820-R-10-017

Fluoride: Dose-Response Analysis For Non-cancer Effects

Fluoride-Related Skeletal Effects: Evaluations of Key Studies

Health and Ecological Criteria Division
Office of Water

January, 2008

U.S. Environmental Protection Agency
Washington, D.C.

TABLE OF CONTENTS

ACKNOWLEDGMENTS 3
INTRODUCTION 4
STUDY SUMMARIES 5

ACKNOWLEDGMENTS

This document was prepared by Oak Ridge National Laboratory, Oak Ridge, Tennessee, under work assignment 2006-014, under the U.S. EPA IAG Number DW-89-9220971. The Principal EPA Scientist is Joyce M. Donohue, Ph.D., Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

The summaries included in this report were prepared by C. Wood, S. Milanez, D. Glass, S. Garcia, S. Goldhaber, and V. Dobozy of Oak Ridge National Laboratory (ORNL). Summary reviewers included J.M. Donohue and T. Duke of the Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U.S. EPA; and D. Glass, D. Opresko and A. Watson of ORNL.

The Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

INTRODUCTION

Prior to initiating the dose-response analysis for fluoride-related skeletal effects, the Office of Water (OW) critically evaluated the studies that had been cited and utilized by the National Research Council (NRC, 2006) in their report *Fluoride in Drinking Water: A Scientific Review of EPA's Standards*. Additional studies identified in the OW initial literature search (2006) were also evaluated. This document is a compilation of the study evaluations arranged alphabetically by the name of the lead author. Critical information fields examined and summarized include endpoint studied, type of study and population studied, exposure period and assessment, characterization of study groups, analytical methods and study design, parameters monitored, statistical methods employed, results (including critical tables and figures) authors' conclusions, critical references and definitions, profiler's appraisal, and critical review of the profiler's assessment. Studies of fluoride-related skeletal effects identified and added to the dose-response analysis for the non-cancer effects document after its external peer review were not evaluated in this fashion.

STUDY SUMMARIES

Fluoride-Related Skeletal Effects: Evaluations of Key Studies

Bharati, P., A. Kubakaddi, M. Rao and R.K. Naik. 2005. Clinical symptoms of dental and skeletal fluorosis in Gadag and Bagalkot districts of Karnataka. J. Hum. Ecol., 18(2): 105-107.

ENDPOINT STUDIED:	Dental and skeletal fluorosis
TYPE OF STUDY:	Case control
POPULATION STUDIED:	India/ 6 villages in Gadag and 2 villages of Bagalkot District: 532 male and female subjects surveyed from 6 villages in the Mundargi taluk (Gadag district) and 300 male and female subjects surveyed from 2 villages in the Hungund taluk (Bagalkot district). Ten percent of the households from each village were chosen for the study with at least one member of the household exhibiting fluorosis. All members of the households chosen were part of the study sample. PROFILER'S NOTE: The ages or range of ages of the participants were not included in the study report.
CONTROL POPULATION:	None described
EXPOSURE PERIOD:	Not described. PROFILER'S NOTE: The profiler assumes since all members of the household were included in the study that some of the participants (i.e. parents) had received long-term exposures to the fluoride levels.
EXPOSURE GROUPS:	Only fluoride levels in drinking water were provided. Water in the Mundargi taluk ranged from 4.0 to 10.5 ppm (Bharati and Meera Rao, 2001; Bharati, 1996) and water in the Hungund taluk ranged from 2.04 to 3.2 ppm (Kubakaddi, 2001). PROFILER'S NOTE: The applicability of this study for use in developing United States' guidelines is limited as the values of fluoride exposure are much higher than those found typically in the U.S. drinking water supply.
EXPOSURE ASSESSMENT:	Participants were only assessed for the exposure to fluoride through the drinking water.
ANALYTICAL METHODS:	Analytical methods were not described. Only ranges for the fluoride level in the water were provided; no other water parameters were measured.
STUDY DESIGN	The study was conducted in 6 villages of Mundargi taluk (Gadag district) and 2 villages of Hungund taluk (Bagalkot district) in India that historically had fluoride levels ranging from 2.04 to 10.5 ppm fluoride. In each village, 10% of the households were selected with the criteria for selection being that one person in the family was affected with fluorosis. A checklist was developed using available literature and consultation with a nutritionist to record the clinical symptoms of fluorosis. The symptoms were recorded by personally interviewing each individual in the families chosen and by observations with the help of local doctors. The symptoms were then tabulated and percentages calculated.
PARAMETERS MONITORED:	No parameters used for scoring either the dental or skeletal fluorosis were described. The dental fluorosis was observed by examination (see Table 1) and the skeletal fluorosis by clinical symptoms described by the participants (see Table 2).
STATISTICAL METHODS:	No statistical methods were described.
RESULTS:	
Dental fluorosis	Table 1 below is copied directly from Bharati et al. (2005). In Mundargi taluk, out of 532 participants, 328 (61.65%) had either dental fluorosis (25%), skeletal fluorosis (5.45%) or

	both (31.20%). Among the 300 participants of Hungund taluk, 194 (64.67%) had either dental fluorosis (35%), skeletal fluorosis (17%) or both (12.67%). In the Mundargi taluk, browning of the teeth was the most common symptom of dental fluorosis followed by pain and pus in teeth. Ninety five subjects had pitting and swelling and 86 participants had lost their teeth. In Hungund taluk, lack of luster was the most common symptom followed by browning of teeth with about 6 participants having lost their teeth. Overall, dental fluorosis was more severe in Mundargi. **Table 1: Symptoms of dental fluorosis among the fluorotic subjects from Mundargi and Hungund taluk** *Symptoms* — *Number of patients/cases* *Male* — *Mundargi* / *Hungund*; *Female* — *Mundargi* / *Hungund*; *Total* — *Mundargi* / *Hungund* Lack of luster 68(37.36) 72(77.42) 43(29.45) 88(87.13) 111(33.84) 160(82.47) White patches 4(2.20) 42(45.16) 1(0.69) 54(53.47) 5(1.52) 96(49.49) Browning of teeth 117(64.29) 51(54.84) 85(58.22) 55(54.46) 202(61.58) 106(54.64) Pitting and swelling 59(32.42) - 36(38.71) - 95(28.96) - Browning with pain - 3(3.23) - 4(3.96) - 7(3.61) Browning with pain and pus 107(58.79) 1(1.07) 66(45.21) 2(1.98) 173(52.74) 3(1.55) Itching and loose teeth 2(1.10) - 4(2.74) 2(1.98) 6(1.83) 2(1.03) Loss of teeth 41(22.53) - 45(30.82) 6(5.94) 86(26.22) 6(3.09) Figures in parenthesis indicate percentages '–' Indicates none of the subjects suffered from that symptom PROFILER'S NOTE: The profiler agrees that the number of more severe findings were observed in the higher fluoride area, Mundargi taluk; however, if the authors had provided the data based on age groups and length of exposure, more useful information for establishing a dose response would have been available for evaluation. Also, more details in how the authors determined signs and symptoms are needed.
Skeletal fluorosis	Table 2 below is copied directly from Bharati et al. (2005). For skeletal fluorosis, tingling and numbness of extremities, back pain and bending were observed in a high number of females in both areas. Males in both areas had more joint and knee pain. A higher percentage of females were unable to walk properly or do normal work compared to males in Hungund but the opposite was true in Mundargi taluk. Overall, skeletal fluorosis was more severe in Mundargi taluk (the high-F communities). **Table 2: Symptoms of skeletal fluorosis among fluorotic subjects from Mundargi and Hungund taluk** *Symptoms* — *Number of patients/cases* *Male* — *Mundaragi* / *Hungund*; *Female* — *Mundaragi* / *Hungund*; *Total* — *Mundaragi* / *Hungund* Tingling and numbing of extremities 21(11.53) 9(9.68) 28(19.18) 11(10.89) 49(14.94) 20(10.31) Joint pain 58(31.87) 21(22.58) 39(26.71) 30(29.70) 97(29.57) 51(26.29) Back pain 58(31.87) 9(9.68) 96(65.75) 24(23.76) 154(46.95) 33(17.01) Knee pain 74(40.66) 29(31.18) 57(39.04) 39(38.61) 131(39.94) 68(35.05) Shoulder pain 5(2.75) 2 (2.16) 15(10.27) 2(1.98) 20(6.10) 4(2.06) Neck pain 6(3.30) - 12(8.22) - 18(5.49) - Pain in limbs 8(4.40) 2(2.16) 1(0.69) 5(4.95) 9(2.74) 6(3.09) Stiff limbs 21(11.54) 1(1.08) 7(4.80) 1(0.99) 28(8.54) 2(1.04) Stiff vertebral column 22(12.09) - 23(15.75) - 45(13.72) - Bent/kyphosis 5(2.75) 1(1.08) 21(14.38) 2(1.98) 26(7.93) 3(1.55) Unable to walk properly 15(8.24) 2(2.16) 10(6.85) 7(6.93) 25(7.62) 9(4.64) Bowed legs - - 4(2.74) 4(1.22) - Can't do normal work 2(1.10) - 1(0.68) 3(2.97) 3(0.92) 3(1.55) Difficult to sit in squatting position - 1(1.08) - - - 1(0.52) Knots on legs 2(1.10) - - - 2(0.61) - Can't cross legs 1(0.55) - - - 1(0.31) - Can't fold hands 1(0.55) - 1(0.68) - 2(0.61) - Can't getup when sits 9(4.95) - 8(5.48) - 17(5.18) - Figures in parenthesis indicate percentages '–' Indicates none of the subjects suffered from that symptom PROFILER'S NOTE: The profiler agrees that the number of more severe findings were observed in the higher fluoride area, Mundargi taluk; however, giving the data based on age groups and length of exposure would have provided more useful information in establishing

		a dose response. Also, more details in how the symptoms were determined are needed.
STUDY AUTHORS' CONCLUSIONS:		The people of Mundargi and Hungund taluk consuming water containing more than 2 ppm of fluoride were suffering from both dental and skeletal fluorosis. Major symptoms of dental fluorosis included lack of luster, browning, pain, pus and untimely loss of teeth. Skeletal fluorotic symptoms observed included tingling and numbing of extremities, pain in joints and knee, bending, stiff limbs, stiff vertebral column and unable to carry out the routine duties. Preventative measures in these villages in the form of a supply of safe drinking water and/or defluoridation of water is needed.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)		Bharati, P. 1996. Nutritional status and occurrence of fluorosis in selected villages of Mundargi Taluk in Dharwad District. PhD. Thesis, University of Agricultural Sciences, Dharwad. Bharati, P. and Meera Rao. 2001. Epidemiology of fluorosis in Dharwad district. Journal of Human Ecology. 14 (1): 37-42. Kubakaddi, A.B. 2001. Epidemiology of fluorosis and educational intervention in Hungund Taluk. M.H. Sc. Thesis, University of Agricultural Sciences, Dharwad. PROFILER'S NOTE: The two references that are thesis publication are not likely to be retrieved.
PROFILER'S REMARKS	***Initials/date DFG/1-07***	The study severely lacked details that could have been used for developing a dose response. The ages of the participants including their length of exposure to the fluoride, actual fluoride levels measured in the water (including analysis techniques), details on other sources of fluoride, using a widely-accepted method for measuring the degree of fluorosis and applying statistical techniques to the data were either not performed or not provided. Application of the findings of this report to exposure conditions in the United States is limited, as the levels of F concentration in US domestic drinking water are usually much lower. Despite the incomplete documentation and limited application of these findings to the US domestic drinking water debate, this paper adds background information to the limited dataset on skeletal fluorosis. No other sources of F, such as food or tea, etc., were reported in Bharati et al (2005). Focus of the study was on documenting the clinical signs of fluorosis. Water fluoride levels for the individual households were not reported, and no evaluation was made of confounding factors. Although the data did show that the community with lower fluoride levels had fewer cases of severe fluorosis, the data are insufficient for a dose-response analysis. Further, the populations studied are not comparable (regarding dental hygiene and diet) to North American domestic water consumers.
PROFILER'S ESTIM. NOEL/NOAEL		The study is not suitable for developing a NOAEL for fluorosis.
PROFILER'S ESTIM. LOEL/ LOAEL		The study is not suitable for developing a LOAEL for fluorosis.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable ( ), Poor (X), Medium ( ), Strong ( ) PROFILER'S NOTE: This study supports the hypothesis that the incidence of decayed and missing teeth is increased when dental fluorosis is severe, especially in areas where access to dental care is poor. There is a dramatic difference between the two populations for decay and other severe dental problems. Although this study lacks details and is incomplete, the results could possibly be combined

	with more robust studies for weight-of-evidence that participants exposed to ≥2 ppm showed signs of dental and skeletal fluorosis, noting that a key piece of information missing was length of exposure.
CRITICAL EFFECT(S):	Dental and skeletal fluorosis

Cao, J., Y. Zhao, J. Liu, R. Xirao, S. Danzeng, D. Daji and Y. Yan. 2003. Brick tea fluoride as a main source of adult fluorosis. Food and Chemical Toxicology, 41: 535-542.

ENDPOINT STUDIED:	Stage 1, 2 and 3 skeletal fluorosis
TYPE OF STUDY:	Cross-sectional survey
POPULATION STUDIED:	Tibet/Naqu County (Northern Tibet plateau): 111 (53 males and 58 females), 30-78 year old adults residing in either one Tibetan Buddhism temple, one nursing home, or two pastoral villages.
CONTROL POPULATION:	none
EXPOSURE PERIOD:	Actual duration that the population was exposed was not included; however, the study stated that the risk of developing early signs of skeletal fluorosis is associated with a fluoride intake of >10 mg/day for > 10 years (Sub-committee on the 10th Edition of the RDAs Food and Nutrition Board Commission on Life Science, National Research Council 1989) and the average estimated intake for the adults in the study was 12 mg/person/day.
EXPOSURE GROUPS:	The 111 participants came from four groups with similar food habits in Naqu County, Tibet. They were either Lamas from a Buddhist temple, elders in a nursing home or herdsmen from two different pastoral villages. The use of brick tea is very prevalent in the area and is an important part of the diet. (Brick tea is a densely compressed block of tea leaves, which is then shaved into hot water and steeped as a beverage, or used as a broth for cooked or parched grains such as barley).
EXPOSURE ASSESSMENT:	The main source of fluoride exposure in the area is through the consumption of brick tea. Tables 1 and 2 are copied directly from Cao et al. (2003) and indicate all factors that are possible contributors to fluoride exposure in the area. Local water supplies and industrial air pollution (nonexistent in this part of Tibet) were eliminated as significant sources of ingested fluoride.

Table 1
Environmental fluoride level in Naqu County (mg/kg or mg/l)

Samples	N	$\chi^2 \pm S$
Drinking water	15	0.10±0.03
Soil	5	0.32±0.11
Cow dung	5	0.12±0.07
Barley flour	5	0.21±0.09
Wheat flour	5	0.38±0.13
Beef and mutton	5	0.07±0.01
Brick tea	4	739±27
Zamba	4	4.5±0.74
Buttered tea	4	3.2±0.65

Table 2
Total daily fluoride intake of adults in Naqu County (mg/person/day)

	Buttered tea	Flour	Beef and mutton	Zamba	Total
F intake Constitutive %	8.03 67	3.84 32	0.10 0.8	0.02 0.2	11.99 100

PROFILER'S NOTE: Both buttered tea and zamba (a parched barley food staple) use

	brick tea in their preparation.
ANALYTICAL METHODS:	The fluoride levels for water, brick tea, brick tea-water, soil, fuel, food and some urine samples were determined with or without pre-treatment by using a fluoride-ion selective electrode method. This method was described by the Chinese Public Health Ministry, "The Manual of Preventing Endemic Fluorosis" (Dept. of Endemic Disease Prevention 1991; Cowell 1977; Itai and Tsunoda 2001)
STUDY DESIGN:	The study was conducted to examine the prevalence of skeletal fluorosis in adults living in the Naqu County area of Tibet where, earlier, Cao et al. (2000) identified dental fluorosis in children. One hundred eleven adults, $\geq$ 30 years old, from four different sites were selected by a randomized sampling method. The level of fluoride in the water, fuel, soil, food, brick tea, brick tea-water and urine were determined. For the fluoride level in food, daily food intake was measured for 3 consecutive days; from these data, a mean daily total fluoride intake was calculated. Physical examinations evaluating for skeletal fluorosis using a standardized set of non-invasive physical signs commonly associated with the disease (e.g., fingers could not touch the shoulder, middle finger could not touch the contralateral ear, etc.) were performed with subjects, and individual radiographs were performed on those that presented with 3 or more physical signs.
PARAMETERS MONITORED:	Environmental sources were measured for fluoride concentrations and included, water, fuel (cow dung), soil, and food. Participants were evaluated for skeletal fluorosis on physical examination for the presence of the following physical limitations according to the Standard of Endemic Fluorosis (Cao, SR 1992): (i) fingers could not touch the shoulder because of restricted elbow flexing; (ii) hands up could not reach 180°; (iii) the middle finger could not reach the contralateral ear; (iv), the thumb could not reach the lower angle of the contralateral scapular; (v) the heels were raised when squatting; (vi) the patient essentially could not squat; (vii) 45–90° kyphosis; (viii) restricted flexing and/or hands up of extremities; (ix) paralysis. Forty two of the 99 patients demonstrating more than three of the physical signs were also radiographed to confirm diagnosis, and some of the 42 also had their urine tested for fluoride concentration. Radiographs were performed according to the recommendations in the Chinese National Standard "Criteria of Skeletal Fluorosis X-Ray Diagnosis" (Public Health Ministry 1976).
STATISTICAL METHODS:	The student's t-test was applied for inter-group comparison.
RESULTS:	
Skeletal fluorosis	Tables 4, 5 and 6 are copied directly from Cao et al. (2003) to indicate the prevalence, age range of those affected, severity of skeletal fluorosis, and the radiographic findings. The tables indicate more prevalence and severity of signs as the adults became older, and that 83% of all the participants had some form of skeletal fluorosis. The most common radiographic change was trabecular changes to the interosseous membrane which occurred in 86% of those diagnosed with skeletal fluorosis.

Table 4
Age distribution for signs of intense skeletal fluorosis

Age groups (years)	Cases with three to nine items of positive signs								Total
	Sex	3 Items	4 Items	5 Items	6 Items	7 Items	8 Items	9 Items	
30–39	M	3	1	0	0	0	0	0	4
	F	3	0	0	0	0	0	0	3
40–49	M	3	3	1	1	0	0	0	8
	F	3	1	1	0	0	0	0	5
50–59	M	4	3	3	2	0	0	0	12
	F	3	5	3	3	0	0	0	14
60–78	M	3	3	6	4	3	2	2	23
	F	6	5	8	6	2	2	1	30

Table 5
Stages of the 35 cases of radiographically diagnosed skeletal fluorosis

	Stage I (early)	Stage II (advanced)	Stage III (late)	Total
Cases	3	13	19	35
Constitutive %	7%	31%	45%	83%

Table 6
Main radiographic features and typing of 35 cases of brick tea-type skeletal fluorosis

	Trabecular changes Interosseous membrane	Ossification and tendon attachment calcification	Articular degeneration	Thick bone syndrome	Increasing (sclerosis type)	Decreasing (porosis type)	Alternating (mixed type)	Total
Cases	30	22	11	12	26	3	6	35
Constitutive %	86%	63%	31%	34%	74%	9%	17%	100%

PROFILER'S NOTE: The prevalence of the skeletal fluorosis with more than 3 positive signs increased with age and was most prominent in those aged 60-78. The radiographic findings correlated well with the physical findings indicating physical signs were a good diagnostic (and non-invasive) tool for screening patients.

STUDY AUTHORS' CONCLUSIONS:

In Naqu County, Tibet, the total daily fluoride intake in adults was estimated to equal 12 mg, with 99% coming from brick-tea containing foods. The occasional urinary fluoride level was 5.73 mg/L and the incidence of adult skeletal fluorosis among subjects examined was 89% by physical examination and 83% by radiographic diagnosis.

DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)

Cao, J, Y. Zhao, and J.W. Liu. 2000. Fluoride in the environment and brick-tea-type fluorosis in Tibet. Journal of Fluoride Chemistry, 106, 93-97.

Cao, S.R. 1992. Standards of endemic fluorosis. Fluoride Research Letters, 7 (1), 29-36.

Cowell, D.C. 1977. Automated fluoride ion determination. Determination of urine fluoride ion levels. Annals of Clinical Biochemistry. 14(5), 269—274.

Department of Endemic Disease Prevention. 1991. Chinese Health Ministry. The manual of preventing endemic fluorosis. Chinese Endemic Disease Preventative Research Center, Harbin.

Itai, K. and H. Tsunoda. 2001. Highly sensitive and rapid method for determination of fluoride ion concentrations in serum and urine using flow injection analysis with a fluoride ion-selective electrode. Clinica Chimica Acta, 308 (1-2), 163-171.

National Research Council. 1989. RDAs: Fluoride subcommittee on the tenth edition of the RDAs food and nutrition board commission on life sciences. Washington D.C., pp. 235-240.

Public Health Ministry, China. 1976. Criteria for bone fluorosis, x-ray diagnosis (GB16397-1996). Chinese Standards Press, Beijing. p. 6.

PROFILER'S REMARKS ***Initials/date DFG 12/15/2006***

The profiler felt the study design was adequate. Based on previous evidence of pediatric dental fluorosis in the region (Cao et al. 2000), the area was revisited to assess skeletal fluorosis in adults. The study used standardized methods for evaluating physical signs, and also relied on two methods of diagnosis (physical limitations and radiographs). The study showed a positive correlation between adult exposures to an estimated 12 mg F/da and adverse skeletal fluorosis. The study helped provide a LOAEL but could not be used for a dose-response study by itself, because only one exposure concentration was identified.

PROFILER'S ESTIM. NOEL/NOAEL	The NOAEL could not be determined in this study.
PROFILER'S ESTIM. LOEL/ LOAEL	It is unclear where the actual LOAEL occurs, as only a single estimate of adult daily ingestion was calculated (e.g., 12 mg F/person/day); no distribution or range of intake was presented by the authors. Adverse skeletal fluorosis findings were identified at 12 mg F/person/day; this estimation is limited by the short-term duration of observation (3 consecutive days of food intake).
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:	Not suitable ( ), Poor (X), Medium (), Strong ( ) Although the study does not use the correlation of fluorosis with a source applicable to the U.S. population, it still provides a thorough study indicating that long-term exposure to fluoride concentrations approximating of 12 mg F/person/da is associated with advanced to late-stages skeletal fluorosis. The study is also a good example of evaluating all sources of possible fluoride intake as the drinking water alone did not possess fluoride concentrations sufficient to cause skeletal fluorosis.
CRITICAL EFFECT(S):	Skeletal fluorosis graded II (advanced) or greater.

Goldman SM, Sievers ML, Templin DW. 1971. Radiculomyopathy in a southwestern Indian due to skeletal fluorosis. Ariz Med. 28(9):675-7.

