually only a small fraction of a country's population is truly exposed or has the risk factor under study. In addition, consistent results showing either similar types of associations or no associations between an environmental factor and cancer, from several different populations and times, can increase confidence that there is or is not a real connection. All of the expert panels have noted the relative strengths and weaknesses of these studies.

Expert panels agree that the available data provide no credible evidence for an association between either natural fluoride or fluoridated drinking water and the risk of human cancer (IARC, 1982; Knox, 1985). The IARC expert working group (IARC, 1982) came to a similar conclusion, namely that "variations geographically and in time in the fluoride content of water supplies provide no evidence of an association between fluoride ingestion and mortality from cancer in humans."

Additional Studies Completed Since The Knox Report

Lynch (1984) studied cancer incidence in Iowa for the years 1969 to 1981, examining the relationship between cancer rates and adjusted or naturally fluoridated municipal drinking water in 158 municipalities with a total 1970 population of 1,414,878. A total of 66,572 cancer cases (including cancer of all sites, the bladder, bone, breast (female), colon, lung, prostate, rectum, and other sites combined) were evaluated in four study groups, two each for controlled fluoridation and natural fluoridation. In addition, the duration of exposure to fluoridated water was evaluated. Univariate and multivariate cancer site sex-specific statistical analyses were performed. Eight sociodemographic variables were evaluated in multivariate models to account for any effect they may have had on the fluoride-cancer relationship. The results showed inconsistent relationships between the exposure variable and cancer incidence rates and did not support a fluoride-cancer association. Although the multivariate analysis indicated higher adjusted cancer rates for the fluoridated areas, the increase was greater for populations with a shorter duration of exposure. Thus, Lynch (1984) concluded that the data did not support an association between water fluoridation and risk of cancer.

National Cancer Institute Study (1990)

In view of the NTP study findings, Hoover and coworkers (1990) expanded and extended an earlier analysis of all U.S. counties (Hoover et al., 1976) to determine the relationship of cancer mortality to the fluoridation status of county drinking water. In the current reports (Appendices E and F), besides evaluating an additional 16 years of cancer mortality data, the investigators examined patterns of cancer incidence between 1973 and 1987. Data on cancer incidence were collected through the National Cancer Institute's (NCI) Surveillance, Epidemiology, and End Results (SEER) Program, a network of nine population-based cancer incidence registries begun in 1973 and covering about 10 percent (over 25,000,000 persons) of the U.S. population (Young et al., 1981; NCI, 1989). Incidence data are especially important for the evaluation of bone osteosarcomas, since histology-specific information is not generally available in mortality data. In addition, the interpretation of the mortality data for bone cancer is limited, since bone is a frequent site of metastases for a number of common malignancies, which may be misclassified as causing primary bone cancer deaths.

Incidence Study: For the incidence study, a county was considered exposed if the proportion of the population served by fluoridated water (either adjusted or natural) increased from less than 10 percent to greater than 60 percent within a 3 year period. Comparison counties were those with less than 10 percent of the population ever served, through 1987, by adjusted or naturally-fluoridated water. In seven of the nine SEER areas fluoride exposure levels did not vary enough to allow meaningful analyses. Two SEER areas, Iowa and the Seattle metropolitan area, included both fluoridated (exposed) and non-fluoridated (nonexposed) counties: 11 exposed and 14 nonexposed counties in Iowa; 1 exposed and 7 nonexposed counties in the Seattle metropolitan area. To avoid potential confounding due to racial differences in site-specific cancer rates, investigators limited both the incidence and mortality analyses to the white population. Expected numbers of cancer cases were derived from the rates in the nonexposed counties specific for age, sex, and region (Iowa or Seattle). The measure of risk was the ratio of observed to expected cases (O/E incidence ratio or O/E ratio). An O/E ratio greater than 1 indicates a possible association between exposure and the risk of a particular cancer. Major emphasis was placed on changes in cancer risk relative to the time of fluoridation in those counties that had adjusted fluoridation of water. For cancer sites that showed an increasing O/E trend with increasing duration of fluoridation, or for those sites where the highest O/E ratios occurred in the longest duration of fluoridation category, further, more detailed analyses were conducted. Investigators further analyzed cancer incidence data from the Iowa and Seattle exposed and nonexposed counties by Poisson regression techniques controlling for age, sex, calendar time, and geographic area. They calculated risk ratios (RRs) by using the referent category of 1.0 for the nonexposed counties. Ninety-five percent confidence intervals (CI) around the RR were calculated and tests for trend in the ordered RRs were performed. Whether or not the CIs encompassed the point estimate for another duration category was used as an indication whether the differences between the RRs could be due to chance alone.

Bone and Joint Cancers: The O/E ratios for bone and joint cancer and for the subset of osteosarcomas by sex and duration of fluoridation are shown in Table 31.

None of the O/E ratios were statistically significant. Overall, 91 osteosarcomas were observed in rapidly fluoridated areas, compared with 93 expected cases based on rates prevailing in non-fluoridated areas. No consistent patterns emerged for the O/E ratios by interval from fluoridation that suggested an effect either in the total group or in men or women separately.

In the regression analysis for bone and joint cancers and for the subset osteosarcomas, no consistent pattern was seen for the RRs by duration of fluoridation for either sex or in the combined data. Furthermore, there were no statistically significant trends in the RRs.

TABLE 31

Observed to expected incidence ratios[a] and observed numbers of cases (N) for bone and joint cancers and osteosarcomas, by duration of fluoridation for counties in the Iowa and Seattle SEER Registries, 1973-1987

		DURATION OF FLUORIDATION (YEARS)				
SITE	SEX	<5	5-9	10-14	15-19	20+
Bone and Joint Cancer	Total[b]	1.2(17)	0.9(47)	1.0(67)	1.1(69)	1.0(90)
	Male	1.9(11)	0.9(24)	1.0(40)	1.0(31)	1.4(60)
	Female	0.7(6)	1.0(23)	1.0(27)	1.2(38)	0.6(30)
Osteosarcoma	Total[b]	1.8(9)	0.8(11)	0.9(20)	1.2(20)	0.9(31)
	Male	2.6(6)	0.8(6)	0.9(14)	0.8(9)	1.2(19)
	Female	1.2(3)	0.8(5)	1.0(6)	1.4(11)	0.7(12)

[a] Relative to non-fluoridated counties (1.00), adjusted for age, calendar time, and geographic area

[b] Also adjusted for sex

Cancers of the Oral Cavity and Pharynx: For cancers of the oral cavity and pharynx, in Iowa, the O/E ratio was 1.2 in counties with water fluoridated for less than 5 years. The O/E ratio rose to 1.4 in counties fluoridated for 5 to 9 years, to 1.7 for 10 to 14 years, and to 1.6 more than 14 years. The increase in O/E ratios followed by a plateau reflected the experience of males, who showed a rise from 0.8 in the shortest interval to about 1.6 for each of the longer duration categories. In the Seattle area, no consistent patterns were observed.

In the regression analyses for cancers of the oral cavity and pharynx, the overall patterns and the RR point estimates themselves were quite similar to the patterns observed for O/E ratios. In Iowa, the RRs for males and the sexes combined were lowest in the shortest duration-of-fluoridation category, and then they increased to about 1.6 for each of the subsequent intervals. Conversely, in Seattle, the RRs were lowest in the longest duration-of-fluoridation category for both males and females. In Seattle, there was a trend towards decreasing RRs with increased duration of fluoridation. However, in Iowa males, there was a positive trend for increasing RRs with increased duration of fluoridation. The statistical significance of these trends was derived primarily from the difference between the value of the RR in an extreme duration category compared with all of the others, rather than from a consistent trend or gradient.

Renal Cancer: The only patterns in the aggregate data that yielded any evidence of a positive relationship between risks and duration of fluoridation were those for kidney cancer. In Iowa, the O/E ratio for renal cancer rose from 0.9 in the less than 5 year category to 1.0 for the next three duration intervals and to 1.2 for the 20-or-more category. In Seattle, the O/E ratio rose from 0.8 in the less than 5 year category, to 0.9 in the next two duration intervals and to 1.1 in the longest category (15 to 19 years). The sex-specific data were much more variable. An increasing trend with duration was seen only among males in Iowa. However, the highest O/E ratio occurred in the longest fluoridation category for three of the four area-sex groupings.

In the regression analysis for renal cancer, the patterns of the RRs and their values were similar to those for the O/E ratios. The RRs for both sexes combined in Seattle rose slightly from 0.9 to 1.0, a trend that was statistically significant ($p = 0.04$). None of the other trends were significant, and for

each registry-sex category, the confidence interval in the less than 5 year category included the point estimate of the RR for the longest duration category, indicating no significant differences between the RRs.

The renal cancer data were further broken down by time of diagnosis (1973-1980, 1981-1987), which is an indicator of the consistency of an association. The consistent increase in the O/E ratios with duration of fluoridation observed in the aggregate data for both sexes combined in Iowa and in Seattle was not seen in any of the nine subgroups defined by sex, calendar-time, and registry. Therefore, the association initially observed in the aggregate data was not corroborated by the time specific analysis.

Cancer Mortality Analysis: For the mortality analysis, only counties with more than 50 percent urbanization in 1980 were included. A county was considered to be exposed if the proportion of the population served by fluoridated water increased from no more than 10 percent to more than two-thirds within a 3-year period. Comparison counties were those with no more than 10 percent of the population ever served by adjusted or naturally fluoridated water through 1985. The mortality analysis thus was based on 123 fluoridated counties with a 1980 population of 40,703,109 persons, accounting for 18 percent of the U.S. population, and 211 non-fluoridated counties with a population of 32,757,238, accounting for 14.5 percent.

Analysis of O/E ratios for bone cancer mortality from 1950 to 1985 indicates no apparent trends that relate to water fluoridation. Analysis of O/E ratios for 11,782 deaths in intervals ranging from 31 to 35 years before fluoridation to 30 to 35 years after fluoridation showed no consistent patterns. For cancer mortality of the oral cavity and pharynx among males, the observed mortality generally ranged from 30 to 50 percent above the expected values, but this excess applied to the periods before and after adjusted fluoridation was begun, without any evidence of a trend. Analyses of cancer mortality for other sites showed no consistent relationship between cancer mortality and time of fluoridation. In fact, there was more evidence for an inverse relationship between water fluoridation and cancer mortality than for a positive one.

Summary of Findings From the NCI Study: The investigators concluded that the long-term survey of counties undergoing rapid fluoridation of community water supplies uncovered no consistent patterns of cancer incidence or mortality that suggested a relationship to fluoridation. Cancer in one site, the kidneys, showed a suggestive relationship between incidence rates and duration of fluoridation in the aggregate data from the registries. However, no such trends were seen when incidence data were examined from two separate periods, and the mortality data for renal cancer actually yielded some evidence of an inverse relationship with duration of fluoridation.

In the report, the investigators said: "Thus in a study of over 2,300,000 cancer deaths in fluoridated counties across the United States, and over 125,000 incident cancer cases in fluoridated counties covered by two population-based cancer registries, we identified no trends in cancer risk that could be ascribed to the consumption of fluoridated drinking water."

Additional NCI Evaluation of Osteosarcoma Incidence Data

Although the NCI evaluation of both the cancer incidence and mortality data indicates no consistent associations with water fluoridation, the small number of osteosarcomas identified in the two registries included in its study (N=91) and the apparent increasing incidence of bone and joint cancers in several international cancer registries (Muir et al., 1987) led to a more detailed evaluation of osteosarcomas using data from the entire SEER incidence data base for 1973 to 1987. The details of this analysis are found in Appendix F. The definition of the fluoridation status for counties included in this analysis is described in the previous section. When the data from two time periods (1973 to 1980 and 1981 to 1987) were compared, researchers found no significant change in the rate of osteosarcoma over time for both sexes combined, reflecting an 18 percent increase for males and an 11 percent decline for females. Most of the increase among males occurred in those younger than 20 years of age at

diagnosis, among whom the annual rate rose from 3.6 cases/10^6 (11 cases per year) from 1973 to 1980, to 5.5 cases/10^6 (14 cases per year) from 1981 to 1987. When the data were further analyzed on the basis of fluoridation status within the SEER registries, researchers found a greater increase in the osteosarcoma rates between the two periods among males younger than 20 years of age in the fluoridated counties than among their counterparts in the non-fluoridated counties. To determine whether this increase was associated with fluoridation or with some other correlate of these fluoridated counties such as increased urbanization, the researchers assessed the relationship between the risk of osteosarcomas and fluoridation while controlling for time. When this was done, there was no increase in the risk of osteosarcoma in young men by duration of fluoridation. If fluoride was exerting an effect on the rate of osteosarcomas the greatest risk would have been expected in those exposed to fluoridation for the longest time period. However, the reverse pattern was observed.

In summary, analyses of incidence data from the SEER program has demonstrated some age- and sex-specific increases over time for bone and joint cancers and for osteosarcomas. These increases are more prominent in fluoridated than in non-fluoridated areas. Results of further analyses, however, have shown that these increases are unrelated to the timing of fluoridation. This finding provides no support for the theory that increases in the rate of osteosarcomas in young males are associated with fluoridation of the water supplies.

The Epidemiology and Etiology of Osteosarcoma

Osteosarcoma rates are highest among persons younger than 20 years of age, a time when bones, especially the long bones, are growing fastest. Among the possible exposures implicated in the etiology of osteosarcoma, ionizing radiation is the best documented. Exposure to bone seeking radioisotopes such as ^{224}Ra and ^{226}Ra, both alpha-ray emitters, has been shown to induce osteosarcomas in radium dial painters and in persons treated with ^{224}Ra for bone tuberculosis (Martland et al., 1925; Mays and Speiss, 1984). High-dose radiotherapy has been shown to induce osteosarcoma (Tucker et al., 1987), but exposure to high doses of external gamma radiation from the atomic bombs in Japan caused no increase in the frequency of osteosarcoma (Shimizu et al., 1990). Alkylating agents used in cancer chemotherapy has induced the neoplasm in children (Tucker et al., 1987). Osteosarcoma is occasionally familial (Miller, 1981; Li et al., 1989), and loss or inactivation of a tumor suppressor gene has been identified as a cause (Knudson, 1989).

The age-specific incidence of osteosarcoma is bimodal with a peak incidence among persons 10 to 19 years of age and a decrease in rates among those between 20 to 50 years of age. Rates increase again beginning in the sixth decade of life. In patients with osteosarcoma older than 60 years of age Huvos, (1986) found that more than half had Paget's disease, a sometimes familial bone disorder among older persons. If fluoridation were a cause of osteosarcoma, one would expect the rates in persons older than 60 years of age (at potentially greater risk for osteosarcoma because of the higher rate of Paget's diseases) to be greater in fluoridated than in non-fluoridated areas. The NCI evaluation of osteosarcoma incidence data, however, indicate that fluorides are not a risk factor in this age group. See Appendix G for a fuller description of osteosarcomas.

Summary

Over 50 epidemiologic studies have evaluated the possibility of an association between cancer mortality and morbidity and fluoride levels in drinking water. With one exception, all available studies are geographic evaluation studies or geographic time-trend correlation studies, in which the measures of both exposure and disease are at the community level. Most epidemiologists consider studies of geographic correlations valuable in indicating the likelihood that positive links do or do not exist or in demonstrating the feasibility of hypotheses. When the results from a number of such studies (conducted in different times and places) converge to indicate an exposure-disease relationship, or consistently fail to indicate such a relationship, one's confidence is bolstered in the collective findings of the studies. The collective body of information derived from more than 50 epidemiologic

studies, conducted in different populations and at different times, demonstrates no credible association between water fluoridation and the risk of cancer.

Although the results of these studies do not show an association between fluoride and osteosarcomas one must understand the limitations of these analyses. The ability of such correlation studies to detect excess risk is limited in several respects, including the following; 1) the inability to measure fluoride exposures among study subjects who migrated into study areas before a diagnosis of cancer or death; and 2) the difficulty or possibility of adjusting these studies to account for differences in risk factors (or in confounding factors) between fluoridated and non-fluoridated areas, e.g., smoking, occupational exposures, and dietary patterns. To the extent that migration is a factor, it is likely to diminish the sensitivity of a geographic correlation study to detect possible excess risk. Confounding can give rise to spurious results, such as positive associations where none are present or alternatively, the masking of positive associations. An additional problem can arise in correlation studies in which mortality statistics are used as end points, since geographic differences in cancer death rates for diseases with good survival rates may not accurately reflect differences in the underlying cancer incidence rates. With a few important exceptions, most of the epidemiologic studies of fluoridated water and cancer are based on mortality statistics, which are also subject to misclassification for specific causes of death because of inaccuracies in death certification. In situations where no associations can be shown, the limitations of such studies in detecting small excesses must be considered. Even when many investigators fail to observe exposure-disease associations, the possibility of small risks, undetected at a population level, cannot be excluded. This is especially true for malignancies with a very low incidence, such as osteosarcoma. If water fluoridation is associated with a few extra cases of bone cancer in tens of millions of people, then no practical methodology exists which would be able to detect an effect this small and attribute a causal interpretation. Findings of 10 to 20 percent differences in rates, such as were measured in the Hoover et al. (1990) study, must be considered at the limits of what can be detected given the incidence and mortality rates for these tumors. Furthermore, the differences in levels of daily fluoride intake in fluoridated and non-fluoridated communities are decreasing rapidly because of the availability of fluoride in dental products, fluoride dietary supplements, foods and beverages. This fact also affects a study's ability to detect a true difference in cancer risk at the community level where individual exposures to fluoride have become more homogeneous. Only by conducting an analytical epidemiologic study of osteosarcomas, where potential exposures and other risk factors are directly measured, can a more precise assessment be made. Therefore using the most sensitive data at this time there is no evidence of a association between fluoridation of water and cancer.

FINDINGS AND CONCLUSIONS

FINDINGS

ASSESSMENT OF FLUORIDE EXPOSURE

In the 1940s and 1950s, fluorides in drinking water and food were the major sources of fluoride exposure. Today, there are numerous sources of fluoride. Fluoride exposure in the United States has expanded significantly because of: the availability of dental products containing fluoride, the consumption of beverages and foods containing fluoride; and fluoride dietary supplements.

Estimates prepared for this report show that while fluoride exposure is greater in fluoridated areas compared to nonfluoridated areas, there is fluoride exposure in both areas. In fact, this report estimates that some children living in nonfluoridated areas who use fluoride dietary supplements can receive about as much fluoride as children living in optimally fluoridated areas. In fluoridated areas, most daily intake of fluoride is derived from drinking water, beverages and food. The inappropriate ingestion by some children of fluoride containing toothpaste and mouthrinses can contribute significantly to their total fluoride intake. Because well over 90 percent of all toothpaste marketed in the United States contains fluoride it is difficult to obtain toothpaste without significant levels of fluoride.

ASSESSMENT OF THE HEALTH BENEFITS OF FLUORIDE

Fluoride has substantial benefits in the prevention of tooth decay. Numerous studies, taken together, clearly establish a causal relationship between water fluoridation and the prevention of dental caries. While dental decay is reduced by fluoridated toothpaste and mouthrinses, professional fluoride treatments and fluoride dietary supplements, fluoridation of water is the most cost-effective method. It provides the greatest benefit to those who can least afford preventive and restorative dentistry and reduces dental disease, loss of teeth, time away from work or school, and anesthesia-related risks associated with dental treatment.

In the 1940s, children in communities with fluoridated drinking water had reductions in caries scores of about 60 percent as compared to those living in non-fluoridated communities. Recent studies still reveal that caries scores are lower in naturally or adjusted fluoridated areas; however, the differences in caries scores between fluoridated and non-fluoridated areas are not as great as those observed in the 1940s. This apparent change is likely explained by the presence, in non-fluoridated areas, of fluoride in beverages, food, dental products and dietary supplements.

Fluoride has been used for nearly 30 years as an experimental therapy to treat osteoporosis, but has only recently been evaluated in controlled clinical trials. Two new U.S. clinical trials showed no significant reduction in the rates of bone fractures related to the administration of fluoride. An FDA advisory panel has concluded that fluoride therapy has not been shown to be effective in reducing the frequency of vertebral fractures.

ASSESSMENT OF THE HEALTH RISKS OF FLUORIDE

Among the more significant health conditions evaluated in relation to fluoride intake are cancer, dental fluorosis, and bone fractures. Other conditions are evaluated in the full report.

Cancer

Two major scientific approaches have been used to determine whether an association exists between the use of fluoride and cancer: carcinogenicity studies in rodents, and human epidemiological analyses which compare cancer incidence and mortality between communities with fluoridated water and those

with negligible amounts of fluoride in drinking water.

Five carcinogenicity studies in animals have been reported in the biomedical literature. Three studies, conducted before 1970 and interpreted as negative, had significant methodological limitations, as judged by current standards of experimental design. Two subsequent studies were conducted using current standards to evaluate the carcinogenicity of sodium fluoride in experimental animals.

One of the two carcinogenicity studies was conducted by the National Toxicology Program (NTP). This peer-reviewed study provided sodium fluoride in drinking water to rats and mice and determined the occurrence of tumor formation in many different organ systems. The peer review panel concluded that, "Under the conditions of these 2-year dosed water studies, there was equivocal evidence of carcinogenic activity of sodium fluoride in male F344/N rats, based on the occurrence of a small number of osteosarcomas in dosed animals. There was no evidence of carcinogenic activity in female F344/N rats receiving sodium fluoride at concentrations of 25, 100, or 175 ppm (0, 11, 45, 79 ppm fluoride) in drinking water for 2 years. There was no evidence of carcinogenic activity of sodium fluoride in male or female mice receiving sodium fluoride at concentrations of 25, 100, or 175 ppm in drinking water for 2 years." The Ad Hoc Subcommittee on Fluoride concurs with this conclusion.

The other carcinogenicity study was sponsored by the Procter and Gamble Company using Cr:CD (Sprague-Dawley) rats and Cr1:CD-1 (ICR) mice both treated with 0, 4, 10, or 25 milligrams/kilogram/day sodium fluoride added to a low fluoride-basal diet. A second control group received powdered rodent chow. There was no evidence of malignant tumors associated with sodium fluoride in mice and rats of either sex in the Procter and Gamble study. While there were two osteosarcomas in the low dose female rats, one osteosarcoma in a high dose male rat, and one fibroblastic sarcoma in a mid-dose male rat, these findings in treated animals were not statistically different from controls. Male and female mice in the study did have a statistically significant increase in benign bone tumors (osteomas). The significance of a Type C retrovirus, detected in the osteomas, remains to be determined. Osteomas and osteosarcomas are different in anatomical site and clinical course. The FDA also noted difficulty in assessing the dose-related aspects of the osteomas in mice (see page 75-6). Furthermore, osteomas and osteosarcomas are so rare normally in rodents that the relationship between these tumors cannot be accurately stated.

When the NTP and the Procter and Gamble studies are combined, a total of eight individual sex/species groups are available for analysis. Seven of these groups showed no significant evidence of malignant tumor formation. One of these groups, male rats from the NTP study, showed "equivocal" evidence of carcinogenicity, which is defined by NTP as a marginal increase in neoplasms--i.e., osteosarcomas--that may be chemically related. Taken together, the two animal studies available at this time fail to establish an association between fluoride and cancer.

There have been over 50 human epidemiology studies of the relationship between water fluoridation and cancer. Epidemiological studies of fluoride usually attempt to identify statistical associations between cancer rates and county or city-wide patterns of water fluoridation. Expert panels which reviewed this international body of literature agree that there is no credible evidence of an association between either natural fluoride or adjusted fluoride in drinking water and human cancer (IARC, 1982; Knox, 1985). Interpretation of these studies is limited by the inability to measure individual fluoride exposures or to measure other individual predictors of cancer risk, such as smoking or occupational exposures.

In March of 1990, the National Cancer Institute (NCI) updated and expanded an earlier analysis of cancer deaths, by county in the United States, to determine whether there is or is not an association between cancer and fluoride in drinking water. The new studies evaluated an additional 16 years of cancer mortality data, and also examined patterns of cancer incidence between 1973 and 1987 in the Surveillance, Epidemiology and End Results (SEER) Program cancer registries. SEER, an NCI sponsored network of population-based cancer incidence registries, started in 1973 and represents

about 10 percent of the U.S. population. The SEER registries were used to obtain incidence data on all cancers, with special emphasis placed on trends in osteosarcoma. Because mortality data do not contain information on tumor-specific pathology, analysis of osteosarcomas is limited to the incidence data.

The NCI study identified no trends in cancer risk which could be attributed to the introduction of fluoride into drinking water. The study examined nationwide mortality data and incidence data from counties in Iowa and the Seattle, Washington metropolitan area. There were no consistent differences in the trends in cancer mortality rates among males and females living in counties having initiated water fluoridation compared with the trends in rates in counties without water fluoridation. The relative mortality rates from cancer, including cancer of the bones and joints, were similar after 20-35 years of fluoridation as they were in the years preceding fluoridation. In addition, there was no relationship between the introduction and duration of fluoridation and the patterns of cancer incidence rates, including those of the bone and joint, and the subset of osteosarcomas (Appendix E). For example, there were 91 observed cases of osteosarcoma in the fluoridated areas, when 93 cases were expected based on rates in non-fluoridated areas..