ENDPOINT STUDIED:	Skeletal fluorosis, fluorotic radiculomyopathy
TYPE OF STUDY:	Case report
POPULATION STUDIED:	Arizona, Gila Bend; 55-year old Papago Indian male admitted to the Phoenix Indian Medical Center on May 2, 1969, for evaluation of possible pulmonary tuberculosis. Long standing severe weakness of both legs was attributed to trauma from an accident 10 years earlier.
CONTROL POPULATION:	Not applicable in case report.
EXPOSURE PERIOD:	Lifetime (55 years)
EXPOSURE GROUPS:	Samples of the water from the patient's drinking source were evaluated by the Arizona State Health Department and found to contain 5.2 to 7.8 ppm fluoride. A random sample of this water, analyzed by two methods by Dr. Leon Singer of the University of Minnesota at the time of the tooth analysis revealed a fluoride content of 4.04 ppm and 4.27 ppm.
EXPOSURE ASSESSMENT:	Subject had a history of drinking large quantities of water with a fluoride concentration 4-8 ppm; he also drank hot tea. Two additional practices further elevated the high water fluoride concentration: boiling water for hot tea, and keeping his drinking water supply in open containers for several days, permitting evaporation in the low humidity climate in Arizona.
ANALYTICAL METHODS:	Method used by Arizona State Health Department to measure fluoride water levels not reported. Two methods were used by Singer; ion electrode method and diffusion isolation with colorimetric analysis.
STUDY DESIGN	A 55-year old Papago Indian male from Arizona was admitted to the Phoenix Indian Medical Center on May 2, 1969 for evaluation of possible pulmonary tuberculosis. He had a lifetime history of drinking large quantities of water with a high fluoride concentration (from 4 to 8 ppm); he also drank hot tea. He was examined and x-rays were taken to determine bone density of the spine, ribs, and pelvis. Laboratory tests included VDRL, hemoglobin, hematocrit, serum calcium, phosphorus, alkaline phosphatase and acid phosphatase. Chemical analysis for fluoride was determined in an extracted tooth. A diagnosis of fluorotic radiculomyopathy was made.
PARAMETERS MONITORED:	The physical examination assessed muscle tone, range of motion, and neurological abnormalities. X-rays were taken to evaluate bone density, evidence of fractures, and sagittal diameters of the cervical and lumbar spine. Laboratory tests included VDRL for syphilis, hemoglobin, hematocrit, serum calcium, phosphorus, alkaline phosphatase and acid phosphatase. Chemical analysis for fluoride was determined in an extracted tooth.
STATISTICAL METHODS:	Statistical analysis was not performed on this one patient.
RESULTS:	
Physical Examination	The subject had bilateral flexion contractures of both knees and elbows. The range of knee motion was 80-95° on the right and 80-140° on the left. Limitation of abduction and rotation of the shoulders was noted. The neck and spine were completely rigid. Muscle tone was normal, but sensation to light touch and pin prick was decreased over the dorsum of the right foot. He was unable to stand without assistance. Laboratory tests revealed nonreactive VDRL, and normal hemoglobin, hematocrit, and serum calcium, phosphorus, alkaline phosphatase, and acid phosphatase.

Skeletal fluorosis	X-ray examination revealed generalized increased bone density of the spine, ribs, and pelvis, suggestive of skeletal fluorosis, and accompanying osteophytosis. No evidence of previous vertebral fracture was noted. The sagittal diameters of both the cervical and lumbar spine were below the 90% tolerance levels, as summarized in Tables 1 and 2 copied directly from Goldman et al. (1971). **TABLE 1** **Sagittal Diameter of Cervical Spinal Canal** 90% minimal tolerance limits for sagittal diameter (mm) (according to Hinck[4]) Lowest normal limits (mm) (according to Wholey[3]) Present patient's measurements (mm) C-1: 16.8; 16; 15 C-2: 16.1; 16; 12 C-3: 14.3; 16; 10 C-4: 14.1; 14; 9.5 C-5: 13.9; 14; 11 **TABLE 2** **Sagittal Diameter of the Lumbar Spine** 90% tolerance limits for sagittal diameter (mm) (according to Hinck[4]) Present patient's measurements (mm) L-1: 16; 14 L-2: 16; 12 L-3: 17; 13.5 L-4: 17; 13 L-5: 16; 12
Dental fluoride content	Chemical analysis of the extracted tooth revealed the following fluoride levels: bulk canal, 614 ppm; a calculus from the crown, 4838 ppm; a supragingival calculus, 5299 ppm. Tooth analysis confirmed fluorosis. PROFILER'S NOTE: Normal tooth values for fluoride were not presented for comparison.
STUDY AUTHORS' CONCLUSIONS:	The patient was exposed to prolonged (55 years) excessive fluoride in drinking water (4-8 ppm). He presented with neurological deficits and severe weakness in both legs. Fluorosis was confirmed in an extracted tooth in which fluoride content ranged from 614 to 5299 ppm, depending on the part of the tooth. Syphilis was ruled out by the VDRL test. The characteristic vertebral changes of skeletal fluorosis and severe osteophytosis were probably the basis for the patient's neurological deficits. Although trauma may have precipitated his radioculomyopathy, the neurological symptoms are adequately explained by the marked narrowing of the sagittal diameter of the cervical and lumbar spinal cord and the vertebral osteophytosis secondary to fluorosis. Neurological deficits occurred as a manifestation of spinal cord and nerve root bony compression. Skeletal fluorosis occurs in only a small percentage of those with prolonged ingestion of water with excessively high fluoride content, and radiculomyopathy is rare among those who develop skeletal fluorosis. This case is of regional importance since fluorosis is endemic to Arizona. The authors stress that water fluoridation programs (at 1 ppm) have no potential for causing skeletal or neurological complications as reported in this case due to the low fluoride concentrations.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)	VDRL: A blood test for syphilis (VDRL stands for Venereal Disease Research Laboratory) Hinck VC, Hopkins CE, Savara BS. 1962. Sagittal diameter of the cervical spinal canal in children. Radiology. 79: 97-108. Wholey MH, Bruwer AJ, Baker HL Jr. 1958. The lateral roentgenogram of the neck. Radiology. 71(3): 350-6.
PROFILER'S REMARKS ***Initials/date SJG/ 10/26/07***	The study design does not aid in the development of a dose response to fluoride with respect to skeletal fluorosis. The objective of the study was to report the second documented case (as of 1971) of fluorotic radiculomyopathy in a single patient with prolonged ingestion of water with a fluoride concentration of 4-8 ppm. The patient's symptoms and neurological deficits are presented well and x-

		ray examination provides evidence of narrowing of the sagittal diameter of the cervical spinal canal and lumbar spine below the tolerance limits. Although the case is interesting and novel, it has some limitations. Laboratory tests did not support or contradict the diagnosis of fluorotic radiculomyopathy, except to rule out syphilis (VDRL test). The authors do not explain the rationale behind the chosen laboratory tests, nor do they present normal values for fluoride in teeth for comparison. Because the diagnosis is so novel, it would have been more compelling if other potential diagnoses were presented for consideration and then ruled out based on the presented evidence.
PROFILER'S ESTIM. NOEL/NOAEL		Study design was not suitable for development of a NOAEL.
PROFILER'S ESTIM. LOEL/ LOAEL		Study design was not suitable for development of a LOAEL.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable (X), Poor (), Medium (), Strong () The study presented a rare case of fluorotic radiculomyopathy as a progression of skeletal fluorosis in one patient with prolonged exposure (55 years) to fluoride in the drinking water at 4-8 ppm.
CRITICAL EFFECT(S):		Skeletal fluorosis and radiculomyopathy

Kurttio, P., N. Gustavsson, T. Vartiainen, and J. Pekkanen. 1999. Exposure to natural fluoride in well water and hip fracture: a cohort analysis in Finland. Am. J. Epidemiol. 150: 817-824.

ENDPOINT STUDIED:	Skeletal (hip) fracture
TYPE OF STUDY:	Retrospective cohort (based on hospital discharge record linkage)
POPULATION STUDIED:	Finland/Southeast and Southwest: 144,627 persons (66,742 men; 77,885 women) from Finland, born in 1900-1930, who from at least 1967-1980 lived at the same address in a rural village or area where >90% of population did not use municipal water (e.g., private well-water users). Their incidence of hip fracture was recorded for 1/1/81-12/31/1994, and is presented in Table 1 according to gender, age on 1/1/81, occupation, and geographic area of residence. Persons were excluded who had a hip fracture between 1/1/78 and 12/31/80 or whose main diagnosis was not hip fracture, and only the first hip fracture was tabulated.

TABLE 1. Description of the study subjects who had lived at least from 1967 to 1980 outside municipal drinking water sources in Finland

	Hip fractures observed in 1981–1994					
	Men (n = 66,742)			Women (n = 77,885)		
	No hip fractures (n = 65,493)	Hip fracture (n = 1,249)	% of hip fractures (1.9%)	No hip fractures (n = 74,685)	Hip fracture (n =3,200)	% of hip fractures (4.1%)
Age (years) on January 1, 1981						
50–54	13,825	77	0.6	13,065	100	0.8
55–59	14,752	128	0.9	15,590	217	1.4
60–64	11,466	172	1.5	14,018	354	2.5
65–69	10,502	234	2.2	12,908	635	4.9
70–74	8,771	310	3.5	10,676	906	8.5
75–80	6,177	328	5.3	8,428	988	11.7
Occupation						
Administrative, service, commercial	2,596	25	1.0	5,926	130	2.2
Construction, industrial, transportation	13,858	200	1.4	3,361	55	1.6
Farming, forestry, fishery	41,023	699	1.7	37,698	1,200	3.1
Unknown	8,016	325	3.9	27,700	1,815	6.6
Geographic area						
1	4,170	54	1.3	4,382	142	3.2
2	3,802	74	1.9	4,207	182	4.3
3	10,033	188	1.8	10,978	437	4.0
4	6,780	118	1.7	7,569	290	3.8
5	6,984	162	2.3	8,113	372	4.6
6	16,119	323	2.0	19,009	901	4.7
7	13,572	260	1.9	15,770	687	4.4
8	4,033	70	1.7	4,657	189	4.1

REVIEWER'S NOTE: It is noted that the study authors report different Ns for the total population studied in various portions of the paper; the alternate N is 144,512 (p. 817), while an N of 144,627 can be obtained from information provided in Table 1above.

CONTROL POPULATION:	Adult Finnish men and women exposed to ≤ 0.1 mg/L estimated drinking water fluoride concentration were used as the reference group for determining effects of drinking water fluoride on the risk of hip fracture.
EXPOSURE PERIOD:	At least 13 years (from 1967 – 1980). Water fluoride exposure prior to 1967 or after 1980 was unknown.
EXPOSURE GROUPS:	Finns who lived in, and obtained their drinking water from, private wells or springs in 8 defined geographic areas located in southern Finland from 1967-1980. To evaluate the effect of water fluoride concentration on the risk of hip fractures, men and women, separately, were subdivided into 6 groups based on their drinking water fluoride concentration (mg/L): ≤0.10, 0.11-0.30, 0.31-0.50, 0.51-1.00, 1.10-1.50, and >1.50.
EXPOSURE ASSESSMENT:	Water concentrations of F were determined potentiometrically from untreated samples. Individual F exposures were estimated for each cohort member based on their location

	and using a database of groundwater fluoride measurements (8,927 wells) by the Geological Survey of Finland (Lahermo et al 1990). The interpolation method of a moving weighted median was used to smooth the concentrations to obtain a regular grid of estimated fluoride concentrations. The grid was 2 x 2 km^2 and the window radius was 42 km. The nearest accepted grid value for each member of the cohort was found using the quad-tree algorithm. The estimates made by this method were compared to fluoride measurements from a national study in 1990-1991of 1,411 wells that geographically represented all of Finland. Other fluoride sources included food and toothpaste, which were estimated in Finland as 0.6 and 0.08 mg/day, respectively, based on work by other investigators. These extraneous fluoride sources were not accounted for in the data analysis.
ANALYTICAL METHODS:	Water fluoride concentrations were measured potentiometrically (no further description provided).
STUDY DESIGN	The study cohort consisted Finns born in 1900-1930, who from at least 1967-1980 lived in the same rural area. Finland was divided into 8 areas along the longitudes of 2.5°, 25.5°, and 28.5°, and latitudes of 62.2° and 65.0° as a crude adjustment for possible effects of the geographic area of residence. The date of birth, gender, and place of residence, residence history, and occupation in 1970, 1975, and 1980 were obtained for each person from the Population Census of Statistics Finland. Occupation was used as a measure of socioeconomic status, although these data was unavailable for 59% of the subjects. The subjects' incidence of hip fracture was recorded for 1/1/81-12/31/1994, based on the Hospital Discharge Registry (linked with personal identification numbers). Persons were excluded who had a hip fracture between 1/1/78 and 12/31/80 or whose main diagnosis was not hip fracture, and only the first hip fracture was tabulated. The effect of the water fluoride concentration on the risk of hip fractures was analyzed for men and women separately, and for either all ages (50-80) combined, or for 5-year age increments. The statistical methods used are described below ("Statistical Methods").
PARAMETERS MONITORED:	First hip fractures, per data obtained from the Hospital Discharge Registry of Finland.
STATISTICAL METHODS:	Age at the beginning of follow-up (i.e. as of 1/1/81) was the basis for subdividing the cohort into age groups. The number of "person-years" (in Tables 2 and 3) was calculated for the period beginning on 1/1/81 and ending with date of the hip fracture diagnosis, the date of death, or 12/31/94. Cox's regression was used to determine the crude and adjusted (age, area, occupation) rate ratios and confidence intervals (CI). Age was adjusted for as a continuous variable (similar risk estimators obtained if age was class variable) and fluoride concentration was analyzed as both a continuous and stratified variable.
RESULTS:	
Fluoride concentration and hip fractures	The estimates of fluoride concentrations ranged from below 0.05 mg/L (detection limit) to 2.4 mg/L, and most of the subjects lived in areas where water fluoride was estimated as <0.1 mg/L. There was a correlation of 0.71 between the analyzed and estimated well water concentration, and the latter "tended to be 0.7 times less than the measured fluoride concentration in a well." This diluted the effects of the highest fluoride concentrations. Hip fracture incidence clearly increased with age, and was higher in females than males in all age groups. When all ages were combined for each gender, there was no correlation (age or area-adjusted) between the rate ratios (RR) of hip fractures and water fluoride concentration, whether fluoride concentration was treated as a stratified variable (Table 2) or a continuous variable (age-adjusted and age-area-adjusted RRs for men were 0.97 and 0.90, respectively, and for women were 1.07 and 1.10, respectively).

TABLE 2. Rate ratios (RR) and 95% confidence intervals (CI) of hip fractures in the categorized fluoride concentration among the Finnish rural population aged 50–80 years in 1981

Fluoride concentration (mg/liter)	No. of hip fractures in 1981–1994	Person-years in 1981–1994	Incidence/ 1,000 person-years	Crude RR	95% CI	Age- and area-adjusted RR	95% CI
Men							
≤0.10	735	442,192	1.66	1.0		1.0	
0.11–0.30	318	165,736	1.92	1.15	1.01, 1.32	1.05	0.90, 1.22
0.31–0.50	38	26,820	1.42	0.85	0.61, 1.18	0.72	0.51, 1.02
0.51–1.00	108	51,347	2.10	1.26	1.03, 1.54	1.03	0.81, 1.32
1.10–1.50	32	22,753	1.41	0.85	0.60, 1.21	0.67	0.46, 0.97
>1.50	18	9,522	1.89	1.13	0.71, 1.81	0.98	0.61, 1.60
Women							
≤0.10	1,850	554,621	3.34	1.0		1.0	
0.11–0.30	775	219,627	3.53	1.06	0.97, 1.15	0.93	0.84, 1.02
0.31–0.50	142	34,617	4.10	1.23	1.04, 1.46	1.12	0.93, 1.34
0.51–1.00	268	66,448	4.03	1.21	1.06, 1.38	1.12	0.96, 1.31
1.10–1.50	118	30,497	3.87	1.16	0.97, 1.40	1.08	0.88, 1.32
>1.50	47	11,759	4.00	1.20	0.90, 1.60	1.08	0.80, 1.46

Analysis of the subjects stratified by age (six 5-year increments), however, found that the crude and adjusted (age, area) RR for men aged 50-59 were below 1.0, whereas for women aged 50-65 were above 1.0, as shown in Figure 3.

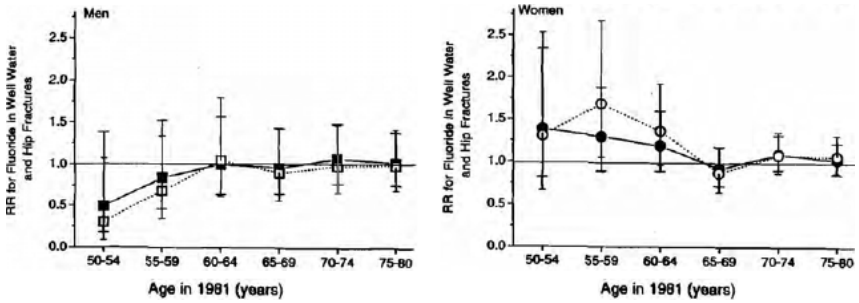


FIGURE 1. Rate ratios and 95 percent confidence intervals of the association of the estimated fluoride concentration in well water (df = 1) and hip fractures in men and women (Cox regression). Age-adjusted (■ and ●) and age- and area-adjusted (□, ○, and the narrower cap of 95 percent confidence intervals)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

Analysis of only subjects aged 50-64 ("younger men and women") suggested that fluoride had a slight, non-significant protective effect against hip fractures in men, but was associated with an increased risk of hip fracture in women. This was seen whether fluoride concentration was treated as a stratified variable (Table 3) or a continuous variable (age-adjusted and age-area-adjusted RRs, respectively, for younger men were 0.85 and 0.75 and for younger women were 1.25 and 1.44). Occupation had no effect on the analysis.

TABLE 3. Rate ratios (RR) and 95% confidence intervals (CI) of hip fractures in the categorized fluoride concentration among Finnish men and women aged 50–65 years ("younger men and women") in 1981

Fluoride concentration (mg/liter)	No. of hip fractures	Person-years in 1981–1994	Incidence/ 1,000 person-years	Crude RR	95% CI	Age- and area-adjusted RR	95% CI
Men							
≤0.1	228	305,816	0.74	1.0		1.0	
0.1–0.3	103	109,615	0.94	1.04	0.99, 1.58	1.20	0.91, 1.56
0.3–0.5	12	17,698	0.68	0.75	0.50, 1.61	0.81	0.44, 1.49
0.5–1.0	21	34,021	0.62	0.82	0.53, 1.29	0.68	0.41, 1.13
1.1–1.5	8	14,409	0.55	0.90	0.37, 1.51	0.60	0.29, 1.25
>1.5	5	6,426	0.78	1.25	0.43, 2.52	0.87	0.35, 2.16
Women							
≤0.1	388	350,847	1.11	1.0		1.0	
0.1–0.3	165	132,505	1.24	1.12	0.94, 1.35	1.16	0.93, 1.43
0.3–0.5	27	20,667	2.66	1.18	0.80, 1.74	1.31	0.86, 1.99
0.5–1.0	57	39,412	1.45	1.31	0.99, 1.73	1.53	1.08, 2.16
1.1–1.5	21	17,875	1.15	1.06	0.68, 1.65	1.24	0.77, 2.01
>1.5	13	6,908	1.88	1.70	0.98, 2.96	2.09	1.16, 3.76

STUDY AUTHORS' CONCLUSIONS:		Kurttio et al. (1999) concluded that fluoride (in the drinking water) had a slight, non-significant protective effect against fractures in men aged 50-64, but was associated with an increased risk of hip fracture in women aged 50-64 as of 1/1/81 (the beginning of the follow-up period). The adjusted RR was 2.09 (95% CI 1.16, 3.76) for women who were exposed to the greatest fluoride concentrations (>1.5 mg/L) as compared to women exposed to the lowest fluoride concentrations (≤ 0.1 mg/L). No correlation was found between fluoride concentration and hip fracture in the older subjects (65-80 years old), possibly due to other more prominent risk factors at higher ages (e.g. age-related changes in calcium absorption, fluoride metabolism, hormonal status, etc.). The weighted median smoothing method of estimating well water fluoride concentrations tended to underestimate the actual fluoride concentrations and diluted the effects at the highest fluoride concentrations, which might have biased the ratio estimates toward the null. Although the effects of possible confounders such as nutrition and physical activity were not addressed, the overall effect of geographic location and occupation were small.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)		Lahermo, P, M Ilmasti, R Juntunen et al. 1990. Geochemical Atlas of Finland, Part 1. Hydrogeochemical survey of Finnish ground waters. (in Finnish). Geological Survey of Finland, Helsinki.
PROFILER'S REMARKS	***Initials/date: SM 1/19/07***	This was a well-conducted study that clearly showed that in the Finnish population, the incidence of hip fracture increased with age, and was particularly higher in younger females (aged 50-65 years) exposed to drinking water >1.5 mg/L than males in all age groups (50-80 years old) or older women. The major confounder was that the subjects, who were up to 80 years old, had to have lived in the same rural location for only 13 years of their life (1967-1980). Other drawbacks were that the water fluoride concentrations were generally underestimated, and that too broad of a concentration range was included in the highest exposure group (i.e. >1.5 mg/L). Information and references provided in Kurttio et al (1999) are pertinent to relative source contribution analysis. Known risk factors such as alcohol consumption/smoking/low body weight were not controlled.
PROFILER'S ESTIM. NOEL/NOAEL		A NOAEL cannot be assigned based on the provided data.
PROFILER'S ESTIM. LOEL/ LOAEL		A LOAEL cannot be assigned based on the provided data. REVIEWER'S NOTE: Nevertheless, the paper points out that drinking water concentrations >1.5 mg F/L are positively associated with an increased relative risk of hip fracture in adult women aged 50-65 (the "younger" cohort). The elevated relative risk for this fluoride water concentration and age class is not statistically significant based on the reported 95% confidence intervals, but is a finding of concern.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable (x), Poor ( ), Medium ( ), Strong ( )
CRITICAL EFFECT(S):		Skeletal (hip) fracture

Leone, N.C., Stevenson, C.A., Hilbish, T.F., Sosman, M.C. 1955. A roentgenologic study of a human population exposed to high-fluoride domestic water: A ten-year study. Am. J. Roentgenol. Radium Ther. Nucl. Med. 74(5):874-85.

ENDPOINT STUDIED:	Bone changes (density; osteoporosis; coarsened trabeculation; hypertrophic change)
TYPE OF STUDY:	Cohort (prospective)
POPULATION STUDIED:	116 white adults, 15 to 63 years old at study initiation (1943), residing for at least 15 years in a high-fluoride area (Bartlett, Texas) where water fluoride level was 8 ppm.
CONTROL POPULATION:	121 white adults, 15 to 63 years old at study initiation (1943), residing for at least 15 years in a low-fluoride area (Cameron, Texas) where water fluoride level was 0.4 ppm.
EXPOSURE PERIOD:	All participants had a minimum of 15 years of residence (exposure) in the respective towns at study initiation in 1943. The average length of exposure was 37 years in the high-fluoride area and 38 years in control area. Follow-up studies were conducted after a 10 year interval, in 1953.
EXPOSURE GROUPS:	In 1943, 237 participants were selected based on minimum residence of 15 years in Cameron or Bartlett, Texas, where naturally occurring fluoride content of the water was 0.4 ppm or 8 ppm, respectively. No other sources of fluoride exposure were considered, besides prolonged fluoride ingestion from drinking water. In 1953, 47 of the original participants moved from the immediate study areas, 22 from Bartlett and 25 from Cameron, predominately of the younger age groups. These 'removed' participants were located and similar roentogenographic studies were made in all but 10 cases.
EXPOSURE ASSESSMENT:	Roentgenograms were made in 1943 and repeated on the same subjects with matching views in 1953. Individual medical histories, physical examinations, and laboratory data were correlated with roentgenograms.
ANALYTICAL METHODS:	The method used for measuring the fluoride concentrations in the water was not reported; no other water quality parameters were measured.
STUDY DESIGN	The primary objective of the study was to present roentogenographic findings of a ten year study of 237 white adults (15 to 63 years old) residing in a high-fluoride area (Bartlett, Texas, 8 ppm F) or in a control area (Cameron, Texas, 0.4 ppm F), and to describe the findings that might be ascribed to prolonged ($\geq$15 years) fluoride ingestion. Roentgenograms were made in 1943 and were repeated on the same individuals with matching views ten years later to enable comparative study of the individuals in each group with themselves and of those in a high-fluoride group with those in a low-fluoride group. The roentogenographic findings were correlated with individual medical histories, physical examinations, and laboratory data to evaluate the presence or absence of any detectable effects in the individual, in the groups, or in the various age categories. Bone fracture histories, arthritis and systemic conditions which might be associated with roentgenographic findings were tabulated. There were no significant differences in these conditions within the study groups. The roentgenograms were made at the Scott and White Clinic, Temple, Texas, under the immediate supervision of the same Chief of Service in 1943 and in 1953. A 500 ma. roentgenographic unit with rotating anode tube was used on both occasions. Emphasis was placed on bone detail. Anteroposterior roentgenograms of the dorsal and lumbar spine and the pelvis, showing the proximal third of each femur, were made for each patient in 1943 and again in 1953. When bone changes were found (e.g., increased

	bone density, coarsened trabeculation, hypertrophic change, ligamentous calcification) a roentogenographic bone survey was made, consisting of the following views: a lateral skull (stereo), cervical spine; left upper arm, forearm and hand; and right femur, lower leg, and foot. These regions represent those in which the earliest or most definite manifestations of fluoride effects might be seen if present. Most of the "removed" participants were transported to the Scott and White Clinic, where they were examined in the same manner and with the same equipment as the other participants. No attempt was made to evaluate the "removed" participants separately because no comparable roentogenographic differences were noted in these persons from either area.
PARAMETERS MONITORED:	Bone density changes refer to increased or decreased density in the presence or absence of coarsened trabeculation. Hypertrophic changes were recorded if they were moderate (2+), severe-limited (3+), or severe-generalized (4+). Some hypertrophic change is a normal finding, especially in aged persons, and was not considered as a possible fluoride effect when change was moderate. Roentgenographic evaluation included a correlation with individual histories and physical examinations.
STATISTICAL METHODS:	Evaluations were made on the basis of age, sex, activity, study area, and elapsed time. Details of data analysis were reported in an earlier paper (Leone et al. 1954) and not included in the current study.
RESULTS:	
Bone changes (*bone density, coarsened trabeculation, osteoporosis*)	Table 1 was copied directly from Leone et al. (1955) and summarizes roentgenographic bone changes in subjects residing in the control (Cameron) and high-fluoride (Bartlett) areas over a ten-year interval. A limited number of subjects from both areas showed some degree of bone change, but these changes were minimal. High concentrations of fluoride in the drinking water did not uniformly produce detectable bone changes. Only one new case of increased bone density was found in the high-fluoride area at the end of the ten-year period. Of the original Bartlett cohort, 16 exhibited bone changes in varying degrees in 1943; in 1953, 9 of the 16 showed no further bone change, 4 showed an increase in bone density, and 3 a decrease in density toward a "normal" appearance. One new case of increased bone density was recorded. Of the cases designated as 'increased density' only 2 could be considered frank abnormalities. In Cameron (control area), there were 4 cases of increased density, 2 cases of increased coarsened trabeculation, and 8 new cases of osteoporosis during the ten year period, as compared with 1 new case of osteoporosis in Bartlett. During the 10-year interval, 4 Bartlett participants showed increased coarsened trabeculation without increased bone density. Increased bone density occurred predominately in persons over the age of 45. Decreased bone density in those who showed an increased density in 1943 is of interest as a transition from a dense bone structure to less dense appearing bone ten years later.

TABLE I

BARTLETT-CAMERON FLUORIDE STUDY

Roentgenographic Bone Changes in Participants Studied in both 1943 and 1953

Abnormality	Bartlett Number Studied: 89		Cameron Number Studied: 101	
	1943	1953	1943	1953
Bone density changes*	16	17	4	4
Increased		14†		3
Decreased‡		3		1
Osteoporosis	4	5	2	10
Coarsened trabeculation (without increased bone density)	12	14	2	3
Hypertrophic change§	3	3	1	1

* Increased or decreased bone density with or without coarsened trabeculation, excluding osteoporotic change.

† Includes one new case of increased bone density in 1953.

‡ Decreased bone density, but not osteoporosis.

§ Only cases with "severe-limited" (3+) and "severe-generalized" (4+) are included.

PROFILER'S NOTE: Results reported in the text do not appear to be clearly tabulated in Table 1. "*In Cameron, there were 4 cases of increased density, 2 cases of increased coarsened trabeculation, and 8 new cases of osteoporosis during the ten year period*." It is unclear whether these values are for 1943 or 1953; there were 4 cases of bone density changes in 1943 with 3 increased cases and 1 decreased case in 1953; similarly, there were 2 cases of coarsened trabeculation in 1943 and 3 cases in 1953. However, it is clear that 8 new cases of osteoporosis were found in 1953 (10 cases in 1953 versus 2 cases in 1943). *"During the 10-year interval, 4 Bartlett participants showed increased coarsened trabeculation without increased bone density."* From Table 1, it seems that there were 2 new cases of coarsened trabeculation in 1953 (14) compared to 1943 (12).

Case reports

Four cases were presented to represent the changes observed.

Increased density and coarsened trabeculation: A 72-year old white male, resident of Bartlett for 33 years showed an increase in the total density of the bone throughout the entire lumbar spine and pelvis. The change involved a coarsening of the trabeculae halfway between the normal pattern and the thickened denser trabeculae in classic Paget's disease. The process was uniform through all bone and not localized. A similar view 10 years later showed no apparent change in the amount or character of this abnormality.

Minimal increased density with coarsened trabeculation: The case of a 71-year old white male, resident of Bartlett for 59 years, was presented as typical of the majority of positive cases in the series. The lumbar spine and pelvis showed a slight, but distinct increase in the total density of the bones with coarsening of trabeculae, most evident in the sacrum and not distinct in any other portion of the pelvic bones. There also was definite ossification of the right sacrotuberous ligament, somewhat more extensive in 1953 than in 1943. There was no change in the degree of density when the 1943 and 1953 films were compared.

Increased bone density and coarsened trabeculation with a decrease in bone density 10 years later: A case of unusual medical interest is that of a woman with increased bone density and coarsened trabeculation in 1943 with a decrease in bone density to a point more closely resembling 'normal' bone 10 years later. Several such cases were noted in

	the series. A 59-year old white female, resident of Bartlett for 39 years, showed a marked increase in the total bone density in 1943, particularly in the vertebrae, sacrum, and around the sacroiliac joints. There was definite coarsening of the trabeculae in the lumbar spine, pelvis, and femurs. In the 1953 films, the increased density previously noted had decreased appreciably and the trabecular pattern has lost much of its coarsening. There were small bony spurs or ossifications in the region of the sacrotuberous ligaments, and one sacrospinous ligament, slightly more pronounced. Changes may have been due to postmenopausal osteoporosis. *50-year fluoride exposure without bone effects:* An 84-year old female resident of Bartlett with known exposure to a high level of naturally occurring fluorides for 50 years, and a resident of the high-fluoride area immediately adjacent to Bartlett for the rest of her life (total exposure of 84 years to 4-8 ppm F) presented with no evidence of increased bone density, coarsened trabeculation, or other significant roentgenographic bone changes. This patient was the mother of one of the participants (not an original subject herself in 1943) and is included to illustrate that a lifetime of exposure to high-fluoride levels does not necessarily produce changes often described as 'fluoride bone effects' or produce recognizable bone changes in all of those who are exposed.
Bone fluoride content	Roentgenographic examinations in 1943 and 1953 on a patient who died after the 1953 examination showed a moderate degree of increased bone density with some coarsened trabeculation. Chemical analysis of the bones showed approximately six times the fluoride content (0.6 mg. per cent) of the same bone from individuals from non-fluoride regions (as determined by F.J. McClure, National Institute of Dental Research). No histologic changes could be linked to fluoride exposure.
STUDY AUTHORS' CONCLUSIONS:	The following types of roentgenographic bone conditions were seen in subjects ingesting water with 8 ppm fluoride for long periods: o Increased bone density with or without coarsened trabeculation, with a 'ground glass' appearance. o Coarsened trabeculation, showing lines of stress, without increased bone density. o Increased thickening of cortical bone and periosteum with equivocal narrowing of bone marrow spaces. The data demonstrate that ingestion of water containing up to 8 ppm fluoride produces no deleterious bone changes: no unusual incidence of bone fractures, arthritis, hypertrophic bone changes or exostoses, or interference with fracture healing; no cases of 'poker spine' and no evidence of associated functional or systemic effects. Excessive fluorides in a water supply may produce roentgenographic evidence of bone changes, but the observable changes: o Occur in only a select few (~10-15% of those exposed) o Are slight, often difficult to recognize, and in most cases equivocal in degree o Are not associated with other physical findings, except for dental mottling in persons who resided in the high-fluoride area during the tooth formative period (birth to 8 years old) o Cannot be definitely ascribed to excessive fluorides alone o Do not necessarily occur even though the fluoride content of bone may be 6 times that of 'normal' bone. There is some indication that the ingestion of excessive fluoride in water may, on occasion, have a beneficial effect in adult bone, as in counteracting the osteoporotic changes of the aged.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT	Not applicable (cited reference is included in NRC fluoride report)

FOUND IN NRC (2006)		
PROFILER'S REMARKS	***Initials/date SJG/11/1/07***	Overall, the study was well-conducted and had adequate study design, with some limitations in the presentation of results. The study design does not aid in the development of a dose response to fluoride with respect to bone changes, such as changes in bone density, coarsened trabeculation, osteoporosis, or hypertrophic change. The objective of the study was to present roentogenographic findings of a ten year study of 237 white adults residing in a high-fluoride area (8 ppm F) or in a control area (0.4 ppm F). Overall, it appears that prolonged (≥15 years) ingestion of water containing up to 8 ppm fluoride produces bone changes such as increased bone density (16-17 vs. 4 cases) and coarsened trabeculation (12-14 vs. 2-3 cases) in a greater number of subjects compared to a group exposed to negligible amounts of fluoride in their water. The paper does not stand alone; that is, statistical methods were presented in an earlier article (Leone et al. 1954). Thus, the statistical significance of the results is unclear. The authors report that bone changes were evident in only about 10-15% of the exposed study population, but whether or not there was a significance difference compared to the control group is not reported. Furthermore, the summarized results in Table 1 did not readily correspond to reported results in the text. Several cases were presented as case reports to illustrate the changes typical in the study; however, this does not provide any statistical power in evaluating the effects of fluoride on bone.
PROFILER'S ESTIM. NOEL/NOAEL		Study design was not suitable for development of a NOAEL.
PROFILER'S ESTIM. LOEL/ LOAEL		Study design was not suitable for development of a LOAEL.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable (X), Poor ( ), Medium ( ), Strong ( ) While the study was well-conducted, the study design was not conducive to provide data for a dose-response. The study suggested that prolonged ingestion of 8 ppm fluoride in water produces bone changes such as increased bone density and coarsened trabeculation in a greater number of subjects compared to a control group (0.4 ppm F), but the effects were sometimes equivocal.
CRITICAL EFFECT(S):		Increased bone density; coarsened trabeculation

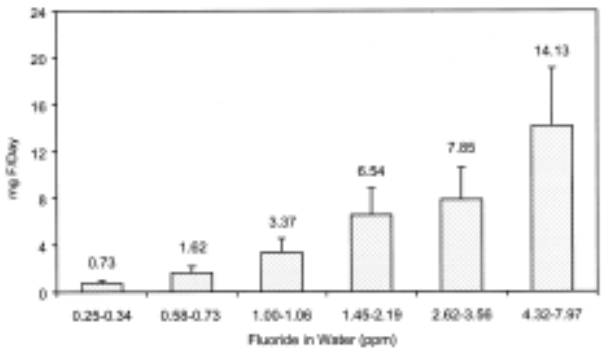
Li, Y., Liang, C., Slemenda, C.W., Ji, R., Sun, S., Cao, J., Emsley, C.L., Ma, F., Wu, Y., Ying, P., Zhang, Y., Gao, S., Zhang, W., Katz, B.P., Niu, S., Cao, S., and Johnston, C.C. 2001. Effect of long-term exposure to fluoride in drinking water on risks of bone fractures. Journal of Bone and Mineral Research. 16(5): 932-939.

ENDPOINT STUDIED:	Bone fracture
TYPE OF STUDY:	Cohort
POPULATION STUDIED:	1363 adults, 62.6 ± 9.3 years old (41.8% male) with long term residence in a Chinese community with **0.25-0.34 ppm** fluoride in the drinking water; mean total daily fluoride intake was 0.73 mg F/day.
POPULATION STUDIED:	1407 adults, 62.7 ± 9.1 years old (47.0% male) with long term residence in a Chinese community with **0.58-0.73 ppm** fluoride in the drinking water; mean total daily fluoride intake was 1.62 mg F/day.
CONTROL POPULATION:	1370 adults, 62.5 ± 9.0 years old (43.7% male) with long term residence in a Chinese community with **1.00-1.06 ppm** fluoride in the drinking water; mean total daily fluoride intake was 3.37 mg F/day.
POPULATION STUDIED:	1574 adults, 63.6 ± 8.8 years old (44.5% male) with long term residence in a Chinese community with **1.45-2.19 ppm** fluoride in the drinking water; mean total daily fluoride intake was 6.54 mg F/day.
POPULATION STUDIED:	1051 adults, 64.0 ± 9.0 years old (43.3% male) with long term residence in a Chinese community with **2.62-3.56 ppm** fluoride in the drinking water; mean total daily fluoride intake was 7.85 mg F/day.
POPULATION STUDIED:	1501 adults, 61.3 ± 8.4 years old (52.4% male) with long term residence in a Chinese community with **4.32-7.97 ppm** fluoride in the drinking water; mean total daily fluoride intake was 14.13 mg F/day.
EXPOSURE PERIOD:	A minimum of 25 years of continuous residence in the study community and a lifelong exposure to the specified fluoride level in drinking water was required of each participant. Mobility in the countryside is almost nonexistent so the history of fluoride exposure in individuals is relatively easy and reliable.
EXPOSURE GROUPS:	Six groups of a total of 8266 male and female subjects, ≥ 50 years old, were recruited randomly from rural communities in China where water fluoride concentration in the drinking water ranged from 0.25 to 7.97 ppm and total daily fluoride intake ranged proportionally from 0.73 to 14.13 mg F/day. There was virtually no fluoride exposure from other sources such as fluoride supplements and fluoride containing dentifrice, mouth rinse, or use of infant formula.

TABLE 1. DEMOGRAPHIC DATA OF SIX CHINESE POPULATIONS RESIDING IN COMMUNITIES OF VARYING FLUORIDE CONCENTRATION IN DRINKING WATER

Group	*Water F (ppm)*	n	*Age (year)*	*Male (%)*
1	0.25–0.34	1363	62.6 ± 9.3*	41.8
2	0.58–0.73	1407	62.7 ± 9.1	47.0
3	1.00–1.06	1370	62.5 ± 9.0	43.7
4	1.45–2.19	1574	63.6 ± 8.8	44.5
5	2.62–3.56	1051	64.0 ± 9.0	43.3
6	4.32–7.97	1501	61.3 ± 8.4	52.4

*Mean ± SD.

	 FIG. 1. Total daily fluoride intake in relation to fluoride concentration in drinking water in six Chinese populations.
EXPOSURE ASSESSMENT:	Data collected from each subject included medical history and demographic information, bone fractures, physical activity as determined using the Chinese standard (National Standards 1995), tea drinking, cigarette smoking, and alcohol consumption. Information regarding bone fracture included: subject age, fracture site, frequency, and circumstances associated with the fracture. Medical records and X-rays were obtained where possible, or an X-ray was taken to verify the self-reported bone fracture.
ANALYTICAL METHODS:	For each site, samples of drinking water were collected and analyzed for fluoride using the direct method with a combination fluoride–specific electrode (no.96-909-00, Orion Research, Inc., Boston, MA). Eight additional elements in drinking water also were analyzed: calcium, selenium, aluminium, lead, cadmium, iron, zinc, and arsenic. A modified International Organization for Standardization ISO method was used to determine the fluoride content in ambient air (Ando et al. 1998). Surveys were conducted to ensure no other potential sources of fluoride exposure (e.g., pollution, dentifrice, etc.) in the study populations. The dietary fluoride and brewed tea samples were analyzed using a modified method of Taves (1968); calcium and protein were determined using Chinese National Standard procedures (1991).
STUDY DESIGN	The purpose of the study was to determine the prevalence of bone fracture, including hip fracture, in six Chinese populations with water fluoride concentrations ranging from 0.25 to 7.97 ppm; a total of 8266 male and female adults, ≥50 years of age, were included in the study. Drinking water was the only major source of fluoride exposure in the study populations. A minimum of 25 years of continuous residence in the study community and a lifelong exposure to the specified fluoride level in drinking water was required of each participant. Residency was determined by three measures: objective assessment by checking the Family Registry Book, an official document issued by the government; a subject survey questionnaire; and confirmation by village officials familiar with the subject. Surveys indicated that the environment, culture, ethnic background, social structure, and economic conditions of these populations had not changed significantly during the past several decades. Ethnic backgrounds and level of physical activity were similar among the six populations. Parameters evaluated included fluoride exposure, prevalence of bone fractures, demographics, medical history, physical activity, cigarette smoking, and alcohol consumption. For those reporting bone fractures, additional information was collected: fracture site (22 sites using an illustrative drawing of the human body); subject age; frequency of each fracture; and circumstances associated with the fracture including cause (eight categories), location (six categories), ground condition, and fall or without fall. Family history of hip fractures and information on falls within the last year also was collected. Medical records and X-rays were obtained where possible. For those without records, an X-ray was taken to verify the self-reported bone fracture.

	A 3-day dietary survey and analysis for dietary intake of calcium, protein, and fluoride were conducted in a randomly selected 10% of subjects to ensure that all study populations had adequate nutrition and to determine fluoride exposure from diet.
PARAMETERS MONITORED:	Parameters evaluated included fluoride exposure, prevalence of bone fractures, demographics, medical history, physical activity, cigarette smoking, and alcohol consumption.
STATISTICAL METHODS:	For each class of fractures, the bivariate relationship was first examined between fracture rate and several demographic and lifestyle variables including gender, current cigarette smoking status, consumption of alcohol, physical activity level on the job, age, and body mass index (BMI). Comparisons were made using χ^2 tests for categorical variables and t-tests for continuous variables. Dose-dependent analyses were performed using a multiple logistic regression model, which was used to compare fracture rates across fluoride levels, while adjusting for demographic and lifestyle variables, which were significant in the bivariate analysis. Adjusted odds ratios (ORs) were calculated based on the coefficients in the multiple logistic regression models. The data analyses were adjusted for water calcium, aluminium, selenium, lead, cadmium, iron, zinc, and arsenic by including them individually in the logistic regression model for overall fractures. Analysis defined subjects as to whether they had the fracture or not and did not use the count of multiple fractures in the same subject. For all statistical tests, the level of statistical significance was set at $p<0.05$.
RESULTS:	
Overall fracture (since age 20 years)	There were 531 subjects reporting fractures; so the prevalence of overall bone fracture in the entire study population was 6.42%; 99.1% of these reported fractures were verified by X-ray. The mean ages of subjects with fracture were 63.4, 64.2, 63.5, 66.1, 64.6, and 62.1 years for groups 1-6, respectively. Statistical analysis showed that group 4 differed significantly from group 6, indicating that subjects with bone fracture were slightly younger in the population of the highest fluoride in drinking water, but the effect was not dose dependent. Table 2 was copied directly from Li et al. 2001 and presents the prevalence of overall bone fracture since age 20 years for each fluoride level as well as the odds ratios (OR) and p values from the multiple logistic regression model adjusted for age and gender. Both the populations with the lowest (0.25-0.34 ppm) and the highest (4.32-7.97 ppm) fluoride concentrations showed a significantly higher prevalence of overall fractures (p=0.01) than those residing in areas where water fluoride was 1.00-1.06 ppm, where the lowest prevalence of overall fractures was found. No significant differences were found among the groups with fluoride levels ranging from 0.58-3.56 ppm (groups 2, 3, 4 and 5). TABLE 2. EFFECT OF FLUORIDE EXPOSURE FROM DRINKING WATER ON PREVALENCE OF OVERALL FRACTURE SINCE THE AGE OF 20 YEARS IN SIX CHINESE POPULATIONS *Water F (ppm)* | n *(surveyed)* | n *(fracture)* | *Prevalence (%)* | *OR*[a] | p *Value*[a] 0.25–0.34 | 1363 | 101 | 7.41 | 1.50 | 0.01 0.58–0.73 | 1407 | 90 | 6.40 | 1.25 | 0.17 1.00–1.06 | 1370 | 70 | 5.11 | 1.00 | — 1.45–2.19 | 1574 | 95 | 6.04 | 1.17 | 0.33 2.62–3.56 | 1051 | 64 | 6.09 | 1.18 | 0.35 4.32–7.97 | 1501 | 111 | 7.40 | 1.47 | 0.01 [a] Values relative to the 1.00- to 1.06-ppm fluoride group, adjusted for age and gender using multiple logistic regression. In general, the trend of fracture prevalence in relation to the water fluoride concentration approximates a U-shaped pattern (Figure 2, copied directly from Li et al. 2001).

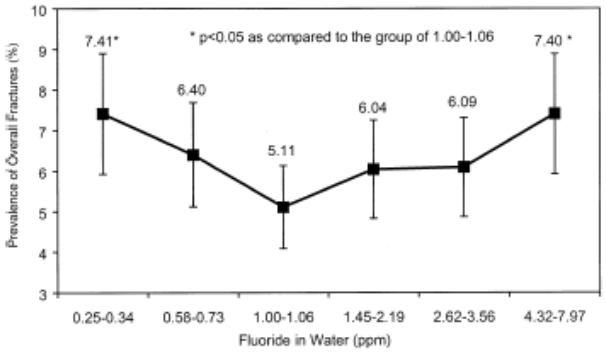


FIG. 2. Prevalence of overall fractures and fluoride concentration in drinking water in six Chinese populations since the age of 20 years.

Table 3 was copied directly from Li et al. 2001 and summarizes results from bivariate analysis. Age, gender, alcohol consumption, and physical activity level were significant factors for the risk of overall bone fractures. Subjects with fractures were significantly older ($p<0.01$) than those without fractures. More males suffered fractures than females ($p<0.01$), and subjects who consumed alcohol had more fractures ($p<0.01$) than non-drinkers. Gender and alcohol consumption were highly correlated, with 46.9% of males and 4.3% of females reported drinking alcohol. The level of physical activity had a significant effect ($p=0.05$); excessively strenuous or lack of activity increased the risk of fractures. No significant effect of cigarette smoking ($p=0.15$) or BMI ($p=0.80$) on overall fracture rates was detected. Only calcium ($p=0.044$) and iron ($p=0.032$) showed a significant relationship with fracture but neither one (nor any of the other analyzed elements) altered the results concerning the six fluoride groups.

TABLE 3. BIVARIATE ASSOCIATIONS OF OVERALL BONE FRACTURE RISKS SINCE THE AGE OF 20 YEARS WITH CATEGORICAL FACTORS

Variable	*Category*	*Subjects*	*Fracture (%)*	p *Value*
Gender	Male	3771	7.48	
	Female	4495	5.54	<0.01
Cigarette smoking	Yes	3100	6.94	
	No	5166	6.12	0.15
Alcohol consumption	Yes	1960	8.52	
	No	6299	5.76	<0.01
Physical activity	Very little	652	7.98	
	Light	2532	5.53	
	Moderate	4157	6.54	
	Heavy	912	7.13	
	Extremely strenuous	11	18.18	0.05

Hip fracture (since age 20 years)

Table 4 was copied directly from Li et al. 2001 and presents the prevalence of hip fractures since age 20 years in the six populations and the results of the multiple logistic regression model. A total of 56 subjects (of 8266) reported hip fractures, resulting in a prevalence of 0.68%. Bivariate analysis showed that subjects with hip fracture were significantly older (mean age 68.5 vs. 62.7 years) and thinner (mean BMI 21.2 vs. 22.6) than those without fractures ($p<0.01$ for both). No significant effects were detected for gender, cigarette smoking, alcohol consumption, and the level of physical activity. After adjusting for age and BMI, the risk of hip fracture was significantly higher in the highest fluoride group (4.32-7.97 ppm) than the population with 1.00-1.06 ppm fluoride, which had the lowest prevalence.