The NCI also conducted a more detailed evaluation of osteosarcomas using nationwide age-adjusted incidence data from the entire SEER database for the years 1973-1987 (Appendix F). Osteosarcoma is a rare form of bone cancer, the cause of which is under study. Approximately 750 newly diagnosed cases occur each year in the United States, representing about 0.1 percent of all reported cancers. Between two time periods, 1973-1980 and 1981-1987, there was an unexplained increase in the annual incidence rates of osteosarcoma in young males under age 20 from 3.6 cases per 1,000,000 people (88 registry cases) to 5.5 cases per 1,000,000 people (100 registry cases). This compares to a decrease in young females of the same age group from 3.8 cases per 1,000,000 people (87 registry cases) to 3.7 cases per 1,000,000 people (63 registry cases). The amount of increase observed in young males was greater in fluoridated than in non-fluoridated areas. Although the reason for the increase in young males remains to be clarified, an extensive analysis reveals that it is unrelated to introduction and duration of fluoridation.

In studying rare cancers, such as osteosarcoma, small increases in risk, on the order of five to 10 percent, would not likely be detected. While descriptive epidemiological studies are useful in determining whether or not there is a credible association, the qualitative nature of any association, if one exists, can best be determined through more refined methods, such as case-control studies.

Dental Fluorosis

Dental fluorosis has been recognized since the turn of the century in people with high exposure to naturally occurring fluoride in drinking water. It has always been more prevalent in fluoridated than non-fluoridated areas. Dental fluorosis only occurs during tooth formation and becomes apparent upon eruption of the teeth. It ranges from very mild symmetrical whitish areas on teeth (very mild dental fluorosis) to pitting of the enamel, frequently associated with brownish discoloration (severe dental fluorosis). The very mild form barely is detectable even by experienced dental personnel. Moderate and severe forms of dental fluorosis, considered by some investigators as presenting a cosmetic problem, do not appear to produce adverse dental health effects, such as the loss of tooth function, and represents less than six percent of the cases of fluorosis nationally.

In the 1940s, about 10 percent of the population displayed very mild and mild dental fluorosis when the concentration of fluoride found naturally in the drinking water was about 1 part per million (ppm). Over the last 40 years, in areas where fluoride has been added to the drinking water to bring the total level of fluoride to about 1 ppm (optimally fluoridated areas), there may have been an increase in the total prevalence of dental fluorosis. In non-fluoridated areas, there is clear evidence that the total prevalence of dental fluorosis has increased over the last 40 years.

The greater the fluoride exposure during tooth development, the greater the likelihood of dental fluorosis. In the 1940s and 1950s, the major sources of fluoride were from drinking water and food. Since then, numerous sources of fluoride have become available, including dental products containing fluoride (e.g., toothpastes and mouthrinses) and fluoride dietary supplements. The inappropriate use of these products can contribute significantly to total fluoride intake.

Increases in the prevalence of dental fluorosis in a population should be taken as evidence that fluoride exposure is increasing. Because dental fluorosis does not compromise oral health or tooth function, an increase in dental fluorosis, by itself, is not as much of a dental public health concern as it is an indication that total fluoride exposure may be more than necessary to prevent tooth decay. Prudent public health practice generally dictates using no more of a substance than the amount necessary to achieve a desired effect.

Bone Fractures

There is some suggestion from epidemiological studies that the incidence of certain bone fractures may be greater in some communities with either naturally high or adjusted fluoride levels. However, there are a number of confounding factors that need resolution to determine whether or not an association exists. Additionally, other studies do not show an increase in the incidence of bone fractures; one study provided evidence of a lower incidence of bone fractures in an optimally fluoridated community as compared to a similar community with trace levels of fluoride in the water. Therefore, further research is required.

Other Effects

Chronic low level fluoride exposure of healthy individuals does not appear to present problems in other organ systems, such as the gastrointestinal system, the genitourinary, and the respiratory systems.

It is not yet clear whether fluoride is essential for reproductive performance. Several species are sensitive to fluoride levels higher than those normally encountered, such that their fertility and reproductive performance is impaired. The potential for adverse reproductive effects of fluoride exposure to humans has not been adequately evaluated.

Fluoride is not a mutagen in standard tests in bacteria. While in rodent and human cells in tissue culture, fluoride is considered to be a mutagen and is considered to cause chromosome breakage, the genotoxicity of fluoride in humans and animals is unresolved despite numerous studies.

CONCLUSIONS

- Extensive studies over the past 50 years have established that individuals whose drinking water is fluoridated show a reduction in dental caries. Although the comparative degree of measurable benefit has been reduced recently as other fluoride sources have become available in non-fluoridated areas, the benefits of water fluoridation are still clearly evident. Fewer caries are associated with fewer abscesses and extractions of teeth and with improved health. The health and economic benefits of water fluoridation accrue to individuals of all ages and socioeconomic groups, especially to poor children.

- Since the addition of fluoride to drinking water in the 1940s, other sources of fluoride have become available, including toothpastes, mouthrinses, and fluoride dietary supplements. These sources of fluoride also have proven to be effective in preventing dental caries.

- Estimates developed for this report show that fluoride exposure is generally greater in

fluoridated areas; however, there is fluoride exposure in both fluoridated and non-fluoridated areas because of the variety of fluoride sources besides drinking water. Beverages and foods are sources of fluoride, especially if they have been prepared with fluoridated drinking water.

- Optimal fluoridation of drinking water does not pose a detectable cancer risk to humans as evidenced by extensive human epidemiological data available to date, including the new studies prepared for this report. While the presence of fluoride in sources other than drinking water reduces the ability to discriminate between exposure in fluoridated as compared to non-fluoridated communities, no trends in cancer risk, including the risk of osteosarcoma, were attributed to the introduction of fluoride into drinking water in these new studies. During two time periods, 1973-1980 and 1981-1987, there was an unexplained increase of osteosarcoma in males under age 20. The reason for this increase remains to be clarified, but an extensive analysis reveals that it is unrelated to the introduction and duration of fluoridation.

- There are two methodologically acceptable studies of the carcinogenicity of fluoride in experimental animals. The Procter and Gamble study did not find any significant evidence of carcinogenicity in rats and mice of either sex. In the NTP study there was no evidence of carcinogenicity in mice and in female rats. Male rats showed "equivocal" evidence of carcinogenicity based on the finding of a small number of osteosarcomas. "Equivocal" evidence is defined by NTP as "...interpreted as showing a marginal increase in neoplasms that may be chemically related" (DHHS, 1990). Taken together, the data available at this time from these two animal studies fail to establish an association between fluoride and cancer.

- By comparison with the 1940s, the total prevalence of dental fluorosis has increased in non-fluoridated areas and may have increased in optimally fluoridated areas. Such increases in dental fluorosis in a population signify that total fluoride exposures have increased and may be more than are necessary to prevent dental caries. For this reason, prudent public health practice dictates the reduction of unnecessary and inappropriate fluoride exposure.

- In the 1940s, drinking water and food were the major sources of fluoride exposure. Since then, additional sources of fluoride have become available through the introduction of fluoride containing dental products. Although the use of these products is likely responsible for some of the declines in caries scores, the inappropriate use of these products has also likely contributed to the observed increases in the prevalence of very mild and mild forms of dental fluorosis.

- Further epidemiological studies are required to determine whether or not an association exists between various levels of fluoride in drinking water and bone fractures.

- Crippling skeletal fluorosis is not a public health problem in the United States, as evidenced by the reports of only five cases in 30 years. Crippling skeletal fluorosis, a chronic bone and joint disease associated with extended exposure to high levels of fluoride, has been more prevalent in some region outside the U.S.

- Well controlled studies have not demonstrated a beneficial effect of the use of high doses of fluoride in reducing osteoporosis and related bone fractures.

- There is no indication that chronic low level fluoride exposure of normal individuals presents a problem in other organ systems, such as the gastrointestinal, the genitourinary, and the respiratory systems. The effects of fluoride on the reproductive system merit

further investigation in animal and human studies.

- Chronic low level fluoride exposure is not associated with birth defects. Studies also fail to establish an association between fluoride and Down Syndrome.

- Genotoxicity studies of fluoride, which are highly dependent on the methods used, often show contradictory findings. The most consistent finding is that fluoride has not been shown to be mutagenic in standard tests in bacteria (Ames Test). In some studies with different methodologies, fluoride has been reported to induce mutations and chromosome aberrations in cultured rodent and human cells. The genotoxicity of fluoride in humans and animals is unresolved despite numerous studies.

RECOMMENDATIONS

POLICY RECOMMENDATIONS

- The U.S. Public Health Service should continue to recommend the use of fluoride to prevent dental caries.

- The U.S. Public Health Service should continue to support optimal fluoridation of drinking water. Currently, the optimal level for water fluoridation is between 0.7 - 1.2 parts per million, depending on mean daily air temperature for a geographic area.

- The U.S. Public Health Service should sponsor a scientific conference(s) to recommend both the optimal level of total fluoride exposure from all sources combined (including drinking water) and the appropriate usage of fluoride containing dental products in order to achieve the benefits of reduced dental caries and to minimize the occurrence of dental fluorosis.

- The U.S. Environmental Protection Agency should review its regulations concerning naturally occurring fluoride in drinking water based on the outcome of the scientific conference(s) recommended above and based on this report.

- In accordance with prudent health practice of using no more than the amount necessary to achieve a desired effect, health professionals and the public should avoid excessive and inappropriate fluoride exposure. For example, health professionals should prescribe fluoride dietary supplements only when the fluoride level of the home water supply is known to be deficient. Parents should educate young children to minimize swallowing of fluoridated toothpaste and to use only small amounts of toothpaste on the brush.

- The U.S. Food and Drug Administration should review the labeling required for toothpaste and other fluoride containing products to ensure that the public has adequate knowledge to make informed decisions about their use, especially for young children (those under six years of age).

- Manufacturers of toothpaste should be encouraged to make the fluoride levels in their products easily known. Manufacturers should determine whether toothpaste can be dispensed in a dose limited container for use by children. Manufacturers of dental products should explore whether the levels of fluoride can be reduced while preserving clinical effectiveness.

- State health and drinking water programs should keep physicians, dentists, pharmacists, physician extenders, and communities informed about the fluoridation status of drinking water. This information will enable residents and health professionals to determine the need for water fluoridation or for supplemental forms of fluoride.

- Communities with high natural fluoride levels in the public drinking water supply should comply with EPA regulations as mandated by the Safe Drinking Water Act. The current primary and secondary maximum contaminant levels are 4 and 2 parts per million, respectively.

- An action plan to implement research and policy recommendations should be developed.

RESEARCH RECOMMENDATIONS

RESEARCH ON THE BENEFITS OF FLUORIDES

- Conduct surveys to evaluate the prevalence of dental caries over time and accurately assess exposure to fluoride.
- Undertake studies to elucidate further the role of fluoride in preventing coronal and root decay in adults. Undertake studies to identify effective means of providing fluoride to individuals at high risk of dental caries.
- Continue longitudinal studies of caries scores in cities after defluoridation or the discontinuation of fluoridation to supplement past information which covers only 2-5 years of follow-up period.
- Document the marginal risks, costs, and benefits of providing multiple fluoride regimens in the prevention of dental caries.
- Determine the relationship among socioeconomic status, water fluoridation status, and the use of fluoride products.
- In scoring dental caries, researchers should count individual surfaces rather than just the number of teeth because such scoring provides more information and greater sensitivity. Researchers should express reductions in caries scores as the number of tooth surfaces saved from caries, in addition to the percentage.

RESEARCH ON THE RISKS OF FLUORIDE

- Continue studies to elucidate the mechanisms of fluoride action on bone and teeth at the molecular and physical chemical levels.
- Develop a method of quantitatively identifying dental fluorosis that is sensitive, specific, reliable and acceptable to the public.
- Continue to study dental fluorosis to determine the etiology and trends in the prevalence of dental fluorosis.
- Conduct analytical epidemiological studies of osteosarcoma to determine the risk factors associated with its development. Fluoride exposure and bone levels of fluoride should be included in the study design.
- Evaluate the scientific merit of conducting further animal carcinogenicity studies which use a wide range of chronic fluoride doses. Industries sponsoring studies of fluoride should be encouraged to make their data publicly available to aid in this evaluation.
- Conduct analytical epidemiological studies to determine the relationship, if any, among fluoride intake, fluoride bone levels, diet, body levels of nutrients such as calcium, and bone fractures.
- Conduct studies on the reproductive toxicity of fluoride using various dose levels including the minimally toxic maternal dose.
- Conduct further studies to investigate whether or not fluoride is genotoxic.

REVIEW OF FLUORIDE BENEFITS AND RISKS

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APPENDIX A

February 16, 1990

Assistant Secretary for Health and
Acting Surgeon General

Review of Fluoride Risks and Benefits

Deputy Assistant Secretary for Health/Science and
Environment

In my capacity as Chairman of the Public Health Service's Committee to Coordinate Environmental Health and Related Programs (CCEHRP), I would like you to serve as Chairman of a new, ad hoc subcommittee of CCEHRP which would respond to heightened concern about water fluoridation and other uses of fluorides.

A new study by the National Toxicology Program (NTP), which is as yet incomplete and uninterpreted, reveals that sodium fluoride may be carcinogenic in one rodent species. Once this study is completed and peer-reviewed, I would like your new Subcommittee to analyze thoroughly its findings in the light of the scientific literature on the risks and benefits of fluoridation and other uses of fluorides. I would like you to provide me with monthly reports of your progress. By July 16, 1990 I would like to have the report finalized with recommendations for PHS policy.

I consider the subcommittee's task a very high priority for the PHS. Accordingly, I have already asked PHS agency heads to identify appropriate Subcommittee members and to allow those individuals to spend a substantial amount of time on this effort.

Given the tangible public health benefits of fluorides in reducing tooth decay, the rarity of the tumor type in humans that is implicated by the study, and the preliminary nature of the NTP findings, our current policy supporting fluoridation must be maintained until your review is finished.

/s/ James O. Mason

James O. Mason, M.D., Dr. P.H.

APPENDIX B

DENTAL FLUOROSIS CLASSIFICATION

DENTAL FLUOROSIS CLASSIFICATION BY H.T. DEAN - 1942[a]

CLASSIFICATION	CRITERIA
Normal	The enamel represents the usual translucent semivitriform type of structure. The surface is smooth, glossy, and usually of a pale creamy white color.
Very Mild	Small, opaque, paper white areas scattered irregularly over the tooth but not involving as much as 25 percent of the tooth surface. Frequently included in this classification are teeth showing no more than about 1-2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars.
Mild	The white opaque areas in the enamel of the teeth are more extensive but do not involve as much as 50 percent of the tooth.
Moderate	All enamel surfaces of the teeth are affected, and the surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature.
Severe	All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded-like appearance.

[a]Dean H.T. 1942. The Investigation of physiological effects by the epidemiological method. In: Moulton FR, ed. Fluorine and dental health. Washington, DC: American Association for the Advancement of Science, Publication No. 19, pp.23-31.

APPENDIX C

SURVEYS OF DENTAL FLUOROSIS PREVALENCE 1939-1987

SURVEYS OF DENTAL FLUOROSIS PREVALENCE, 1939-1987

TABLE C-1

DEAN'S 21 City Survey, 1940s

CITY	APPRX YEAR	N	F PPM	VERY MILD	MILD	MODER	SEVERE	TOTAL PREV	AUTHOR
Waukegan IL	1939	423	0.0	0.2	0.0	0.0	0.0	0.2	Dean et al.
Oak Park IL	1939	329	0.0	0.6	0.0	0.0	0.0	0.6	Dean et al.
Evanston IL	1939	256	0.0	1.6	0.0	0.0	0.0	1.6	Dean et al.
Mi City, IN	1940	236	0.1	0.0	0.0	0.0	0.0	0.0	Dean et al.
Quincy IL	1940	330	0.1	0.3	0.0	0.0	0.0	0.3	Dean et al.
Elkhart IN	1940	278	0.1	0.4	0.0	0.0	0.0	0.4	Dean et al.
Portsmouth OH	1940	469	0.1	1.3	0.0	0.0	0.0	1.3	Dean et al.
Middletown OH	1940	370	0.2	1.1	0.0	0.0	0.0	1.1	Dean et al.
Zanesville OH	1940	459	0.2	1.5	0.0	0.0	0.0	1.5	Dean et al.
Lima OH	1940	454	0.3	2.2	0.0	0.0	0.0	2.2	Dean et al.
Marion OH	1940	263	0.4	5.3	0.8	0.0	0.0	6.1	Dean et al.
Elgin IL	1939	403	0.5	3.5	0.7	0.0	0.0	4.2	Dean et al.
Pueblo CO	1940	614	0.6	6.2	0.3	0.0	0.0	6.5	Dean et al.
Kewanee IL	1939	123	0.9	10.6	1.6	0.0	0.0	12.2	Dean et al.
Aurora IL	1939	633	1.2	13.9	1.1	0.0	0.0	15.0	Dean et al.
Joilet IL	1940	447	1.3	22.2	3.2	0.0	0.0	25.3	Dean et al.
E Moline IL	1940	152	1.6	29.6	2.0	0.0	0.0	32.0	Dean et al.
Maywood IL	1939	171	1.6	29.2	4.1	0.0	0.0	33.3	Dean et al.
Elmhurst IL	1939	170	1.8	30.0	8.8	1.2	0.0	40.0	Dean et al.
Galesburg IL	1940	273	1.9	40.3	6.2	1.1	0.0	48.0	Dean et al.
C Springs CO	1940	404	2.6	42.1	21.3	8.9	1.5	73.8	Dean et al.

TABLE C-2

Studies of the 1980s

CITY	YEAR	N	CONC F	MILD	VERY MILD	MODER	SEVERE	TOTAL PREV	AUTHOR
BP, Du, Ma, MV, I	1980	316	0.0*	1.9	1.0	0.0	0.0	2.9	Driscoll et al.
Kingston, NY	1985	425	0.0	2.2	7.0	0.0	0.0	7.3	Kumar et al.
West NY State	1981	564	0.3	2.7	1.1	0.6	0.0	4.4	Leverett
San Marcos, TX	1981	223	0.3*	8.5	0.0	0.0	0.0	8.5	Segretto et al.
N Braunfels, TX	1981	103	0.3*	6.8	1.9	0.0	0.0	8.7	Segretto et al.
San Antonio, TX	1981	126	0.4*	2.4	0.0	0.0	0.0	2.4	Segretto et al.
Kewanee, IL	1980	336	1.0*	7.4	4.8	1.8	0.6	14.6	Driscoll et al.
Rochester, NY	1982	729	1.0	22.6	3.2	1.1	0.0	26.9	Leverett
Kingsville, TX	1981	361	1.0*	36.6	2.5	0.3	0.0	39.4	Segretto et al.
Newburgh, NY	1985	459	1.0	2.0	7.0	0.0	0.0	7.7	Kumar et al.
Alvin, TX	1981	211	1.3*	22.3	5.7	0.9	0.0	28.9	Segretto et al.
Angleton, TX	1981	187	1.3*	21.4	10.2	1.1	0.0	32.7	Segretto et al.
Kerrville, TX	1981	128	1.4*	14.8	0.8	0.0	0.0	15.6	Segretto et al.
Monmouth, IL	1980	143	2.0*	23.1	16.8	8.4	4.9	53.2	Driscoll et al.
Littlefield, TX	1981	109	2.3*	37.6	25.7	14.7	0.0	78.0	Segretto et al.
Ft.Stockton, TX	1981	301	2.5*	38.2	24.2	3.3	0.0	65.7	Segretto et al.
Monahans, TX	1981	170	2.7*	32.4	30.0	13.5	0.0	75.9	Segretto et al.
Perryton, TX	1981	90	2.7*	42.2	33.3	6.7	0.0	82.2	Segretto et al.
Hillsboro, TX	1981	200	2.7*	52.0	34.5	4.0	0.0	90.5	Segretto et al.
Abernathy, TX	1981	67	2.9*	19.4	41.8	32.8	0.0	94.0	Segretto et al.
Abi, Elm, Ipava	1980	192	3.0*	15.1	19.8	7.8	8.3	51.0	Driscoll et al.
Gatesville, TX	1981	113	3.1*	44.2	28.3	4.4	0.0	76.9	Segretto et al.
Bushnell, TGrv	1980	136	4.0*	16.9	25.0	7.4	22.8	72.1	Driscoll et al.
Taylor, TX	1981	190	4.3*	32.6	30.5	31.1	5.0	94.7	Segretto et al.

* Indicates "level of optimal" fluoride concentration, not an exact mg F/L concentration

TABLE C-3

Dental Fluorosis Studies of the 1980s Using TSIF Index

Percentages distribution for all tooth surfaces

CITY	YEAR	AGE	N	LEVEL F	TSIF 1	TSIF 2	TSIF 3	TSIF 4	TSIF 5	TSIF 6	TSIF 7	TPREV (surfs)	TPREV (indivs)	AUTHOR
Cadillac, MI	1987	6-12	131	0.0	11.7	0.3	0.1	0.1	0.0	0.1	0.0	12.2	15.0	Szpunar & Burt
Mich Comm	1987	9-13	543	0.0	6.1	0.6	0.2	0.0	0.0	0.0	0.0	6.9	22.3	Woolfolk et al.
Hudson, MI	1987	6-12	133	0.8	31.2	0.3	0.0	0.1	0.0	0.0	0.0	31.6	32.5	Szpunar & Burt
Redford, MI	1987	6-12	249	1.0	49.7	1.3	0.0	0.0	0.0	0.0	0.0	49.0	48.4	Szpunar & Burt
Richmond, MI	1987	6-12	43	1.2	35.4	0.7	0.2	0.0	0.0	0.0	0.1	36.3	60.1	Szpunar & Burt
Kewanee, IL	1980	8-10	113	1.0	14.8	2.3	1.6	0.0	1.0	0.0	0.0	18.8		Heifetz et al.
Kewanee, IL	1980	13-15	111	1.0	9.1	1.5	0.8	0.0	0.0	0.0	0.0	11.4		Heifetz et al.
Kewanee, IL	1985	8-10	156	1.0	20.6	5.6	1.8	0.0	1.0	0.0	0.0	28.0		Heifetz et al.
Kewanee, IL	1985	13-15	94	1.0	21.6	4.9	2.8	1.0	0.0	0.0	0.0	29.3		Heifetz et al.
Monmouth, IL	1980	8-10	61	2.0	33.0	6.9	6.8	2.0	2.0	0.0	0.0	47.0		Heifetz et al.
Monmouth, IL	1980	13-15	39	2.0	25.4	7.8	5.0	0.0	1.0	0.0	0.0	38.3		Heifetz et al.
Monmouth, IL	1985	8-10	102	2.0	30.4	11.6	8.7	0.0	1.3	0.0	0.0	52.0		Heifetz et al.
Monmouth, IL	1985	13-15	23	2.0	32.5	18.6	13.8	0.3	1.3	0.0	0.0	65.5		Heifetz et al.
Abi, Elm, Ipava	1980	8-10	82	3.0	30.6	10.9	8.1	0.5	1.0	1.0	3.0	51.5		Heifetz et al.
Abi, Elm, Ipava	1980	13-15	50	3.0	21.6	13.7	9.6	0.2	0.7	0.0	1.0	46.0		Heifetz et al.
Abi, Elm, Ipava	1985	8-10	112	3.0	29.4	12.3	8.2	0.2	1.5	0.0	4.0	52.0		Heifetz et al.
Abi, Elm, Ipava	1985	13-15	47	3.0	34.9	18.2	13.6	3.0	1.2	1.0	9.0	69.2		Heifetz et al.
Bushnell, TGrv	1980	8-10	59	4.0	28.5	17.1	19.7	0.3	2.8	1.0	1.2	69.7		Heifetz et al.
Bushnell, TGrv	1980	13-15	34	4.0	25.6	16.7	18.6	0.3	1.3	0.1	0.5	63.1		Heifetz et al.
Bushnell, TGrv	1985	8-10	62	4.0	32.2	18.7	19.7	6.0	3.1	1.0	1.4	75.8		Heifetz et al.
Bushnell, TGrv	1985	13-15	29	4.0	30.8	18.8	22.1	5.0	3.9	0.0	1.5	77.6		Heifetz et al.

* For the Heifetz et al. studies, level of F represents "factor of optimal" F, not an exact water fluoride concentration.

TABLE C-4

Dental Fluorolsis Studies Using T-F Index

CITY	YEAR	N	F	TF1	TF2	TF3	TF4	PREV	AUTHOR
Toronto,Canada	1987	633	1.0	10.0	2.2	0.6	0.2	12.9	Osuji et al.

TABLE C-5

Dental Fluorosis Study Index Not Reported

CITY	YEAR	N	F	TF1	TF2	TF3	TF4	PREV	AUTHOR
Conn, Mass	1986	4222	0.3	NK	NK	NK	NK	25.2	Pendrys

* For the Heifetz et al. studies, level of F represents "factor of optimal" F, not an exact water fluoride concentration.

APPENDIX - D

CDER CEAC - 90-06

FINAL REPORT

Dose Determination and Carcinogenicity Studies of Sodium Fluoride in Crl:CD-1 Mice and Crl:CD (Sprague Dawley)BR Rats
June 28, 1990

FINAL REPORT

Dose Determination and Carcinogenicity Studies of Sodium Fluoride
in Crl:CD-1 Mice and Crl:CD (Sprague Dawley)BR Rats

ABSTRACT - In studies of fluoride in CD-1 mice and Sprague Dawley rats, there was no increase in malignant tumors of bone or other organs. There was a compound-related increase in benign osteomas in CD-1 mice, with a clear increase seen only in the high dose group. The mouse study was flawed by the lack of a dose-determination study, by diet and water that were outside specifications, and by viral outbreaks. Incomplete histologic examination of the low and mid dose bones from terminal sacrifice animals in the mouse study leaves it indeterminate with respect to whether there were increase osteomas at the lower doses. The rat study was flawed by an inappropriate dose determination study, by diet and water that were outside specifications, possibly by viral outbreaks, and by relatively high mortality.

CAC Members -

HFD-502/Weissinger (chair)	HFD-715/Fairweather
HFD-110/Resnick	HFD-510/Jordan
HFD-120/Contrera	HFD-520/Osterberg
HFD-150/Hamrell	HFD-530/Green
HFD-160/DeWitt	HFD-180/Choudary

CAC 90-01 Ad Hoc Members
HFD-715/Lin, D. HFD-160/Yu

Consultants
NIEHS/Eustis AFIP/Slayter CVM/O'Neill

Advisors

HFD-100/Temple	HFD-500/Bilstad
HFD-101/Botstein	HFD-501/Burlington
HFD-007/Harter	HFD-150/Palmer

Dr. Bilstad, Botstein, and Burlington were unable to attend. Dr. Jean attended for Dr. Harter.

SUMMARY OF CAC DELIBERATIONS - Only preliminary data from the draft reports were available for evaluation of the mouse study. The slides are currently being reread in toto, and the final report is pending.

I. INTRODUCTION

The Division of Medical Imaging, Surgical, and Dental Drug Products made available to the Carcinogenicity Assessment Committee (CAC) their reviews, the statistical reviews, and background data on the carcinogenicity studies on sodium fluoride, submitted under a Procter and Gamble IND, for evaluation. Two year mouse and rat studies were conducted. According to the sponsor, the doses were selected based upon the results of a 91 day rat dose-determination study, among other considerations.

II. STUDY DESCRIPTION

II. STUDY DESCRIPTION

A. Mouse Dose-Determination Study

No study conducted.

B. Rat dose-determination study

A 91-day oral gavage study was conducted at doses of 8.7, 19, and 43 mg/kg/day, using a slurry of sodium fluoride dentifrice in distilled water. The doses for the carcinogenicity study were selected based upon 1) the results of this study, 2) published information in the scientific literature, and 3) the anticipated level of human exposure.

C. Mouse Carcinogenicity Study

Male and female CD-1 mice (60/sex/dose) received sodium fluoride mixed into a basal low-fluoride (Teklad) diet at 0, 4, 10, or 25 mg/kg/day for 95 and 97 weeks, respectively. An additional control group received a powdered Purina rodent chow.

D. Rat Carcinogenicity Study

Sprague Dawley rats (70/sex/ group) received sodium fluoride added to a synthetic low-fluoride (Teklad) diet at doses of 0, 4, 10, or 25 mg/kg/day for 95 and 99 weeks (males and females, respectively). An additional control group received powdered purina rodent chow.

III. STUDY RESULTS

A. Mouse Dose-Determination Study

No study conducted

B. Rat Dose-Determination Study

In life observations, gross necropsy, and clinical chemistry revealed no effects at 43 mg/kg. There was, however, a 7% decrease in body weight gain in females at 19 mg/kg and 11% decrease at 43 mg/kg. Males showed a 13% decrease in body weight at 43 mg/kg. Overall food consumption did not decrease in treated animals by more than 11% in any week. The average kidney weight and kidney weight to body weight ratios were increased 28 and 45% at the high dose in females and 5 and 25% in males, respectively. Interstitial nephritis and/or dilated tubules were observed at all doses. Reversible forestomach gastritis was observed.

C. Mouse Carcinogenicity Study

There was a clear increase in high dose animals, male and female, in characteristic lesions of fluoride exposure (hyperostoses, osteoscleroses, and enostoses) and in osteomas, a benign lesion that is sometimes hard to distinguish from the typical fluoride lesions. The incidence of osteomas is reviewed in the table below:

Relevant bone	Male (mg/kg)					Female (mg/kg)				
lesions	0	0	4	10	25	0	0	4	10	25
osteomas	1	1	0	3	21	3	4	7	4	17
	2%	2%	0%	7%	35%	5%	9%	17%	9%	28%

Based on actual number of animals examined

The non-neoplastic lesions are not always easily demarcated from osteomas and an AFIP consultant pathologist felt there was a diagnostic drift during the day's readings. Nonetheless, while the exact number of each lesions could be debated, the increase in osteomas and non-neoplastic lesions is unequivocal. In low and mid-dose animals, the bones of terminal sacrifice animals were not examined. It is possible that a full analysis of these groups would reveal an increased rate of osteomas in those groups.

The osteomas were reviewed by an AFIP consultant pathologist who agreed with the initial reader that none were malignant.

Results of one-tailed statistical analyses for testing "positive dose-response relationships" using the method described by Peto, et al. 1980, and a pairwise comparison are presented below for the Teklad controls (C1), the Purina controls (C2), and the low, mid and high dose animals (L,M,H):

Tumors	Statistical Significance		
osteoma	Peto(trend)	(M,F)	Pairwise (M,F)
C1, L, M, H	p<0.00001, p=0.000357	C1,H	p=0.000001, p=0.00031
C2, L, M, H	p<0.00001, p=0.00359	C2,H	p=0.000024, p=0.00707
C_1+C_2, L, M, H	p<0.00001, p=0.000138	C_1+C_2,H	p<0.000001, p=0.00007

Fluoride levels were measured in bone to confirm exposure using a fluoride ion specific electrode analysis and the following levels were reported:

		ug F/gram ash (ppm)	
Treatment	Group	Males	Females
Control Diet	1	1582±309	970±69
Control Chow	2	1676±38	1294±79
4 mg/kg F	3	4405±828	3380±503
10 mg/kg F	4	7241±621	6189±610
25 mg/kg F	5	13177±565	10572±1717

There was an apparent effect of the Teklad diet. All Teklad fed animals had pale livers and gastric hairballs. The Purina rodent chow control mice did not.

D. Rat Carcinogenicity Study

There were four tumors found in bone, three were of osteogenic origin while the fourth was a fibroblastic sarcoma with areas of osteoid formation. Dental and bone lesions (gross and histologic) consistent with chronic fluoride toxicity were increased in all dose groups. Diet-related lesions included pale livers and gastric hairballs. There was also dose-dependent chronic gastritis.

	Male (mg/kg)					Female (mg/kg)				
Relevant lesions	0	0	4	10	25	0	0	4	10	25
osteosarcoma	0	0	0	0	1	0	0	2	0	0
	0%	0%	0%	0%	2%	0	0	6%	0%	0%
fibroblastic	0	0	0	1	0	0	0	0	0	0
sarcoma	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%
glandular stomach										
ulceration	2	0	3	6	2	3	1	5	3	5
	3%	0%	6%	9%	3%	4%	2%	9%	5%	9%
acanthosis	11	6	13	24	46	18	4	14	39	40
	16%	13%	24%	38%	66%	26%	9%	25%	59%	58%

Based on actual number of animals examined

The osteosarcoma in the high dose male and one of the low dose female osteosarcomas were identified by AFIP but not by the sponsor, who considers the lesions debatable. Whether they are included or not, however, there is no significant increase in tumors in rats in this study.

Results of one-tailed statistical analyses for testing "positive dose-response relationships" using the Peto prevalence method and counting all four tumors are:

Tumors	Statistical Significance	
osteosarcoma	Peto	(M,F)
C1, L, M, H	p=0.2965	p=0.7778
C2, L, M, H	p=0.2695	p=0.7780
C1+C2, L, M, H	p=0.2287	p=0.6351
fibroblastic sarcoma		
C1, L, M, H	p=0,4709	
C2, L, M, H	p=0.4709	C1+C2, L, M, H p=0.3632
osteosarcoma and fibroblastic sarcoma		
C1, L, M, H	p=0.1907	
C2, L, M, H	p=0.1907	
C1+C2, L, M, H	p=0.1133	

Tumors	Statistical Significance	
glandular	Peto	(M,F)
stomach ulceration		
C1, L, M, H	p=0.618	p=0.3408
C2, L, M, H	p=0.4997	p=0.2895
C_1+C_2, L, M, H	p=0.3832	p=0.2235
glandular stomach acanthosis		
C1, L, M, H	p<0.000001	p=0.000006
C2, L, M, H	p<0.000001	p<0.000001
C1+C2, L, M, H	p<0.000001	p<0.000001

Fluoride levels were measured in bone to confirm exposure using ion specific electrode analysis and the following levels were reported:

		ug F/gram ash (# samples)	
Treatment	Group	Males	Females
Control Diet	1	467 (10)	505 (10)
Control Chow	2	691 (10)	785 (10)
4 mg/kg F	3	5014 (10)	4541 (10)
10 mg/kg F	4	8849 (10)	8254 (10)
25 mg/kg F	5	16761 (10)	14428 (10)

According to the sponsor, the bone radiographs of the female rats at terminal sacrifice were negative.

IV. GENERAL DISCUSSION

A. Study Design and Validity

Experimental animals, including Teklad control animals, showed generally poor survival and studies were terminated prior to two years. As provided for prospectively in the protocol, the experiment was terminated when any group reached 10 survivors; this occurred in the high dose male mice, mid dose female mice, low dose male rats, and control female rats . Thus the male and female mouse and rat

studies were 95, 97, 95, and 99 weeks, respectively.

The rat dose-determination study was conducted using a dentifrice slurry administered by gavage. Dentifrice slurry by gavage, is not an appropriate design in preparation for a carcinogenicity study giving sodium fluoride as a dietary admix. Nonetheless, as all rat groups showed signs of chronic fluoride toxicity and the treated animals showed decreased weight gain compared to Teklad controls, the study was probably conducted with doses in close proximity to the MTD in the rat study. The bone showed clear toxicity. The mice had a lower incidence of gross signs of fluorosis in teeth than the rats. Although there was histologic evidence of target organ toxicity in bone, there was no decrease in weight gain compared to Teklad diet controls and no effect of fluoride on mortality in mice was observed, suggesting the MTD may not have been used in mice.

B. Study Performance

The studies had a number of limitations with uncertain effects on the observations made. The diet used may not have allowed normal growth and development. Diet-related lesions were observed in all rats and mice except those fed the Purina rodent chow. Pale livers and gastric hairballs were found in all animals fed the Teklad diet, even in groups receiving added fluoride and the Teklad animals had increased weight with respect to rodent chow controls, especially in mice. The diet and water were often outside specifications (high) for minerals, ions, and vitamins.

A Type C retrovirus was present in the mice used in the study and a Sendai viral outbreak was identified during about weeks 32-36 of the study. Viral outbreaks were confirmed during the pretest and were suspected to occur (based upon antemortem evidence) during the study in rats. There is some suggestion that Type C retroviruses may be the cause of osteogenic lesions. Active virus was found in the osteomas but not in animals that did not have osteomas. It is clear, nonetheless, that if they had a role it was only in the presence of fluoride. The fact that viral particles were associated with osteoma could suggest that virus caused the osteoma but could also reflect activation of virus by rapid growth. The relationship of the virus to the lesions with respect to causation is not clear in this case, because there were increased osteomas observed at the high fluoride dose.

The adequacy of the gross examination at necropsy was questioned based upon the rat bone tumors that were not identified by the contract laboratory. Bone sections from low and mid dose animals probably should have been
included regardless of the high-dose findings, since bone and teeth are the target organs. Given the high mortality in the rat study, most (50- 80%) of the animals were included in the examinations.

C. Study Conclusions

Mouse carcinogenicity study:

There was clearly a compound-related increase in osteomas in both male and female mice. It is not clear whether these are merely extensions of the well-known proliferative effect of fluoride on bone or represent something more, but it is reassuring that in the presence of so many benign tumors, no osteosarcomas were found. The study has limitations. The presence of a Type C retrovirus confuses the issue somewhat and it is possible the tumor response was related to this factor (i.e. an interaction with fluoride rather than a direct effect). It is also possible that the retrovirus played no role at all and its presence in tumors, but not normal bone, may mean only that the retrovirus was expressed in the presence of rapid growth. The study was flawed by the lack of a dose-determination study in mice and the diet and water were often outside the specifications; Sendai viral contamination was also reported so the health status of the animals is questionable. We can conclude that in this study fluoride did not produce any statistically significant increase in osteosarcomas or any other malignancy, despite the clear increase in a benign lesion, osteoma.

Rat Carcinogenicity Study:

Procter and Gamble identified two tumors in rats; a premaxillary osteosarcoma in a low dose female and a fibroblastic sarcoma in a high dose male rat. Review of over 4000 slides by AFIP (Drs. Slayter, Pletcher, and Johnson) identified two additional tumors; a fibroblastic sarcoma in a mid dose male and a maxillary osteosarcoma in a low dose female. Even with the added tumors, there is no evidence of carcinogenicity as there were just 2 tumors in males (one high dose and one mid dose) and two tumors in low dose females. Bony proliferative lesions consistent with chronic fluoride exposure were observed in this study. The study may have been flawed by 1) the unidentified gross pathology (some histopathologically identified sizable tumors were identified by AFIP), and 2) high mortality, 3) an inappropriate dose-determination study to assess the adequacy of the dose, 4) diet and water often outside the specifications, 5) incomplete histologic examination in low and mid dose bone tissue (for terminal sacrifice animals) and 6) pretest viral outbreaks that may have affected the health status of the rats.

Under the conditions of the studies, malignant tumors related to dietary fluoride exposure in rodents were not observed. An increased frequency of benign tumors was observed in mice at the high dose (osteomas).

V. CAC INTERPRETATION AND RECOMMENDATIONS

There are flaws and uncertainties in the studies that keep them from providing strongly reassuring data. These included the role of the synthetic Teklad diet, incomplete tissue examination, and possible viral influences, etc. The CAC noted the difficulty in assessing the dose-related aspect of the osteomas in mice when all bones from mice sacrificed at termination were not evaluated histopathologically. It may be possible that this can be corrected. The CAC recommended that the sponsor attempt to obtain these data.

The mouse data from the Procter and Gamble study can be considered along with the NTP mouse study. The NTP study was conducted in another strain of mouse at slightly lower doses of fluoride administered in the drinking water and showed no evidence of carcinogenicity. It should be noted, however, that the NTP mice had little bone histopathologic effects and definite mottling of teeth (consistent with a lower exposure to fluoride) compared to the Procter and Gamble study, which showed histopathologic bone and teeth effects associated with chronic fluoride toxicity. Together the studies do not add to the concerns raised by the NTP rat study. While osteomas were seen in the Procter and Gamble study, their distinction from other bone effects of fluoride is unclear and they did not appear in the NTP study. No malignant bone tumors were seen.

The results of the Procter and Gamble rat study, based upon the data submitted and slides reviewed, do not reinforce the equivocal finding in the NTP study male rats, and appears to reaffirm the negative finding in females.

APPENDIX E

FLUORIDATION OF DRINKING WATER AND SUBSEQUENT CANCER INCIDENCE AND MORTALITY

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INTRODUCTION

For over 40 years, fluoridation of municipal drinking water supplies to prevent dental caries has sparked controversy. The issues involved have been political and public policy in nature, with claims of possible health effects sometimes evoked to buttress the arguments. Among the many adverse effects raised by opponents of fluoridation as being possibly related to fluoride ingestion is the risk of malignancy. For this reason, the relationship between natural and artificial fluoridation of drinking water and cancer incidence and mortality has been the subject of numerous epidemiologic studies. These investigations, employing a variety of methods, have been conducted in many regions around the world. The results have been intensively reviewed by a number of expert panels (1-3), and the conclusions reached have been remarkably consistent. That is, when appropriate methods are applied to high-quality data, the resulting analyses have not found any evidence of an association between fluoridation of drinking water and an increased risk of cancer.

In March of 1990, a preliminary report of a bioassay study conducted in rodents under the auspices of the National Toxicology Program suggested a relationship between the dose of fluoride and the risk of osteosarcomas and possibly the risk of oral tumors (4). The Draft Technical Report (5) prepared subsequently for a peer review committee concluded that: "Under the conditions of these 2-year dosed water studies, there was equivocal evidence of carcinogenic activity of sodium fluoride in male F344/N rats, based on the occurrence of a small number of osteosarcomas in dosed animals. There was no evidence of carcinogenic activity in female F344/N rats receiving sodium fluoride at concentrations of 25, 100, or 175 ppm (11, 45, or 79 ppm fluoride) in drinking water for 2 years. There was no evidence of carcinogenic activity of sodium fluoride in male or female mice receiving sodium fluoride at concentrations of 25, 100, or 175 ppm in drinking water for 2 years." The suggestion of a relationship with oral cancer was discounted in this report: "The squamous cell neoplasms in rats receiving sodium fluoride were not considered chemical related because a squamous cell carcinoma was observed in one control male (paired control group) and in one control female, the incidence rates in the dosed groups were not significantly greater than in concurrent controls and were within the range of historical controls, and there was no supporting evidence of focal hyperplasia of the oral mucosa."

In 1976, the National Cancer Institute (NCI) evaluated 20 years of U.S. cancer mortality and a limited amount of cancer incidence data, and found no pattern consistent with an adverse effect of fluoridation (6). In the face of the new laboratory findings, we felt it was appropriate to update this study. The passage of time has provided 16 additional years of mortality data, enabling an evaluation of the effects of up to 35 years of fluoridation. In addition, the establishment in 1973 of the NCI Surveillance, Epidemiology and End Results (SEER) program, a network of population-based cancer incidence registries, has resulted in more relevant incidence data than were previously available. Incidence statistics are especially important for the evaluation of bone osteosarcoma, since histology-specific information is not generally available in mortality data. In addition, mortality data for bone cancer are limited since bone is a frequent site of metastases for a number of common malignancies, which may be misclassified as bone cancer deaths.

MATERIALS AND METHODS

Population-based cancer incidence data for 1973-87 were obtained from the nine registries participating in the SEER program (7). This program provides continuous incidence coverage for about 10% of the U.S. population. National mortality data from 1950-85 were obtained from the Center for Health Statistics, and populations at risk were estimated based on decennial censuses conducted by the Census Bureau (8).

Data regarding fluoridation of municipal water supplies and levels of natural fluoride were based on records from a series of published fluoridation censuses provided by the Centers for Disease Control (CDC) (9-12). The communities served by these water systems were identified and their populations related to the total population of the counties in which they were located. The listings of counties were reviewed with the assistance of the CDC, then modified, and finally validated by state public health or drinking water supply officials. The proportion of each county's population that received fluoridated water was used to categorize counties as non-fluoridated (control or non-exposed), fluoridated (exposed), or out-of-scope (not qualifying as a fluoridated or non-fluoridated county). A small proportion of the digitized records of the 1985 Fluoridation Census, from which our lists were first generated, do not include populations of served communities. While we made extensive efforts to complete this information, it is possible that a small number of fluoridated counties which should have been included in our analysis were inadvertently omitted. However, even if this occurred, it would not have biased the results of the analyses.

For the mortality analysis, we focused attention on areas likely to have centralized water systems, and thus we included only those counties that were more than 50% urban in 1980. Due to difficulties in county definitions, we excluded Alaska, Hawaii, and Virginia from the analysis. A county was considered exposed if the proportion of the population served by fluoridated water increased from no more than 10% to more than two-thirds within a three-year period. Control counties were those with no more than 10% of the population through 1985 ever served by artificially-fluoridated water or by water that was naturally fluoridated above 0.3 ppm. The mortality analysis thus is based on 131 fluoridated counties with a 1980 population of 40,362,091 persons, accounting for 22% of the U.S. population, and 195 non-fluoridated counties with a population of 30,216,689, accounting for 13% (Appendix Table E).

Since many fewer counties were available for the incidence analyses, slightly more liberal criteria were used for defining exposure. Selection was not limited to counties that were >50% urban. Levels of natural fluoride were not considered. This is reasonable since the evaluation was based entirely on time trends--an analysis that would be sensitive to changes in fluoridation and not influenced by the baseline levels. Finally the proportion of the population served that would qualify it as an exposed county was lowered to 60%. Thus, for the incidence analysis, a county was considered exposed if the proportion of the population served by artificially-fluoridated water (usually about 1 ppm) increased from no more than 10% to more than 60% within a three-year period. If fluoridation occurred over more than one year, the single year that the largest proportion of the population was affected was used as the index year for fluoridation. Control counties were those with less than 10% of the population ever served, through 1987, by artificially-fluoridated water. Counties which did not meet the criteria for being either fluoridated or non-fluoridated were excluded from the study. Seven of the nine SEER areas had inadequate variation in fluoridation exposure levels to allow for meaningful analysis. Two SEER areas, Iowa and the Seattle metropolitan area, included both fluoridated and control counties: 11 exposed and 14 non-exposed counties in Iowa, and one exposed and seven non-exposed counties in Seattle (Appendix Table A). Incidence data for 1973 were not available for the Seattle area, as the registry commenced operation in 1974.

Our study was restricted to the white population to avoid confounding by the substantial racial variation in cancer incidence and mortality rates (13). Definitions of the groupings of cancers are presented for the incidence analysis in Appendix Table B and for the mortality analysis in Appendix

Table F. Major emphasis was placed on changes in cancer risk relative to the time of fluoridation in those counties that were artificially fluoridated, comparing ever-fluoridated with never-fluoridated counties for baseline purposes only. Observed cases (or deaths) in fluoridated counties were compared to those expected based on age-, sex-, time-, and region-specific rates in non-fluoridated counties. These were aggregated into five-year periods relative to time of fluoridation, and the ratio of observed-to-expected (O/E) events was then calculated (14). Poisson regression models were used to estimate corresponding relative risks (RR), 95% confidence intervals (CI), and the significance of trends in RRs according to duration of fluoridation (15,16). For the Poisson analysis, data were cross classified by county, year (1973-77, 78-82, 83-87), age (0-4, 5-9 ..... 85+), sex and time since fluoridation (never, 0-4, 5-9, 10-14, 15-19, $\geq$20). The mean duration of fluoridation within each cell was used as the quantitative value for the trend tests. All regression analyses controlled for categories of age and year, at least, and for area and sex where appropriate.

RESULTS

INCIDENCE ANALYSIS

Bone Cancer: Table 1 presents the observed numbers of incident cancers of the bones and joints and the subset of osteosarcomas occurring in fluoridated counties of the Iowa and Seattle SEER areas, and the O/E ratios based on expected values generated from the age-, sex-, time-, and area-specific rates in the non-fluoridated counties. The O/E ratios are presented by duration of fluoridation from <5 years through $\geq$20 years. Overall, the risks in fluoridated and non-fluoridated areas were quite similar. A total of 290 cases of bone and joint cancer occurred in fluoridated areas compared to 279 expected based on the rates in non-fluoridated areas. The comparable numbers for osteosarcomas were 91 observed and 93 expected. There were no patterns in the O/Es by interval from fluoridation that suggested an effect either in the total group or in men and women separately. The O/E ratio was slightly higher in the <5 year interval from fluoridation for bone and joint cancers, based on nine observed cases of osteosarcoma vs. 4.89 expected. While examination of patterns in each of the two areas was hampered by small numbers, all nine cases were observed in Seattle compared to 2.91 expected during this short interval from fluoridation.

The categorization of duration of fluoridation was revised for cancers which have a substantial childhood component, such as bone malignancies. Obviously, for example, in counties fluoridated for 20 or more years, individuals under 20 years of age could not attain 20 years of exposure. Thus for bone malignancies and osteosarcomas, the observed and expected numbers were also classified by duration of fluoridation or age, whichever was less. The results of this analysis resembled the patterns seen in Table 1. For all bone and joint cancers, the O/Es by duration of fluoridation were 1.3, 1.0, 1.1, 1.1, and 0.9 for <5, 5-9, 10-14, 15-19 and 20+ years, respectively. For osteosarcomas, the corresponding O/Es were 1.8, 1.0, 0.8, 1.0, and 0.9. Because of the age curve for these tumors and the timing of fluoridation, we also compared the incidence among those under age 30 who resided in counties with fluoridated water for their entire lifetime to the incidence among those exposed for only part of their life. Using this approach, the observed number of bone and joint cancers among those with lifetime exposure was 77 compared to an expected value of 58.16 based on age-, sex-, time- and area-specific rates in the non-fluoridated counties (O/E=1.3). Among those exposed less than one-half of their life, the observed number was 23 compared to an expected of 23.11 (O/E=1.0). While this ratio was slightly lower than that for the group with lifetime exposure, the two O/Es were not significantly different from one another (95% confidence interval for the O/E of 1.0 is 0.6-1.5). The observed and expected values for osteosarcoma associated with lifetime exposure were 30 and 32.65 (O/E=0.9), while the corresponding values associated with less than one-half lifetime exposure were 10 and 11.15 (O/E=0.9). Thus, these results do not provide any evidence of an adverse effect of fluoridation on the risk of bone tumors.

While not under *a-priori* suspicion, other histologic types of bone cancer were also evaluated for their relationship to timing of fluoridation. There was an excess of Ewing's sarcoma in the fluoridated

counties (observed = 49, expected = 30.33, O/E=1.6). Virtually all of this excess was derived from the 20+ years of fluoridation category, where 16 cases were observed versus 1.64 expected (O/E=9.8). Upon further evaluation, it was noted that the expected value for the 20+ year category was extremely unstable, since the rate used to generate it was based on only one case occurring in the non-fluoridated portions of Iowa during the relevant time period. The question arose as to whether there was a real excess, or whether the rate of this rare tumor was low simply by chance in the relatively small population of the non-fluoridated portions of Iowa during this time. To address this issue, we expanded the comparison population by adding to the non-fluoridated counties in Iowa all counties in the rest of the SEER program where less than 10% of the population was exposed to artificially or naturally fluoridated drinking. When the resulting rates were applied to the person-years in the 20+ years-of-fluoridation category, the expected value was 20.45, yielding an O/E ratio of 0.8. Thus, the early observation of an excess of Ewing's sarcoma associated with 20+ years of fluoridation appears to be an artifact due to chance based on an unstable rate in the initial comparison population.