TABLE 4. EFFECT OF FLUORIDE EXPOSURE FROM DRINKING WATER ON PREVALENCE OF HIP FRACTURES IN SIX CHINESE POPULATIONS SINCE THE AGE OF 20 YEARS

Water F (ppm)	n *(surveyed)*	n *(fracture)*	*Prevalence (%)*	*OR*[a]	p *Value*[a]
0.25–0.34	1363	5	0.37	0.99	0.99
0.58–0.73	1407	6	0.43	1.12	0.85
1.00–1.06	1370	5	0.37	1.00	—
1.45–2.19	1574	14	0.89	2.13	0.15
2.62–3.56	1051	8	0.76	1.73	0.34
4.32–7.97	1501	18	1.20	3.26	0.02

[a] Values relative to the 1.00- to 1.06-ppm fluoride group, adjusted for age and BMI using multiple logistic regression.

In general, the hip fracture prevalence was stable up to 1.06 ppm of fluoride and then appeared to rise, although it did not attain statistical significance until the water fluoride concentration reached 4.32-7.97 ppm (Figure 3, copied directly from Li et al. 2001).

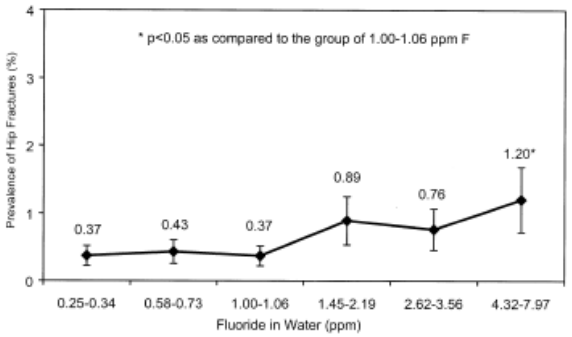


FIG. 3. Prevalence of hip fractures and fluoride concentration in drinking water in six Chinese populations since the age of 20 years.

Overall fracture (since age 50 years)

Table 5 was copied directly from Li et al. 2001 and summarizes prevalence of overall bone fracture since the age of 50 years, including odds ratios (ORs) and p values. There were 311 people with fractures, resulting in 3.76% overall prevalence. There was a similar trend in the relationship between water fluoride level and overall fractures when evaluated from age 20 years, but less pronounced. Only the highest fluoride group (4.32-7.97 ppm) had a significantly higher risk for fractures, after adjusting for age, than the group with 1.00-1.06 ppm of fluoride.

TABLE 5. EFFECT OF FLUORIDE EXPOSURE FROM DRINKING WATER ON PREVALENCE OF OVERALL FRACTURES IN SIX CHINESE POPULATIONS SINCE THE AGE OF 50 YEARS

Water F (ppm)	n *(surveyed)*	n *(fracture)*	*Prevalence (%)*	*OR*[a]	p *Value*[a]
0.25–0.34	1363	59	4.33	1.33	0.16
0.58–0.73	1407	45	3.20	0.97	0.87
1.00–1.06	1370	45	3.28	1.00	—
1.45–2.19	1574	52	3.30	0.96	0.85
2.62–3.56	1051	38	3.62	1.04	0.87
4.32–7.97	1501	72	4.80	1.59	0.02

[a] Values relative to the 1.00- to 1.06-ppm fluoride group, adjusted for age using multiple logistic regression.

Table 6 was copied directly from Li et al. 2001 and summarizes results from the bivariate analysis. Age remained a risk factor; subjects with fractures were significantly older than subjects without fractures. The level of physical activity also was significant (p=0.03) in relation to fractures. No significant effects were observed for gender, cigarette smoking, alcohol consumption, and BMI.

TABLE 6. BIVARIATE ASSOCIATION OF OVERALL BONE FRACTURE RISKS SINCE THE AGE OF 50 YEARS WITH CATEGORICAL FACTORS

Variable	*Category*	*Subjects*	*Fracture (%)*	p *Value*
Gender	Male	3771	3.61	
	Female	4495	3.89	0.52
Cigarette smoking	Yes	3100	3.29	
	No	5166	4.05	0.08
Alcohol consumption	Yes	1960	3.98	
	No	6299	3.70	0.59
Physical activity	Very little	652	5.21	
	Light	2532	3.71	
	Moderate	4157	3.58	
	Heavy	912	3.51	
	Extremely strenuous	11	18.18	0.03

STUDY AUTHORS' CONCLUSIONS:

Based on the data collected from this investigation, it is concluded that long-term fluoride exposure from drinking water containing 4.32 ppm or more increases the risk of overall fracture as well as hip fracture. The prevalence of overall bone fractures was lowest for populations living in areas of approximately 1.00 ppm of fluoride. Thus, water fluoride levels of 1.00-1.06 ppm decrease the risk of overall fractures relative to negligible fluoride in water; however, there does not appear to be a similar protective benefit for the risk of hip fractures. The U-shaped effect of water fluoride observed in bone fractures was not observed in the hip fracture data. The prevalence of hip fractures was stable until the water concentration reached 1.45-2.19 ppm. Additionally, fractures are influenced by other factors, such as age, gender, alcohol consumption, and physical activity.

DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)

National Standards (1995). Levels of labor intensity. Chinese National Standard GB 3869-83, Beijing, China.

Ando, M., Tadano, M., Asanuma, S., Tamura, K., Matsushima, S., Watanabe, T., Kondo, T., Sakurai, S., Ji, R., Liang, C., and Cao, S. (1998). Health effects of indoor fluoride pollution from coal burning in China. Environmental Health Perspectives 106: 239-244.

Taves, DR. (1968). Separation of fluoride by rapid diffusion using hexamethyldisiloxane. Talanta 15: 969-974.

Standard Department of Chinese Academy of Preventative Medicine (1991). Standard compilation of environment, school and radiation health. Chinese Standard Publishing House, Beijing, China, pp. 278-338.

PROFILER'S REMARKS ***Initials/date SJG/ 9/25/07***

Overall, the study was well-conducted and had adequate study design, considering several aspects to confirm exposure, reported fractures, and confounding factors. The study design aids in the development of a dose response to fluoride with respect to risk of overall bone fracture and hip fracture. The objective of the study was to determine the prevalence of bone fractures in six cohorts of older adults (≥50 years old) from rural communities in China with long-term exposure to different fluoride concentrations in the drinking water (0.25-0.34 ppm; 0.58-0.73 ppm; 1.00-1.06 ppm; 1.45-2.19 ppm; 2.62-3.56 ppm; and 4.32-7.97 ppm). Analysis of water samples confirmed fluoride concentrations and considered eight other elements (calcium, selenium, aluminium, lead, cadmium, iron, zinc, and arsenic). There were no significant differences among groups concerning other potential sources of fluoride (e.g., diet, supplements, or air), ethnic backgrounds, level of physical activity, or occupation.

The data suggest an optimal beneficial window of fluoride intake for bone health as approximately 1.00 ppm. There was an increased risk of overall bone fractures and hip fractures in the highest exposure group (4.32-7.97 ppm). Further, the risk for overall fractures decreased in the population with 1.00-1.06 ppm compared to the lowest exposure group with negligible fluoride in the water (0.25-0.34 ppm). Factors that influenced bone fracture risk included age, gender, alcohol consumption, and physical activity.

When considering odds ratios (ORs) presented in the tables above, it may not be appropriate to conclude that the risk of hip fracture is more sensitive to water fluoride concentration as

	compared to overall fractures the number of hip fractures in the present study is relatively small. Further, the total number of people with fractures was relatively small, making it impossible to sort out all potential confounding factors individually.
PROFILER'S ESTIM. NOEL/NOAEL	Study design was suitable for development of a NOAEL for bone fracture. No statistically significant effects on overall bone fracture or hip fracture prevalence were observed at fluoride levels **≤3.56 ppm** in the drinking water. Water fluoride levels at 1.00 -1.06 ppm decreases the risk of overall fractures relative to negligible fluoride in water (**≥0.58 ppm** resulted in statistically similar results).
PROFILER'S ESTIM. LOEL/ LOAEL	Study design was suitable for development of a LOAEL for bone fracture. Long-term fluoride exposure from drinking water containing **≥4.32 ppm** fluoride increases the risk of overall bone fractures and hip fractures. Water fluoride levels at 1.00 -1.06 ppm decreases the risk of overall fractures relative to negligible fluoride in water (**≤0.34 ppm** resulted in increased prevalence of overall fractures).
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:	Not suitable (), Poor ( ), Medium ( ), Strong (X) The study was well-conducted, and the study design was conducive to provide data for a dose-response bone fracture risk. The study indicated that long-term fluoride exposure from drinking water containing 4.32 ppm or more increases the risk of overall fracture as well as hip fracture. The prevalence of overall bone fractures was lowest for populations living in areas of approximately 1.00 ppm of fluoride.
CRITICAL EFFECT(S):	Prevalence of overall bone fracture and hip fracture

Reid IR, Cundy T, Grey AB, Horne A, Clearwater J, Ames R, Orr-Walker BJ, Wu F, Evans MC, Gamble GD, and King A. 2007. Addition of monofluorophosphate to estrogen therapy in postmenopausal osteoporosis: a randomized controlled trial. **J Clin Endocrinol Metab.** 92(7): 2446-52. Epub 2007 Apr 17**.**

<table>
<tr><td></td><td></td></tr>
<tr><td>ENDPOINT STUDIED:</td><td>Bone mineral density (BMD)</td></tr>
<tr><td></td><td></td></tr>
<tr><td>TYPE OF STUDY:</td><td>Randomized control trial (double-blind)</td></tr>
<tr><td></td><td></td></tr>
<tr><td>POPULATION STUDIED: (MFP)</td><td>41 postmenopausal women with osteoporosis. Subjects received daily doses of 20 mg elemental fluoride and 600 mg calcium. 15 subjects discontinued the study due to personal reasons or illness.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>CONTROL POPULATION: (Placebo)</td><td>39 postmenopausal women with osteoporosis. Subjects received daily doses of placebo containing 600 mg calcium. 14 subjects discontinued the study due to death, personal reasons or illness.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>EXPOSURE PERIOD:</td><td>Duration of follow-up in the study was 3.1±1.3 years in the placebo group and 2.9±1.7 in the MFP group.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>EXPOSURE GROUPS:</td><td>80 postmenopausal women with osteoporosis were recruited from a hospital clinic. They were required to have at least one vertebral fracture (i.e., a reduction in the anterior, middle, or posterior relative height of a vertebra of more than 3 standard deviations in relation to the vertebra-specific normal values or a bone mineral density (BMD) T-score in the lumbar spine (L2-4) of <-2.5). All subjects had been receiving estrogen for at least 12 months prior to study entry. Subjects were ineligible if they had disorders of calcium metabolism other than osteoporosis, thyroid or hepatic dysfunction, or serum creatinine >0.20 mmol/L. No subjects had previously used calcitonin or fluoride and none had used bisphosphonates in the previous year. Subjects received monofluorophosphate (20 mg F/day) with calcium (600 mg/day, 50% citrate and 50% gluconate) or placebo (calcium only), in addition to 500 mg calcium carbonate supplement and estrogen therapy.

Table 1: Characteristics of Study Subjects at Baseline
<table>
<tr><th></th><th>Placebo</th><th>MFP</th></tr>
<tr><td>n</td><td>41</td><td>39</td></tr>
<tr><td>Age (years)</td><td>65.0 (7.1)</td><td>65.5 (7.3)</td></tr>
<tr><td>Weight (kg)</td><td>60.0 (9.8)</td><td>60.4 (11)</td></tr>
<tr><td>Height (cm)</td><td>157.9 (6.6)</td><td>157.8 (4.8)</td></tr>
<tr><td>Calcium intake (mg/d)</td><td>890 (460)</td><td>1030 (570)</td></tr>
<tr><td>Current smokers</td><td>4 (10%)</td><td>7 (19%)</td></tr>
<tr><td>BMD T-score</td><td></td><td></td></tr>
<tr><td>Lumbar spine</td><td>-2.56 (0.92)</td><td>-2.49 (1.19)</td></tr>
<tr><td>Femoral neck</td><td>-1.98 (0.83)</td><td>-1.78 (0.82)</td></tr>
<tr><td>Total body</td><td>-2.50 (1.07)</td><td>-2.31 (0.94)</td></tr>
<tr><td>Prevalent vertebral fractures</td><td>26 fractures in12 women</td><td>23 fractures in 7 women</td></tr>
<tr><td>Duration of estrogen use (years)</td><td>2.5 (2.2)</td><td>2.3 (2.0)</td></tr>
</table>
Data are mean (SD). There were no significant differences between the groups.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>EXPOSURE ASSESSMENT:</td><td>Serum fluoride concentrations were monitored. Bone mineral density (BMD) was assessed at trial entry and annually. Bone turnover markers were assessed using standard methods (Reid 2004). Lateral radiographs of the thoracic and lumbar spines were performed at trial entry and annually. Bone biopsies were performed at the end of the study in 7-9 subjects from each group.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>ANALYTICAL METHODS:</td><td>Fluoride levels were maintained by tablet and not analyzed for content.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>STUDY DESIGN</td><td>Prior to study entry, a full medical history was taken, dietary calcium intake was assessed using a food</td></tr>
</table>

	frequency questionnaire and physical activity was assessed by questionnaire. Height and weight were measured at study entry and every 6 months using a Harpenden stadiometer and an electronic balance, respectively. Women were randomized to receive tablets of calcium (TRIDIN, RottapharmSpA, Monza, Italy) with or without glutamine monofluorophosphate. Tablets were taken with the morning and evening meals and provided daily doses of 20 mg of elemental fluoride and 600 mg of calcium. Compliance was checked at each clinic visit by tablet counts. All patients also took nightly supplements of 500 mg of calcium carbonate. Subjects continued on their estrogen regimen, usually continuous conjugated equine estrogens (03.-0.625 mg/day) plus medroxyprogesterone acetate (2.5-5 mg/day). Vitamin D_3 (400-800 IU/day) was given to any patient whose serum 25-hydroxyvitamin D level was <50 nmol/L, either initially or at the annual checks. Patients were seen at trial entry, at 3 and 6 months, and then semi-annually to 4 years. Mean trial medication compliance (based on tablet counts) was 86% (±17.3) in the placebo group and 81% (±15.2) in the MFP group. Serum fluoride concentrations (at least 12 hours after last MFP dose) were monitored at each visit, with the intention of maintaining levels <12.5 μmol/L. Results were monitored by a staff member who had no contact with the participants; all other study personnel and the study participants were blinded to treatment allocation. Bone mineral density (BMD), bone turnover markers, and lateral radiographs of the thoracic and lumbar spines were monitored. Bone biopsies were performed at the end of the study in 7-9 subjects from each group.
PARAMETERS MONITORED:	Bone mineral density (BMD) was assessed at trial entry and annually using a Lunar DPX-L dual energy x-ray absorptiometer. Separate scans of the total body, lumbar spine in the anteroposterior projection, third lumbar vertebra in the lateral projection, proximal femur and distal forearm were undertaken. For lumbar spine scans, only those vertebral bodies demonstrated not to be fractured on plain radiographs were included in the analysis. Bone turnover markers were assessed using standard methods (Reid 2004). Lateral radiographs of the thoracic and lumbar spines were performed at trial entry and annually, using a film-tube distance of 100 cm. An incident vertebral fracture was defined as a decrease in an anterior, middle, or posterior vertebral height of ≥20% *and* ≥4 mm.
STATISTICAL METHODS:	Continuous variables (e.g., BMD, biochemical measurements) were analyzed using a mixed models approach to repeated measures (Proc Mixed). Significant interaction effects were further explored using the method of Tukey to preserve an overall 5% significance level. Time to first fracture was compared between treatment and control arms using a proportional hazards model, and results presented as the hazard ratio and 95% confidence interval (CI). Fractures were expressed as fractures per 1000 patient-years at risk, and the incidences compared between groups assuming a Poisson distribution. All analyses were performed using procedures of SAS version 9.1 (SAS Institute Inc, Cary NC). The study was powered to assess effects on lumbar spine BMD and on vertebral fractures. A study of this size has >90% power to detect a difference in the absolute change in lumbar spine BMD between treated and control groups of at least 5%. Based on figures from Riggs (1982), 80 subjects yield a power of 90% to detect this difference (α=0.05) and a power of 80% to detect a halving of fracture numbers. PROFILER'S NOTE: The information regarding power of sample size does not specify whether the original sample size (n =39, 41) or the final sample size after subjects discontinued participation (n=25, 26) was used for the calculations.
RESULTS:	
Bone Mineral Density	Figure 1 was copied directly from Reid et al. (2007) and shows an increase in BMD for lumbar spine

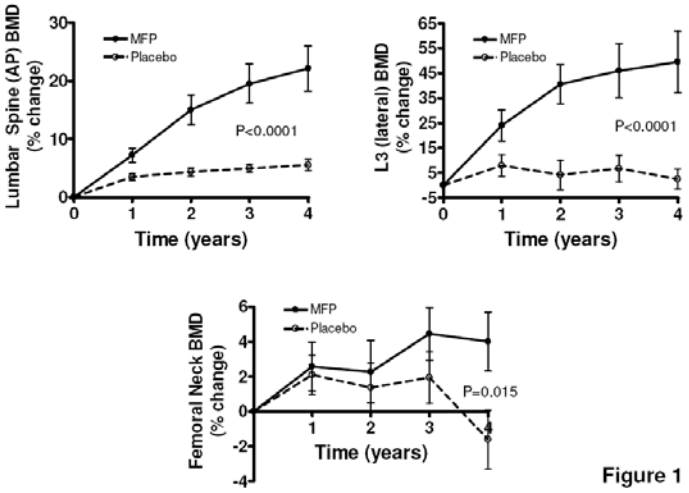
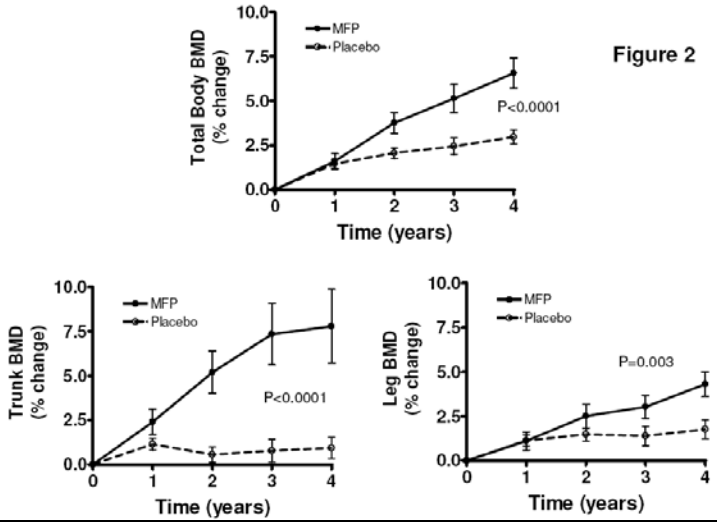
	(L2-4 in the anteroposterior projection, AP) ($p<0.0001$), third lumbar vertebra in the lateral projection (L3 lateral) ($p<0.0001$), and femoral neck ($p=0.015$). P values are for the treatment-time interaction over the trial period. In the AP projection, the MFP group increased 22% whereas the placebo group was only 6% above baseline at the end of the study. These changes were most marked in the trabecular bone, as reflected in the L3 (lateral) projection; MFP group increased 49%, compared to 2.5% in placebo group. In the femoral neck, BMD increased 4.6% above baseline by year 4 in the MFP group and decreased slightly in the placebo group.  Figure 1 Figure 2 also was copied directly from Reid et al. (2007) and shows the increase in total body ($p<0.0001$), trunk ($p<0.0001$), and leg ($p=0.003$) BMD. P values are for the treatment-time interaction over the trial period. There were significant increases in BMD over baseline in both groups, but the increase was greater in the MFP group. In the trunk the difference between MFP and placebo groups was 6.9%, whereas in the legs it was only 2.5%.  Figure 2
Biochemical Parameters	Figure 3 was copied directly from Reid et al. (2007) and indicates effects on bone turnover markers from a randomly selected 20 subjects per group (βCTX, β C-terminal telopeptide of type I collagen; P1NP, procollagen type-I N-terminal propeptide; ALP, total alkaline phosphatase). P values are for the treatment-time interaction over the trial period. There was a significant stimulation of bone formation in year 1 (osteocalcin $p<0.0005$, P1NP $p<0.03$) with no change in bone resorption (βCTX $p=0.2$). Total ALP (bone formation marker) showed a sustained effect of MFP treatment ($p<0.03$). The stimulation of bone formation was substantially attenuated at year 4.

Figure 3

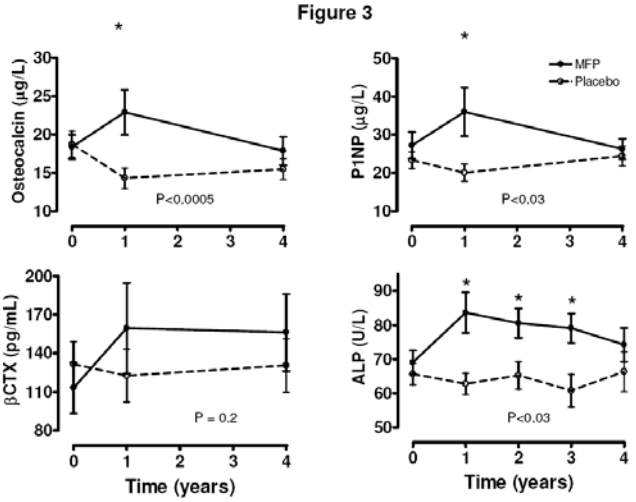


Table 2 was copied directly from Reid et al. (2007) and summarizes serum biochemistry and urine calcium during the study. Serum calcium, phosphate, 25-hydroxyvitamin D and 1,25-hydroxyvitamin D and urine calcium showed no significant between-group changes during the study. Parathyroid hormone levels were comparable at baseline, subsequently tended to be higher in the MFP group (p=0.06), but only reached significance at year 3 (placebo value slightly lower in year 3). Serum fluoride levels were maintained at ~7 µmol/L in the MFP group.

Table 2: Serum Biochemistry and Urine Calcium During Study

Analyte	Baseline		Year 1		Year 2		Year 3		Year 4	
	Placebo	MFP	Placebo	MFP	Placebo	MFP	Placebo	MFP	Placebo	MFP
Fluoride (µmol/L)	2.7 (0.7)	2.8 (1.1)	3.2 (1.2)	6.7 (2.2)	3.1 (1.2)	6.9 (2.9)	3.0 (0.8)	7.2 (2.6)	3.1 (1.3)	7.3 (2.3)
Total Calcium (mmol/L)	2.29 (0.12)	2.29 (0.08)	2.31 (0.09)	2.32 (0.09)	2.30 (0.09)	2.32 (0.14)	2.31 (0.09)	2.30 (0.07)	2.27 (0.07)	2.31 (0.12)
Phosphate (mmol/L)	1.1 (0.1)	1.1 (0.1)	1.1 (0.2)	1.1 (0.2)	1.2 (0.2)	1.2 (0.2)	1.2 (0.2)	1.2 (0.2)	1.2 (0.1)	1.2 (0.2)
25-hydroxyvitamin D (nmol/L)	73 (34)	63 (30)	71 (30)	70 (26)	72 (33)	70 (29)	60 (27)	59 (27)	59 (24)	51 (21)
1,25-hydroxy-vitamin D (pmol/L)	105 (33)	105 (35)	94 (31)	102 (36)	90 (41)	91 (31)	84 (29)	93 (23)	79 (34)	87 (43)
Parathyroid Hormone (pmol/L)	3.6 (1.7)	3.6 (1.5)	3.0 (1.3)	3.7 (1.6)	3.1 (1.7)	3.8 (1.7)	2.7 (1.1)	3.7 (1.6)*	3.0 (1.3)	3.4 (1.5)
Urine Calcium (mmol/day)	4.3 (2.7)	4.1 (2.4)	4.3 (2.3)	4.9 (3.1)	4.4 (2.2)	4.2 (2.4)	4.2 (1.8)	4.7 (2.6)	4.3 (2.3)	4.6 (2.5)

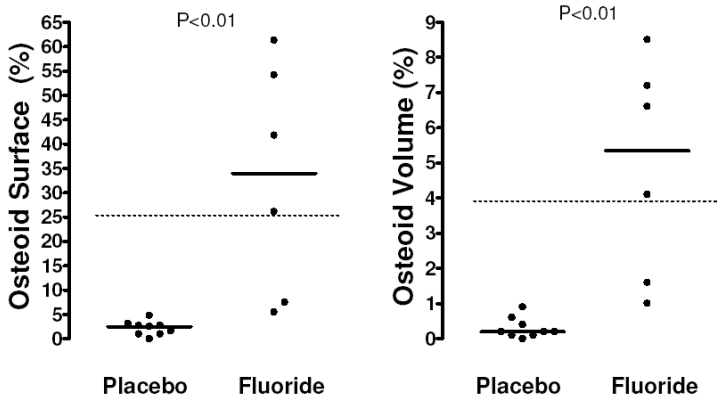
Data are mean (SD)

* significantly different between-groups, P=0.01.

Serum fluoride concentrations were significantly higher in the MFP group at all timepoints after baseline.

There were no other significant differences between-groups

PROFILER'S NOTE: Data points are not evident for years 2 and 3 for osteocalcin, P1NP or βCTX in Figure 3. It is unclear whether data was collected for these time points and whether 'sustained' effects on all bone formation markers would become significant if these values were included since the general trend is similar for ALP.

Fractures	The vertebral fracture rate for the placebo group was 60.3 per 1000 patient-years compared to 9.8 per 1000 patient-years for the MFP group. A Poisson regression gives an incidence rate ratio of 0.12 (95% CI, 0.06-0.23, p<0.01). Analysis of the time to first vertebral fracture showed a hazards ratio of 0.20 (95% CI, 0.05-1.30). Six non-vertebral fractures occurred in the MFP group and 2 in the placebo group, giving a hazards ratio of 3.3 (95% CI, 0.8-12.0). Height loss tended to be greater in the placebo group (0.46±0.10 cm) compared to the MFP group (0.24±0.10 cm) at 4 years, but this was not significant over the whole study period (p=0.45).
Bone Biopsies	Figure 4 was copied directly from Reid et al. (2007) and indicates histomorphometric assessments of bone biopsies after 4 years of treatment with either placebo (n=9) or MFP (n=6). Medians for each group are shown as solid lines, and the upper limit of normal in postmenopausal women as horizontal dotted lines. Values for both parameters were different between-groups (p<0.01). In one MFP subject whose biopsy was not quantifiable, there was evidence of hyperosteoidosis. Osteoid surface and osteoid volume were above the reference ranges in 4 of the remaining 6 MFP subjects, but were within normal range in all placebo subjects. Thus, 5 MFP-treated subject and none of the placebo-treated subjects had hyperosteoidosis (significantly different between-groups, p=0.005). Osteomalacia was evident in 3 of the MFP-treated subjects. **Figure 4** 
STUDY AUTHORS' CONCLUSIONS:	*Bone mineral density*: The present data re-emphasize the anabolic action of fluoride ion on bone. The most dramatic changes are in the lumbar spine, where BMD in the AP projection is 22% above baseline (17% higher than placebo) at the trial's end. Substantial increments in BMD were seen at sites rich in trabecular bone, including the 'trunk' region of the total body scan. In contrast, cortical bone effects were modest or non-existent. Legs (predominately but not exclusively cortical bone) had a 2.5% higher BMD in the MFP group compared to placebo, and there was no therapeutic benefit in the mid-forearm (exclusively cortical bone). This study reiterates substantial long-term benefits of estrogen on the skeleton and indicates additivity of the effects of fluoride and estrogen. *Bone turnover markers*: The changes in bone turnover markers are as expected with an anabolic agent; three osteoblast indices showed evidence of significant stimulation, whereas there was no change in bone resorption. The stimulation of indices of osteoblast function confirms that fluoride activates osteoblast activity and is not artifactually changing BMD as a result of changes in the crystal structure of bone. The difference between-groups for all markers tended to decreased over time, which may account for the slower rate of increase in bone density later in the study. *Fractures*: Despite the small number of events, fracture data suggested a trend towards fewer vertebral fractures, but a trend in the opposite direction for non-vertebral fractures. Together with results from other studies, this study is consistent with evidence indicating that doses of fluoride <20 mg/day are likely to demonstrate anti-fracture efficacy. *Bone biopsies*: The biopsies demonstrate accumulation of osteoid in the majority of MFP-treated

		subjects evaluated. In 2 of 7, these values were within normal range, but in 2 others the diagnostic criteria for osteomalacia were met. The present findings indicate that despite increases in BMD, abnormal mineralization contributing to fracture risk still occurs with elemental fluoride doses as low as 20 mg/day. Therefore, it is inappropriate to recommend the widespread use of the dosing regimen employed in the current study. Much lower doses should be assessed to find a safe dose window for the use of this powerful anabolic agent.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)		Reid IR, Davidson JS, Wattie D, Wu F, Lucas J, Gamble GD, Rutland MD, and Cundy T. (2004). Comparative responses of bone turnover markers to bisphosphate therapy in Paget's disease of bone. Bone 35: 224-230. Riggs BL, Seeman E, Hodgson SF, Taves DR, O'Fallon WM. (1982). Effect of the fluoride/calcium regimen on vertebral fracture occurrence in postmenopausal osteoporosis. N Engl J Med 306:444-450.
PROFILER'S REMARKS	***Initials/date SJG/ 10/12/07***	Overall, the study was well-conducted and had adequate study design. The study design aids in the development of a dose response to fluoride with respect to increased bone mineral density and interference with bone mineralization. The objective of the study was to determine the anti-fracture efficacy of fluoride in low doses combined with an antiresorptive agent in postmenopausal women with osteoporosis who had been taking estrogen for ≥1 year. Serum fluoride was elevated in the MFP-treated group after 1 year of treatment without changes in other serum biochemistry parameters. There were progressive increases in BMD in the MFP-treated group as measured in the trabecular bone of L3 (49%), the lumbar spine (22%), and the femoral neck (4.6%), as well as in total body scans and their subregions (particularly trunk). The increases in BMD are well documented and confirm fluoride's anabolic action on bone. Bone formation markers (osteocalcin, P1NP, ALP) increased during the study in the MFP group whereas no change was observed in bone resorption (βCTX). Data values for years 2 and 3 were missing from the graphs for all bone markers except ALP, so the effects at theses time points are unclear. The hazards ratio for vertebral fractures was 0.20, suggesting decreased fracture risk, and 3.3 for non-vertebral fractures, suggesting increased fracture risk. However, the sample size for these events was small so the authors cited similar results from the literature to support the findings. Moreover, in a few subjects (n=7-9) bone biopsies in the MFP group indicated hyperosteoidosis in 5/7 subjects and osteomalacia in 2/7 subjects. Therefore, fluoride at 20 mg/day is beyond the therapeutic window due to interference with bone mineralization.
PROFILER'S ESTIM. NOEL/NOAEL		Study design was not suitable for development of a NOAEL.
PROFILER'S ESTIM. LOEL/ LOAEL		Study design was suitable for development of a LOAEL for bone mineral density and fracture risk. Fluoride at 20 mg/day increases BMD in the total body as well as in specific bones (trabecular bone of L3, the lumbar spine, and femoral neck) and subregions (e.g., trunk). Interference with bone mineralization contributing to fracture risk also occurs at 20 mg fluoride/day.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable (), Poor (X), Medium (), Strong () The study was well-conducted, but only one dose level was used (20 mg/day); the study design was conducive to contribute data for LOAEL for BMD and fracture risk together with other data from the literature. Effects were noted at 20 mg/day but a lower dose level also may increase fracture risk.
CRITICAL EFFECT(S):		Bone mineral density; osteomalacia

Riggs BL, Hodgson SF, O'Fallon WM, Chao EY, Wahner HW, Muhs JM, Cedel SL, Melton LJ III. 1990. Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. N Engl J Med. 322(12):802-9.

ENDPOINT STUDIED:	Skeletal fracture, bone mineral density
TYPE OF STUDY:	Prospective, randomized, double-blind, placebo-controlled clinical trial
POPULATION STUDIED:	101 white women, 50 to 75 years of age, with postmenopausal osteoporosis who were patients at the Mayo Clinic (Rochester, MN) received 75 mg sodium fluoride and 1500 mg elemental calcium per day.
CONTROL POPULATION:	101 white women, 50 to 75 years of age, with postmenopausal osteoporosis who were patients at the Mayo Clinic (Rochester, MN) received placebo tablets and 1500 mg elemental calcium per day.
EXPOSURE PERIOD:	The women were treated for 4 years.
EXPOSURE GROUPS:	The 202 patients who were enrolled in the study were fully ambulatory, postmenopausal women with documented osteoporosis but no evidence of an associated disease or a history of use of any drug known to cause osteoporosis. The criteria for osteoporosis were diffuse osteopenia on spinal x-rays, one or more vertebral fractures, and a bone mineral density (BMD) value for the lumbar spine below the normal range for premenopausal women. About 1000 patients or patients' medical records at the Mayo Clinic were screened for the above criteria to find volunteers to enroll in the study. At the time of recruitment, 153 of the women were receiving treatment for their osteoporosis: calcium supplements ± vitamin D ± estrogen; vitamin D ± estrogen. None of the women had ever received sodium fluoride or diphosphonate drugs. Before the start of the study, treatment was discontinued for three months in women receiving calcium supplements, vitamin D, or both and for six months in those receiving estrogen. The treatment group received 75 mg sodium fluoride and 1500 mg elemental calcium daily and the control group received placebo and 1500 mg elemental calcium daily.
EXPOSURE ASSESSMENT:	Treatment was by the oral route.
ANALYTICAL METHODS:	Serum and urinary calcium levels were measured by atomic-absorption spectrophotometry. Serum levels of phosphate, creatinine, and alkaline phosphatase and urinary levels of phosphate and creatinine were measured by routine methods. Serum bone Gla-protein (BGP, osteocalcin) and parathyroid hormone levels and urinary cyclic AMP were measured by radioimmunoassay. Serum and urinary fluoride levels were measured by a glass-electrode method. Urinary hydroxyproline levels were measured colorimetrically after fractionation by high performance liquid chromatography (HPLC). The glomerular filtration rate was estimated by measuring creatinine clearance. The BMDs of the lumbar spine, femoral neck, and femoral intertrochanteric region were measured by dual photon absorptiometry. The bone mineral content of the shaft of the radius was measured by single photon absorptiometry. Vertebral fractures from T4 through L5 were assessed by quantitative biplanar radiography. Total vertebral height and total lateral area of the vertebral bodies were summed and recorded as continuous variables. All measurements were made by a person unaware of the patient's treatment assignment.
STUDY DESIGN	The 202 women were randomly assigned to treatment or placebo groups, with 101 women in each group. The composition of the groups was balanced according to the number of vertebral fractures at base line, the BMD of the lumbar spine, the dosage of any previous estrogen treatment, and the interval after the discontinuation of previous treatment for osteoporosis. The treatment group received sodium fluoride (75 mg per day, given as 30-mg tablets three times daily and twice daily on alternate days) and the control group received a placebo tablet identical in appearance. Both groups received divided doses of calcium carbonate equivalent to 1500 mg of elemental calcium per day. The women were treated for 4 years, during which they were evaluated every six months. There was no formal exercise program, though they

	were encouraged to be active. Dietary calcium intake was assessed in interviews with a dietician and by review of a 7-day diet diary. Changes in calcium intake were evaluated at the end of the study. BMD measurements of the lumbar spine and radius were made at base line and every six months, and those for femur every two years. Roentgenography of the lumbar and thoracic vertebra was performed at base line and yearly. Blood and 24-hour urine samples were collected for various biochemical measurements. Side effects were assessed during evaluations, specifically gastric pain or distress, nausea or vomiting, joint pain, stiffness or swelling, neurological symptoms, and hair loss. When side effects occurred, fluoride treatment was stopped until the symptoms cleared and then treatment was resumed with a 15 mg reduction in the daily dose of fluoride or placebo (Profiler's note: composition of placebo was not reported).
PARAMETERS MONITORED:	Serum and urinary biochemical measurements, side effects, BMD, vertebral and nonvertebral fractures were monitored.
STATISTICAL METHODS:	Fracture rates and confidence limits were expressed as fractures per 100 person-years. Relative risk was defined as the ratio of the number of fractures per person-year in the patients receiving fluoride to those receiving placebo. Binary logistic analysis was used to assess differences between groups with respect to the number of women in whom new fractures developed during treatment. The dependent variable in this analysis was whether at least one new fracture occurred during treatment. Patients were grouped into categories according to the rate of occurrence of new fractures, and an ordered categorical analysis (proportional–odds model) with the logistic-regression model was performed. To assess the effect of treatment on the rate of occurrence of first new fractures, the Cox proportional-hazards model was used. Side effects were assessed by binary logistic regression and Cox proportional–hazards modeling. The rates of change in BMD (% change per year) at each site were calculated as the slope of a least-squares regression line of successive measurements of BMD for each patient, divided by its intercept and multiplied by 100. This ratio was used as the dependent variable in weighted multiple regression models to assess the effect of treatment on these rates of change. The weights were the variance estimates of the ratios. In each analysis, the results were adjusted for stratification variables and significant ($p<0.05$) covariates and interactions. Covariates included: base line values for age; number of years since menopause; height and weight; calcium intake; type of previous treatment; number of fractures; initial BMD at various sites; serum and urinary fluoride, calcium, phosphorous, and creatinine levels; serum parathyroid hormone, bone Gla-protein, and alkaline phosphatase levels; urinary excretion of cyclic AMP and hydroxyproline; and hemoglobin. In all analyses, the results are for all available data for the 202 patients who entered the trial, regardless of full 4 year completion of the study.
RESULTS:	
Bone mineral density	The following changes in BMD (or bone mineral content in the case of the radial shaft) were reported (Table created by Profiler from text results). The difference between treatment and placebo rates was significantly different ($p<0.0001$) from zero at all sites reported.

Site	**% Change per year (95% CI)**		**Rate difference (95% CI)**
	Fluoride	**Placebo**	
Lumbar spine	8.2 (5.5 to 10.9)	0.4 (-1.6 to 2.5)	7.8 (6.0 to 9.5)
Femoral neck	1.8 (-0.7 to 4.2)	-0.9 (-3.4 to 1.6)	2.6 (1.7 to 3.6)
Femoral intertrochanteric region	-1.8 (-1.4 to 5.1)	-0.7 (-3.7 to 2.3)	2.5 (1.1 to 4.0)
Radial shaft	1.8 (-3.3 to 0.3)	0.4 (-1.7 to 1.1)	-1.4 (-2.0 to 0.08)

Figures 2 and 3 were copied directly from Riggs et al. 1990. During the four years of the trial, there was a cumulative increase in BMD of 35% for the lumbar spine (Figure 2), 12% for the femoral neck, and 10% for the femoral intertrochanteric area, and a decrease of 4% for the radial shaft (Figure 3) in the fluoride group compared to the placebo group.

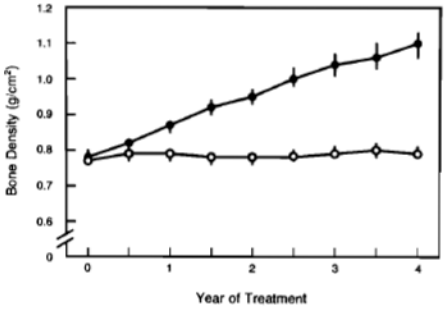


Figure 2. Mean (±SE) Bone Density of the Lumbar Spine in the Fluoride Group (Solid Circles) and the Placebo Group (Open Circles).

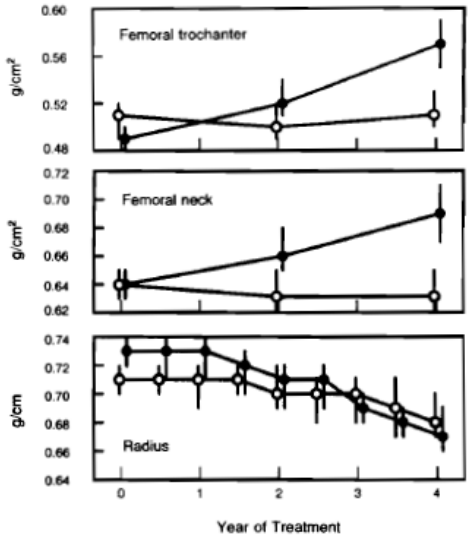


Figure 3. Mean (±SE) Bone Density of the Femoral Trochanter, Femoral Neck, and Radial Shaft in the Fluoride Group (Solid Circles) and the Placebo Group (Open Circles).

Skeletal fractures (nonvertebral)	Table 4 was copied directly from Riggs et al. 1990 and summarizes all nonvertebral fractures that occurred during treatment. In the fluoride group, a total of 72 fractures occurred in 61 women; of these, 40 fractures were complete and 32 were incomplete. In the placebo group, there were 24 fractures in 24 women; 22 were complete, and 2 were incomplete. The rates of fracture per 100 person-years in the fluoride group were 23.2, 12.9, and 10.3 for total, complete, and incomplete fractures, respectively; the rates in the placebo group were 7.4, 6.8, and 0.6, respectively. The fluoride-treated patients had nonvertebral fractures 3.2 times more often than those given placebo. The total nonvertebral fractures occurred more frequently ($p<0.02$) and in more women ($p<0.01$) in the fluoride group than the placebo group. The number of incomplete fractures ($p<0.0005$) and the number of women who had them ($p<0.0005$) were higher in the fluoride group than in the placebo group. There were no differences between groups for complete fractures.

Table 4. Nonvertebral Fractures during the Study in the Women with Osteoporosis in the Fluoride and Placebo Groups.

Site	Incomplete Fractures		Complete Fractures		Total Fractures	
	Fluoride	Placebo	Fluoride	Placebo	Fluoride	Placebo
	no. of patients (no. of fractures)					
Radius (Colles' fracture)	0 (0)	0 (0)	1 (1)	4 (4)	1 (1)	4 (4)
Humerus	0 (0)	0 (0)	5 (6)	1 (1)	5 (6)	1 (1)
Rib	1 (1)	0 (0)	10 (13)	8 (8)	11 (14)	8 (8)
Pelvis	3 (4)	0 (0)	3 (3)	1 (1)	6 (7)	1 (1)
Proximal femur	4 (5)	1 (1)	7 (8)	3 (3)	11 (13)	4 (4)
Tibia	10 (11)	0 (0)	2 (2)	0 (0)	12 (13)	0 (0)
Metatarsus or calcaneus	7 (10)	1 (1)	2 (2)	2 (2)	9 (12)	3 (3)
Other*	1 (1)	0 (0)	5 (5)	3 (3)	6 (6)	3 (3)
All sites	26 (32)	2 (2)	35 (40)	22 (22)	61 (72)	24 (24)
Relative risk (95% confidence interval)†	16.8 (3.9–71.7)		1.9 (1.1–3.4)		3.2 (1.8–5.6)	

*Other fractures involved the clavicle (two), the shaft or distal femur (two), and the small bones of the wrist or foot (two) in the fluoride group and the ulna, fibula, and hand in the placebo group.

†The fluoride group had 310 person-years of follow-up, and the placebo group had 325 person-years of follow-up. Patients were evaluated every six months for nonvertebral fractures.

Skeletal fractures (vertebral)	Table 5 was copied directly from Riggs et al. 1990 and shows the rates of vertebral fracture per person-year of follow-up, according to year of treatment and over the entire 4-year trial. The risk of vertebral fracture was similar in both groups. The 15% reduction in the number of women over the entire trial who had new vertebral fractures in the fluoride group compared to the placebo group was not significant ($p=0.32$).

Table 5. Vertebral Fractures during the Study Period in the Women with Osteoporosis in the Fluoride and Placebo Groups.*

Year	Fluoride				Placebo				Relative Risk (95% CI)
	No. of Patients	No. of Fractures	No. of Person-Years	Rate	No. of Patients	No. of Fractures	No. of Person-Years	Rate	
1	87	56	88.7	63.1	89	39	91.1	42.8	1.47 (0.4–5.5)
2	81	22	81.4	27.0	82	36	77.8	46.3	0.58 (0.3–1.1)
3	67	26	66.2	39.3	71	54	72.7	74.3	0.53 (0.3–1.0)
4	66	32	68.9	46.4	69	34	68.7	49.5	0.94 (0.5–1.8)
Entire period	301	136	305.2	44.6	311	163	310.3	52.5	0.85 (0.6–1.2)

*The number of vertebral fractures was assessed only at yearly visits. Rates shown are per 100 person-years. CI denotes confidence interval.

Figure 4 also was copied directly from Riggs et al. 1990 and shows the proportion of women who did not have new vertebral fractures during the treatment period. There were no significant differences with treatment to the time of first new vertebral fracture (proportion-hazards model, p=0.34; proportional-odds model, p=0.41).

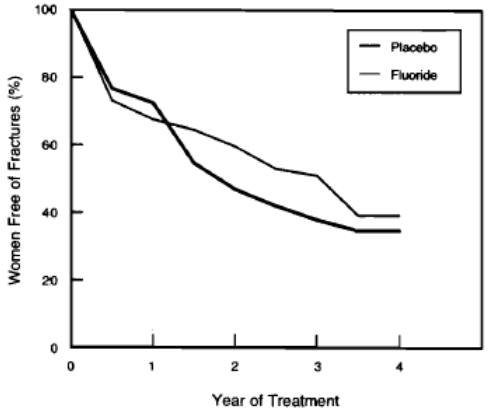


Figure 4. Proportion of Women in the Fluoride and Placebo Groups Who Did Not Have New Vertebral Fractures during the Four-Year Study Period.

In a proportional-hazards model of the time to the first fracture, with adjustment for the variables used as covariates to balance the composition of the groups at treatment assignment, the estimated relative risk in the fluoride group as compared with the placebo group was 0.82 (95 percent confidence interval, 0.55 to 1.23).

Clinical findings	The clinical characteristics of the 202 women at baseline are provided in Table 1. There were no significant differences between the two groups for covariates at baseline. Of the 202 enrolled, 135 (66 in the fluoride group, 69 in the placebo group) completed the 4-year trial. Reasons for discontinuation were side effects (n=10), personal reasons (39), development of other illnesses (10), death (6), or loss to follow-up (2). As assessed by tablet count, the median dosage of sodium fluoride at the end of the study was 71 mg/day. The dosage distribution was as follows: ≥75 mg/day (n=25), 6574 mg/day (36), 55-64 mg/day (18), <55 mg/day (13), not determined (9). The median dosage of supplemental calcium was 1329 and 1305 mg/day in the fluoride and placebo groups, respectively (not significant).

Table 1. Clinical Characteristics of the Women in the Fluoride and Placebo Groups at Base Line.