Oral Cancers: Table 2 gives the observed numbers of malignancies of the oral cavity and pharynx occurring in fluoridated counties and the corresponding O/Es by duration of fluoridation for the Iowa and Seattle SEER areas. In Iowa, the observed number of cases in counties fluoridated less than five years was 1.2 times that expected based on the rates in the non-fluoridated counties. The ratio rose to 1.4 in counties fluoridated for 5-9 years, 1.7 for 10-14 years, and 1.6 for the longer intervals. The increase in O/E followed by a plateau reflected the experience of males, which showed a rise from 0.8 in the shortest interval to about 1.6 for each of the longer duration categories. There was no discernable trend among females.

In the Seattle area, no consistent patterns were noted. Overall, the O/Es were 1.0, 1.2, 1.2, and 0.8 for the four duration categories available, and the variations were similar for males and females.

Other Cancer Sites: A series of anatomic sites commonly used in describing cancer patterns was examined for trends by duration of fluoridation. Attention was initially centered on sites for which the expected rates for each sex and duration-of-fluoridation category were based on at least 50 cases. The O/Es were evaluated to 2 decimal places, and are given in Appendix Table C. All other cancer sites were evaluated to one decimal place, and the results given in Appendix Table D.

Only one site, renal cancer [including cancers of the renal parenchyma (kidney) and pelvis], suggested a consistent relationship with duration of fluoridation over the entire time period. In Iowa, the O/E for renal cancer rose from 0.9 in the <5 year category, to 1.0 for the next three intervals, to 1.2 for the 20+ year category. In Seattle, the O/E rose from 0.8 in the <5 year category, to 0.9 in the next two intervals, to 1.1 in the longest category (15-19 years). The sex-specific data were much more variable. An increasing trend with duration was seen only among males in Iowa. However, the highest O/E occurred in the longest fluoridation category for three of the four area-sex groupings (the exception being females in Seattle).

While renal cancer was the only site indicating the need for more detailed analysis, it was recognized that we may be looking for a low-level risk and that we needed to maximize our opportunities to detect one. Therefore, we developed some liberal guidelines in selecting cancer sites to investigate further. We chose any site showing an increasing O/E trend (including ties) with increasing duration of fluoridation for any two of the six area-sex analyses (two of the two for sex-specific sites) in Appendix Tables C and D. Also included were cancer sites where the highest O/E (including ties) occurred in the longest duration-of-fluoridation category in both geographic areas for the same sex group. This resulted in the inclusion of five additional sites for more detailed analysis: cancers of the colon and rectum, soft tissue, prostate, and urinary bladder, and non-Hodgkin's lymphoma. The weakest evidence in this group was for prostate and bladder cancers. For prostate cancer, there was no consistent trend in either Iowa or Seattle; the highest O/E occurred in the longest duration-of-fluoridation categories in both areas, but in each instance, this was by one-hundredth of the O/E ratio (Appendix Table C). For bladder cancer, there was an increasing trend in the O/E for males

in Iowa, ranging from 1.0 in the <5 year interval to 1.2 in the 20+ year group (Appendix Table D). No other area-sex group showed a consistent trend, but for males in Seattle the highest O/E was in the longest duration category. However, this O/E was 1.0, which is identical to the ratios seen in two of the three other duration categories.

For cancers of the colon and rectum, the O/E in Iowa was highest in the longest duration category for both sexes combined, resulting primarily from an upward trend among males. In Seattle, the highest O/E for both sexes combined also occurred in the longest interval, although the ratio (1.03) was identical to that seen in two previous duration categories. For soft tissue cancer, the O/Es in Seattle increased with duration of fluoridation for both sexes combined. This was due to the trend among females, which rose from 0.7 in the <5 year interval to 1.7 in the 15-19 year group. However, no increasing trends were seen among males in Seattle or among any groups in Iowa. In addition, the highest O/E in these groups did not occur in the longest duration category, and some suggestion of an inverse trend was seen among females in Iowa. For non-Hodgkin's lymphoma, the O/Es for the combined sexes in Seattle increased from 1.0 in the <5 year interval to 1.2 in the 15-19 year interval. The trend was apparent among females only, although in males the highest O/E (1.1) was in the longest duration category. In Iowa, no increasing trends were noted with duration of fluoridation in any group, and the O/Es for the 20+ year category were all less than or equal to the O/Es for the <5 year category.

For the sites of *a-priori* interest and for the six sites chosen for further analysis, Poisson regression analyses were performed to estimate risk ratios (RRs) by duration of fluoridation after control for age, sex, calendar time, and geographic area. Data from all of the fluoridated and non-fluoridated counties in the study were used, and for all of the RRs the referent category was an RR of 1.00 in the non-fluoridated counties. Ninety-five percent confidence intervals were calculated for each RR and a test for trend in the ordered RRs was performed. The confidence intervals are useful in several ways. If they exclude 1.0, then a test of the hypothesis that the rates are the same as the referent rates in the non-fluoridated counties would likely be rejected at the $p<0.05$ level. In addition, whether or not the confidence intervals encompass the point estimate for another category is a conservative indication of the possibility that chance events may have accounted for differences between the point estimates of the RRs for these categories. For example, the difference between the RRs for the 20+ year fluoridation category and the <5 year category could more readily be due to chance if each confidence interval includes the other point estimate.

The lack of any consistent pattern for all bone and joint cancers and for osteosarcomas by duration of fluoridation was also seen with the Poisson regression analysis (Table 3). There was no consistent pattern in the RRs by duration of fluoridation for either sex or in the combined data. Not only were there no consistent associations or patterns, but also there were no statistically significant trends in the RRs. In addition, the confidence intervals for the longest duration category in every instance encompassed both 1.00 and the point estimate of the RR for the <5 year category.

For cancers of the oral cavity and pharynx, the overall patterns, as well as the RR point estimates themselves, are quite similar to the O/E ratios (Table 4). In Iowa, the RRs for males and the sexes combined were lowest in the shortest duration-of- fluoridation category, and then increased to around 1.6 for each of the subsequent intervals. The confidence interval for the shortest duration group among males in Iowa excludes the point estimates of each of the subsequent categories and vice-versa. Conversely, in Seattle, the RRs were lowest in the longest duration-of-fluoridation category for males, females, and both sexes combined. The only significant trend in the ordered RRs was the negative trend ($p=0.02$) for the combined sexes in Seattle. Trends of borderline significance ($0.05\leq p<0.10$) in opposite directions occurred for males in Iowa (positive trend, $p=0.05$) and Seattle (negative trend, $p=0.09$). As can be seen from the distribution of the RRs, the statistical significance of these trends was derived primarily from the difference between the value of the RR in an extreme duration category compared to all of the others, rather than from a consistent trend or gradient.

For renal cancer, the only site in the initial analysis suggesting any consistent evidence of a trend in O/E ratios, the patterns of the RRs and their values (Table 5) are similar to those for the O/E ratios. The RRs for both sexes combined in Seattle rose slightly from 0.9 to 1.0, a trend that was statistically significant (p=0.04). None of the other trends was significant, and for each registry-sex category, the confidence interval in the <5 year category included the point estimate of the risk for the longest duration category.

Table 6 gives the modeled RRs by registry and sex for the two more common cancers chosen for further evaluation from the O/E analyses. For colorectal cancers, the results are essentially the same as the O/E analysis. In Iowa, there was a significant upward trend in the ratios with increasing duration of fluoridation, for the sexes combined and among males. In Seattle, the highest ratio occurred in the longest duration category, but the difference was not statistically significant and there was no evidence of a consistent trend. For prostate cancer, the modeled estimates are similar to the O/E ratios except for the 15-19 year interval in Seattle (O/E=1.02, RR=1.13). For both registries, the trends in risk ratios were statistically significant.

Similar analyses for the less common sites are presented in Table 7. In general, the RRs are similar to the O/E analysis. For cancers of the urinary bladder and soft tissue, there were no significant trends with duration of fluoridation, and the confidence intervals for the shortest intervals included the RRs for the longest intervals. For non-Hodgkin's lymphoma in the Seattle registry, the p-value for trend for the sexes combined was 0.01, and the confidence interval around the RR for the 15-19 year interval did not include the RR for the <5 year interval. Among females, the trend was not significant, but the RRs for the longest and shortest intervals were significantly different from each other.

Time-Specific Analyses: For each of the six cancer sites selected for further analyses on the basis of O/E trends, or the presence of the highest O/E in the longest duration-of-fluoridation category in both geographic areas for the same sex, the consistency of these patterns was evaluated by assessing them for two separate time periods (1973-80 and 1981-87). In Iowa, this is an informative test for consistency of associations, since there are a number of fluoridated counties that contribute differentially to the various intervals of fluoridation in these two time periods. For completeness, these time-period data are presented for Seattle also. However, since there is only one fluoridated county in the Seattle registry, separation of the data by time period does not result in a different mix of counties in each grouping but is simply a finer breakdown of duration of fluoridation (i.e., the 10-14 year interval for 1973-80 is actually year 10, and the 10-14 year interval for 1981-87 corresponds to years 11-14).

For renal cancer, the consistent increase in O/E ratios with duration of fluoridation observed in the aggregate data for both sexes combined in Iowa and Seattle, and for males in Iowa, was not seen in any of the nine location-sex-time groupings where trends can be evaluated (those that have data for more than two categories) (Table 8). Indeed, the highest O/E (including ties) occurred in the longest duration-of-fluoridation category in only four out of all 12 groupings. This would have been expected to occur by chance alone about four times, given the number of categories involved in Table 8.

Table 9 presents the time-specific data for the two common cancer sites that warranted further analysis. In the aggregate (1973-87) data for colorectal cancer, the highest O/E ratio occurred in the longest duration-of-fluoridation category (with ties) for the sexes combined in both areas, and there was also a consistently increasing trend in the O/E ratios for males in Iowa. When data are examined by time period, this same pattern prevailed during 1973-80. However, there was no consistent trend during 1981-87 for the sexes combined or for males in Iowa, and in neither instance was the O/E ratio highest in the longest duration category. The O/E for the 15-19 year interval in Seattle was higher than that for 10-14 years, but only marginally so (one-hundredth of the O/E ratio). In the aggregate (1973-87) data for prostate cancer, the O/E ratio was highest in the most extreme duration category for both geographic areas, but the differences were very small. In the time-specific data, this pattern was not seen for either Iowa or Seattle in the earlier period, or for Iowa in the later period.

Table 10 presents corresponding data for the less frequent sites singled out for further analysis. For bladder cancer, the increasing O/E trend among males in Iowa in the overall data was not present in either time period, and only in the earlier period was the highest O/E ratio seen in the longest duration category. In Seattle, where the highest O/E ratio was in the longest interval for males in the aggregate data, the ratio for the longest interval was lowest in the earlier period and highest only in the later period.

For soft-tissue cancer, the O/E increased with duration of fluoridation for both sexes combined in Seattle, which was derived from the trend for females. The trend for the sexes combined was seen in both time periods. However, there was no consistent trend among females in the earlier time period, although in the later period the O/E ratio for 15-19 years was higher than that for 10-14 years. Similar patterns in the overall data were noted for non-Hodgkin's lymphoma (trends in both sexes combined and females, in Seattle only). In addition, the ratio among males in Seattle was highest in the longest duration category. In the time-specific data, the trend for the sexes combined was not seen in the earlier period, but the O/E ratio for 15-19 years was higher than that for the 10-14 category (1.2 vs. 1.1) in the later period. The trend toward increases in O/E ratios for females in Seattle was seen in both time periods.

Thus, for the few cancer sites with any suggestive overall patterns in O/E ratios, the trends were either absent or not consistently seen when examined within separate calendar-time periods. The only exceptions were for soft tissue cancer and non-Hodgkin's lymphoma among females in Seattle. For both soft tissue cancer and non-Hodgkin's lymphoma, there was actually some suggestion of a negative association (lower O/E ratios with longer duration of fluoridation) among females in Iowa.

MORTALITY ANALYSIS

Bone cancer: Table 11 gives the observed numbers of deaths ascribed to malignancies of the bones and joints from 1950-1985 in counties having undergone rapid fluoridation, and O/E ratios based on expected values generated from the age-, sex-, time-, and region-specific rates in non-fluoridated counties. These data are presented in intervals according to time of fluoridation, ranging from 31-35 years prior to fluoridation to 30-35 years post-fluoridation. Overall, 12,363 deaths were observed compared to 12,130 expected. There were no apparent trends that might relate to fluoridation. The highest O/E ratio for males (1.29) occurred 15-19 years post-fluoridation, but ratios for the subsequent 15 years resembled those for the 15-year interval preceding fluoridation. The highest O/E ratio for females (1.30) occurred 16-20 years prior to fluoridation, while the ratios for 20-35 years post-fluoridation were similar or lower than those for the 15-year interval preceding fluoridation.

Oral Cancers: Table 11 also displays mortality data from cancers of the oral cavity and pharynx. Among males, the observed mortality generally ranged from 30 to 50 percent above the expected values, but this applied to the years prior to fluoridation as well as to those afterward, without any evidence of a trend. Females also showed elevated O/E ratios of around ten to 30 percent that were similarly unrelated to the timing of fluoridation.

Other Cancer Sites: Appendix Table G presents the observed deaths and O/E ratios in relation to time of fluoridation for the remaining cancers. Because of the large populations covered and consequent large numbers of deaths, data can be evaluated for even rare malignancies. Similar to the previous mortality analyses through 1969, no consistent patterns with increasing duration of fluoridation were noted for any of these forms of cancer. While this analysis incorporates substantial improvements (larger numbers, control for geographic region, and removal of counties with substantial levels of natural fluoride from the comparison group) over the original study (6), the results of the two analyses are obviously not independent of one another. The major advantage is 16 additional years of mortality data, so we could evaluate longer durations of fluoridation. By comparing three time intervals from 20 to 35 years post-fluoridation to three intervals prior to fluoridation, we found more evidence of an

inverse relationship between fluoridation and cancer risk than a positive one. For seven site groupings, the O/E ratios in the three longer intervals were all below the lowest ratio for the 15 years prior to fluoridation among both males and females (all sites combined, lung, colon and rectum, pancreas and brain cancers, non-Hodgkin's lymphoma, and multiple myeloma). Inverse relationships were seen among males for esophagus, stomach, larynx and thyroid cancers, and among females for nasopharyngeal, ovarian and renal cancer and malignant melanoma. The converse, higher O/E ratios in the three longer intervals than any of those seen during the 15 years prior to fluoridation was not seen for any form of cancer.

Once again, we applied some liberal guidelines as to which cancer sites (in addition to bone and oral cancers) to single out for further scrutiny. Focusing on the three time intervals from 20 to 35 years of fluoridation, we searched for such sites where any one of these three O/E ratios surpassed the highest O/E ratio in the 15 years prior to fluoridation. This criterion was met for cancers of the lip in both sexes, for "other" cancers and for Hodgkin's disease in males only, for salivary gland, stomach, larynx and thyroid cancers in females only, and for uterine and testicular cancers. On the basis of chance alone, one would expect that 50 percent of the time the highest O/E ratio for the 20 to 35 year categories would be greater than the highest ratio for the categories 1 to 15 years prior to fluoridation. Thus, among the 46 site-sex comparisons excluding bone and oral cancers in Appendix Table G, we should have identified 23 such instances on the basis of chance alone, compared to the ten we did identify. Not only is the total number selected consistent with chance, so are the individual patterns by site. Lip cancer was the only site where our criterion was met for both sexes, was the rarest site investigated, and gave the most evidence of random variation. Among males, the highest O/E ratio was in the longest duration-of-fluoridation category, but was based on only 17 deaths, while the ratios for 15 to 29 years post fluoridation were consistent with those for the 15 years prior to fluoridation. Among females, the highest post-fluoridation O/E ratio for lip cancer was in the 20-24 year interval, while much lower ratios were seen in the 15-19 and 30-35 year intervals.

A similar lack of consistency was noted for cancer sites in which our criterion for further inspection applied to only one sex. For Hodgkin's disease among males, the O/E ratio in the 20-24 year post-fluoridation group was higher than the O/E ratios in the three groups 15 years prior to fluoridation. However, this ratio was lower than those seen 26-35 years prior to fluoridation, while the ratio for the longest post-fluoridation group was the lowest of the 14 investigated. For "other" cancers in males, the O/E ratio 30-35 years post-fluoridation was higher than the ratios in the 15 years prior to fluoridation. However, this was only marginally so, 1.11 versus 1.10 for the 11-15 years preceding fluoridation, while the ratios for 21-35 years prior to fluoridation were higher. The O/E ratio for thyroid cancer in females for the 25-29 year interval was 1.46 compared to 1.17-1.25 for the 15 years prior to fluoridation, but it decreased to 1.23 for the longest duration group. Among females with stomach cancer, the largest O/E ratio was also in the 25-29 year interval, but this was followed by a ratio that was lower than any during the fluoridated time periods, and lower than the pre-fluoridation values up to 20 years prior to fluoridation. For laryngeal cancer among women, the O/E ratio for 30-35 years post fluoridation was greater than those for the 15 years prior to fluoridation, but only marginally (1.30 vs. 1.29), and was lower than the O/E ratio within the 5 years following fluoridation. As noted above, there was evidence of an inverse relationship between the O/E ratios and fluoridation for each of these last 3 sites (thyroid, stomach, and larynx) among males.

The O/E ratio for testicular cancer was also elevated for the 25-29 year post-fluoridation period, but the value for the longest duration group was lower than the ratio for the period 21-25 years prior to fluoridation. The O/E ratio for cancer of the uterus excluding cervix in the longest post-fluoridation period (1.02) was slightly higher than that for the five-year period prior to fluoridation (0.96). However, there was no evidence of a trend, and the ratio for the 25-29 year interval was the lowest of any group following fluoridation.

Finally, it is noteworthy that none of the six cancer sites singled out for further analysis based on the incidence data showed any evidence of a positive relationship with fluoridation in the mortality

analyses. For three of these six sites (renal and colorectal cancers, and non-Hodgkin's lymphoma), there was actually evidence of an inverse relationship between duration of fluoridation and cancer mortality.

DISCUSSION

This updated survey of the risk of cancer related to artificial fluoridation of community water supplies offers several improvements over our earlier study (6). With respect to cancer mortality, several technical advances in methodology were possible and, in particular, the inclusion of 16 additional years of data enhanced the statistical power of the study and permitted us to evaluate the influence of up to 35 years of fluoridation. In addition, for the first time, an evaluation of cancer incidence could be performed with reasonable numbers of cases available for analysis. Cancers of the bones and joints, with emphasis on osteosarcomas, and cancers of the oral cavity and pharynx were evaluated in detail because of *a-priori* suspicion of a carcinogenic hazard, based on a preliminary report of the National Toxicology Program's bioassay of the effects of sodium fluoride administration in rodents (4).

For cancers of the bones and joints, there were no mortality or incidence patterns that suggested an effect of fluoridation. Analysis of over 12,000 deaths from bone and joint cancers in fluoridated counties indicated a very similar risk to that seen in non-fluoridated counties, in various periods before and after fluoridation. However, mortality data for this site are not generally of high quality, because deaths certified as bone cancer often include a substantial number of deaths due to metastatic cancers arising from other primary sites. Using population-based cancer registries in the SEER program, we were able to evaluate with greater precision the relation of water fluoridation to the incidence of bone cancer and specifically to osteosarcoma. Overall, and among males (to which the effect was restricted in rodents), there were no patterns of increasing risks of bone cancers or osteosarcomas with increasing duration of fluoridation.

The patterns of oral cancer also revealed no evidence of a fluoridation effect. Overall, mortality from oral cancer was somewhat higher in fluoridated counties than in non-fluoridated counties. However, this excess was evident in the study counties for 30 years prior to their becoming fluoridated, as well as afterwards. This negative finding is statistically stable, since it was based on over 46,000 oral cancer deaths in the fluoridated counties and 24,000 deaths in the control counties. Analysis of over 2,600 incident cases of oral cancer in fluoridated counties and 1,100 cases in control counties in two SEER registry areas also failed to reveal any patterns consistent with a fluoridation effect. An increasing trend in observed-to-expected ratios for oral cancer by increasing duration of fluoridation was seen for males in Iowa, but this pattern was not seen for males in Seattle or for females in either area.

While there was no epidemiologic evidence of a carcinogenic effect of fluoride for cancer sites under suspicion (bone and oral cavity), the availability of data on cancer of all forms allowed us to search for patterns among various cancer sites that might be associated with fluoridation status. This *a-posteriori* exploration would be expected to reveal some associations on the basis of chance alone in a certain number of instances. Therefore, our main criterion for an effect that might warrant pursuit in more analytic studies was that of consistency. When risks increased with duration of fluoridation, or were highest in the longest duration categories, the patterns were examined for consistency between the sexes, across geographic areas, and in different time periods. Even if there were a sex-specific effect, one would expect to see consistency across the other parameters. Since for most cancer sites, epidemiologic variation is similar for incidence and mortality rates, one would also expect to see similar patterns by fluoridation in both data sets. Extremely liberal criteria were used to identify sites which might be showing a fluoridation effect, so that further evaluation could be carried out for indications of consistency. The result was that no site showed any consistent evidence of a fluoridation effect.

The only hint of a possible effect was seen in the incidence data for renal cancer. There was an increasing trend in the O/E ratios by duration of fluoridation for the sexes combined in both registries. The patterns in the sex-specific data were more variable, but the highest ratio was in the longest duration-of-fluoridation category for three of the four sex-registry groups. Beyond this point, however, there was no consistency. When the 15-year incidence data were divided into two calendar-time groups, trends were not seen within the periods. In addition, the number of ratios that were highest in the longest intervals of fluoridation was consistent with the number expected on the basis of chance. Finally, evaluation of mortality data for renal cancer revealed generally lower risk in the years after fluoridation than in those immediately before, particularly for the longest durations of fluoridation.

Thus, this long-term survey of counties undergoing rapid fluoridation of community water supplies uncovered no consistent patterns of cancer incidence or mortality that suggested a relation to fluoridation. Indeed, no evidence was found to even suggest that further, more analytic epidemiologic investigation of a fluoride effect is warranted for any cancer site.

To avoid over-interpretation of these negative results, certain limitations of these analyses need discussion. While this survey deals with areas in which virtually all of the population was exposed to the agent of interest, it is still an ecologic or correlation study. That is, exposure and disease information is available for aggregate county populations, not for individuals. Thus, some subjects in both the fluoridated and non-fluoridated areas have not spent their entire lives in these areas, and may have been exposed or unexposed to fluoridated drinking water for the varying periods spent outside of these areas. There is no reason to believe that this misclassification of the exposure would be biased (i.e., different for those who developed cancer vs. those who did not), but the resulting random misclassification may tend to obscure real associations. Another potential source of random misclassification is the opportunity for exposure to fluoride from other sources (e.g., dentifrice, vitamins, foods), which might tend to restrict our ability to detect real differences associated with water treatment. However, the major source of systemic exposure to fluoride is the drinking water in areas having fluoridated water systems (17).

The method of analysis used in this study, while having many advantages, also has some potential disadvantages. Different counties at differing time periods were grouped according to their relation to the time of fluoridation. Thus, the same counties do not contribute to all of the time-to-fluoridation categories, and when counties contribute to multiple categories, their relative contribution will be different from category to category. This was done purposefully, since the aim of the analysis was to determine if any temporal patterns of risk seemed related to fluoride exposure, rather than to other underlying factors (e.g., change in the mix of populations being compared) which are assumed to act randomly with respect to time of fluoridation. It is possible, however, that if a large county or grouping of counties had an unusual cancer experience, and appeared only at one end or the other of the time-to-fluoridation groupings, then the patterns in O/E ratios could be biased. This was in fact the main reason for the registry- and time-specific incidence analyses. If a pattern in the total series represents a fluoride effect, then it should appear consistently in these subgroup analyses. However, if the pattern is an artifact of the mixture of counties being compared, then it should not appear consistently in the subcategories, since different groupings of counties are being compared. We would have done similar subgroup analyses by geographic region and calendar time for the mortality data, if any consistent or provocative patterns were seen in the aggregate data, but none were. The marked similarity of the O/E ratios by type of cancer for different time-to-fluoridation categories provided further evidence that the results were not likely to be biased.

In this survey, factors other than age, geographic area, calendar time and sex were not explicitly adjusted for in the analysis. However, the time-trend analysis should have controlled for most of these differences, since it allowed fluoridated and non-fluoridated areas to have different risks due to other factors and looked for changes in baseline differences that might relate to the timing of fluoridation. It is possible, however, that the analyses may have been influenced by time trends in other risk factors for cancer that varied between fluoridated and non-fluoridated areas. Explicit control for

socio-demographic and other potential risk factors at the county level with the Poisson regression analyses would have been the next step, if any type of cancer emerged from the main analyses with any consistent evidence of a relationship to fluoridation. But none did. We will be pursuing some of these analyses over the next several months to explore the reasons for baseline differences in cancer rates between fluoridated and non-fluoridated areas prior to fluoridation. In this process we will also be able to evaluate whether any provocative patterns were obscured by these differences.

Finally, as in any epidemiologic investigation, very small increases in risk would not be detectable by studies in human populations. If fluoridation of water supplies is associated with a few extra cases of bone cancer in tens of millions of people, then no methodology would be able to detect this and attribute a causal interpretation to the difference. Even explorations of the 10% to 20% differences in ratios that took place in this analysis must be considered at the limits of what could be detected relative to the background forces of incidence and mortality for these tumors.