Characteristic	Fluoride	Placebo	P Value*
	median (range†)		
Age (yr)	68 (58–74)	68 (57.2–74)	0.96
Years since menopause	21.5 (9–33)	21 (10–35)	0.69
Years since diagnosis of osteoporosis	3.5 (0.2–12.9)	2.5 (0.2–11.7)	0.47
Years since initial vertebral fracture	4 (0.5–15.8)	3 (0.8–13.28)	0.16
Dietary calcium intake (mg/day)	961 (436–1510)	983 (419–1566)	0.76
Height (cm)	159 (149–166)	157 (149–165)	0.12
Weight (kg)	61 (49.6–75.5)	61 (50.2–77)	0.84
Vertebral fractures (median no./person)	4 (1–6)	3 (1–7)	0.72
Total vertebral area (cm^2)	82.0 (71.3–95.1)	82.8 (70.9–96.3)	0.94
Bone mineral density (g/cm^2)			
Lumbar spine	0.77 (0.60–0.98)	0.78 (0.59–0.99)	0.60
Femoral neck	0.66 (0.47–0.77)	0.64 (0.51–0.75)	0.43
Femoral intertrochanter	0.50 (0.38–0.63)	0.50 (0.37–0.64)	0.44
Bone mineral content of radius (g/cm^2)	0.72 (0.59–0.88)	0.68 (0.55–0.89)	0.20

*P<0.05 by the rank-sum test.
†Ranges shown are from the 10th to the 90th percentile.

Biochemical measurements

The biochemical characteristics of the 202 women at baseline are provided in Table 2.

Table 2. Biochemical Characteristics of the Women in the Fluoride and Placebo Groups at Base Line.

Characteristic*	Fluoride	Placebo	P Value†
	median (range‡)		
Serum calcium (mmol/liter)	2.42 (2.30–2.52)	2.40 (2.27–2.54)	0.76
Serum phosphate (mmol/liter)	1.19 (1.03–1.39)	1.16 (1.00–1.42)	0.23
Alkaline phosphatase (μkat/liter)	0.4 (0.3–0.6)	0.4 (0.2–0.5)	0.72
Bone Gla-protein (ng/ml)	7.7 (3.9–11.4)	7.4 (3.8–11.9)	0.68
Parathyroid hormone (ml-eq/liter)	36 (22–61)	35 (22–60)	0.68
Serum fluoride (μmol/liter)	1.5 (0.9–2.3)	1.4 (0.8–2.2)	0.26
Hemoglobin (g/liter)	139 (128–150)	139 (126–151)	0.63
Creatinine clearance (ml/min)	83.4 (56.3–114.1)	84.8 (59.5–111.7)	0.93
Urinary calcium (mmol/day)	3.1 (1.2–5.1)	3.1 (1.6–6.1)	0.53
Urinary phosphate (mmol/day)	20 (12–29)	21 (12–31)	0.28
Urinary fluoride (μmol/liter)	76 (39–127.4)	80 (36.3–131.6)	0.89
Cyclic AMP (nmol/dl of GF)	3.4 (2.24–5.07)	3.5 (2.22–5.45)	0.49
Urinary hydroxyproline (nmol/dl of GF)	19.8 (13.6–29.5)	20.3 (13.7–30.8)	0.97

*GF denotes glomerular filtrate.
†P<0.05 by the rank-sum test.
‡Ranges shown are from the 10th to the 90th percentile.

During the trial, the mean serum alkaline phosphatase concentration increased by 15.6 U/liter ($p<0.0001$) (Profiler's note: the units provided in the baseline table are not the same), and the mean serum bone Gla-protein concentration increased by 5.2 ng/liter ($p<0.0001$) in fluoride-treated women compared to those taking placebo. Serum and urinary fluoride levels increased ($p<0.0001$) in the fluoride group (Figure 1, copied directly from Riggs et al. 1990). Serum calcium, phosphorous, and parathyroid hormone concentrations, the urinary excretion of calcium, and the creatinine clearance did not change significantly.

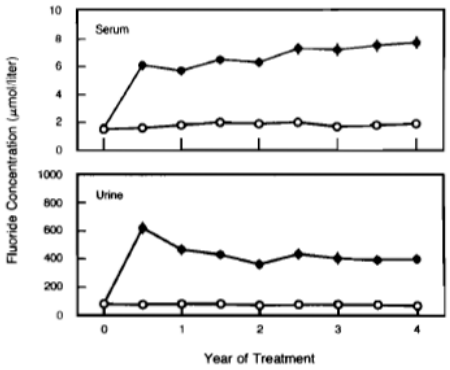


Figure 1. Mean (±SE) Serum Fluoride and Urinary Fluoride Levels in the Fluoride Group (Solid Circles) and the Placebo Group (Open Circles).

Safety

Table 3 was copied directly from Riggs et al. 1990 and summarizes the side effects of women in the study. Fifty–four of the women receiving fluoride and 24 women receiving placebo had side effects during the study that were severe enough to require a decrease in dosage. In the fluoride and placebo groups, 65% and 26%, respectively, had side effects during the 4-year period (Kaplan-Meier method). After adjustment for stratification variables and covariates, more women receiving fluoride had side effects than did those receiving placebo ($p<0.0001$). The fluoride-treated women had about 3 times as many side effects as women receiving placebo. The side effects were categorized as gastric irritation or pain in the lower extremities. The gastric symptoms consisted of nausea, epigastric pain and/or vomiting and were 2.9 times more frequent in the fluoride group than placebo group. The episode of lower extremity pain developed relatively acutely, associated at times with local pain and erythema and sometimes requiring the use of crutches, and was 9.9 times more frequent in fluoride-treated women than in those receiving placebo.

Table 3. Untoward Symptoms and Signs in the Women with Osteoporosis in the Fluoride and Placebo Groups.

Symptom	No. of Patients		No. of Episodes		Relative Risk (95% CI)*
	Fluoride	Placebo	Fluoride	Placebo	
Gastric pain, nausea, vomiting	17	7	22	8	2.88 (1.2–7.1)
Gastrointestinal bleeding, anemia	9	9	10	12	0.87 (0.3–2.3)
Lower-extremity pain	37	5	47	5	9.85 (4.0–24.2)
Bone spurs	3	4	3	4	0.79 (0.2–3.4)
Fracture, nonunited	1	0	1	0	—
Any of the above†	54	24	83	29	3.00 (1.9–4.8)

*The fluoride group had 310 person-years of follow-up, and the placebo group had 325 person-years of follow-up. Patients were evaluated every six months for side effects. CI denotes confidence interval.

†Some patients had more than one untoward symptom.

STUDY AUTHORS' CONCLUSIONS:

The data from this study suggests that fluoride treatment increases cancellous bone mass (e.g., lumbar spine), but deceases cortical-bone mass (e.g., radial shaft) and increases skeletal fragility. Combined, these effects result in a slight trend toward a decrease in fracturing of the vertebral bodies (predominately composed of cancellous bone) but an increase in fracturing at sites containing either predominantly cortical bone or similar amounts of cortical and cancellous bone. Thus, although there was an increase in cancellous-bone mass, the findings do not support the clinical efficacy of the fluoride-calcium regimen for the treatment of osteoporosis.

DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)

None to report.

PROFILER'S REMARKS *Initials/date SJG/12/15/07*

This prospective, randomized, controlled, double-blind study followed 202 postmenopausal women with osteoporosis who were treated with sodium fluoride or placebo over the course of 4 years. The study found

		that when compared to women in the placebo group, women treated with 75 mg/day of fluoride had more side effects (placebo: 24; fluoride: 54 women), including gastric symptoms and lower extremity pain; increased serum alkaline phosphate and bone Gla-protein, and serum and urinary fluoride levels; increased BMD in the lumbar spine (35%; predominately cancellous bone), femoral neck (12%), and femoral intertrochanteric region (10%; both sites of mixed cortical and cancellous bone); decreased bone mineral content in the radial shaft (-4%; predominately cortical bone); and increased incidence of nonvertebral fractures (placebo, 24; fluoride: 72 fractures). The slight decrease in the relative risk (0.85) of vertebral fractures in the fluoride group compared to the placebo group was not significant. Thus, although bone mass increased, particularly in the lumbar spine, the risk of nonvertebral fractures also increased. One-third (67) of the patients failed to complete the full treatment period; however, the dropout rate was similar in both groups and the data analysis included all randomized patients, so the impact was unlikely to bias the results. Because there have been conflicting conclusions from other studies, the conclusions drawn here are for the conditions of this study only.
PROFILER'S ESTIM. NOEL/NOAEL		Study design was not suitable for development of a NOAEL.
PROFILER'S ESTIM. LOEL/ LOAEL		Study design was suitable for development of a LOAEL for bone mineral density and fracture risk. Fluoride at 75 mg/day increases BMD in sites with predominately or mixed cancellous bone (lumbar spine, femoral neck, femoral intertrochanteric region). Nonvertebral fracture risk also occurs at 75 mg fluoride/day.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable (), Poor (X), Medium (), Strong () The study was well-conducted, but only one dose level was used (75 mg/day); the study design was conducive to contribute data for LOAEL for BMD and fracture risk. Effects were noted at 75 mg/day but a lower dose level also may increase fracture risk.
CRITICAL EFFECT(S):		Skeletal fractures, bone mineral density

Sauerbrunn BJ, Ryan CM, Shaw JF. 1965. Chronic fluoride intoxication with fluorotic radiculomyelopathy. Ann Intern Med. 63(6):1074-8.

ENDPOINT STUDIED:	Skeletal fluorosis and Fluorotic radiculomyelopathy
TYPE OF STUDY:	Case report
POPULATION STUDIED:	64-year old white male with the following history: birth to 7 years in Calhoun, GA; 43 years on farms in southwestern Ellis County and the Grand Prairie area of Dallas County, TX, drinking well water containing 2.4 to 3.5 ppm fluoride; at age 50, he moved to the Grapevine area of Tarrant County, TX. The patient suffered from persistent polydipsia.
CONTROL POPULATION:	Not applicable in case report.
EXPOSURE PERIOD:	The patient's exposure from birth until age 7 was unknown, but records showed the water supply in Calhoun, GA was not artificially fluoridated at the time he lived there. Drinking water seems to have been his only source of fluoride intake, with exposure for 43 years to water with fluoride concentrations ranging from 2.4 to 3.5 ppm.
EXPOSURE GROUPS:	A 64-year old white male was admitted to Veterans Administration Hospital in McKinney, TX on May 11, 1962 because of severe respiratory distress and semi-coma. He had been a complete invalid and in a nursing home for a year.
EXPOSURE ASSESSMENT:	Medical records, including physical examinations, laboratory findings and x-rays, were evaluated from hospitalizations at McKinney in 1950, 1954, 1955, 1959, 1961 and finally in 1962. An autopsy was performed upon death. Medical history was obtained by questioning the patient's sister who had lived with him for 35 years. The records of the patient's brother were reviewed following admission to the VA hospital at McKinney in 1955 at age 61.
ANALYTICAL METHODS:	Records from the GA State Department of Health showed the water supply in Calhoun, GA was not artificially fluoridated until 1957; natural fluoride content was not reported. Data from the TX State Department of Health on chemical analyses of public water systems (1953- 1959) showed that wells in southwestern Ellis County may contain up to 3.5 ppm fluoride; in the Grand Prairie area, 2.8 ppm (90% of wells above 2.4 ppm); and in the Grapevine area, 2.4 ppm in wells and 2.2 ppm in the distribution system. Analytical methods were not reported.
STUDY DESIGN	A 64-year old white male was admitted to Veterans Administration Hospital in McKinney, TX on May 11, 1962 because of severe respiratory distress and semi-coma. He had been a complete invalid and in a nursing home for a year. Medical records, including physical examinations, laboratory findings and x-rays, were evaluated from hospitalizations at McKinney from 1950 until his final admission in 1962. An autopsy was performed upon death, with attention to the skeletal system, urinary system, brain and lung. Medical history, including potential fluoride exposure, was obtained by questioning the patient's sister who had lived with him for 35 years. Drinking water seems to have been his only source of fluoride intake, with exposure for 43 years to water with fluoride concentrations ranging from 2.4 to 3.5 ppm. The records of the patient's brother were reviewed for comparison.
PARAMETERS MONITORED:	The following parameters were monitored: skeletal x-rays; neurological signs (e.g., pain, cramping, stiffness, or weakness of limbs or joints; Babinski and Hoffmann's reflexes; muscular atrophy); laboratory tests (e.g., serum calcium and phosphorus, acid phosphatase, alkaline phosphatase, nonprotein nitrogen, blood urea nitrogen, creatinine; cerebrospinal fluid examination and electroencephalogram, and renal function tests); and bone and liver fluoride content upon death.
STATISTICAL METHODS:	Statistical analysis was not performed on this one patient.

RESULTS:	
Physical Examination	In 1950, the patient complained of pain and cramping in the left leg and of weakness and stiffness of all limbs for several years. He had polydipsia and polyuria for at least 20 years. Physical findings included moderate kyphosis, stiffness of the knee and ankle joints, spastic weakness of all extremities, and ankle and patellar clonus. Bilateral Bibinski's and Hoffmann's signs were present. Hypalgesia was found over both feet. Fasciculations were noted in both shoulders and arms. A cystometrogram revealed a hypertonic bladder and spastic external sphincter. The patient's fluid intake and output of urine varied from 4 to 10 liters/24-hour period. (Profiler note: it is unclear whether this amount of fluid intake was only during the hospital admission period or an indication of past consumption over the patient's lifetime). The principal disorder was thought to be due to amyotrophic lateral sclerosis. In 1954, the patient was readmitted due to acute epididymitis and intermittent retention of urine of 1 month's duration. His bladder was distended and the neurological signs of spastic quadraparesis had increased since 1950. The patient's neurological disorder was thought to be caused by spinal cord compression due to extrinsic pressure from an unidentified disease of bone. The polyuria was considered to represent diabetes insipidus. Admissions in 1955, 1959, and 1961 showed increasing functional urinary obstruction and progression of crippling skeletal disease. On the final admission in 1962, the patient was critically ill with pneumonia and shock; his condition deteriorated and he died on the third day after admission.
Skeletal findings	Skeletal x-rays in 1950 revealed increased size and density of long bones and of the vertebrae. Calcification of the paravertebral and sacrospinous ligaments was noted. Skull films showed a normal sella turcica and diffuse internal hyperostosis with obliteration of diploe. Minimum to moderate narrowing of the foramen magnum was described. X-rays in 1954 showed increasing bone density. Autopsy revealed the following: the sternum, calvarium, and vertebrae were extremely dense. There was no spongy bone between the tables of the skull; the cranial vault was 2.5 cm thick and uniformly white and dense throughout. The anterior fosse showed massive protuberances of bone. The clinoid processes were white, thick, and homogenous. The vertebral column was massive. The spinal cord showed no gross abnormalities. Multiple sections of vertebral bone showed marked thickening of trabeculae. The marrow spaces were markedly narrowed. Osteoblastic reaction was noted in several areas.
Laboratory findings	Laboratory findings in 1950: serum calcium, 9.7 to 11.6 mg/100 ml; serum phosphorus, 3.1 to 3.7 mg/100 ml; acid phosphatase, 0.3 Bodansky units/100 ml; alkaline phosphatase, 3.1 Bodansky units/100 ml; nonprotein nitrogen, 29 to 54 mg/100 ml; blood urea nitrogen, 11 to 12 mg/100 ml; creatinine, 1.9 mg/100 ml; a phenolsulphonphthalein test showing 36% excretion in the first 30 minutes and 52% in 1 hours. A Fishberg concentration test showed a maximum specific gravity of 1.008. Maximum urea clearance was 44 ml/min. Cerebrospinal fluid examination and electroencephalogram showed normal findings. Laboratory findings in 1955, 1959, and 1961: Serum calcium, phosphorous and potassium levels were normal in each of these admissions. In 1961, serum alkaline phosphatase was elevated on three occasions at 15.1, 11.3, and 12.3 Bodansky units/100 ml. Serum creatinine was 2.54 mg/100ml. Maximum urine specific gravity was 1.010. Autopsy revealed the following: The pituitary gland, anterior and posterior lobes, and parathyroids appeared normal. A culture of the lung revealed *Staphylococcus aureus*. The bladder, ureters and calyces were dilated. Acute inflammatory changes were noted in the renal pelves. Chronic inflammation was noted in the renal parenchyma, and hyaline casts were seen in dilated tubules.
Bone and liver fluoride content	A toxicological study (from the laboratory of Morton Mason, University of Texas Southwestern Medical School, Dallas, Texas) indicated an elevated bone fluoride content of 610 mg/100 g of dried bone and a liver fluoride content of 6.1 mg/100 g of dried liver. The analytical method was a microdiffusion method for determination of fluorides, according to Frere (1962).

	PROFILER'S NOTE: The normal values for fluoride content ion the bone and liver were not presented for comparison.
Supporting evidence of fluoride exposure	The patient's brother was admitted to the VA hospital at McKinney in 1955 at age 61. A review of records and x-rays of the spine showed evidence of osteoarthritis but none of osteosclerosis. His teeth were described as discolored, pitted, ridged, and worn, but there was no evidence of caries. His sister, who lived with him for 35 years, also had mottled teeth.
STUDY AUTHORS' CONCLUSIONS:	The patient reported here showed most of the features of the cases of fluorotic radiculomyelopathy reported from India by Singh et al. (1963) and Siddiqui (1955), notably crippling fluorosis with marked neurological complications. Acroparesthesias, root pain, and muscular wasting are attributed to compression of anterior roots. Spastic weakness, exaggerated deep tendon reflexes, and extensor plantar reflexes follow spinal cord compression. Patchy sensory changes and occasionally a definite sensory level are found. The laboratory findings indicated defective renal concentrating function, possibly as a consequence of functional urinary tract obstruction resulting from cord compression. Abnormalities of bladder function have been noted by others and are not unusual when advanced fluorosis is complicated by radiculomyelopathy. In the post-mortem inquiry, no evidence was found to suggest self-medication, industrial exposure, or dietary idiosyncrasy. Drinking water with fluoride concentrations from 2.4 to 3.5 ppm for 43 years seems to have been his only source of fluoride intake. Although these levels of fluoride have not been thought to result in clinically detectable fluorosis except for mottled teeth, this relationship appears to be for individuals with normal water consumption. The risk and degree of fluorosis may also depend on the quantity of water consumed. This is suggested by the findings in this patient who developed severe crippling fluorosis while his brother, who drank the same water, showed only mottling of the teeth. The brothers were exposed to the same water for the same period of time, had the same diet, lived under similar environmental conditions, but differed by the excessive water intake by this patient. The bone content of fluoride in this patient is also much greater than the amount predicted from the fluoride content of his drinking water (Profiler's note: normal value not indicated). Thus, it appears that the probable cause for fluoride intoxication was prolonged polydipsia. Prolonged polydipsia may be hazardous to persons living in areas where the levels of fluoride in drinking water are not those usually associated with significant fluorosis.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)	Frere, FJ. 1962. A microdiffusion method for determination of fluorides. *Microchem. J*. 6: 167. Singh A, Jolly SS, Bansal BC, Mathur CC. 1963. Endemic fluorosis. Epidemiological, clinical and biochemical study of chronic fluorine intoxication in Panjab (India). *Medicine* 42:229-46.
PROFILER'S REMARKS ***Initials/date SJG/ 11/12/07***	The study design does not aid in the development of a dose response to fluoride with respect to skeletal fluorosis. The objective of the study was to report the case of fluorotic radiculomyelopathy in a single patient with prolonged and excessive ingestion of water with a fluoride concentration of 2.4 to 3.5 ppm. The patient's symptoms and neurological deficits are presented well and x-ray examination provides evidence of increased vertebral size, marked osteoarthritis, and characteristic calcification in the distal portion of the both sacrospinous ligaments. Autopsy confirmed increased bone density and bone protuberances; thickening of trabeculae and narrowed marrow spaces; and elevated bone and liver fluoride content. Decreased renal function is evident and, together with excess water intake (polydipsia), may have increased the risk of fluorosis. Although the case is interesting and novel, it has some limitations. The authors do not explain the rationale behind the chosen laboratory tests, nor do they present normal values for fluoride in bone and liver for comparison. Furthermore, it is not stated whether or not the patient also had mottled teeth, indicative of fluoride exposure and dental fluorosis, like his siblings.
PROFILER'S ESTIM. NOEL/NOAEL	Study design was not suitable for development of a NOAEL.

PROFILER'S ESTIM. LOEL/ LOAEL	Study design was not suitable for development of a LOAEL.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:	Not suitable (X), Poor (), Medium (), Strong () The study presented a rare case of fluorotic radiculomyelopathy as a progression of skeletal fluorosis in one patient with prolonged exposure (43 years) to fluoride in the drinking water at 2.4 to 3.5 ppm.
CRITICAL EFFECT(S):	Skeletal fluorosis and radiculomyelopathy

Sowers, M., Whitford, G.M., Clark, M.K., and Jannausch, M.L. 2005. Elevated Serum Fluoride Concentrations in Women Are Not Related to Fractures and Bone Mineral Density. J Nutr. 135: 2247-2252.