With all of these caveats in mind, however, it should be pointed out that while this was an ecologic study, it was a strong ecologic study. Many such investigations look for differences in risk related to differences in average values for social class-related or other variables where there is considerable overlap between the populations being compared. Others investigate the influence of exposures that apply to only a small minority of the populations investigated (e.g., occupations). The current investigation was able to evaluate the influence of an abrupt switch from non-fluoridated to fluoridated drinking water on the part of the vast majority of the populations under study. Ecologic studies of general environmental exposures that were much more crudely measured (18), or applied to much smaller segments of the population (19), have generated evidence of associations, which have been confirmed as low-level hazards by subsequent analytic investigations (20-21). Thus, the failure to detect any evidence of a consistent relationship between water fluoridation and cancer risk, either for cancer types suggested by the rodent studies or for any other forms of cancer, should be reassuring. The findings should also help put into perspective what the maximum magnitude of any potential risk might be.

SUMMARY

In response to concerns over the possible carcinogenicity of fluoride compounds added to drinking water raised by the results of a recent animal experiment, we evaluated 36 years of U.S. cancer mortality data and 15 years of cancer incidence data from two population-based cancer registries, in relation to the fluoridation status of drinking water supplies in the populations under study. Osteosarcomas of the bone were singled out for detailed analysis based on the results of the animal experiment. Among both males and females residing in counties having undergone rapid fluoridation, the relative risk of death from cancers of the bones and joints was the same after 20-35 years of fluoridation as it was in the years immediately preceding fluoridation. A similar lack of a relationship to timing of fluoridation was noted for the incidence of bone and joint cancers, and osteosarcomas. The relative risk of developing these cancers 20 or more years after fluoridation was lower than the risk associated with less than five years of fluoridation among both males and females.

The mortality and incidence data in this survey allowed an evaluation of the patterns of risk for virtually all forms of cancer in relation to the timing of fluoridation of drinking water supplies. For no type of malignancy was there consistent evidence of a relationship with the patterns of fluoridation. One site, renal cancer, showed a suggestive relationship between incidence rates and duration of fluoridation in the aggregate data from the registries. However, no such trends were seen when incidence data were examined for two separate periods, and the mortality data for renal cancer actually yielded some evidence of an inverse relationship with duration of fluoridation.

Thus, in a study of over 2,300,000 cancer deaths in fluoridated counties across the United States, and over 125,000 incident cancer cases in fluoridated counties covered by two population-based cancer registries, we identified no trends in cancer risk that could be ascribed to the consumption of fluoridated drinking water.

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TABLE 1

Observed to Expected Incidence Ratios[a] and observed number of cases( ) for bone and joint cancers and osteosarcomas, by duration of fluoridation for counties in the Iowa and Seattle SEER areas, 1973-87.

		Duration of Fluoridation (Yrs.)				
Site	Sex	<5	5-9	10-14	15-19	20+
Bone and Joint Cancer	Total[b]	1.2(17)	0.9(47)	1.0(67)	1.1(69)	1.0(90)
	Male	1.9(11)	0.9(24)	1.0(40)	1.0(31)	1.5(60)
	Female	0.7(6)	1.0(23)	1.0(27)	1.2(38)	0.6(30)
Osteosarcoma						
	Total[b]	1.8(9)	0.8(11)	0.9(20)	1.1(20)	0.9(31)
	Male	2.6(6)	0.8(6)	0.9(14)	0.8(9)	1.2(19)
	Female	1.2(3)	0.8(5)	1.0(6)	1.4(11)	0.7(12)

[a] Relative to non-fluoridated counties (1.00), adjusted for age, calendar time, and geographic area.
[b] Adjusted also for sex.

TABLE 2

Observed to Expected Incidence Ratios[a] and observed numbers of cases( ) of cancers of the oral cavity and pharynx, by duration of fluoridation for counties in the Iowa and Seattle SEER areas, 1973-1987.

		Duration of Fluoridation (Yrs.)				
Registry	Sex	<5	5-9	10-14	15-19	20+
Iowa	Total[b]	1.2(31)	1.4(38)	1.7(116)	1.6(210)	1.6(848)
	Male	0.8(15)	1.6(25)	1.5(77)	1.6(135)	1.6(555)
	Female	2.3(16)	1.2(13)	2.0(39)	1.7(75)	1.6(293)
Seattle	Total[b]	1.0(81)	1.2(500)	1.2(577)	0.8(292)	----
	Male	1.0(50)	1.3(296)	1.2(360)	0.9(184)	----
	Female	1.1(31)	1.1(204)	1.2(217)	0.7(108)	----

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time.
[b] Adjusted also for sex.

TABLE 3

Risks Ratios[a] and 95% confidence intervals ( ) from Poisson regression modeling of incidence rates for bone and joint cancer and osteosarcoma by duration of fluoridation and sex in the Iowa and Seattle SEER areas, 1973-87.

		Duration of Fluoridation (Yrs.)					
Site	Sex	<5	5-9	10-14	15-19	20+	Z for Trend [P]
Bone & Joint Cancer	Total	1.1 (0.7-1.9)	0.9 (0.6-1.2)	1.1 (0.8-1.5)	1.3 (0.9-1.8)	1.0 (0.7-1.4)	0.37 [0.71]
	Male	1.2 (0.6-2.4)	0.7 (0.5-1.2)	1.3 (0.9-2.0)	1.2 (0.8-1.9)	1.3 (0.8-2.1)	1.13 [0.26]
	Female	0.9 (0.4-2.2)	1.1 (0.6-1.8)	0.9 (0.6-1.4)	1.3 (0.9-2.1)	0.7 (0.4-1.1)	-0.76 [0.44]
Osteosarcoma	Total	2.0 (0.9-4.4)	0.7 (0.3-1.4)	1.0 (0.6-1.8)	1.2 (0.7-2.1)	1.2 (0.6-2.2)	0.43 [0.67]
	Male	1.7 (0.7-4.6)	0.5 (0.2-1.3)	1.3 (0.6-2.6)	0.9 (0.4-2.0)	1.4 (0.6-3.1)	0.48 [0.63]
	Female	2.5 (0.6-9.7)	1.2 (0.4-3.6)	0.7 (0.3-2.0)	1.6 (0.7-3.7)	0.9 (0.4-2.5)	0.07 [0.94]

[a] Adjusted for age, calendar time, geographic area and, for the "Total" row, for sex.

TABLE 4

Risk Ratios[a] and 95% confidence intervals from Poisson regression modeling of incidence rates for cancers of the oral cavity and pharynx, by duration of fluoridation and sex in two SEER registry areas, 1973-87.

		Duration of Fluoridation (Yrs.)					
Registry	Sex	<5	5-9	10-14	15-19	20+	Z for Trend [P]
Iowa	Total	1.2 (0.8-1.8)	1.6 (1.1-2.3)	1.5 (1.2-1.9)	1.7 (1.4-2.1)	1.6 (1.4-1.9)	1.07 [0.28]
	Male	0.8 (0.5-1.4)	1.6 (1.1-2.4)	1.5 (1.1-2.4)	1.6 (1.3-2.0)	1.6 (1.4-1.9)	1.95 [0.05]
	Female	2.2 (1.3-3.8)	1.6 (0.9-2.9)	1.6 (1.1-2.3)	2.0 (1.4-2.8)	1.6 (1.2-2.0)	-0.86 [0.39]
Seattle	Total	1.0 (0.8-1.3)	1.2 (1.1-1.4)	1.2 (1.1-1.3)	0.9 (0.8-1.0)	--- ---	-2.26 [0.02]
	Male	1.0 (0.6-1.4)	1.2 (1.0-1.4)	1.3 (1.1-1.5)	0.9 (0.7-1.1)	--- ---	-1.71 [0.09]
	Female	0.9 (0.6-1.4)	1.2 (1.0-1.4)	1.1 (0.9-1.3)	0.8 (0.7-1.1)	--- ---	-1.53 [0.13]

[a] Relative to a risk of 1.0 in non-fluoridated counties of these same areas, adjusted for age, calendar time and for the "Total" row, for sex.

TABLE 5

Risk Ratios[a] and 95% confidence intervals () from Poisson regression modeling of incidence rates for cancers of the kidney and renal pelvis, by duration of fluoridation and sex in two SEER registry areas, 1973-87.

		Duration of Fluoridation (Yrs.)					
Registry	Sex	<5	5-9	10-14	15-19	20+	Z for Trend [P]
Iowa	Total	1.0 (0.7-1.4)	1.0 (0.7-1.4)	1.0 (0.8-1.3)	1.1 (1.0-1.3)	1.2 (1.1-1.3)	1.63 [0.10]
	Male	0.8 (0.5-1.3)	1.1 (0.7-1.6)	1.1 (0.8-1.4)	1.1 (0.8-1.3)	1.2 (1.0-1.4)	1.43 [0.15]
	Female	1.3 (0.8-2.2)	1.0 (0.6-1.7)	0.9 (0.6-1.3)	1.2 (0.9-1.6)	1.2 (1.0-1.5)	0.82 [0.41]
Seattle	Total	0.9 (0.7-1.1)	0.8 (0.7-1.0)	1.0 (0.9-1.1)	1.0 (0.9-1.2)	--- ---	2.05 [0.04]
	Male	1.0 (0.7-1.4)	0.9 (0.7-1.0)	1.0 (0.9-1.1)	1.1 (0.9-1.3)	--- ---	1.65 [0.10]
	Female	0.6 (0.3-1.0)	0.8 (0.7-1.1)	0.9 (0.8-1.1)	0.9 (0.7-1.2)	--- ---	1.24 [0.22]

[a] Relative to a risk of 1.0 in non-fluoridated counties of these same areas, adjusted for age, calendar time and for the "Total" row, for sex.

TABLE 6

Risk Ratios[a] and 95% confidence intervals from Poisson regression modeling of incidence rates for colorectal and prostate cancers, by duration of fluoridation in selected sex and Registry specific groupings, 1973-87.

Anatomic site	Registry	Sex	Duration of Fluoridation(Yrs.) <5	5-9	10-14	15-19	20+	Z for Trend [P]
Colon & Rectum	Iowa	Total	0.91 (0.80-1.03)	0.97 (0.86-1.10)	1.06 (0.98-1.14)	1.06 (0.99-1.13)	1.13 (1.08-1.18)	5.25 [<0.001]
		Male	0.85 (0.70-1.03)	1.02 (0.86-1.22)	1.07 (0.96-1.20)	1.15 (1.05-1.27)	1.1 (1.12-1.27)	4.19 [<0.001]
	Seattle	Total	0.97 (0.88-1.07)	1.03 (0.98-1.08)	1.00 (0.95-1.04)	1.06 (1.00-1.12)	---- ----	0.98 [0.32]
Prostate	Iowa	Male	0.82 (0.69-0.97)	0.99 (0.86-1.15)	0.88 (0.80-0.98)	0.86 (0.79-0.94)	0.95 (0.90-1.01)	2.33 [0.02]
	Seattle	Male	0.86 (0.76-0.97)	1.01 (0.95-1.06)	0.91 (0.86-0.95)	1.13 (1.07-1.20)	---- ----	2.44 [0.01]

[a] Relative to a risk of 1.0 in non-fluoridated counties of these same areas, adjusted for age, calendar time and for the "Total" row, for sex.

TABLE 7

Risk Ratios[a] and 95% confidence intervals from Poisson regression modeling of incidence rates for bladder cancer, soft-tissue cancer and non-Hodgkin's lymphoma, by duration of fluoridation in selected sex and Registry-specific groupings, 1973-87.

Anatomic site	Registry	Sex	Duration of Fluoridation (Yrs.) <5	5-9	10-14	15-19	20+	[P]
Urinary Bladder								
	Iowa	Male	1.0 (0.8-1.3)	1.2 (0.9-1.5)	1.1 (0.9-1.2)	1.1 (0.9-1.2)	1.1 (1.0-1.3)	1.78 [0.08]
	Seattle	Male	1.1 (0.9-1.3)	1.0 (0.9-1.1)	0.9 (0.9-1.0)	1.0 (0.9-1.1)	--- ---	-1.48 [0.14]
Soft Tissue								
	Seattle	Total	1.1 (0.7-1.8)	1.0 (0.8-1.3)	1.0 (0.8-1.3)	1.1 (0.8-1.4)	--- ---	-0.07 [0.95]
		Female	0.8 (0.4-1.6)	1.1 (0.8-1.6)	1.2 (0.9-1.7)	1.3 (0.8-2.0)	--- ---	0.97 [0.33]
Non-Hodgkin's Lymphoma								
	Seattle	Total	1.0 (0.8-1.2)	1.0 (0.9-1.2)	1.1 (1.0-1.2)	1.2 (1.1-1.4)	--- ---	2.45 [0.01]
		Male	1.1 (0.8-1.5)	1.0 (0.8-1.1)	1.1 (1.0-1.3)	1.2 (1.0-1.4)	--- ---	1.79 [0.07]
		Female	0.8 (0.6-1.1)	1.1 (0.9-1.3)	1.2 (1.0-1.4)	1.2 (1.1-1.5)	--- ---	1.69 [0.09]

[a] Relative to a risk of 1.0 in non-fluoridated counties of these same areas, adjusted for age, calendar time and for the "Total" row, for sex.

TABLE 8

Observed to Expected Incidence Ratios[a] and observed numbers of cases ( ) of cancers of the kidney and renal pelvis, by duration of fluoridation, time period and sex in two SEER registry areas, 1973-87.

			Duration of Fluoridation (Yrs.)				
Registry	Sex	Time Period	<5	5-9	10-14	15-19	20+
Iowa							
	Total[b]						
		1973-80	0.9(35)	1.0(27)	0.8(57)	1.1(194)	1.0(306)
		1981-87	---	0.9(14)	1.4(55)	0.8(11)	1.4(677)
	Male						
		1973-80	0.7(17)	1.1(18)	0.8(40)	1.0(111)	0.9(170)
		1981-87	---	0.9(8)	1.4(31)	0.7(5)	1.5(412)
	Female						
		1973-80	1.2(18)	1.0(9)	0.7(17)	1.2(83)	1.1(136)
		1981-87	---	0.8(6)	1.5(24)	0.9(6)	1.3(265)
Seattle							
	Total[b]						
		1973-80	0.8(67)	0.9(346)	0.7(75)	---	---
		1981-87	---	---	1.0(383)	1.1(336)	---
	Male						
		1973-80	0.9(51)	0.9(222)	0.8(53)	---	---
		1981-87	---	---	0.9(242)	1.2(221)	---
	Female						
		1973-80	0.6(16)	0.8(124)	0.5(22)	---	---
		1981-87	---	---	1.2(141)	0.9(115)	---

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time.
[b] Adjusted also for sex.

TABLE 9

Observed to Expected Incidence Ratios[a] and observed numbers ( ) of cases of colorectal and prostate cancers, by duration of fluoridation, and time period for selected sex groupings in two SEER registry areas, 1973-87.

Anatomic site	Time Registry	Sex	Period	Duration of Fluoridation (Yrs.) <5	5-9	10-14	15-19	20+
Colon & Rectum								
	Iowa	Total[b]	1973-80	0.93(277)	0.95(190)	1.06(575)	1.06(1472)	1.12(2580)
			1981-87	---	0.95(125)	1.16(383)	1.02(116)	1.11(4469)
		Males	1973-80	0.90(115)	0.99(88)	1.04(239)	1.15(691)	1.22(1201)
			1981-87	---	0.99(55)	1.28(190)	1.15(57)	1.16(2073)
	Seattle	Total[b]	1973-80	0.88(508)	1.03(2819)	1.07(611)	---	---
			1981-87	---	---	1.02(2451)	1.03(2123)	---
Prostate								
	Iowa	Males	1973-80	0.82(151)	1.01(123)	0.75(251)	0.87(701)	0.96(1233)
			1981-87	---	0.86(80)	1.15(243)	0.62(51)	0.96(2462)
	Seattle	Males	1973-80	1.00(316)	1.01(2018)	1.00(433)	---	---
			1981-87	---	---	0.94(1851)	1.02(2026)	---

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time.
[b] Adjusted also for sex.

TABLE 10

Observed to Expected Incidence Ratios[a] and observed numbers ( ) of cases of bladder cancer, soft tissue cancer, and non-Hodgkin's lymphoma, by duration of fluoridation, time period, for selected sex groupings in two SEER Registry areas, 1973-87.

Anatomic site	Registry	Sex	Time Period	Duration of Fluoridation (Yrs.) <5	5-9	10-14	15-19	20+
Urinary Bladder								
	Iowa	Males	1973-80	1.0(72)	1.0(45)	0.9(122)	1.1(330)	1.2(583)
			1981-87	---	1.2(37)	1.3(96)	1.0(25)	1.2(1026)
	Seattle	Males	1973-80	1.0(143)	1.0(708)	0.9(160)	---	---
			1981-87	---	---	0.9(626)	1.0(535)	---
Soft Tissue								
	Seattle	Total[b]	1973-80	0.8(26)	1.0(122)	1.4(25)	---	---
			1981-87	---	---	1.1(101)	1.2(77)	---
		Females	1973-80	0.7(10)	1.0(67)	0.9(13)	---	---
			1981-87	---	---	1.3(50)	1.7(39)	---
Non-Hodgkin's Lymphoma								
	Seattle	Total[b]	1973-80	1.0(102)	1.1(581)	1.0(126)	---	---
			1981-87	---	---	1.1(662)	1.2(597)	---
		Male	1973-80	1.1(61)	1.0(281)	0.9(60)	---	---
			1981-87	---	---	1.1(342)	1.2(316)	---
		Female	1973-80	0.9(41)	1.1(300)	1.1(66)	---	---
			1981-87	---	---	1.1(320)	1.3(281)	---

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time.
[b] Adjusted also for sex.

TABLE 11

Observed to Expected Mortality Ratios[a] and number of observed deaths ( ) from bone and oral cancers in rapidly fluoridated counties by years before (Pre) and after (Post) fluoridation, 1950-85.

Time (yrs) in Relation to Start of Fluoridation	Site of Cancer: Bones and Joint		Site of Cancer: Oral Cavity	
	Male	Female	Male	Female
35-31 Pre	0.81 (17)	1.20 (13)	1.04 (46)	1.16 (15)
30-26 Pre	0.87 (69)	1.25 (65)	1.36 (296)	1.55 (77)
25-21 Pre	1.21 (160)	0.95 (93)	1.52 (580)	1.19 (129)
20-16 Pre	1.04 (301)	1.30 (225)	1.45 (1088)	1.27 (273)
15-11 Pre	0.92 (707)	1.09 (513)	1.24 (2744)	1.31 (656)
10-6 Pre	1.00 (768)	1.01 (517)	1.30 (3220)	1.17 (786)
5-1 Pre	0.98 (1042)	1.09 (758)	1.30 (4536)	1.15 (1219)
0-4 Post	0.95 (1082)	1.02 (782)	1.32 (5012)	1.23 (1481)
5-9 Post	1.07 (901)	1.09 (724)	1.31 (4758)	1.06 (1510)
10-14 Post	0.99 (777)	0.87 (555)	1.27 (4399)	1.05 (1521)
15-19 Post	1.29 (610)	1.09 (454)	1.23 (3639)	1.10 (1444)
20-24 Post	0.97 (381)	1.02 (293)	1.27 (2382)	1.19 (1005)
25-29 Post	1.02 (242)	0.90 (173)	1.31 (1813)	1.15 (772)
30-35 Post	0.87 (78)	0.97 (63)	1.37 (739)	1.10 (344)

[a] Relative to non-fluoridated counties, adjusted for age, calendar-time, and geographic regions.

APPENDIX TABLE A.
Counties Used in the Incidence Fluoride Analysis

	Year Fluoridation[a]	County Name
IOWA (1973-1987):		
	1951	Dubuque
	1952	Linn
	1952	Scott
	1953	Johnson
	1957	Story
	1958	Wapello
	1959	Marshall
	1959	Polk
	1962	Pottawattami
	1963	Des Moines
	1973	Woodbury
	NF*	Bremer
	NF	Cherokee
	NF	Chickasaw
	NF	Fayette
	NF	Floyd
	NF	Fremont
	NF	Grundy
	NF	Hamilton
	NF	Iowa
	NF	Plymouth
	NF	Poweshiek
	NF	Shelby
	NF	Washington
	NF	Webster
SEATTLE (1974-1987):	1970	King
	NF	Grays Harbor
	NF	Jefferson
	NF	Mason
	NF	Pierce
	NF	San Juan
	NF	Thurston
	NF	Whatcom

[a] non-fluoridated

APPENDIX TABLE B.
Definition of Cancer Sites[a] Used in the Incidence Analysis

ICDO codes[b]	Site
400:999	All sites combined
410:419, 430-469, 480-499	Oral (excluding lip, salivary gland, nasopharynx)
500:509	Esophagus
510-519	Stomach
530:539, 590	Colon
540:541	Rectum
550	Liver (excluding intrahepatic bile ducts)
570:579	Pancreas
610:619	Larynx
622:629	Lung and bronchus
700:709	Bones and joints
700:709	Osteosarcoma (morphology 9180:9200)
700:709	Ewing's sarcoma (morphology 9260)
641, 710:719	Soft tissue (including heart)
730:739	Melanoma of the skin (morphology 8720:8790)
740:749, 759	Breast
800:809	Cervix uteri
799, 820:828	Corpus uteri and uterus, NOS
830	Ovary
859	Prostate
880:889	Urinary bladder
890:891	Kidney and renal pelvis
910:929	Brain and other nervous systems
939	Thyroid
All	Hodgkin's disease (morphology 9650:9667)
All	Non-Hodgkin's disease (morphology 9590:9642, 9670:9710, 9750)
All	Multiple myeloma (morphology 9730:9731)
All	Leukemias (morphology 9800:9940, 9951)

[a] Site-specific lymphomas have been regrouped with lymphomas.

[b] Source: (22). The leading "1" and the decimal point have been ommitted from the ICD-O topography (site) codes for brevity.

APPENDIX TABLE C. Observed over expected incidence ratios[a] and observed numbers of cases for the more common forms of cancer by duration of fluoridation for counties in the Iowa and Seattle SEER areas. 1973-1987

			DURATION OF FLUORIDE (YEARS)									
			00-04		05-09		10-14		15-19		20+	
SITE	REGISTRY	SEX	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT
ALL SITES	IOWA	BOTH SEXES[b]	1.00	1799	1.10	2100	1.08	5864	1.11	9933	1.13	43592
		MALE	0.98	873	1.08	1033	1.09	2891	1.13	4916	1.15	21549
		FEMALE	1.02	926	1.11	1067	1.08	2973	1.09	5017	1.12	22043
	SEATTLE	BOTH SEXES	1.05	3880	1.06	20318	1.01	22434	1.04	15885	.	.
		MALE	1.07	1858	1.04	9865	0.98	10905	1.04	7933	.	.
		FEMALE	1.03	2022	1.07	10453	1.05	11529	1.05	7952	.	.
COLON AND RECTUM	IOWA	BOTH SEXES	0.93	277	0.95	315	1.10	958	1.06	1588	1.11	7049
		MALE	0.90	115	0.99	143	1.14	429	1.15	748	1.18	3274
		FEMALE	0.95	162	0.92	172	1.07	529	0.98	840	1.06	3775
	SEATTLE	BOTH SEXES	0.88	508	1.03	2819	1.03	3062	1.03	2123	.	.
		MALE	1.00	260	1.12	1384	1.02	1483	1.06	1108	.	.
		FEMALE	0.78	248	0.96	1435	1.04	1579	1.01	1015	.	.
LUNG AND BRONCHUS	IOWA	BOTH SEXES	1.26	244	1.45	314	1.32	836	1.54	1490	1.46	6680
		MALE	1.17	188	1.27	216	1.24	622	1.47	1156	1.36	4778
		FEMALE	1.71	56	2.14	98	1.64	214	1.84	334	1.77	1902
	SEATTLE	BOTH SEXES	1.20	528	1.00	2901	0.94	3383	0.97	2300	.	.
		MALE	1.26	395	0.98	1982	0.91	2099	0.92	1348	.	.
		FEMALE	1.06	133	1.06	919	1.00	1284	1.04	952	.	.
FEMALE BREAST	IOWA	FEMALE	1.02	262	1.10	289	1.07	836	1.06	1405	1.08	6166
	SEATTLE	FEMALE	1.11	601	1.17	2876	1.14	3333	1.14	2601	.	.
CERVIX UTERI	IOWA	FEMALE	1.57	48	1.83	47	1.35	116	1.12	171	1.28	682
	SEATTLE	FEMALE	0.75	68	0.69	245	0.70	219	0.80	145	.	.
CORPUS AND UTERUS, NOS	IOWA	FEMALE	0.66	59	0.93	71	0.82	191	1.01	428	0.98	1517
	SEATTLE	FEMALE	1.32	281	1.20	1214	1.03	966	0.93	507	.	.
OVARY	IOWA	FEMALE	0.77	37	0.88	38	0.95	134	1.11	256	1.01	928
	SEATTLE	FEMALE	0.95	90	1.11	435	1.08	474	1.20	340	.	.
PROSTATE	IOWA	MALE	0.82	151	0.95	203	0.91	494	0.85	752	0.96	1895
	SEATTLE	MALE	1.00	316	1.01	2018	0.95	2284	1.02	2026	.	.

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time period.
[b] Adjusted also for sex.