ENDPOINT STUDIED:	Skeletal fractures; bone mineral density; serum fluoride levels
TYPE OF STUDY:	Prospective cohort
POPULATION STUDIED: (High-fluoride)	526 women (54.8±0.80 years old) residing in a small, predominately Caucasian, American (USA) community with high-fluoride concentration (210.4 μmol/L) and below national average calcium concentration (0.375 mmol/L) in the water supply.
POPULATION STUDIED: (High-calcium)	406 women (54.1±0.91 years old) residing in a small, predominately Caucasian, American (USA) community with high-calcium concentration (9.375 mmol/L) in the water supply and fluoride levels (52.6 μmol/L) consistent with the national average.
CONTROL POPULATION:	368 women (55.9±0.96 years old) residing in a small, predominately Caucasian, American (USA) community with fluoride (52.6 μmol/L) and calcium (1.500 mmol/L) concentrations in the water supply consistent with the national average.
EXPOSURE PERIOD:	The exposure period was based on the number of years residing in each community (categorized into tertiles: 0-13 years; 14-27 years; 27-79 years). Fluoride levels in the 210.4 μmol/L communities have been augmented by water treatment since 1958. Bone fracture incidence and site were recorded every 6 months for 4 years.
EXPOSURE GROUPS:	Data collection was initiated in 1300 women, aged 20-92 years, living in 3 communities with diverse mineral content in the water supplies. The communities were similar with respect to size (<2000 residents per community), racial and ethnic mix (>95% Caucasian), mean income, and primary occupations. A community census identified women ≥18 years old who were ambulatory (able to climb 3 steps without assistance), and able to provide informed consent. There were no additional selection criteria. Study participation rates among eligible women were 70%, 79%, and 81% in the high-fluoride, high-calcium, and control communities, respectively. There was no significant difference in mean ages among the 3 communities. The fluoride and calcium concentrations in each community were as follows: *High-fluoride community*: F: 210.4 μmol/L = 4 mg/L; Ca: 0.375 mmol/L = 15 mg/L *High-calcium community*: F: 52.6 μmol/L = 1 mg/L; Ca: 9.375 mmol/L = 375 mg/L *Control community*: F: 52.6 μmol/L = 1 mg/L; Ca: 1.500 mmol/L = 60 mg/L The fluoride content in the high-fluoride community was naturally occurring due to the geology of the area. In the other communities, fluoride levels were augmented by water treatment. The communities are small, so blending and processing of waters from more than one source do not occur.
EXPOSURE ASSESSMENT:	Individual serum fluoride concentrations and bone mineral density (BMD) of the femoral neck, lumbar spine, and distal radius were measured. Self-reported fractures were confirmed by medical record abstraction. Fluoride intakes were based on reported water and water-based beverage consumption and duration of residence in the community. Additional sources of calcium intake (water, diet, and supplement) were considered.
ANALYTICAL METHODS:	The University of Iowa Hygienic Laboratory, the state public health laboratory, has monitored the calcium and fluoride concentrations in these communities since 1938; the concentrations have varied only slightly over that period. The analytical methods were not

	reported.
STUDY DESIGN	This study relates serum fluoride concentrations, which reflect individual fluoride exposures, to BMD and bone fractures. The study population consisted of 1300 female residents, ≥18 years old, of 3 small, predominately Caucasian, communities in which the water fluoride and calcium concentrations were as follows: *control*: 52.6 µmol F/L; 1.50 mmol Ca /L; *high-calcium*: 52.6 µmol F/L; 9.375 mmol Ca/L; or *high-fluoride*: 210.4 µmol F/L; 0.375 mmol Ca/L. Subjects were interviewed for fluoride and calcium intake. Fluoride intakes were based on water and water-based beverage consumption and duration of residence in the community. Additional sources of calcium intake (water, diet, and supplement) were considered. Serum fluoride and osteocalcin concentrations, bone mineral density (BMD), and fracture incidence and site were assessed as follows: Serum fluoride was analyzed using an ion-specific electrode (Model 9409, Orion Research) and a miniature calomel reference electrode coupled to a potentiometer after overnight diffusion using a modification of the hexamethyldisiloxane-facilitated diffusion of Taves (Taves 1969, Whitford 1996). The CV was <5%. Serum osteocalcin concentrations were measured using the Instar™ RIA (radio-immunoassay). The inter- and intra-assay variation was <10%. BMD of the femoral neck and lumbar spine and total body bone calcium were measured by dual X-ray densitometry (DEXA-Lunar; DPX-L™, analysis software version 1.3y). BMD of the distal radius was measured using single-photon densitometry. Measurements of BMD at the various sites allowed for assessment of different effect of fluoride in bone that is more cortical, as in the radius, or more trabecular, as in the lumbar spine. Calibration was performed daily and a lumbar spine phantom was scanned weekly. The CV for DEXA was <1.5% for the femoral neck site. Participants reported the site of any bone fracture, date of occurrence and, if appropriate, the facility where the fracture was treated using a postal card every 6 months for 4 years. Fracture status was confirmed at treatment facilities by abstracting medical records and securing available copies of images. Other measures included: height and weight to calculate body mass index (BMI; (weight (kg)/height (m^2)); self-reported menopause status (≥12 months of amenorrhea); medication use; and total time (min) per week of activity.
PARAMETERS MONITORED:	Individual serum fluoride concentrations were analyzed by ion-specific electrode. Serum osteocalcin concentrations were measured by radio-immunoassay (RIA). Bone mineral density (BMD) of the femoral neck and lumbar spine (predominately trabecular bone) and total body bone calcium were measured by dual X-ray densitometry. BMD of the distal radius (predominately cortical bone) was measured using single-photon densitometry. Self-reported fractures were confirmed by medical record abstraction.
STATISTICAL METHODS:	Continuous variables were evaluated for normality and transformations undertaken when appropriate. General linear models were used to estimate group means and test for pair-wise significant differences between groups. To show the association of serum fluoride with duration of residence in the community, serum fluoride values were categorized into quartiles with the lowest quartile acting as the reference category. Duration of residence was classified into tertiles (1-13, 14-26, 27-77 years) with the first category acting as the reference. Serum fluoride concentrations were calculated for each cell and compared using ANOVA. Multiple variable regression models were fit with quadratic terms centred about their means. These models were built by identifying the relation between fluoride exposure and BMD and then including other variables (e.g., age, BMI, and menopause status) based on p-values for individual terms <0.05. Logistic regression analyses were used to assess the association between risk of osteoporotic fractures and serum fluoride concentration, BMD, age, body size, and medications. In all regression analyses, serum fluoride was a log-transformed

	continuous variable; indicator variables represented the high-calcium and high-fluoride communities, with the control community as the reference.
RESULTS:	
Serum fluoride	Tables 1 and 2 were copied directly from Sowers et al. (2005). Table 1 summarizes the characteristics of the study populations according to water mineral content. Serum fluoride concentrations were highest in the high-fluoride community. The mean serum fluoride concentration was 32% greater in the high-fluoride community (2.11 μmol/L) compared with that in the control community (1.60 μmol/L) and 73% greater than that in the high-calcium-community (1.22 μmol/L).

TABLE 1

Characteristics of women, aged 20–92 y, in communities according to water mineral concentration, with comparisons across community designations[1]

	Community			
Variable	Control	High-calcium	High-fluoride	*P*-value
n	368	406	526	
Age, *y*	55.9 ± 0.96	54.1 ± 0.91	54.8 ± 0.80	NS[2]
Serum fluoride, *μmol/L*	1.60 ± 0.04[b]	1.22 ± 0.05[c]	2.11 ± 0.05[a]	0.0001
Daily calcium intake, *mg*	754 ± 20[b]	1001 ± 25[a]	679 ± 16[c]	<0.0001
Daily water fluoride intake, *μmol/L*	63.65 ± 2.63[b]	40.50 ± 1.58[c]	192.52 ± 6.84[a]	<0.0001
BMD, *g/cm²*				
Lumbar spine	1.179 ± 0.0110	1.197 ± 0.0104	1.195 ± 0.009	NS
Femoral neck	0.914 ± 0.0084	0.912 ± 0.0083	0.912 ± 0.007	NS
Distal radius	0.651 ± 0.0053[b]	0.656 ± 0.0057[ab]	0.667 ± 0.004[a]	0.05
BMI, *kg/m²*	27.85 ± 0.33	28.56 ± 0.31	28.30 ± 0.27	NS
Osteocalcin, *nmol/L*	0.385 ± 0.012[c]	0.446 ± 0.011[a]	0.434 ± 0.010[b]	0.0005
Fracture, *n (%)*				
Osteoporotic	5 (1.4)	14 (2.3)	15 (2.9)	0.01
Nonosteoporotic	11 (3.2)	14 (3.4)	16 (3.1)	NS
Current smoking, *n (%)*	31 (10.8)	51 (16.2)	58 (15.4)	0.02
Thiazide antihypertensive, *n (%)*	22 (7.7)	22 (7.0)	38 (10.1)	0.02
Use in previous 12 mo, *n (%)*	13 (4.5)	16 (5.1)	23 (6.1)	0.01
Hormone replacement, *n (%)*	2 (0.7)	17 (5.4)	16 (4.3)	0.01
Physical activity, *n (%)*				<0.0001
<40 min/wk	98 (27)	79 (19)	248 (47)	
40–150 min/wk	142 (38)	137 (34)	156 (30)	
>150 min/wk	128 (35)	190 (47)	122 (23)	

[1] Values are means ± SE or *n* (%). Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] NS, nonsignificant; $P \geq 0.05$.

Table 2 ranks serum fluoride concentrations related to years of residence in each community. Serum fluoride concentrations increased with greater years of residency in the high-calcium community (4th quartile) and in the high-fluoride community (3rd quartile).

TABLE 2

Quartiles of serum fluoride concentration related to years of residence in the community (by tertiles) according to community water supply mineral concentration[1]

	Serum fluoride concentration			
Years of residence (tertiles)	Quartile 1 <25%	Quartile 2 25–50%	Quartile 3 50–75%	Quartile 4 >75%
		μmol/L		
Control community				
T1 0–13 (30%)	0.76 ± 0.04	1.32 ± 0.03	1.68 ± 0.03	2.60 ± 0.18
T2 13–27 (31%)	0.80 ± 0.04	1.29 ± 0.02	1.69 ± 0.02	2.58 ± 0.14
T3 27–77 (38%)	0.79 ± 0.04	1.33 ± 0.02	1.73 ± 0.02	2.54 ± 0.11
High-calcium community				
T1 0–13 (38%)	0.55 ± 0.02	0.92 ± 0.02	1.23 ± 0.02	1.84 ± 0.13*
T2 13–27 (31%)	0.57 ± 0.02	0.89 ± 0.02	1.27 ± 0.02	2.27 ± 0.13
T3 27–79 (30%)	0.55 ± 0.03	0.93 ± 0.02	1.27 ± 0.02	2.24 ± 0.10
High-fluoride community				
T1 0–13 (33%)	0.84 ± 0.03	1.47 ± 0.03	2.09 ± 0.05	3.87 ± 0.33
T2 13–27 (33%)	0.91 ± 0.04	1.43 ± 0.03	2.15 ± 0.04	3.78 ± 0.24
T3 27–78 (33%)	0.91 ± 0.04	1.45 ± 0.03	2.27 ± 0.04**	3.97 ± 0.18

[1] Values are means ± SE, total $n = 1300$. * Different from T2, * $P < 0.01$. ** Different from T2, $P < 0.001$.

PROFILER'S NOTE: The profiler agrees that the serum fluoride concentrations in the high-fluoride community were higher than the values from the control and high calcium communities; however, serum fluoride levels do not appear to be related to the number of years of residency in the communities. If serum fluoride concentrations increased with the number of years of exposure in a given community, greater values in each serum fluoride quartile in the 27-77 year tertile compared to the 0-13 or 13-27 year tertiles would be expected. Only one quartile in each of the high-calcium (4th quartile) and high-fluoride (3rd quartile) communities showed statistical significance, thus the toxicological relevance is

	questionable.
Bone mass density (BMD)	The BMD of the distal radius, mainly cortical bone, was significantly higher (2.5%) in the high-fluoride community compared with the control community. BMD of the lumbar spine or femoral neck did not differ among communities (Table 1). Table 3 was copied directly from Sowers et al. (2005) and lists results from multiple-variable regression models relating serum fluoride and BMD. There were no statistically significant associations of serum fluoride concentrations and BMD measures. Other variables (age, BMI, osteocalcin concentration, thiazide antihypertensive use, oral contraceptive use, hormone therapy use, menopause status and physical activity) explained 50% of the variation in the BMD at the femoral neck and distal radius, and 32% of the variation in the BMD at the lumbar spine. **TABLE 3** *β-Coefficients and variance (R^2) from multiple-variable regression models relating serum fluoride and BMD of the femoral neck, radius, and lumbar spine*[1] Femoral neck BMD model (R^2 = 50%) / Radius BMD model (R^2 = 51%) / Lumbar spine BMD model (R^2 = 32%) Variable — β — *P*-value — β — *P*-value — β — *P*-value Serum fluoride[2] — 0.011 ± 0.0072 — 0.13 — 0.005 ± 0.006 — 0.37 — 0.019 ± 0.0121 — 0.12 High calcium community — — — 0.020 ± 0.008 — 0.011 — 0.028 ± 0.015 — 0.07 High fluoride community — — — 0.018 ± 0.007 — 0.011 — — — Age — −0.0056 ± 0.0003 — 0.0001 — −0.008 ± 0.003 — 0.005 — 0.0001 ± 0.0001 — 0.004 Age × age quadratic term — 0.0001 ± 0.0001 — 0.0049 — — — — −0.014 ± 0.0056 — 0.01 BMI[2] — 0.2742 ± 0.0179 — 0.0001 — 0.07 ± 0.014 — 0.0001 — 0.256 ± 0.029 — 0.0001 Osteocalcin[3] — −0.0513 ± 0.0097 — 0.0001 — −0.050 ± 0.008 — 0.0001 — −0.068 ± 0.016 — 0.0001 Natural postmenopause — — — — — −0.07 ± 0.013 — 0.0001 — −0.1237 ± 0.028 — 0.0001 Surgical menopause — — — — — −0.08 ± 0.013 — 0.0001 — −0.1361 ± 0.029 — 0.0001 Thiazide use — — — — — — — — 0.048 ± 0.021 — 0.03 Current hormone use — — — — — 0.021 ± 0.016 — NS[4] — 0.072 ± 0.03 — 0.02 Oral contraceptive use — −0.038 ± 0.0189 — 0.04 — — — — −0.077 ± 0.03 — 0.01 Moderate physical activity — — — — — −0.017 ± 0.007 — 0.02 — — — High physical activity — — — — — −0.017 ± 0.007 — 0.02 — — — Age × postmenopause — — — — — −0.005 ± 0.0007 — 0.0001 — −0.008 ± 0.0028 — 0.004 Age × surgical menopause — — — — — −0.004 ± 0.0007 — 0.0001 — −0.005 ± 0.0025 — 0.05 Age × BMI — — — — — 0.003 ± 0.0008 — 0.0002 — 0.004 ± 0.0016 — 0.008 Age × osteocalcin — — — — — −0.001 ± 0.0004 — 0.006 — — — [1] Values are β-coefficients ± SE. [2] Values were log-transformed. [3] Expressed in μg/L square-root transformed, where 1 μg/L = 0.171 nmol/L. [4] NS, nonsignificant; $P \geq 0.05$. PROFILER'S NOTE: Although statistically insignificant, femoral neck and lumbar spine BMD was weakly associated with serum fluoride levels (β-coefficient 0.011, p=0.13; and β-coefficient 0.019, p=0.12, respectively). It is unclear whether smoking status, which differed among communities, was included in the model.
Skeletal fractures	Table 5 was copied directly from Sowers et al. (2005) and lists the association of fluoride-based measures and osteoporotic fracture. Fluoride measures were not associated with a greater risk of osteoporotic fracture after adjustment for BMD levels. Although there was an association of osteoporotic fracture frequency with type of community (see Table 1), this association was no longer significant after adjustment for covariates. Calcium intake was not predictive of osteoporotic fracture. Greater femoral neck BMD was associated with a lower fracture risk. **TABLE 5** *Association of fluoride-based measures and osteoporotic fracture after adjustment for BMD, site, and age (not shown) based on logistic regression modeling* Model — Variable — Reference unit — Risk ratio — Variable *P*-value — Model *P*-value 1 — Log of serum fluoride — μmol/L — 1.16 — 0.66 — 0.001 Femoral neck BMD — g/cm^2 — 0.008 — 0.0024 Calcium intake (diet + water) — mg/d — 0.999 — 0.11 Age — y — 1.01 — 0.49 2 — Duration of community residence — y — 1.03 — 0.73 — 0.001 Femoral neck BMD — g/cm^2 — 0.005 — 0.0001 High-calcium community — Control community — 3.01 — 0.04 High-fluoride community — — 2.55 — 0.07 PROFILER'S NOTE: The risk ratio (RR) was 1.16 (p=0.66) for osteoporotic fracture versus log of serum fluoride. RR was 2.55 for osteoporotic fractures in the high-fluoride community, but this association was not significant after adjustment for covariates (p=0.7). RR for osteoporotic fractures was 3.01 (p=0.04) in the high-calcium community.
Serum osteocalcin	Osteocalcin concentrations were 13 and 16% higher in women living in the high-calcium

		and in the high-fluoride communities, respectively, compared with women in the control community (Table 1).
STUDY AUTHORS' CONCLUSIONS:		This study measured multiple variables including serum fluoride concentration, fluoride exposure, assessment of bone metabolism, and fluoride interactions with other important bone factors including age, body size, menopause status, and medications. Neither serum fluoride concentrations nor the duration of residency in communities with known water fluoride concentrations predicted incident osteoporotic fractures in women 20-92 years old. A substantial fluoride exposure gradient was found among the communities, as indicated by both the serum concentration and the duration of residency in the communities. The measures of fluoride exposure used in this study, and at the amounts identified, were not associated with osteoporotic fractures or with BMD, particularly after adjustment of covariates including age, body size (BMI), thiazide use, hormone use, and menopause status. There were no independent associations of fluoride exposure with BMD at the 3 measured bone sites. BMD was higher in the distal radius of residents of the high-fluoride community; BMD of the vertebrae or femoral neck did not differ among communities. The authors conclude that within the range of these exposures and variables, the risk of deleterious bone-related outcomes was not related to fluoride exposure. At 210.4 μmol F/L in the drinking water, there is little evidence of a bone demineralization defect associated with low BMD or fracture.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)		Taves, D.R. 1969. Determination of submicromolar concentrations of fluoride in biological samples. Talanta 15: 1015-1023.
PROFILER'S REMARKS	***Initials/date SJG/2/19/07***	This was a well-conducted and designed study. Serum fluoride, BMD and skeletal fractures were monitored. The study indicated higher individual serum fluoride levels in the community with 210.4 μmol F/L in the water supply. There was an increase in distal radius BMD in the high-fluoride community and increased osteoporotic fracture frequency in the high-fluoride and high-calcium communities, with an association between the high-fluoride or high-calcium communities and BMD of the distal radius (β-coefficient: 0.020, p=0.011; and β-coefficient 0.018, p=0.011, respectively). There was only a weak association between serum fluoride and BMD in the femoral neck and lumbar spine (β-coefficient 0.011, p=0.13; and β-coefficient 0.019, p=0.12, respectively). After adjustment for covariates, no association was found between serum fluoride levels and osteoporotic fractures (RR 1.16, p=0.66) or between the high fluoride community and fractures (RR=2.55, p=0.07). Thus, serum fluoride levels in subjects of the community with 210.4 μmol F/L in the water supply were not statistically associated with fracture incidence. Some weaknesses of the study included the following: Serum fluoride levels may not be a suitable biomarker for bone fluoride levels. The profiler disagrees that a substantial fluoride exposure gradient was found, as indicated by the duration of residency in the communities. Further, it is unclear whether smoking status, which differed among communities, was included in the regression models. Other unidentified confounders may be contributing to the results since some effects (i.e., increased osteoporotic fracture frequency; an association with BMD of the distal radius) were observed in both the high-fluoride and high-calcium communities; moreover, the RR for osteoporotic fractures was 3.01 (p=0.04) in the high-calcium community. Finally, fracture incidence and risk associated with fluoride-based measures was not broken down by age; although mean age among communities did not differ, it is unclear whether limiting regression models to certain ages (e.g., 20-40, 41-60, 61-80) would yield different risk ratios. Limited data for dose-response analysis; however, results indicate that fluoride levels of 4 ppm in drinking water may not increase risk of fractures.
PROFILER'S ESTIM. NOEL/NOAEL		Based on the risk of osteoporotic fracture, the estimated NOAEL is 210.4 μmol F/L (4

	mg/L) in the drinking water.
PROFILER'S ESTIM. LOEL/ LOAEL	After adjustment for covariates, an estimated LOAEL could not be established.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:	Not suitable ( ), Poor ( ), Medium (x), Strong () This was a well-conducted and designed study, with only a few limitations. The study indicated the following results in the community with 210.4 μmol F/L in the water supply: higher individual serum fluoride levels; increased distal radius BMD; and increased osteoporotic fracture frequency (also noted in the community with 52.6 μmol F/L and 9.375 mmol Ca/L); an association between the high-fluoride (or high-calcium) communities and BMD of the distal radius. Nevertheless, after adjustment for covariates, no association was found between serum fluoride levels and osteoporotic fractures (RR 1.16, p=0.66) or between the high fluoride community and fractures (RR=2.55, p=0.07).
CRITICAL EFFECT(S):	Skeletal fracture incidence and site; bone mineral density

Sowers, M.R., Wallace, R.B., and Lemke, J.H. 1986. The relationship of bone mass and fracture history to fluoride and calcium intake: a study of three communities. Am. J. Clin. Nutr. 44:889-898.

ENDPOINT STUDIED:	Skeletal fractures and bone mass in adult women
TYPE OF STUDY:	Cross-sectional baseline survey (May – August, 1983 and 1984) of bone mass and skeletal fractures in women supplied with drinking water of high and low F content and different calcium concentrations. REVIEWER'S NOTE: This study was followed during 1988-89 and published in Sowers et al. (1991) PROFILER'S NOTE: The study authors' hypothesis was that higher fluoride intake would result in greater bone mass and fewer fractures. The findings did not support the hypothesis.
POPULATION STUDIED:	US/Iowa: 827 adult women in three rural communities in northwest Iowa; participants had lived in the community at least 5 years; ages at the beginning were 20-80 years for the high fluoride group and 20-35 and 55-80 years for both low and high calcium groups; all individuals were ambulatory, not knowingly pregnant, and had not experienced wrist or forearm fractures in the previous two years. The communities were similar with respect to population size (less than 2000 persons), age distribution, proportion foreign born, mean income, and occupational categories; all eligible women were of northern European heritage. Completion rates were high, with ≥77% of eligible women completing the study in each community.
CONTROL POPULATION:	Community with relatively low fluoride (1 mg F/L) and low calcium (67 mg Ca/L) in the municipal water. Demographics were similar to the study population. PROFILER'S NOTE: 1 mg/L F is optimally fluoridated water and is not really considered low fluoride except in comparison to 4 mg/L F.
EXPOSURE PERIOD:	Participants had lived in the high fluoride community for 5-77 years at the beginning of the study.
EXPOSURE GROUPS:	The communities were chosen based on municipal water supplies containing either high natural fluoride (4.0 ± 0.1 mg F/L with 15 ± 3 mg Ca/L), high calcium (375 ± 8 mg Ca/L), or low calcium (60 ± 4 mg Ca/L as reported); the low and high calcium water supplies were treated to 1 mg fluoride/L.
EXPOSURE ASSESSMENT:	The exposure assessment consisted solely of measured fluoride concentrations in drinking water and estimated fluoride concentrations of water-based beverages (frozen juices, powdered drink mixes, coffee, and tea). Water and water-based beverage intake was assessed in a 24-hour recall survey and in a water intake section of a questionnaire. Fluoride content in foods was not evaluated and non-dietary sources of fluoride were not measured. Each individual was asked to recall her previous 24-hour intake of food and beverage during an interview. Nutrient values, including calcium, were assigned to coded foods and beverages using the US Department of Agriculture Food Composition Tape # 456. A supplemental program was developed to assign vitamin D values to foods and beverages based on information from published food composition tables (Southgate and Southgate 1978) and other information sources about fortified products.
ANALYTICAL METHODS:	Inorganic constituents of the community water supplies were determined at the University of Iowa Hygienic Laboratory (the state public health laboratory) according to most current methods (APHA 1976). No further information was given regarding the methods for analyzing fluoride and calcium in drinking water. Water sampling and testing had been performed approximately every 5 years since 1938.
STUDY DESIGN:	Bone mass was studied in adult women in three rural communities supplied with water of differing mineral (F and Ca) content. Mid-radius bone mass of women whose community

	drinking water contained naturally occurring high fluoride (4 mg F/L) and low calcium (15 mg Ca/L) was compared to bone mass of women living in two demographically similar communities where the fluoride level of the treated drinking water was 1 mg F/L but the Ca concentration varied considerably (375 mg Ca/L or 60 mg Ca/L).
PARAMETERS MONITORED:	Bone mass was measured using a Norland 278 photon absorptiometer with an I^{125} source (Cameron and Sorensen 1963, Cameron et al. 1968). Bone mass was expressed as the bone mineral to bone width ratio (g/cm^2) of the radius. Each participant was measured for height, weight, triceps skinfold thickness, and mid-arm circumferences. Responses to questions regarding demographic information, reproductive history, medication and nutritional supplement use, smoking, alcohol use, medical history, fracture history, and sunlight exposure were recorded.
STATISTICAL METHODS:	Normality of variance was evaluated with univariate analysis. Variables with highly skewed distributions were $\log_{10}$ transformed (nutrient intakes) or categorized. Chi-square tests were used to determine if subjects were demographically homogeneous. Analysis of covariance with multiple comparison tests was used to generate and compare mean nutrient intakes and physical measurements by community. Associations between levels of bone mass and factors such as medication use and dietary fluoride and calcium intakes were tested using multiple regression analysis. Probability of fracture history in relation to community fluoride exposure considering important covariates such as perimenopausal estrogen and current thiazide use as well as interactions was evaluated using stepwise multiple logistic regression analysis. PROFILER'S NOTE: Thiazide is a diuretic used in the prevention of urinary calculi.
RESULTS:	
Diet assessment	Composite diet analysis performed by means of a 24-hr recall interview indicated that water was the primary source of fluoride intake.
Drinking water characteristics	TABLE 1 Selected mineral characteristics of wells providing the drinking water of three rural demographically-similar communities in northwest Iowa* Community / Date drilled / Depth (ft) / Mean calcium content (mg/L) / Calcium range (min–max) (mg/L) / Natural fluoride level (mg/L) / Treated to 1 mg/L / Frequency of evaluation since 1938 High fluoride: < 1938 / 1211 / 15 / 14–19 / 4.00 / No / 6 High calcium Well #1: 1960 / 600 / 351 / 336–370 / 0.35 / Yes / 6 Well #2: 1938 / 660 / 360 / 345–390 / 0.40 / / 11 Low calcium Well #1: 1960 / 230 / 70 / 66–71 / 0.20 / Yes / 6 Well #2: 1948 / 230 / 65 / 62–68 / 0.20 / / 9 * Data Source: University of Iowa Hygienic Laboratory.
Bone mass in baseline study (Sowers et al. 1986)	Mid-radius bone mass did not differ among women aged 20-35 years in the three communities. However, women 55-80 yr living in the high fluoride community had significantly less (25%) mean mid-radius bone mass than women living in either the low or high calcium communities. When adjusted for estrogen and thiazide use as well as total calcium intake (including water, food, and supplement), vitamin D intake and muscle area, levels of bone mass from women in the high fluoride community were slightly lower (< 2%) but did not attain statistical significance (Fig 2). Findings were similar using bone mineral (g) or bone width (cm) instead of bone mass (g/cm^2) as the variable of interest. PROFILER'S NOTE: Total calcium and Vit D intake were estimated from respondent questionnaire, as described in the methods section above.