APPENDIX TABLE D. Observed over expected incidence ratios[a] and observed numbers of cases for the less common forms of cancer by duration of fluoridation for counties in the Iowa and Seattle SEER areas. 1973-1987

			DURATION OF FLUORIDE (YEARS)									
			00–04		05–09		10–14		15–19		20+	
SITE	REGISTRY	SEX	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT
ESOPHAGUS	IOWA	BOTH SEXES [b]	0.9	12	1.1	14	1.0	38	1.30	80	1.30	336
		MALE	0.9	8	1.1	10	1.2	30	1.30	57	1.30	243
		FEMALE	1.0	4	1.0	4	0.7	8	1.20	23	1.30	93
	SEATTLE	BOTH SEXES	1.4	33	1.2	185	1.1	202	1.20	158	.	.
		MALE	1.8	22	1.3	129	1.0	129	1.20	107	.	.
		FEMALE	0.9	11	1.1	56	1.4	73	1.30	51	.	.
STOMACH	IOWA	BOTH SEXES	1.3	57	1.3	47	0.8	110	0.90	179	1.10	768
		MALE	1.4	36	1.4	30	0.9	65	1.10	117	1.10	471
		FEMALE	1.1	21	1.1	17	0.8	45	0.70	62	1.10	297
	SEATTLE	BOTH SEXES	0.9	87	1.0	424	1.2	481	1.00	267	.	.
		MALE	1.2	58	1.0	257	1.3	297	1.20	180	.	.
		FEMALE	0.6	29	1.0	167	1.2	184	0.80	87	.	.
LIVER MINUS IHBD	IOWA	BOTH SEXES	1.3	10	1.1	9	1.6	35	1.00	45	1.00	171
		MALE	1.0	6	2.0	7	1.2	19	0.80	26	1.00	106
		FEMALE	2.8	4	0.4	2	2.8	16	1.30	19	0.90	65
	SEATTLE	BOTH SEXES	0.9	17	1.1	87	1.4	102	1.10	78	.	.
		MALE	1.2	9	1.3	60	1.3	66	1.20	56	.	.
		FEMALE	0.7	8	0.9	27	1.6	36	0.80	22	.	.
PANCREAS	IOWA	BOTH SEXES	1.3	63	0.9	60	1.1	165	1.10	251	1.10	1089
		MALE	1.6	38	0.8	23	1.3	95	1.30	139	1.20	562
		FEMALE	1.0	25	1.0	27	0.9	70	0.90	112	1.00	527
	SEATTLE	BOTH SEXES	1.1	100	1.2	519	0.9	591	1.10	363	.	.
		MALE	0.9	47	1.3	272	1.0	292	1.00	153	.	.
		FEMALE	1.3	53	1.1	247	0.9	299	1.20	210	.	.
LARYNX	IOWA	BOTH SEXES	1.7	31	1.2	25	1.6	84	1.60	154	1.50	619
		MALE	1.6	27	1.0	20	1.6	76	1.40	129	1.40	519
		FEMALE	2.9	4	3.0	5	1.5	8	3.40	25	2.10	100
	SEATTLE	BOTH SEXES	0.9	39	1.0	240	1.0	257	1.10	161	.	.
		MALE	0.7	28	1.1	209	1.0	200	1.10	129	.	.
		FEMALE	1.7	11	0.5	31	1.0	57	1.00	32	.	.

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time period.
[b] Adjusted also for sex.

APPENDIX TABLE D (continued). Observed over expected incidence ratios[a] and observed numbers of cases for the less common forms of cancer by duration of fluoridation for counties in the Iowa and Seattle SEER areas. 1973-1987

			DURATION OF FLUORIDE (YEARS)									
			00–04		05–09		10–14		15–19		20+	
SITE	REGISTRY	SEX	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT
SOFT TISSUE	IOWA	BOTH SEXES [b]	0.9	10	0.8	9	1.1	34	0.9	54	1.0	254
		MALE	0.7	4	0.6	3	1.1	18	0.9	30	1.1	141
		FEMALE	1.3	6	1.0	6	1.0	16	0.8	24	0.9	113
	SEATTLE	BOTH SEXES	0.8	26	1.0	122	1.1	126	1.2	77	.	.
		MALE	0.9	16	0.9	55	1.0	63	0.9	38	.	.
		FEMALE	0.7	10	1.0	67	1.2	63	1.7	39	.	.
MELANOMA OF THE SKIN	IOWA	BOTH SEXES	1.1	31	1.1	42	0.9	97	1.2	178	1.3	1017
		MALE	1.2	17	1.1	17	1.0	45	1.1	75	1.5	502
		FEMALE	1.0	14	1.1	25	0.9	52	1.2	103	1.1	515
	SEATTLE	BOTH SEXES	1.1	77	1.2	492	1.3	639	1.1	408	.	.
		MALE	1.1	38	1.3	250	1.3	325	1.0	210	.	.
		FEMALE	1.2	39	1.0	242	1.4	314	1.3	198	.	.
URINARY BLADDER	IOWA	BOTH SEXES	1.0	92	1.2	110	1.1	304	1.1	460	1.2	2186
		MALE	1.0	72	1.1	82	1.1	218	1.1	355	1.2	1609
		FEMALE	0.8	20	1.3	28	1.2	86	1.0	105	1.2	577
	SEATTLE	BOTH SEXES	1.0	185	1.1	993	1.0	1110	0.9	714	.	.
		MALE	1.0	143	1.0	708	0.9	786	1.0	535	.	.
		FEMALE	0.9	42	1.3	285	1.2	324	0.9	179	.	.
KIDNEY AND RENAL PELVIS	IOWA	BOTH SEXES	0.9	35	1.0	41	1.0	112	1.0	205	1.2	983
		MALE	0.7	17	1.0	26	1.0	71	1.0	116	1.2	582
		FEMALE	1.2	18	0.9	15	1.0	41	1.1	89	1.2	401
	SEATTLE	BOTH SEXES	0.8	67	0.9	346	0.9	458	1.1	336	.	.
		MALE	0.9	51	0.9	222	0.9	295	1.2	221	.	.
		FEMALE	0.6	16	0.8	124	1.0	163	0.9	115	.	.
BRAIN AND NERVOUS SYSTEM	IOWA	BOTH SEXES	0.8	22	0.9	31	0.9	84	1.0	155	1.0	737
		MALE	0.7	15	0.9	18	0.8	44	0.8	79	0.9	405
		FEMALE	0.9	7	1.0	13	1.2	40	1.5	76	1.0	332
	SEATTLE	BOTH SEXES	1.5	68	1.2	379	0.9	337	1.1	250	.	.
		MALE	2.6	40	1.1	212	0.9	193	1.4	144	.	.
		FEMALE	0.9	28	1.2	167	0.8	144	0.9	106	.	.

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time period.
[b] Adjusted also for sex.

APPENDIX TABLE D (continued). Observed over expected incidence ratios[a] and observed numbers of cases for the less common forms of cancer by duration of fluoridation for counties in the Iowa and Seattle SEER areas. 1973-1987

			DURATION OF FLUORIDE (YEARS)									
			00–04		05–09		10–14		15–19		20+	
SITE	REGISTRY	SEX	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT	O/E RATIO	OBSERVED COUNT
THYROID	IOWA	BOTH SEXES[b]	0.8	12	0.9	13	1.1	56	1.6	123	1.2	492
		MALE	0.8	4	2.1	4	0.8	12	2.1	38	1.1	118
		FEMALE	0.8	8	0.7	9	1.3	44	1.5	85	1.2	374
	SEATTLE	BOTH SEXES	2.2	42	1.1	201	0.9	228	1.1	169	.	.
		MALE	1.0	9	1.2	67	0.9	59	1.9	53	.	.
		FEMALE	3.2	33	1.1	134	0.9	169	0.9	116	.	.
HODGKIN'S DISEASE	IOWA	BOTH SEXES	1.1	19	0.5	10	1.0	53	1.0	92	0.9	390
		MALE	1.2	10	0.5	5	1.1	30	1.2	59	0.9	206
		FEMALE	1.0	9	0.5	5	0.8	23	0.7	33	0.9	184
	SEATTLE	BOTH SEXES	1.2	45	1.0	172	0.8	170	1.1	128	.	.
		MALE	1.0	27	0.9	102	0.8	93	1.2	71	.	.
		FEMALE	1.8	18	1.1	70	0.8	77	1.0	57	.	.
NON-HODGKIN'S LYMPHOMA	IOWA	BOTH SEXES	1.0	58	1.2	77	1.1	198	1.1	313	1.0	1488
		MALE	1.0	29	1.1	37	1.0	97	1.2	169	0.9	746
		FEMALE	1.1	29	1.3	40	1.2	101	1.0	144	1.1	742
	SEATTLE	BOTH SEXES	1.0	102	1.0	581	1.1	788	1.2	597	.	.
		MALE	1.1	61	1.0	281	1.1	402	1.1	316	.	.
		FEMALE	0.8	41	1.1	300	1.1	386	1.3	281	.	.
MULTIPLE MYELOMA	IOWA	BOTH SEXES	1.2	25	1.2	32	1.3	79	1.0	113	1.1	518
		MALE	0.5	6	0.9	14	1.5	48	0.9	67	0.8	243
		FEMALE	2.0	19	1.8	18	1.0	31	1.0	46	1.3	275
	SEATTLE	BOTH SEXES	1.0	45	1.0	225	1.1	253	1.1	166	.	.
		MALE	0.9	22	0.9	111	1.2	136	1.1	88	.	.
		FEMALE	1.2	23	1.1	114	1.0	117	1.1	78	.	.
LEUKIMIAS (TOTAL)	IOWA	BOTH SEXES	0.9	64	1.1	88	1.2	226	1.0	369	1.0	1352
		MALE	0.8	32	1.3	51	1.1	122	1.0	204	0.9	742
		FEMALE	1.1	32	1.0	37	1.3	104	1.1	165	1.0	610
	SEATTLE	BOTH SEXES	1.2	128	0.9	573	1.0	639	1.0	385	.	.
		MALE	1.2	73	0.9	322	1.1	371	0.9	203	.	.
		FEMALE	1.3	55	1.0	251	1.0	268	1.0	182	.	.

[a] Relative to non-fluoridated counties (1.00), adjusted for age and calendar time period.
[b] Adjusted also for sex.

APPENDIX TABLE E. List of counties used in the mortality analysis

	Year Started Fluoridation[a]	County Name
Region=West:	1947	Nez Perce, ID
	1952	San Francisco, CA
	1954	Denver, CO
	1954	Hot Springs, WY
	1955	Custer, MT
	1956	Colfax, NM
	1966	Albany, WY
	1968	Los Alamos, NM
	1970	King, WA
	1974	Bernalillo, NM
	1974	Laramie, WY
	1982	Pueblo, CO
	-NF-	Coconino, AZ
	-NF-	Pima, AZ
	-NF-	Fresno, CA
	-NF-	Imperial, CA
	-NF-	Kern, CA
	-NF-	Kings, CA
	-NF-	Monterey, CA
	-NF-	Napa, CA
	-NF-	Riverside, CA
	-NF-	Sacramento, CA
	-NF-	San Bernardino, CA
	-NF-	San Diego, CA
	-NF-	San Joaquin, CA
	-NF-	Santa Barbara, CA
	-NF-	Santa Cruz, CA
	-NF-	Shasta, CA
	-NF-	Sonoma, CA
	-NF-	Stanislaus, CA
	-NF-	Sutter, CA
	-NF-	Tulare, CA
	-NF-	Ventura, CA
	-NF-	Yolo, CA
	-NF-	Yuba, CA
	-NF-	Gunnison, CO
	-NF-	Logan, CO
	-NF-	Bannock, ID
	-NF-	Bonneville, ID
	-NF-	Power, ID
	-NF-	Washington, ID
	-NF-	Deer Lodge, MT
	-NF-	Fergus, MT
	-NF-	Hill, MT
	-NF-	Lewis and Clark, MT

[a] "NF" = Not Fluoridated

APPENDIX TABLE E. List of counties used in the mortality analysis (cont.)

Year Started Fluoridation[a]	County Name
-NF-	Missoula, MT
-NF-	Park, MT
-NF-	Powell, MT
-NF-	Silver Bow, MT
-NF-	Toole, MT
-NF-	Yellowstone, MT
-NF-	Clark, NV
-NF-	Lander, NV
-NF-	Mineral, NV
-NF-	Ormsby, NV
-NF-	Washoe, NV
-NF-	White Pine, NV
-NF-	Dona Ana, NM
-NF-	Baker, OR
-NF-	Clackamas, OR
-NF-	Jackson, OR
-NF-	Klamath, OR
-NF-	Lane, OR
-NF-	Multnomah, OR
-NF-	Cache, UT
-NF-	Davis, UT
-NF-	Grand, UT
-NF-	Iron, UT
-NF-	Juab, UT
-NF-	Salt Lake, UT
-NF-	Tooele, UT
-NF-	Utah, UT
-NF-	Wasatch, UT
-NF-	Washington, UT
-NF-	Weber, UT
-NF-	Benton, WA
-NF-	Columbia, WA
-NF-	Douglas, WA
-NF-	Franklin, WA
-NF-	Grays Harbor, WA
-NF-	Pierce, WA
-NF-	Spokane, WA
-NF-	Thurston, WA
-NF-	Walla Walla, WA
-NF-	Whatcom, WA
-NF-	Converse, WY
-NF-	Johnson, WY
-NF-	Natrona, WY
-NF-	Sheridan, WY
-NF-	Sweetwater, WY

[a] "NF" = Not Fluoridated

APPENDIX TABLE E. List of counties used in the mortality analysis (cont.)

	Year Started Fluoridation[a]	County Name
	-NF-	Washakie, WY
Region = North Central:	1949	Rock, WI
	1951	Marion, ID
	1951	Brown, SD
	1951	Clay, SD
	1952	Scott, IA
	1952	Cass, ND
	1952	Stark, ND
	1953	Douglas, KS
	1953	Codington, SD
	1953	Milwaukee, WI
	1954	Davison, SD
	1955	Cape Girardeau, MO
	1955	Marion, MO
	1955	St. Louis City, MO
	1955	Burleigh, ND
	1955	Lucas, OH
	1956	Cook, IL
	1956	Morgan, IL
	1956	Stutsman, ND
	1956	Cuyahoga, OH
	1956	Beadle, SD
	1957	Shawnee, KS
	1958	Wapello, IA
	1958	Grand Forks, ND
	1959	Lyon, KS
	1962	Pottawattamie, IA
	1962	Ramsey, MN
	1963	Vanderburgh, IN
	1963	St. Louis, MO
	1964	Wyandotte, KS
	1964	Eau Claire, WI
	1965	Clay, MN
	1967	Wayne, MI
	1967	Nicollet, MN
	1968	Logan, IL
	1968	Macon, IL
	1968	Douglas, NE
	1968	Hughes, SD
	1969	Wabash, IL
	1969	Summit, OH
	1969	Champaign, IL
	1969	Saline, KS

[a] "NF" = Not Fluoridated

APPENDIX TABLE E. List of counties used in the mortality analysis (cont.)

	Year Started Fluoridation[a]	County Name
	1970	Minnehaha, SD
	1973	Woodbury, IA
	1973	Franklin, OH
	1974	Adair, MO
	1983	Greene, MO
	-NF-	Fayette, IN
	-NF-	Vigo, IN
	-NF-	Clay, KS
	-NF-	Cloud, KS
	-NF-	Harvey, KS
	-NF-	McPherson, KS
	-NF-	Mitchell, KS
	-NF-	Norton, KS
	-NF-	Pratt, KS
	-NF-	Reno, KS
	-NF-	Russell, KS
	-NF-	Sedgwick, KS
	-NF-	Seward, KS
	-NF-	Stevens, KS
	-NF-	Dickinson, MI
	-NF-	Buchanan, MO
	-NF-	Adams, NE
	-NF-	Dakota, NE
	-NF-	Dawson, NE
	-NF-	Dodge, NE
	-NF-	Gage, NE
	-NF-	Hall, NE
	-NF-	Kimball, NE
	-NF-	Lincoln, NE
	-NF-	Madison, NE
	-NF-	Phelps, NE
	-NF-	Red Willow, NE
	-NF-	Scott Bluff, NE
	-NF-	York, NE
	-NF-	La Crosse, WI
Region=South	1949	Mecklenburg, NC
	1950	Ohio, WV
	1951	Tuscaloosa, AL
	1951	Pulaski, AR
	1951	Clarke, GA
	1951	Jefferson, KY
	1951	Montgomery, MD
	1951	Prince Georges, MD
	1951	Brooke, WV

[a] "NF" = Not Fluoridated

APPENDIX TABLE E. List of counties used in the mortality analysis (cont.)

Year Started Fluoridation[a]	County Name
1952	Washington, D.C.
1952	Boyd, KY
1952	Baltimore City, MD
1952	Lamar, TX
1953	Ouachita, AR
1953	Dougherty, GA
1953	Daviess, KY
1953	Nueces, TX
1953	Cabell, WV
1954	De Kalb, GA
1954	Fayette, KY
1954	Kanawha, WV
1957	Franklin, KY
1959	Houston, AL
1959	Madison, AL
1960	Maury, TN
1960	Montgomery, TN
1961	Bradley, AR
1962	Cobb, GA
1962	Henderson, KY
1962	Durham, NC
1962	Madison, TN
1963	Gwinnett, GA
1964	Simpson, KY
1965	Tarrant, TX
1966	Mc Cracken, KY
1966	Dallas, TX
1967	Garfield, OK
1967	Richland, SC
1967	Hopkins, TX
1968	Warren, KY
1969	Jefferson, AR
1969	Fulton, GA
1969	Hinds, MS
1970	Shelby, TN
1971	Comanche, OK
1972	Travis, TX
1973	Greenville, SC
1974	Chatham, GA
1974	Muscogee, GA
1974	Orleans, LA
1976	Forrest, MS
1979	Montgomery, AL
1980	Caddo, LA
1981	Autauga, AL

[a] "NF" = Not Fluoridated

APPENDIX TABLE E. List of counties used in the mortality analysis (cont.)

Year Started Fluoridation[a]	County Name
1981	Jefferson, AL
1981	Bossier, LA
1982	Bibb, GA
1982	Harris, TX
1982	Zavala, TX
1983	Jefferson, AL
1983	St. John the Baptist, LA
1983	Jackson, OK
1983	Calhoun, TX
1983	Comal, TX
1985	Victoria, TX
-NF-	Garland, AR
-NF-	Miller, AR
-NF-	Sebastian, AR
-NF-	Union, AR
-NF-	Bay, FL
-NF-	Charlotte, FL
-NF-	Clay, FL
-NF-	Escambia, FL
-NF-	Hillsborough, FL
-NF-	Leon, FL
-NF-	Martin, FL
-NF-	Palm Beach, FL
-NF-	Pasco, FL
-NF-	Pinellas, FL
-NF-	Chattahoochee, GA
-NF-	Acadia, LA
-NF-	Iberia, LA
-NF-	Lafayette, LA
-NF-	Lincoln, LA
-NF-	Madison, LA
-NF-	Ouachita, LA
-NF-	Plaquemines, LA
-NF-	St. Bernard, LA
-NF-	Allegany, MD
-NF-	Coahoma, MS
-NF-	Grenada, MS
-NF-	Leflore, MS
-NF-	Canadian, OK
-NF-	Okmulgee, OK
-NF-	Woodward, OK
-NF-	Bailey, TX
-NF-	Bexar, TX
-NF-	Bowie, TX
-NF-	Cameron, TX

[a] "NF" = Not Fluoridated

APPENDIX TABLE E. List of counties used in the mortality analysis (cont.)

	Year Started Fluoridation[a]	County Name
	-NF-	Coleman, TX
	-NF-	Collingsworth, TX
	-NF-	Crane, TX
	-NF-	Dimmit, TX
	-NF-	Eastland, TX
	-NF-	Erath, TX
	-NF-	Fort Bend, TX
	-NF-	Frio, TX
	-NF-	Hardeman, TX
	-NF-	Hartley, TX
	-NF-	Howard, TX
	-NF-	Kimble, TX
	-NF-	Kleberg, TX
	-NF-	Lampasas, TX
	-NF-	La Salle, TX
	-NF-	Limestone, TX
	-NF-	Palo Pinto, TX
	-NF-	Scurry, TX
	-NF-	Sutton, TX
	-NF-	Taylor, TX
	-NF-	Titus, TX
	-NF-	Tom Green, TX
	-NF-	Uvalde, TX
	-NF-	Young, TX
	-NF-	Brown, TX
Region=North-East:	1952	Bristol, RI
	1954	Philadelphia, PA
	1964	Mercer, NJ
	1965	New York, NY
	1967	New Haven, CT
	1970	Androscoggin, ME
	1978	Suffolk, MA
	-NF-	Berkshire, MA
	-NF-	Hampden, MA
	-NF-	Hampshire, MA
	-NF-	Nantucket, MA
	-NF-	Hillsborough, NH
	-NF-	Sullivan, NH
	-NF-	Bergen, NJ
	-NF-	Camden, NJ
	-NF-	Cape May, NJ
	-NF-	Cumberland, NJ
	-NF-	Essex, NJ
	-NF-	Hudson, NJ

[a] "NF" = Not Fluoridated

APPENDIX TABLE E. List of counties used in the mortality analysis (cont.)

Year Started Fluoridation[a]	County Name
-NF-	Morris, NJ
-NF-	Ocean, NJ
-NF-	Passaic, NJ
-NF-	Salem, NJ
-NF-	Union, NJ
-NF-	Albany, NY
-NF-	Blair, PA
-NF-	Cambria, PA
-NF-	Delaware, PA
-NF-	Erie, PA
-NF-	Lackawanna, PA
-NF-	Lehigh, PA
-NF-	Luzerne, PA
-NF-	Montgomery, PA
-NF-	Washington, PA

[a] "NF" = Not Fluoridated

APPENDIX TABLE F. Definition of Cancer Sites Used in the Mortality Analysis

Site	6th, 7th[a]	8th[a]	9th[a]
Lip	140	140	140
Salivary Glands	142	142	142
Other Oral	141, 143-5 147-8	141,143-6, 148-9	141, 143-6, 148-9
Nasapharynx	146	147	147
Esophagus	150	150	150
Stomach	151	151	151
Colon and Rectum	153, 154	153, 154	153, 154 ex. 154.3, 159.0
Liver	155	155, 156	155, 156 ex. 155.2
Pancreas	157	157	157
Larynx	161	161	161
Lung (inc. bronchus and pleura)	162, 163	162, 163.0	162, 163
Bones and joints	196	170	170
Soft tissue	197	171	171
Melanoma of the skin	190	172	172
Breast	170	174	174
Cervix uteri	171	180	180
Corpus uteri and uterus NOS	172-4	181-182	179, 181, 182
Ovary	175	183	183
Prostate gland	177	185	185
Testis	178	186	186
Bladder	181	188, 189.9	188, 189.3-9
Kidney (inc. renal and ureter)	180	189.0-2	189.0-2
Brain and nervous system	193	191, 192	191, 192
Thyroid	194	193	193
Hodgkin's disease	201	201	201
Non-Hodgkin's lymphomas	200, 202, 205	200, 202	159.1, 200, 202 ex. 202.2-.6
Multiple myeloma	203	203	203 ex. 203.1
Leukemias	204	204-207	202.4, 203.1 204-208 ex 207.1
Other cancers	152, 156, 158-160, 164, 165, 176, 179, 191, 192 195, 198, 199	152, 158-160, 163.1-9, 173, 184, 187, 190, 194-198	152, 154.3, 155.2, 158, 159.2-160.9, 164, 165, 173, 184, 187, 190, 194-198
All cancer sites	140-205	140-207	140-208 ex. 202.2, 202.3, 202.5, 202.6, 207.1

[a] Sources: (23-26).

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly flucridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	All Cancer Sites				Lip				Salivary Glands			
	Males		Females		Males		Females		Males		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	1.07	1604	1.05	1593	0.71	4	1.15	3	3.31	9	1.08	3
30-26 PRE	1.12	8114	1.04	7352	0.81	16	2.46	3	1.08	25	1.28	14
25-21 PRE	1.14	15303	1.05	13663	1.14	40	1.14	6	1.32	41	1.18	28
20-16 PRE	1.13	31988	1.07	28813	0.99	85	0.50	6	0.98	79	0.95	59
15-11 PRE	1.14	88560	1.10	79784	0.66	137	0.86	17	0.79	188	1.03	131
10-6 PRE	1.12	104354	1.09	91725	0.81	136	0.83	11	0.94	242	0.99	144
5-1 PRE	1.12	149807	1.06	133040	0.56	171	0.30	15	0.97	336	0.77	183
0-4 POST	1.09	169656	1.07	151525	0.72	178	1.27	29	0.90	409	1.16	263
5-9 POST	1.07	161113	1.05	144945	0.84	137	0.79	13	0.97	354	1.08	226
10-14 POST	1.04	156744	1.03	142537	0.67	110	0.80	14	0.89	315	0.96	223
15-19 POST	1.01	137693	1.02	127267	0.89	82	0.40	9	0.90	252	1.07	192
20-24 POST	1.01	87436	1.03	79870	0.68	54	1.65	12	0.76	163	0.84	105
25-29 POST	1.03	73510	1.03	67844	0.58	31	1.41	12	0.94	112	1.04	98
30-35 POST	1.05	31557	1.04	29655	1.50	17	0.82	4	0.91	43	1.01	36

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	Nasopharynx				Esophagus				Stomach			
	Males		Females		Males		Females		Males		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	1.19	8	0.00	0	0.91	28	1.47	15	0.98	179	0.92	105
30-26 PRE	1.40	26	1.20	7	1.41	254	1.47	74	0.99	800	1.02	529
25-21 PRE	1.31	50	1.06	15	1.38	448	1.20	121	1.02	1343	0.99	892
20-16 PRE	1.47	106	1.02	25	1.35	842	1.33	272	1.10	2982	1.08	1922
15-11 PRE	1.24	211	1.31	75	1.28	2622	1.49	768	1.16	9372	1.13	5806
10-6 PRE	1.28	230	1.24	82	1.29	2934	1.33	894	1.16	9404	1.17	5979
5-1 PRE	1.18	327	1.32	114	1.49	4597	1.21	1223	1.18	12954	1.14	8224
0-4 POST	1.14	285	1.17	105	1.39	4718	1.21	1424	1.13	12226	1.18	8193
5-9 POST	1.14	193	0.87	66	1.34	4433	1.23	1478	1.17	10294	1.16	6857
10-14 POST	1.27	211	1.23	89	1.22	3974	1.21	1520	1.14	8206	1.15	5876
15-19 POST	1.09	143	0.93	66	1.23	3522	1.14	1326	1.13	6278	1.15	4564
20-24 POST	1.18	59	0.83	23	1.26	2236	1.32	848	1.14	3763	1.18	2716
25-29 POST	1.13	116	0.86	69	1.25	1835	1.06	663	1.15	2822	1.25	2018
30-35 POST	0.99	55	1.03	32	1.13	738	1.10	325	0.98	968	1.02	735

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	Colon and Rectum				Liver				Pancreas			
	Males		Females		Males		Females		Males		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	0.80	146	1.08	219	2.12	40	1.57	50	1.04	95	1.34	67
30-26 PRE	0.97	942	1.06	1138	1.59	181	1.23	199	1.09	431	1.12	307
25-21 PRE	1.06	1932	1.05	2182	1.27	99	1.05	358	1.17	856	1.10	614
20-16 PRE	1.14	4203	1.09	4533	1.25	591	1.00	724	1.09	1754	1.08	1254
15-11 PRE	1.18	13591	1.12	13735	1.17	1501	1.11	2191	1.13	4748	1.18	3427
10-6 PRE	1.18	15641	1.08	15361	1.24	1877	1.08	2540	1.12	5780	1.12	4286
5-1 PRE	1.19	22262	1.08	22293	1.30	2908	1.03	3668	1.10	8180	1.12	6357
0-4 POST	1.19	24917	1.09	24873	1.16	3055	1.04	4124	1.09	9370	1.10	7497
5-9 POST	1.16	23129	1.06	24047	1.17	3010	1.01	3778	1.07	9053	1.08	7523
10-14 POST	1.09	21726	1.02	22672	1.18	3054	1.00	3627	1.05	8622	1.06	7538
15-19 POST	1.07	18829	1.01	19734	1.20	2852	1.02	3227	1.00	7268	1.04	6961
20-24 POST	1.11	11656	1.02	12216	1.20	1741	1.02	1913	0.97	4519	1.02	4274
25-29 POST	1.07	9578	1.04	10024	1.27	1671	1.04	1814	1.03	3625	1.01	3840
30-35 POST	1.08	3872	1.03	4125	1.10	700	0.95	708	1.05	1507	0.97	1564

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	Larynx				Lung				Soft Tissue			
	Males		Females		Males		Females		Males		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	1.12	24	0.67	2	1.25	322	0.92	58	0.93	5	0.67	4
30-26 PRE	1.34	165	1.19	16	1.24	1684	1.11	288	1.91	32	0.92	21
25-21 PRE	1.45	298	0.72	17	1.25	3570	1.16	634	1.13	56	1.23	53
20-16 PRE	1.28	550	1.07	54	1.21	7359	1.07	1300	1.00	117	1.10	95
15-11 PRE	1.37	1696	1.09	134	1.21	19807	1.20	3764	0.97	299	1.40	282
10-6 PRE	1.29	1830	1.29	202	1.16	26196	1.22	5494	1.10	406	1.07	299
5-1 PRE	1.37	2660	1.27	285	1.15	38185	1.15	8657	0.90	581	1.21	513
0-4 POST	1.24	2799	1.33	355	1.11	46200	1.14	11619	0.96	707	1.09	614
5-9 POST	1.32	2685	1.13	348	1.06	46541	1.16	12878	0.98	662	1.12	673
10-14 POST	1.21	2540	1.19	413	1.03	48266	1.09	15604	1.00	739	0.94	670
15-19 POST	1.19	2122	1.21	387	0.99	43905	1.08	16466	0.96	671	0.96	672
20-24 POST	1.20	1336	1.01	251	1.00	28885	1.08	10793	0.95	367	0.87	387
25-29 POST	1.14	1071	1.18	236	1.01	24966	1.09	10725	1.04	397	0.90	407
30-35 POST	1.10	378	1.30	104	1.05	11219	1.07	5450	1.05	182	1.09	211

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	Melanoma of the Skin				Breast		Cervix Uteri	
	Males		Females		Females		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	1.03	18	1.38	17	0.94	240	1.16	1.74
30-26 PRE	1.54	113	1.05	70	1.06	1363	1.08	614
25-21 PRE	1.00	155	1.04	119	1.08	2573	1.02	1035
20-16 PRE	1.11	343	1.16	268	1.10	5599	1.10	2209
15-11 PRE	1.03	749	1.03	577	1.13	16063	1.02	4773
10-6 PRE	0.97	898	1.05	753	1.10	18599	0.94	4631
5-1 PRE	0.92	1328	0.98	1032	1.08	27029	0.87	6201
0-4 POST	1.01	1711	0.95	1308	1.10	30974	0.89	6543
5-9 POST	0.92	1663	0.95	1302	1.07	30044	0.86	5496
10-14 POST	0.95	1809	0.93	1345	1.07	29357	0.84	4567
15-19 POST	0.96	1686	0.88	1255	1.04	25991	0.82	3284
20-24 POST	0.90	1061	0.86	759	1.04	16219	0.83	1935
25-29 POST	0.90	895	0.81	674	1.05	13375	0.83	1355
30-35 POST	0.93	434	0.89	329	1.08	5783	0.97	542

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

Time rel. to start of fluor.	Uterus (Corpus and NOS) Females O/E Ratio	Uterus (Corpus and NOS) Females Obs. Deaths	Ovary Females O/E Ratio	Ovary Females Obs. Deaths	Prostate Males O/E Ratio	Prostate Males Obs. Deaths
35-31 PRE	0.92	107	0.97	78	0.96	141
30-26 PRE	0.73	344	1.11	455	1.12	767
25-21 PRE	0.92	689	1.04	847	1.07	1362
20-16 PRE	0.88	1340	1.01	1786	1.09	2948
15-11 PRE	0.82	3387	1.05	5070	1.02	7009
10-6 PRE	0.94	3807	1.05	6030	0.98	8375
5-1 PRE	0.96	5840	1.02	8778	0.98	12240
0-4 POST	0.96	5958	1.03	10162	0.97	14236
5-9 POST	0.99	5336	0.99	9588	0.94	13795
10-14 POST	1.01	4996	0.99	9311	0.94	13926
15-19 POST	0.96	4026	0.99	8039	0.92	12512
20-24 POST	0.99	2474	0.98	5187	0.90	7708
25-29 POST	0.94	1992	0.97	4033	0.93	6905
30-35 POST	1.02	815	1.01	1639	0.98	2982

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	Brain and Nervous System				Thyroid				Hodgkin's Disease			
	Males		Females		Males		Females		Males		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	0.90	43	1.40	31	1.19	5	1.76	10	1.42	31	0.92	16
30-26 PRE	1.18	242	1.16	167	1.16	27	0.82	29	1.15	147	1.12	88
25-21 PRE	1.16	466	1.12	304	0.73	29	1.07	68	1.07	234	1.12	153
20-16 PRE	1.14	975	1.17	662	0.93	73	1.00	130	1.05	512	1.06	307
15-11 PRE	1.16	2491	1.21	1743	1.17	272	1.25	458	1.07	1328	1.17	843
10-6 PRE	1.14	2918	1.12	2034	1.15	288	1.17	519	1.02	1367	1.14	984
5-1 PRE	1.06	3941	1.09	2923	1.18	411	1.17	714	0.99	1907	1.13	1291
0-4 POST	1.06	4578	1.02	3314	1.19	440	1.26	805	0.97	2004	1.01	1368
5-9 POST	1.02	4231	1.03	3276	1.01	353	1.04	677	0.99	1692	1.07	1235
10-14 POST	0.99	4055	.94	3194	1.00	317	0.98	557	0.93	1376	1.01	1038
15-19 POST	0.90	3378	0.94	2813	0.97	250	1.17	481	1.00	1139	1.10	905
20-24 POST	0.90	2251	0.94	1866	1.04	148	1.07	295	1.09	636	0.97	430
25-29 POST	0.90	1803	0.96	1512	0.79	113	1.46	227	1.07	413	1.15	319
30-35 POST	0.95	749	0.98	657	0.83	47	1.23	89	0.83	134	1.01	109

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

Time rel. to start of fluor.	Testis Males		Bladder Males		Bladder Females		Kidney Males		Kidney Females	
	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	1.04	8	1.07	63	0.92	26	0.83	28	1.35	32
30-26 PRE	1.00	45	1.06	317	0.89	123	1.05	178	1.02	106
25-21 PRE	1.06	88	0.98	520	0.99	249	1.08	340	0.94	189
20-16 PRE	0.99	185	1.13	1230	1.08	536	1.11	717	0.98	403
15-11 PRE	0.97	434	1.08	3488	1.09	1479	1.13	2077	1.08	1153
10-6 PRE	0.87	445	1.07	3954	1.09	1713	1.10	2360	1.08	1434
5-1 PRE	0.81	610	1.12	5873	1.13	2475	1.02	3286	0.98	2028
0-4 POST	0.99	760	1.09	6430	1.08	2789	1.06	3976	0.96	2306
5-9 POST	0.95	680	1.08	6121	1.10	2629	1.06	3844	0.92	2192
10-14 POST	0.93	582	1.06	5675	1.11	2619	1.03	3697	0.95	2221
15-19 POST	0.79	416	1.03	4831	1.00	2173	0.96	3173	0.88	1995
20-24 POST	0.83	285	1.05	3029	1.09	1402	1.02	2083	0.92	1283
25-29 POST	1.11	175	1.06	2405	1.01	1073	1.03	1694	0.85	1106
30-35 POST	1.03	67	1.08	960	1.05	484	1.02	721	0.95	485

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	Non-Hodgkin's Lymphoma				Multiple Myeloma				Leukemias			
	Males		Females		Males		Females		Males		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	1.28	43	2.06	44	0.93	12	1.49	13	1.26	107	0.93	61
30-26 PRE	1.12	215	1.16	144	1.27	80	0.84	40	1.05	413	1.01	309
25-21 PRE	1.17	443	1.13	296	1.30	160	1.05	104	1.09	797	1.00	565
20-16 PRE	1.11	908	1.33	740	1.22	302	1.21	272	1.07	1673	1.07	1258
15-11 PRE	1.11	2405	1.23	1820	1.19	810	1.20	641	1.08	4180	1.08	3242
10-6 PRE	1.12	2964	1.22	2376	1.20	1093	1.04	893	1.03	4904	1.11	3876
5-1 PRE	1.08	4248	1.14	3480	1.17	1500	1.05	1327	1.01	6888	0.99	5250
0-4 POST	1.10	5127	1.14	4282	0.97	1705	1.04	1725	0.95	7664	1.03	6209
5-9 POST	1.06	4833	1.10	4352	0.99	1898	0.92	1703	0.96	7191	0.98	5650
10-14 POST	1.03	4833	1.07	4571	0.97	1914	1.01	1898	0.98	7006	1.01	5608
15-19 POST	1.02	4360	1.04	4294	0.96	1796	0.93	1766	0.96	5971	0.97	4877
20-24 POST	1.00	2666	0.98	2573	0.85	1118	0.95	1196	0.92	3620	0.99	3035
25-29 POST	0.99	2215	1.01	2324	0.85	956	0.87	989	1.02	3078	0.99	2588
30-35 POST	1.06	1048	1.08	1069	0.97	451	0.97	483	0.95	1212	0.96	1085

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX TABLE G. Observed over Expected mortality ratios[a] and number of observed deaths in rapidly fluoridated counties by years before (PRE) and after (POST) fluoridation by site of cancer, 1950-85.

	Other Cancer Sites			
	Males		Females	
Time rel. to start of fluor.	O/E Ratio	Obs. Deaths	O/E Ratio	Obs. Deaths
35-31 PRE	1.19	232	0.95	207
30-26 PRE	1.12	922	0.98	846
25-21 PRE	1.12	1559	1.03	1476
20-16 PRE	1.06	3097	0.98	2872
15-11 PRE	1.10	7897	1.07	7526
10-6 PRE	1.04	8328	1.02	7754
5-1 PRE	1.03	11533	0.99	11213
0-4 POST	1.01	11669	1.01	11417
5-9 POST	1.01	9304	0.97	9421
10-14 POST	1.02	8196	1.06	8382
15-19 POST	0.99	5425	1.00	5636
20-24 POST	1.00	3359	1.00	3493
25-29 POST	1.01	2189	0.99	2279
30-35 POST	1.11	943	0.96	859

[a]Relative to non-fluoridated counties (1.00), adjusted for age, calendar time period, and region.

APPENDIX F

TIME TRENDS FOR BONE AND JOINT CANCERS AND OSTEOSARCOMAS IN THE SURVEILLANCE, EPIDEMIOLOGY AND END RESULTS (SEER) PROGRAM NATIONAL CANCER INSTITUTE

AUGUST, 1990

At the request of the Committee, we have enclosed a brief description of the time trends for bone and joint cancers and for osteosarcomas in the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute (NCI), and the relationship of these trends to fluoridation of drinking water supplies. The SEER Program, begun in 1973, is a group of population-based cancer registries that covers approximately 10% of the U.S. population. In contrast to our main report which focused on observed/expected events, in this report we also present age-adjusted rates. As cancer rates generally increase with age, to properly compare rates among populations which may differ in age distribution, a statistical procedure called age adjustment applies a standard set of weights to age-specific rates to derive age-adjusted rates; this allows meaningful comparison while minimizing potential confounding. Table 1 presents the data for the entire SEER program split into 2 time periods (1973-80 and 1981-87). The incidence of all bone and joint cancers over all ages increased slightly between these two time periods. When examined by age, the only increase occurred for the rates among those under age 20, where an 18% rise occurred for the sexes combined, reflecting a 23% rise in males and a 13% rise in females. When osteosarcomas are considered separately, there was essentially no change in the incidence rate over time for the sexes combined, reflecting the averaging of an 18% rise for males and an 11% decline among females. Among males, the upward trend resulted mainly from the experience of those under age 20, whose rates rose from 0.36 to 0.55 (53%).

It was possible to evaluate these same trends for groupings of counties within the SEER areas that were "non-fluoridated" as well as for those undergoing abrupt fluoridation at some time before the establishment of the SEER program. The definitions of "non-fluoridated" and "fluoridated" counties were similar to those used in our previously submitted study. It should be noted that a substantial number of counties within the SEER program do not fit under either definition and thus are not informative with respect to a time-trend analysis of fluoridation. Tables 2, 3, and 4 present rates for these groupings of counties which do meet the inclusion criteria. As shown in Table 2, the pattern for the entire SEER program of a rising rate of bone and joint cancers at all ages combined, due mainly to trends under age 20, was seen in the "fluoridated" counties but not in the "non-fluoridated" counties. Tables 3 and 4 are restricted to the patterns among males. Once again, the larger increase in males under age 20 seen in the aggregate data for all bone and joint cancers is seen only in the "fluoridated" counties (Table 3). For osteosarcomas among males, increases were seen for those under age 20 in both the "fluoridated" and "non-fluoridated" areas, although more prominently in the "fluoridated" counties (Table 4).

Based on these data, one could conclude that summarized over all ages and both sexes, there were no meaningful time trends in incidence of these tumors. However, for bone and joint cancers, temporal increases were seen among those under age 20 in both sexes. For osteosarcomas, there were some increases, but only among young males. In addition, these patterns were associated with the fluoridation status of the counties for which these trends were assessed.

To determine whether these incidence trends are associated with fluoridation of water supplies, or with some other correlate of fluoridated counties, one needs to assess the relationship of risk of these tumors to fluoridation while controlling for calendar-time. This analysis was carried out in our earlier report covering the Iowa and Seattle registries. In brief, after control for age, area and calendar-time, there was no evidence of an increased risk of these tumors with increasing duration of fluoridation. For example, the observed-to-expected ratios for osteosarcomas among those under age 30 who had "lifetime" exposure to fluoridated drinking water was 0.9, compared to a value of 0.9 for those who had less than one- half of their lifetime spent in areas with fluoridated water. The expected values for both of these ratios were based on the age, sex, time and area-specific rates in non-fluoridated areas.

Our analyses utilizing all available data for the Iowa and Seattle registries did not reveal positive correlations, which should have appeared if an association existed between fluoridation and risk of bone cancers. However, since the evaluation of temporal patterns in the SEER data noted some age

and sex-specific increases, we reanalyzed our study to further investigate these subgroups. First of all, the age and sex-specific time trends and their relation to the fluoridation status of the counties covered by the Iowa and Seattle registries are similar to those described for the total SEER program. When restricted to persons under age 20, the rates for bone and joint cancers in both sexes rose 47% from 1973-80 to 1981-87 in the fluoridated areas of Seattle and Iowa and declined 34% in the non-fluoridated areas. For osteosarcomas in males under 20, the rates increased 79% in the fluoridated areas and decreased 4% in the non-fluoridated areas. With this background, Table 5 presents for the Iowa and Seattle registries, the relationship of duration of fluoridation to the risk of bone and joint cancers and osteosarcomas for those under age 20. As seen when data for all ages were analyzed in our submitted report, there was no evidence of an increase in the incidence ratios with increasing duration of fluoridation. Indeed, the ratios for osteosarcomas are lowest in the longest duration categories. Similarly, when incidence ratios were calculated for those under age 20 with "lifetime" exposure to fluoridation versus those exposed less than one-half of their life, there was no evidence of a significant positive relationship. The ratios for bone and joint cancer for both sexes were 1.3 for "lifetime" exposure and 1.2 for <1/2 "lifetime" exposure. The corresponding ratios for osteosarcomas were 1.1 and 1.5 for both sexes, and 0.6 and 1.3 for males. While the observed numbers on which some of these incidence ratios are based are relatively small, they are the same numbers which yielded the time-trend patterns. Thus, the differences in time trends between fluoridated and non-fluoridated areas for those under age 20 in these two registries is not related to the timing of fluoridation.

While we believe our analysis is the appropriate way of assessing this relationship, the conclusions revealed are supported by another methodologic approach presented in Tables 2, 3, and 4, using data for the entire Seer program. For this analysis we considered two subgroups of the "fluoridated" counties, those fluoridated early (before 1955) and those fluoridated late (after 1965). The time trends of most concern, those under age 20, are all greater for the counties fluoridated in the earlier time-period. For this group, you would expect to see no influence of fluoridation, since essentially all persons under age 20 in both time periods from these early-fluoridated counties would have been exposed for their entire life-times. Other inconsistencies with a fluoridation effect are also present. This includes a marked decline in osteosarcoma rates in males aged 20-39 between these two time-periods for the early-fluoridation countries, although one might anticipate that this age group would experience a marked impact of fluoridation if there was one.

One can quantify the relationship between interval from fluoridation of drinking water and risk of bone malignancies by analyzing all of the fluoridated and non-fluoridated counties in the SEER Program summarized in Tables 2-4 in the same manner as the Iowa and Seattle counties were analyzed. The difficulty with this approach, as noted in our earlier report, is that Iowa and Seattle were the only areas with enough of both fluoridated and non-fluoridated areas by our definitions to allow an analysis which controlled for geographic area. Thus, the expanded analysis involves adding data to the non-fluoridated areas mainly from Utah and New Mexico, and adding to the fluoridated areas data from Detroit, Atlanta, San Francisco and Connecticut. The resulting comparison of trends between these different areas could be suspect. However, since this is the comparison yielding the time-trend differences noted in the SEER program, it is useful to see if the patterns observed are indeed explained by the timing of fluoridation in these areas. Table 6 presents observed-to- expected incidence ratios by time since fluoridation (duration of fluoridation) for those under age 20 in the "fluoridated" and "non-fluoridated" counties in the entire SEER program. For none of the categories revealing differences in time trends between fluoridated and non-fluoridated areas is there any evidence of an increase in incidence ratios by duration of fluoridation. For osteosarcomas, there is even some evidence of a decline in the ratio with duration of fluoridation.

In summary, analysis of incidence data from the SEER program has revealed some age- and sex-specific increases over time for bone and joint cancers, and for osteosarcomas, which are more prominent in fluoridated than in non-fluoridated areas. However, on further analysis these increases are unrelated to the timing of fluoridation, and thus are not linked to the fluoridation of water supplies.

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TABLE 1

Average annual age-adjusted incidence rates[a] (# of cases) for bone and joint cancers and osteosarcomas for 2 time periods (1973-80 and 1981-87) and percent change between periods by sex and age, all SEER areas combined.

	All Ages			<20			20 - 39			40 - 79		
	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change
Bone & Joint												
Both sexes	0.84 (1162)	0.88 (1099)	+5	0.80 (373)	0.94 (334)	+18	0.60 (254)	0.61 (265)	+2	1.00 (472)	0.97 (445)	-3
Males	1.00 (666)	1.04 (628)	+4	0.87 (209)	1.07 (195)	+23	0.75 (159)	0.77 (168)	+3	1.28 (276)	1.19 (248)	-7
Females	0.69 (496)	0.73 (471)	+6	0.72 (164)	0.81 (139)	+13	0.46 (95)	0.44 (97)	-4	0.76 (196)	0.81 (197)	+7
Osteosarcoma												
Both sexes	0.30 (404)	0.31 (370)	+3	0.37 (175)	0.46 (163)	+24	0.20 (85)	0.19 (82)	-5	0.27 (128)	0.24 (109)	-11
Males	0.33 (215)	0.39 (226)	+18	0.36 (88)	0.55 (100)	+53	0.21 (45)	0.25 (53)	+19	0.37 (79)	0.32 (68)	-14
Females	0.27 (189)	0.24 (144)	-11	0.38 (87)	0.37 (63)	-3	0.19 (40)	0.14 (29)	-26	0.19 (49)	0.17 (41)	-11

[a] Cases per 100,000 population per year, age-adjusted based on the 1970 U.S. Population.

TABLE 2

Average annual age-adjusted incidence rates[a] (# of cases) for bone and joint cancers in both sexes for 2 time periods (1973-80 and 1981-87) and percent change between periods by age and fluoridation status, all SEER areas combined.

	All Ages			<20			20 - 39			40 - 79		
	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change
"Non-Fluoridated" SEER	0.90 (156)	0.85 (153)	-6	0.85 (56)	0.88 (55)	+4	0.62 (34)	0.49 (31)	-21	1.13 (61)	1.04 (61)	-10
"Fluoridated" SEER	0.84 (553)	0.91 (511)	+8	0.82 (175)	1.08 (166)	+32	0.57 (116)	0.54 (114)	-5	0.99 (228)	0.94 (221)	-5
<1955	0.90 (91)	0.86 (76)	-4	0.57 (16)	0.91 (18)	+60	0.78 (26)	0.55 (19)	-29	1.35 (47)	0.93 (29)	-26
≥1966	0.83 (395)	0.93 (373)	+12	0.89 (141)	1.07 (118)	+20	0.55 (79)	0.60 (84)	+9	0.88 (150)	0.96 (149)	+9

[a] Cases per 100,000 population per year, age-adjusted based on the 1970 U.S. Population.

TABLE 3

Average annual age-adjusted incidence rates[a] (# of cases) for bone and joint cancers in males for 2 time periods (1973-80 and 1981-87) and percent change between periods by age and fluoridation status, all SEER areas combined.

	All Ages			<20			20 - 39			40 - 79		
	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change
"Non-Fluoridated SEER	0.98 (83)	0.83 (73)	-15	0.71 (24)	0.69 (22)	-5	0.78 (22)	0.57 (18)	-28	1.38 (35)	1.17 (32)	-15
"Fluoridated" SEER	1.02 (318)	1.12 (299)	+10	0.97 (106)	1.35 (107)	+39	0.72 (73)	0.70 (73)	-3	1.26 (130)	1.13 (110)	-10
<1955	1.23 (58)	1.18 (48)	-4	0.88 (12)	1.22 (12)	+39	0.84 (15)	0.85 (15)	+1	1.97 (31)	1.39 (20)	-29
≥1966	1.00 (228)	1.10 (209)	+10	1.03 (84)	1.35 (77)	+31	0.70 (50)	0.71 (50)	+1	1.13 (86)	1.06 (75)	-6

[a] Cases per 100,000 population per year, age-adjused based on the 1970 U.S. Population.

TABLE 4

Average annual age-adjusted incidence rates[a] (# of cases) for osteosarcoma in males for 2 time periods (1973-80 and 1981-87) and percent change between periods by age and fluoridation status, all SEER areas combined.

	All Ages			<20			20 - 39			40 - 79		
	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change	73-80	81-87	Change
"Non-Fluoridated" SEER	0.29 (25)	0.30 (26)	+3	0.30 (10)	0.42(13)	+40	0.29 (8)	0.15 (5)	-48	0.29 (7)	0.25 (7)	-14
"Fluoridated" SEER	0.33 (103)	0.44 (112)	+3	30.39 (43)	0.66 (52)	+69	0.21 (21)	0.25 (25)	+19	0.37 (37)	0.34 (33)	-8
<1955	0.32 (16)	0.42 (14)	+31	0.22 (3)	0.73 (7)	+232	0.32 (6)	0.18 (3)	-44	0.44 (7)	0.27 (4)	-39
≥1966	0.35 (98)	0.45 (82)	+29	0.44 (36)	0.66 (38)	+50	0.19 (13)	0.29 (19)	+53	0.37 (27)	0.32 (23)	-14

[a] Cases per 100,000 population per year, age-adjusted based on the 1970 U.S. Population.

TABLE 5

Observed to expected[a] incidence ratios (# of observed) by duration of fluoridation or age, whichever is less, for selected sex and cancer-site groupings for those under age 20 at diagnosis ("fluoridated" counties, Seattle and Iowa SEER registries).

		Duration of Fluoridation (yrs.)			
Site	Sex	<5	5-9	10-14	15-19
Bones & Joints	Both	1.7 (11)	1.1 (21)	1.4 (38)	1.3 (25)
Osteosarcoma	Both	1.6 (4)	1.8 (11)	1.0 (16)	0.9 (13)
	Males	1.0 (1)	2.9 (9)	0.7 (8)	0.4 (5)

[a] Expected based on the rates in "non-fluoridated" counties adjusted for age, calendar time, and geographic area.

TABLE 6

Observed to expected[a] incidence ratios (# of observed) by duration of fluoridation or age, whichever is less, for selected sex and cancer-site groupings for those under age 20 at diagnosis ("Fluoridated" counties, all SEER areas).

		Duration of Fluoridation (yrs.)			
Site	Sex	<5	5-9	10-14	15-19
Bones & Joints	Both	1.3 (27)	0.9 (104)	1.2 (142)	1.0 (67)
Osteosarcoma	Both	1.4 (10)	1.2 (52)	1.2 (70)	1.0 (34)
	Males	1.7 (4)	1.5 (30)	1.7 (41)	1.0 (20)

[a] Expected based on the rates in "non-fluoridated" counties adjusted for age and calendar time.

APPENDIX G

OSTEOSARCOMA

OSTEOSARCOMA

Bone cancers are of several cell types that differ etiologically. Osteosarcoma, which originates most often at the growing end of bones, is most prevalent among males 10 to 19 years of age; girls whose adolescent growth spurt occurs before that of boys are at greater risk earlier (Johnson, 1952). The cancer cells, which come from mesenchymal tissue, form bone in a wild disorderly fashion. The incidence rates are equal among children of all races (Miller, 1981). According to recent evidence (Cavazzana et. al, 1987) the other main bone tumor, Ewing's sarcoma, arises from primitive nervous tissue, most commonly in the shaft of the bone (Coley, 1960). Its incidence in young whites is similar to that of osteosarcoma, but it is very rare among youths of other races who presumably are either not exposed or not susceptible to whatever causes the neoplasm in whites.

In a large series of cases at the Mayo Clinic, osteosarcoma occurred 1.4 times more often in males than in females of all ages (Dahlin, 1978). Although osteosarcoma occurs at all ages, the rates are much lower among the elderly. Ewing's sarcoma differs, since it rarely occurs among people older than 30 years of age. In adolescence a slight preponderance of boys is affected. Lagging in frequency behind osteosarcoma and Ewing's sarcoma are cancers of cartilage (chondrosarcomas) and miscellaneous rare forms of bone cancer.

Mortality data for 1950 to 1959, which closely reflected incidence data because effective treatment had not yet been developed, showed that death rates for children with bone cancer rose as a function of their stature. Since boys on the average grew taller than girls, their bone cancer mortality exceeded that for girls and remained elevated over a longer interval (Figure G-1) (Miller, 1981).

Veterinarians have shown that giant breeds of dogs, such as the St. Bernard and Great Dane, have much higher rates of osteosarcoma than do dogs of small or medium size (Tjalma, 1966). In humans, however, children with osteosarcoma were taller than children with other forms of cancer, but the difference was not statistically significant (Fraumeni, 1967).

Osteosarcoma has been induced by radium-226, first used early in this century by women who painted radium-dials on watches (Martland et al., 1924). Radium was deposited in bone, especially the jaw, where over time its radioactivity constantly bombarded the surrounding tissue. Shortly after World War II, West German patients with tuberculosis and bone diseases other than osteosarcoma were treated with radium-224, also a bone-seeker. The treatment caused 30 cases of osteosarcomas among 220 children treated (Mays and Spiess, 1984). Six more children developed other forms of bone cancer, and 20 developed benign bone tumors, mostly osteochondromas. Radiation therapy, in the thousands of rad has also caused bone cancer (Tucker et al., 1987). Lower doses, in the hundreds of rad (such as those received by Japanese atomic-bomb survivors) have not caused an excess of bone cancer (Shimizu et al., 1990).

Bone cancer occurred excessively after patients received alkylating agents as therapy for childhood cancer (Tucker et al., 1987). This effect was observed in cases in which no radiotherapy had been given: in other cases, alkylating agents enhanced the capacity of radiotherapy to induce bone cancers, mostly osteosarcomas.

Osteosarcoma is rarely familial. It occurs in certain genetic syndromes that affect bone (reviewed by Miller, 1981) and in the Li-Fraumeni family cancer syndrome, in which certain dissimilar cancers aggregate in certain families, because of mutation of the p53 gene on chromosome 17 (Malkin, et al, 1990).

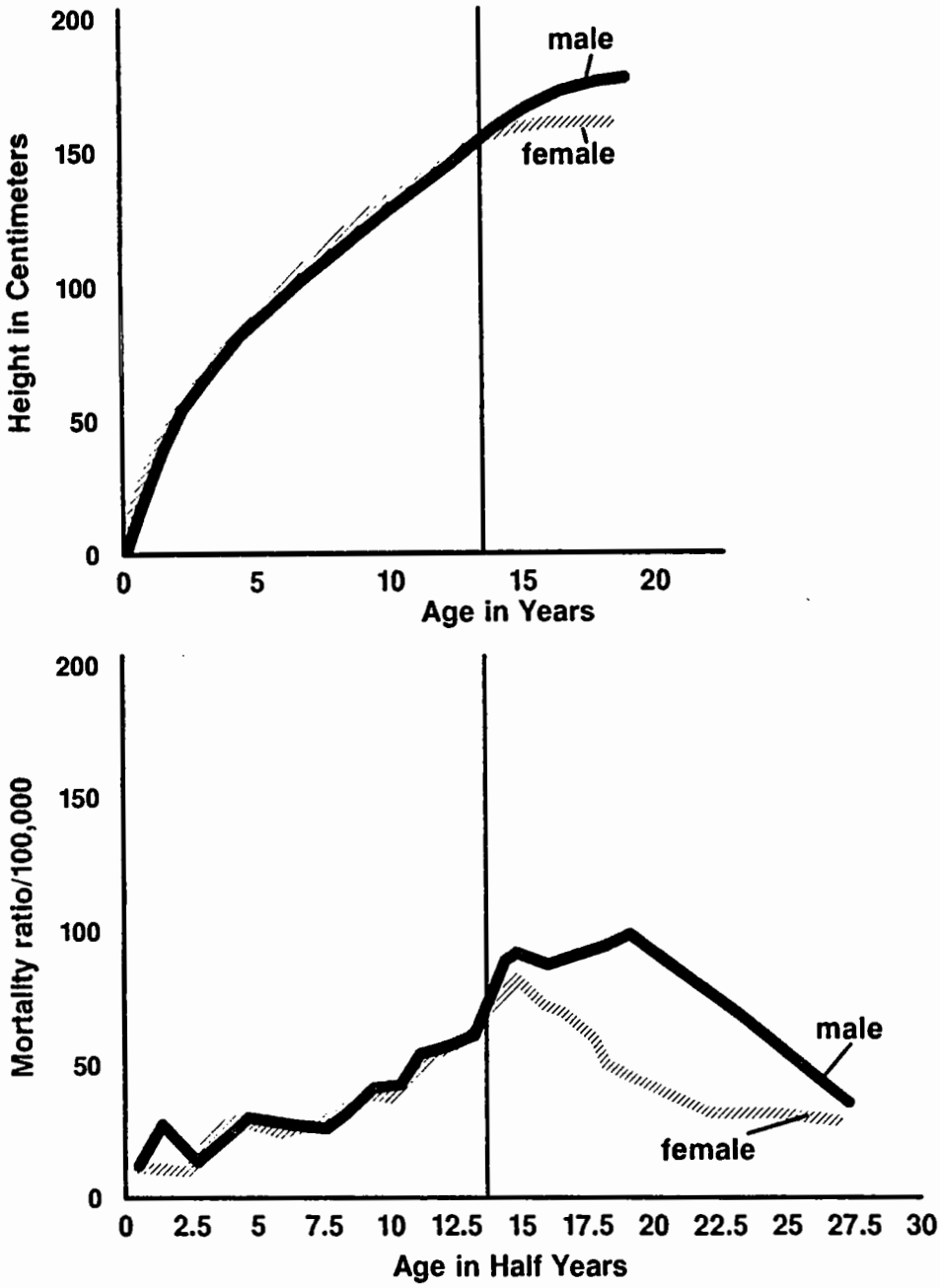

Figure G-1. Comparison of stature with bone cancer mortality by single year of age among United States whites. 1950-59. (Miller, 1981)

Paget's disease of bone, transmissible as an autosomal dominant trait, occurs late in life, perhaps because of the "wear and tear" on the bone, and predisposes the person to osteosarcoma and other skeletal tumors (McKusick, 1966). Localized bone destruction occurs for an unknown reason; repair occurs almost simultaneously with destruction, and the bone swells (Fallon and Schwamm, 1989). The skull is commonly involved, and one sign of the disease is the need for a larger hat size than before. Of 101 osteosarcomas in a series of patients older than 60 years of age, 55 percent were associated with Paget's disease (Huvos, 1986). This association between osteosarcoma and Paget's disease is thought to account for the rise in bone sarcoma late in life (Fraumeni and Boice, 1982). In the United Kingdom, a radiologic survey showed that 5.4 percent of people older than 55 years of age had Paget's disease (Barker et al., 1977). The disorder is usually silent. In one study, only 3 of 85 patients with the disease had complaints referable to bone (Monroe, 1951). Men are affected twice as often as women. Cancer may result from Paget's disease that is limited to one bone, such as a vertebra (Groh, 1988).

Osteosarcoma occurs excessively as a second primary cancer in children who have survived retinoblastoma and whose only treatment was surgery. (The bone cancer is inherent; it occurs outside the field of radiotherapy in patients who do not receive chemotherapy for the eye tumor). The (tumor suppressor) gene for retinoblastoma has been cloned, and current evidence indicates that the same gene induces some cases of osteosarcoma. For this reason both tumors occur in the same patient or family (reviewed by Knudson, 1989).

APPENDIX H

GENOTOXICITY OF FLUORIDE

GENOTOXICITY OF FLUORIDE

There are many published reports, and a number of recent reviews (Aardema et al., 1989; IARC, 1987, Li et al., 1988; Smith, 1988), on the genotoxic effects of fluoride on cultured cells and in animals. Many of the reports present only a positive or negative result without supporting data, or with insufficient information to permit the reader to draw definitive conclusions. With few exceptions, these reports are not included here. Other reports have sufficient detail concerning the methods used, but the reported results, are questioned because of unusually high spontaneous control values or the use of nonstandard endpoints or effects. Such reports were included or excluded on a case-by-case basis; where they have been included, comments about the data have been added.

There are also a number of reports on sodium fluoride being used as an antimutagen or anticlastogen in bacteria, cultured mammalian cells, and Drosophila. It was effective in some studies for reducing the responses to chemical mutagens (Buchi and Burki, 1975; Israelewski and Obe, 1977; Francis et al., 1988; Gebhart et al., 1984; Ivashchenko and Grozdova, 1972; Obe and Slacik-Erben, 1973; Vogel, 1973). These effects are not addressed here.

MICROBIAL TEST SYSTEMS

Sodium fluoride was not mutagenic in Salmonella typhimurium when tested with or without metabolic activation in the standard plate or preincubation tests (Arlauskas et al., 1985; Gocke et al., 1981; Haworth et al., 1983; Li et al., 1987a; Litton Bionetics, 1975; Martin et al., 1979; Tong et al., 1988). Nikiforova (1982) reported positive responses, however, in strains TA98 and TA1535 in a suspension assay. The protocol used in that study could have produced artifactual positive responses because of cell killing and the presence of histidine on the selection plates. In only one laboratory was stannous fluoride (SnF_2) positive when tested by an atypical protocol. Sodium fluoride and sodium monofluorophosphate (Na-MFP) were not mutagenic under the same atypical conditions (Gocke et al., 1981); the investigators gave no explanations for these differences. Sodium fluoride was negative in the Bacillus subtilis REC assay (Matsui, 1980) and did not induce point mutation or gene conversion in the yeast Saccharomyces cerevisiae (Litton Bionetics, 1975).

IN VITRO MAMMALIAN TEST SYSTEMS

Mutation

Sodium fluoride and potassium fluoride induced gene mutations at the tk locus in mouse lymphoma cells (Caspary et al., 1987; Cole et al., 1986) in the dose range of 100 to 600 µg/ml; the investigators speculated that these effects resulted from chromosome damage rather than point mutations. This hypothesis was supported by the lack of induction of ouabain-resistant mutants (Cole et al., 1986) in the same cells. Sodium fluoride was also positive at the tk and hgprt loci in human lymphoblastoid cells treated with 100 to 600 µg/ml for 28 hours and at the tk locus in cells treated with 65 µg/ml for up to 20 days (Crespi et al., 1990). No mutagenicity was seen at the hgprt locus in a rat liver epithelial cell line treated with up to 160 ug sodium fluoride/ml (Tong et al., 1988).

Chromosome Aberrations and Sister Chromatid Exchange (SCE)

Sodium fluoride caused chromosome damage in a variety of *in vitro* mammalian cell systems. The effects reported were primarily chromatid deletions and achromatic regions (gaps). These effects were not always clearly demonstrated and appeared to be highly protocol-dependent (Li et al., 1988; Aardema et al., 1989).

Animal Cells

Chromosome aberrations were reported in the dose range of 25 to 100 µg sodium fluoride/ml in a variety of cells (Aardema et al., 1989; Tsutsui et al., 1984b; He et al., 1983; Bale and Mathew, 1987). During the NTP studies (DHHS, 1990) in one laboratory where CHO cells were tested at NaF doses up to 200 ug/ml, the cells showed no response; in a second laboratory where the cells were tested at NaF doses of 1,2000 to 1,6000 ug/ml, with a longer harvest time to accommodate NaF-induced cell cycle delay, the cells showed a positive response. No aberrations were induced in CHO cells by sodium fluoride at doses up to 500 µg/ml, or by Na-MFP at doses up to 4,000 µg/ml (Ishidate, 1987). Contradictory results were reported for the induction of SCE in cultured mammalian cells (Li et al., 1987c).

Human Cells

Chromosomal aberrations were induced in cultured human lymphocytes and fibroblasts at doses in the range of 20 to 40 µg sodium fluoride/ml (Albanese, 1987; Scott and Roberts, 1987; Tsutsui et al., 1984c) and in leukocytes at doses in the range of 0.03 to 3.15 mM fluoride ion [1-132 µg sodium fluoride per ml] (Jachimczak and Skotarczak, 1978). In contrast, others (Gebhart et al., 1984; Kralisz and Szymaniak, 1978; Matsuda, 1980; Slacik-Erben and Obe, 1976; Thomson et al., 1985; Voroshilin et al., 1973) reported no aberration induced in leukocytes or lymphocytes that were treated with sodium fluoride *in vitro* at doses up to 3 mM [126 µg/ml] and 16 ppm [16 µg/ml]. No sister chromatid exchange were induced in lymphocytes treated with up to 160 µg/ml (Tong et al., 1988) or 10 mM [420 µg/ml] (Thomson et al., 1985). Potassium fluoride did not induce aberrations at up to 3 mM [193 µg/ml] or sister chromatid exchange at up to 10 mM [580 µg/ml] in lymphocytes (Thomson et al., 1985).

DNA Damage and Repair

Sodium fluoride inhibits both protein and DNA synthesis in cultured mammalian cells; the inhibition of DNA synthesis may be a secondary effect of the inhibition of protein synthesis or a result of the direct inhibition of DNA polymerase (Skare et al., 1986b). Reports conflict on the ability of sodium fluoride to produce unscheduled DNA synthesis (UDS) (which is one measure of DNA repair) in cultured mammalian cells, as measured by incorporation of ^{3}H-thymidine into DNA. Skare and coworkers (1986b) have suggested that false positive responses that are due to precipitated complexes of Mg^{++}:F^{-}:^{3}H-thymidine may be seen during studies that measure total cellular radioactivity by scintillation counting; this artifact would not affect autoradiographic scoring procedures.

Sodium fluoride was negative for UDS in primary rat hepatocytes at doses up to 160 µg/ml (Tong et al., 1988) when scored by autoradiography but was positive in human fibroblasts and oral keratinocytes at doses up to 400 µg/ml and 300 µg/ml, respectively, when scored by scintillation counting (Tsutsui et al., 1984a,c). UDS was not found in Wistar-38 human fibroblasts when measured by density gradient ultracentrifugation (Skare et al., 1986b).

Cell Transformation

Dose-related increases in the frequencies of transformed colonies were observed in SHE cells within a concentration range of 10 TO 125 ug NaF/ml (Jones et al., 1988a,b; Lasne et al., 1988; Tsutsui et aL., 1984b). Morphological transformation was not induced in the BALB/3T3 cell line at similar concentrations (Lasne et al., 1988). SHE cells transformed by 75 or 100 µg NaF/ml and injected into newborn Syrian hamsters produced anaplastic fibrosarcomas at the site of injection after 28 to 39 days (Tsutsui et al., 1984b). Sodium fluoride showed promoter activity in SHE cells *in vitro* when used in conjunction with benzo(a)pyrene in a two-step exposure study (Jones et al., 1988a).

IN VIVO MAMMALIAN TEST SYSTEMS -- SOMATIC CELLS

Like the *in vitro* cytogenetics test results, results from *in vivo* tests for chromosomal effects of NaF are mixed. In numerous instances, similar protocols performed in different laboratories produced different results and conclusions, and the descriptions of test protocols often did not contain dose selection criteria and toxicity information, thus precluding an assessment of the adequacy of the test concentrations used.

Martin and coworkers found no chromosome aberrations in the bone marrow of mice given 50 ppm F^-, as NaF, in their drinking water for several generations or in those given doses up to 100 ppm for 6 weeks (Martin et al., 1979). The administration of up to 200 ppm F^- in their drinking water for 3 or 6 weeks however led to dose-related increases in aberrations in bone marrow cells at doses of 1 ppm and higher (Mohamed and Chandler, 1982). Because of unusually high frequencies of aberrant cells in the control animals and questions about the nature of the aberrations scored, the interpretation of these studies is uncertain. Pati and Bhunya (1987) found that mice injected or gavaged with 40 mg NaF/kg, in a variety of dose-exposure combinations, had increased aberrations in their bone marrow cells. This study included gaps in the analysis of aberrations and relied largely on these gaps for the conclusion that sodium fluoride was clastogenic. The significance of gaps is not well understood and this lesion is not usually included in data analyses.

Aliev and Babaev (1981) reported aberrations in rat bone marrow cells after the rats received an acute dose of about half the LD_{50} dose which the authors defined as 20 mg F^-/kg [44 mg NaF/kg], or a daily administration of 3.2 mg F^-/kg [7 mg NaF/kg] for 3 months. Voroshilin and coworkers (1973) reported no aberrations in the bone marrow of rats inhaling 0.1 mg/M^3 HF for 5 months, but in a subsequent study in which rats inhaled 1.0 mg/M^3 HF for 1 month, they reported an increase in damaged cells. This increase was characterized by the authors as resulting principally from age-related hyperploidy; they also reported that the fluoride content of the bones was lower in the older than in the younger treated animals (Voroshilin et al., 1975).

Kram and coworkers (1978) found no increase in SHE in the bone marrow of mice administered 50 ppm NaF in their drinking water for at least 7 generations. In the first of two bone marrow SHE studies conducted on male Chinese hamsters (Li et al., 1987c), 0.1 to 130 mg NaF/kg was administered as a single dose by gavage. The 130 mg/kg dose was toxic, and cell cycle inhibition was seen at doses of 60 mg/kg and higher. In the second study (Li et al., 1989), 1 to 75 ppm NaF was administered in drinking water for 21 weeks, with no apparent effect on cell proliferation kinetics. Li and coworkers found no significant increases in bone marrow SCE in either study, although fluoride concentrations in the rats' bone and plasma increased with the amount of sodium fluoride they received.

Micronuclei were not induced in the bone marrow of mice injected (i.p.) with 30 mg NaF/kg or 80 mg Na-MFP/kg in a single treatment (Hayashi et al., 1988), or with two injections of 39.5 mg SnF_2/kg (Gocke et al., 1981), or with 144 mg Na-MFP/kg (Gocke et al., 1981). When Li and coworkers (1987b) administered sodium fluoride by gavage to male and female mice in doses ranging from 0.1 mg/kg to 0.5 maximum tolerated dose (MTD) (40 and 57.5 mg/kg, respectively), and the MTD (80 and 115 mg/kg, respectively), the mice did not have increased micronucleated polychromatic erythrocytes in their bone marrow. In bone marrow the polychromatic erythrocytes of mice that received two i.p. injections of 10 to 40 mg NaF/kg increased (Pati and Bhunya, 1987). No micronuclei were induced in the bone marrow of rats that received 500 or 1,000 mg NaF/kg by gavage (Albanese, 1987).

IN VIVO TEST SYSTEMS -- GERM CELLS

Drosophila

NaF, SnF_2, and HF produced dose-related increases in sex-linked recessive lethal mutations in postmeiotic germ cells of Drosophila males treated by inhalation (Gerdes, 1971) and feeding (Mitchell and Gerdes, 1973). Chromosome breakage was seen in postmeiotic germ cells of males fed sodium fluoride (Vogel, 1973). Though other researchers have reported that fluoride did not induce sex-linked recessive lethal mutations, most researchers found that the genetic systems they used or the numbers of flies that they treated or examined were judged inadequate.

Rodents

Reports of chromosome aberrations in testicular cells of mice administered sodium fluoride are conflicting. Martin and coworkers (1979) found no aberrations in the testes of mice maintained for "several generations" on 0.5 or 50 ppm F^- in their drinking water, or in those of BALB/c mice administered up to 100 ppm F^- in their drinking water for 6 weeks. Voroshilin and coworkers (1973) found no aberrations in the testes of white mice that inhaled 1.0 mg/M3 HF 6 hours a day, 6 days a week, for 2 months. In contrast, BALB/c mice that were administered up to 200 ppm in their drinking water for 3 or 6 weeks showed dose-related increases in chromosome aberrations in spermatocytes in anaphase and telophase at doses of 1 ppm and more (Mohamed and Chandler, 1982). In this study, the control mice had unusually high frequencies of aberrations. No DNA single-strand breaks were seen in the testicular cells of rats administered up to 84 mg NaF/kg/day for 5 days by gavage (Skare et al., 1986a). Dominant lethal mutations were not induced in male mice that inhaled 1 mg HF/M3 for 2 or 4 weeks (Voroshilin et al., 1975).

HUMAN STUDIES *IN VIVO*

No published studies were found on the genetic or cytogenetic effects of fluoride ion in exposed humans.

PROPOSED MECHANISMS OF GENOTOXICITY

Although the mechanism(s) by which these cellular effects result from exposure to fluoride is not known, a number of possible mechanisms have been proposed to explain the genetic activity observed. These mechanisms have been based on the observed reactions of fluoride in solution with divalent cations or nucleotides, or the physiological and biochemical responses or nucleotide, or the physiological and biochemical responses of cells treated with fluoride. Sodium fluoride inhibits both protein and DNA synthesis in cultured mammalian cells. The inhibition of DNA synthesis may be a secondary effect of the inhibition of protein synthesis, or a result of the direct inhibition of DNA polymerase. Fluoride can react with divalent cations in the cell so as to affect enzyme activities that are necessary for DNA or RNA synthesis, or chromosome metabolism or maintenance; it may react directly with DNA as part of a complex; or it can disrupt other cellular processes such as cell differentiation or energy metabolism (Edwards and Parry, 1986; Harper et al., 1974; Hellung-Larsen and Klenow, 1969; Holland, 1979a,b; Imari et al., 1983; Srivastava, et al., 1981).

A hypothesis of indirect or 'secondary' effects on DNA or chromosomes is attractive in light of the fact that there is no apparent mechanism by which many of the genotoxic effects observed can be induced by direct interaction of fluoride with DNA.

SUMMARY

A number of conclusions can be drawn concerning the genetic toxicity of fluoride in vitro and in vivo, despite the apparently contradictory reports appearing in the published literature. Fluoride is not

mutagenic in bacteria (Ames test) but does induce gene mutations in some cultured rodent and human cells. Fluoride can also transform rodent cells *in vitro*. Although there is disagreement in the literature concerning the ability of fluoride to be a clastogen (induce chromosome aberrations) in cultured cells, it has been suggested that fluoride can cause chromosome aberrations in cultured rodent and human cells. It induced primarily chromatid gaps and chromatid breaks, indicating that the cells are most responsive in the G2 stage of the cell cycle, i.e., after chromosome duplication in preparation for cell division. Negative results reported in some cytogenic studies are likely the effect of an inadequate test protocol.

The disagreements among the *in vivo* tests for chromosome damage in rodents can not yet be reconciled. There are a few reports of positive results for chromosome aberrations in rodent bone arrow and testes, but other studies, using similar protocols and dose ranges, have reported no induced chromosome damage. In the majority of studies reporting positive results, the background frequencies of chromosome damage were significantly higher than the accepted levels, or the investigators reported categories of chromosome damage that may be associated with procedural artifacts. Other reports have presented insufficient information to allow adequate evaluations. Therefore, at this time, the *in vivo* clastogenicity of fluoride should be considered unresolved.

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