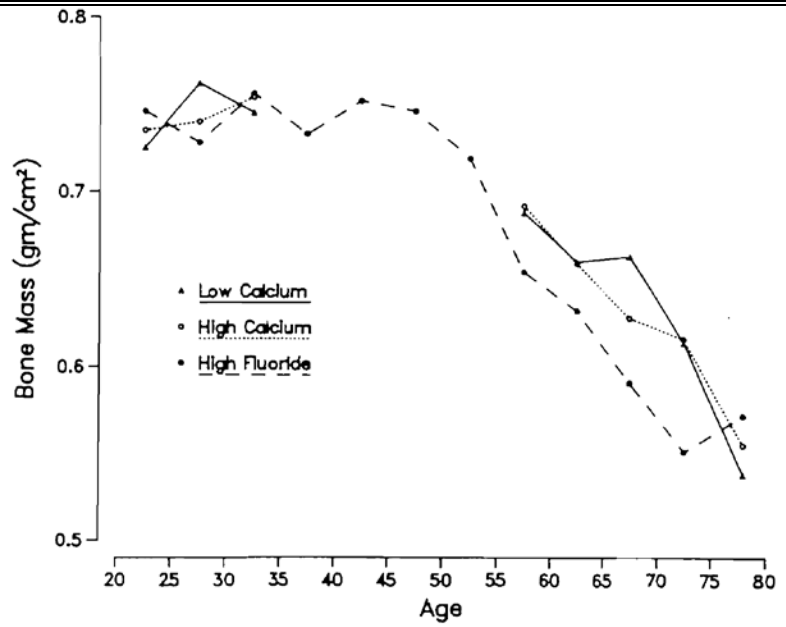


FIG 2. A comparison of mean mid-radius bone mass of women (adjusted for age, humeral muscle area, estrogen use, and thiazide use) living in communities with differing mineral content of municipal waters.

Skeletal fractures

Fracture history was determined from subject interview and was evaluated by community of residence (Table 3). Among women aged 20-35 yr, no community differences were observed in the history of fracture frequency. Women 55-80 yr living in the high fluoride community reported significantly more lifetime and current fractures than did women in the high calcium or low calcium communities.

REVIEWER'S NOTE: Fracture history and medical history information was obtained by subject interview; there is no evidence that individually reported fracture history was confirmed with medical records or other more objective evidence.

TABLE 3
Frequency of fractures among women living in three communities whose drinking waters have differing levels of fluoride and calcium

Type of fracture	High fluoride		High calcium		Low calcium		Significance level
	Number	%	Number	%	Number	%	
Women 55–80	*n* = 200		*n* = 173		*n* = 151		
Ever had a fracture	92	46%	61	35%	55	36%	p = 0.0660
Sites of fracture							
hip, wrist, spine	31	16%	15	9%	18	12%	p = 0.1408
other sites	61	30%	46	26%	46	24%	
Fracture in past 10 yr	57	29%	24	14%	20	13%	p = 0.0001
Sites of fracture in the past 10 yr							
hip, wrist, spine	3	2%	2	1%	3	2%	p = 0.0006
other sites	54	27%	22	13%	17	11%	
Women 20–35	*n* = 85		*n* = 43		*n* = 43		
Ever had a fracture	27	31%	12	28%	9	21%	p = 0.4359
Sites of fracture							
hip, wrist, spine	6	7%	1	2%	1	2%	p = 0.5436
other sites	21	24%	11	26%	8	19%	
Fracture in past 10 yr	8	9%	3	7%	5	11%	p = 0.7599
Sites of fracture in past 10 yr							
hip, wrist, spine	—	—	—	—	—	—	
other sites	8	9%	3	7%	5	11%	p = 0.7599

Dose Response

To estimate fluoride intake, years of residence multiplied by reported typical beverage consumption (expressed as quartiles of the distribution) was related to bone mass. Mean bone mass of women in the upper quartile of fluoride exposure tended to be significantly less (p = 0.0665) than mean bone mass of women in the third quartile and slightly, but not statistically, less than the mean value of women in the second quartile. This relationship is observed following adjustment for age, humeral muscle area, current thiazide use, history of estrogen use, and menopausal state. The quartile categorization of fluoride exposure was not associated significantly with an increased probability of recent or

	lifetime fracture history after considering the important covariants.
Interaction with calcium and vitamin D	Premenopausal women with lower calcium and vitamin D intakes (determined by recall interviews) and greater fluoride intake (n = 16) had significantly lower mean bone mass than mean values of women in the low fluoride/low calcium, low fluoride/high calcium, and high fluoride/high calcium intake groupings (0.715±0.017 vs 0.744±0.004, p = 0.0470). No consistent interactions of the three nutrients in relation to bone mass were observed in postmenopausal women after considering important covariants.
Demographics	No differences in demographics were found among the women in the three communities.
STUDY AUTHORS' CONCLUSIONS:	"We observed no protective effect with higher fluoride intake. Bone mass was lower in older women from the high fluoride community though not statistically so; these women reported significantly more fractures. There was no observed community difference in young women's bone mass or fracture history. Young women in the high fluoride community consuming calcium and vitamin D in excess of 800 mg/day and 400 IU/day, respectively, had significantly better bone mass ($p < 0.05$) than their peers." PROFILER'S NOTE: The study authors' hypothesis was that higher fluoride intake would result in greater bone mass and fewer fractures. The findings did not support the hypothesis.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)	American Public Health Association. Standard methods for the examination of water and wastewater, 14th ed. Washington, DC: American Public Health Association, 1976. Cameron, J.R. and Sorenson, J.A. 1963. Measurements of bone mineral in vivo: an improved method. Science 142:230-232. Cameron, J.R., Mazess, R.B., and Sorensen, J.A. 1968. Precision and accuracy of bone mineral determination by direct photon absorptiometry. Invest. Radiol. 3:141-150. Southgate, P.P.A. and Southgate, D.A.T. 1978. McCance and Widdowson's the composition of foods, 4th ed. Amsterdam: Elsevier/North-Holland Biomedical Press.
PROFILER'S REMARKS ***Initials/date CSW 12/18/2006 and 1/22/2007***	This study was well conducted and controlled for factors such as age and estrogen use. The most pronounced effect appeared to be in older women (≥55 years) with high fluoride (4 mg/L) content of their drinking water. This group had the lowest bone mass and the greatest number of fractures. Good comparison to communities with low fluoride and either high or low calcium content of the drinking water. Doses could not be reconstructed based on the data presented. In the baseline study, overall mean drinking water calcium levels in the low and high communities were reported in the text as 60 and 375 mg/L, respectively. However, the mean ranges (based on 6-11 measurements in two wells) given in the table were 65-70 and 351-360 mg/L so clearly the overall mean is outside the range. In the follow-up study (Sowers et al. 1991) the overall mean for the low calcium water is 67 mg/L which is reasonable based on the data presented in the first report (1986). It may be that the overall mean for the high calcium value is a typo as it appears that it should be about 355 mg/L. This discrepancy does not affect the conclusions of either paper.
PROFILER'S ESTIM. NOEL/NOAEL	Could not be determined
PROFILER'S ESTIM. LOEL/ LOAEL	Could not be determined.
POTENTIAL	Not suitable ( ), Poor (X), Medium ( ), Strong ( )

SUITABILITY FOR DOSE-RESPONSE MODELING:	A positive correlation was found between years of residence and lower bone mass and number of fractures. Dose was estimated by years of residence and reported beverage consumption. However, the study authors stated only that significance was found for "women in the upper quartile of fluoride exposure" but corresponding doses were not given for each quartile. Thus, a range of doses could be calculated from the residence time and estimated intake data presented, but it is unknown at which dose statistical significance is attained.
CRITICAL EFFECT(S):	Skeletal fracture, bone mass

Sowers, M.R., Clark, M.K., Jannausch, M.L., and Wallace, R.B. 1991. A prospective study of bone mineral content and fracture in communities with differential fluoride exposure. Am. J. Epidemiol. 133:649-660.

ENDPOINT STUDIED:	Skeletal fractures and bone mass in adult women
TYPE OF STUDY:	Five-year follow-up study (1988 and 1989; N = 684) after the initial cross-sectional baseline survey (May – August, 1983 and 1984; N = 827) of bone mass and skeletal fractures (see Sowers et al 1986 for baseline study documentation).
POPULATION STUDIED:	US/Iowa: Adult women in three rural communities in northwest Iowa; participants had lived in the community at least 5 years and consumed the community water supply; ages at the beginning were 20-80 years for the high fluoride group and 20-35 and 55-80 years for both low and high calcium groups; all individuals were ambulatory, not knowingly pregnant, and had not experienced wrist or forearm fractures in the previous two years. The communities were similar with respect to population size (less than 2000), age distribution, proportion foreign born, mean income, and occupational categories; all eligible women were of northern European heritage. Of those participating in the baseline study (Sowers et al. 1986), 81.5-85% participated in the follow-up.
CONTROL POPULATION:	Community with low fluoride (1 mg/L) and low calcium (67 mg/L) concentrations in the community water. Demographics were similar to the other study populations.
EXPOSURE PERIOD:	Participants had lived in the high fluoride (4.0 mg F/L with 15 mg Ca/L) community for 5-77 years at the beginning of the baseline study (Sowers et al. 1986). The current study was a follow-up five years after the baseline study. Residence duration for the other 2 communities was not reported.
EXPOSURE GROUPS:	The communities were chosen based on community water supplies with either naturally high fluoride (4.0 mg F/L with 15 mg Ca/L), high calcium (375 mg Ca/L), or low calcium (67 mg Ca/L); the low and high calcium water supplies were treated to 1 mg fluoride/L.
EXPOSURE ASSESSMENT:	The exposure assessment consisted solely of measured fluoride concentrations in drinking water and estimated fluoride concentrations of water-based beverages (frozen juices, powdered drink mixes, coffee, and tea). Water and water-based beverage intake was assessed in a 24-hour recall survey and in a water intake section of a questionnaire. Fluoride content in foods was not evaluated and non-dietary sources of fluoride were not measured. Each individual was asked to recall her previous 24-hour intake of food and beverage during an interview. Nutrient values, including calcium, were assigned to coded foods and beverages using the US Department of Agriculture Food Composition Tape #456. A supplemental program was developed to assign vitamin D values to foods and beverages based on information from published food composition tables (Southgate and Southgate 1978) and other information sources about fortified products.
ANALYTICAL METHODS:	Inorganic constituents in community water supplies were determined at the University of Iowa Hygienic Laboratory (the state public health laboratory) according to methods recommended in APHA (1976). No further information was given regarding the methods for analyzing fluoride and calcium in drinking water.
STUDY DESIGN	Bone mass and relative fracture risk were studied in 684 adult women aged 20-80 years and residing in three rural communities supplied with differing mineral (F and Ca) content in community water, as follow-up to a baseline study of similar design (Sowers et al 1986; N = 827). Mid-radius bone mass and fracture history of women living in a community whose municipal drinking water had naturally-occurring high fluoride (4 mg F/L) were compared to those of women living in two demographically similar communities where the fluoride level of the treated drinking water was 1 mg F/L and the Ca content varied (375 or 67 mg Ca/L).

PARAMETERS MONITORED:	Bone mass was measured using a Norland 278 photon absorptiometer with an I^{125} source (Cameron and Sorensen 1963, Cameron et al. 1968). Bone mass was expressed as the bone mineral to bone width ratio (g/cm^2) of the radius. In addition, femoral bone mass was measured using a Norland 2600 Dichromatic dual-photon densitometer with a gadolinium-153 source; femoral bone mass was measured only in women who were post menopausal, were capable of reclining to a flat position, did not have hip pins, and were sufficiently lean to fit under the scanner arm. Each participant was measured for height, weight, triceps skinfold thickness, and mid-arm circumferences. Responses to questions regarding demographic information, reproductive history, medication and nutritional supplement use, smoking, alcohol use, medical history, fracture history, and sunlight exposure were recorded.
STATISTICAL METHODS:	Normality of variance was evaluated with univariate analysis. Variables with highly skewed distributions were $\log_{10}$ transformed (nutrient intakes) or categorized. Chi-square tests were used to determine if subjects were demographically homogeneous. Analysis of covariance with multiple comparison tests was used to generate and compare mean nutrient intakes and physical measurements by community. Associations between levels of bone mass and factors such as medication use and dietary intakes were tested using multiple regression analysis. Probabilities of fracture between baseline and follow-up in relation to community fluoride exposure were evaluated using stepwise logistic regression analysis (important covariates such as age, Quetelet index (wt/ht^2), calcium intake, vitamin D intake, and interactions were evaluated). Estimates of relative risk with 95 percent CIs were calculated from beta coefficients and standard errors.
RESULTS:	
Diet assessment	Composite diet analysis performed by means of a 24-hr recall interview indicated that water was the primary source of fluoride intake. Calcium and vitamin D intakes were assessed as described above in "Exposure Assessment."
Drinking water characteristics	Drinking water in the higher fluoride community had a naturally occurring fluoride concentration of 4 mg F/L and a calcium concentration of 15 mg Ca/L. Drinking water in the higher calcium community had a calcium concentration of 375 mg Ca/L and was fluoridated to a concentration of 1 mg F/L. The "control" community had drinking water that was fluoridated to 1 mg F/L and an average calcium concentration of 67 mg Ca/L.
Bone mass	Mean radial bone mass, by community, in adult women age 20-35 yr, adjusted for age and Quetelet index is given in Table 2. No significant differences by community in mean radial bone mass measurement were observed at baseline. At follow-up, young women in the higher fluoride community had significantly lower mean bone mass values than did women in the control and higher-calcium communities. The mean loss of radial bone (absolute difference or percentage of loss) was greater in women of the higher-fluoride community than in women of the control and higher-calcium communities.

TABLE 2. Mean radial bone mass in women aged 20–35 years at baseline (1983/1984), by community, in rural Iowa communities with different water mineral characteristics

	Adjusted* value	p for difference in means	
Baseline (1983/1984) radial bone mass (g/cm²)			
Control (n = 37)	0.75 ± 0.008†		
Higher-calcium (n = 33)	0.75 ± 0.008		NS‡
Higher-fluoride (n = 67)	0.74 ± 0.006		
Follow-up (1988/1989) radial bone mass (g/cm²)			
Control	0.73 ± 0.008		
Higher-calcium	0.74 ± 0.009	0.02	0.04
Higher-fluoride	0.71 ± 0.006		
Absolute difference in radial bone mass in 5 years (g/cm²)			
Control	−0.015 ± 0.005		
Higher-calcium	−0.011 ± 0.005	0.03	0.08
Higher-fluoride	−0.027 ± 0.004		
% loss of radial bone mass in 5 years			
Control	−2.1 ± 0.7		
Higher-calcium	−1.6 ± 0.7	0.03	0.08
Higher-fluoride	−3.6 ± 0.5		

* Adjusted for age and Quetelet index (weight (kg)/height (m)²).
† Mean ± standard error.
‡ NS, not significant.

The mean radial bone mass values, by community, for women in the 55-80-year age group are shown in table 3. The values are adjusted for age and Quetelet index. At both baseline and follow-up, mean radial bone mass was significantly lower in the higher-fluoride community than in the control and higher-calcium communities. However, the rates of change in radial bone mass were not significantly different among the communities during this 5-year period. The mean bone mass of the femur was consistently lower in the higher fluoride community than in the higher calcium community; however the mean femoral bone mass measures were not significantly lower than mean values in the control community (femur bone mass data not copied to profile).

TABLE 3. Mean radial bone mass in women aged 55–80 years at baseline (1983/1984), by community, in rural Iowa communities with different water mineral characteristics

	Adjusted* value	p for difference in means	
Baseline (1983/1984) radial bone mass (g/cm²)			
Control (n = 121)	0.63 ± 0.008†		
Higher-calcium (n = 148)	0.63 ± 0.007	0.006	0.02
Higher-fluoride (n = 163)	0.60 ± 0.007		
Follow-up (1988/1989) radial bone mass (g/cm²)			
Control	0.59 ± 0.008		
Higher-calcium	0.59 ± 0.007	0.003	0.01
Higher-fluoride	0.56 ± 0.007		
Absolute difference in radial bone mass in 5 years (g/cm²)			
Control	−0.039 ± 0.004		
Higher-calcium	−0.043 ± 0.003		NS‡
Higher-fluoride	−0.046 ± 0.003		
% loss of radial bone mass in 5 years			
Control	−6.4 ± 0.6		
Higher-calcium	−6.9 ± 0.5		NS
Higher-fluoride	−7.4 ± 0.5		

* Adjusted for age and Quetelet index (weight (kg)/height (m)²).
† Mean ± standard error.
‡ NS, not significant.

Skeletal fractures	Women aged 20-35 yr in the higher fluoride community had an increased probability of any fracture and of fracture at the spine, hip or wrist as compared with the control community; however, the confidence interval included 1. Women in the 55-80 yr group in the higher-fluoride community, had an increased relative risk of 2.1 (95 percent confidence interval (CI) 1.0-4.4) for any fracture, 2.2 (95 percent CI 1.1-4.7) for fracture at the spine, hip or wrist, and 2.2 (95 percent CI 1.0-4.6) for multiple fractures during the 5-year period compared with the control community. No significant differences in the 5-year fracture relative risk; risk of fracture occurring at the

	wrist, spine, or hip; or for multiple fractures were found between the higher-calcium community vs the control community. **TABLE 6. Risk of fracture in a 5-year period (1983/1984–1988/1989) among women of three rural Iowa communities with differences in the mineral content of their community water supplies, by age group and community** Relative risk* (95% confidence interval) Community / Any fracture / Fracture of hip, wrist, or spine / Fractures at multiple sites *Women aged 20–35 years at baseline†* Control: —‡ / — / Higher-calcium: 0.36 (0.03–3.63) / 0.30 (0.04–3.39) / Higher-fluoride: 1.81 (0.45–8.22) / 2.70 (0.16–8.28) / *Women aged 55–80 years at baseline* Control: — / — / — Higher-calcium: 1.54 (0.70–3.37) / 1.60 (0.71–3.40) / 1.60 (0.71–3.41) Higher-fluoride: 2.11 (1.01–4.43)§ / 2.20 (1.07–4.69) / 2.20 (1.04–4.57) * Adjusted for age and Quetelet index (weight (kg)/height (m)^2). † There were no multiple fractures in this age group. ‡ Referent. § Relative risk adjusted for baseline radial bone mass = 1.99 (95 percent confidence interval 0.95–4.20).
Dose response	In the higher fluoride community, fluoride dose (years of residence multiplied by daily intake from beverages) was positively correlated with increased risk of fracture. The relative risk of fracture in postmenopausal women with a fluoride exposure less than the median was 1.9 (95 percent CI 0.88-4.0), while those postmenopausal women with an exposure greater than the median had a relative risk of 2.6 (95 percent CI 1.2-6.0) when compared with premenopausal women. These relative risks were adjusted for age and Quetelet index. Bone mass and fracture risk were similar between the control and higher-calcium communities such that a dose-response could not be evaluated. PROFILER'S NOTE: These data were given in the text with no additional details on dose calculation or the median value.
STUDY AUTHORS' CONCLUSIONS:	Residence in the higher-fluoride community was associated with a significantly lower radial bone mass in premenopausal and postmenopausal women, an increased rate of radial bone mass loss in premenopausal women, and significantly more fractures among postmenopausal women. For women in the higher fluoride community, aged 55-80 yr, the 5-year relative risk of any fracture or of wrist, spine, or hip fracture was increased compared to the control community. REVIEWER'S NOTE: Sowers et al (1986) tested alternate explanations (mean body size, oral contraceptive use, Ca intakes), which were all found to be similar between observed communities. The authors admit that skeletal observations may be related "either to fluoride exposure or other unique but unknown factors." The authors further observe that the increased incidence (not statistically significant) of fractures in young adult women further suggests that the fracture observation in older women is not an artifact or due to the age cohort.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)	American Public Health Association. Standard methods for the examination of water and wastewater, 14th ed. Washington, DC: American Public Health Association, 1976. Cameron, J.R. and Sorenson, J.A. 1963. Measurements of bone mineral in vivo: an improved method. Science 142:230-232. Cameron, J.R., Mazess, R.B., and Sorensen, J.A. 1968. Precision and accuracy of bone mineral determination by direct photon absorptiometry. Invest. Radiol. 3:141-150.
PROFILER'S ***Initials/date***	This study was well conducted and controlled for such factors as age and estrogen use; a large

REMARKS	***CSW 12/19/2006***	percentage of those women participating in the baseline study also participated in the follow-up study (Sowers et al 1991). The most pronounced effect appeared to be in older women (≥55 years) with high fluoride (4 mg/L) content of their drinking water. This group had the lowest bone mass and an increased risk of fractures. However, younger women in the high fluoride community had an increase in bone loss over the five years between baseline and follow-up. Good comparison to communities with low fluoride and either high or low calcium content of the drinking water. Doses could not be reconstructed based on the data presented. In the baseline study, overall mean calcium levels in the low and high communities were reported in the text as 60 and 375 mg/L, respectively. However, the mean ranges (based on 6-11 measurements in two wells) given in the table were 65-70 and 351-360 mg/L; clearly, the overall means for the low and high communities are outside the reported range. In the follow-up study (Sowers et al. 1991) the overall mean for the low calcium water is 67 mg/L which is reasonable based on the data presented in the first report (1986). It may be that the overall mean for the high calcium value is a typo as it appears that it should be about 355 mg/L. This discrepancy does not affect the conclusions of Sowers et al (1986, 1991). "Control" community would be more accurately termed the "reference" community.
PROFILER'S ESTIM. NOEL/NOAEL		Could not be determined.
PROFILER'S ESTIM. LOEL/ LOAEL		Could not be determined.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable ( ), Poor (X), Medium ( ), Strong ( ) A positive correlation was found between years of residence and lower bone mass and number of fractures. Dose was estimated by years of residence and reported beverage consumption. However, the study authors stated only that an increased risk of fracture was found for "postmenopausal women with an exposure greater than the median" but a corresponding dose was not given.
CRITICAL EFFECT(S):		Skeletal fracture, fracture risk, bone mass

Stevenson, C.A. and Watson, A.R. 1957. Fluoride osteosclerosis. Am. J. Roentgenol. Radium Ther. Nucl. Med. 78(1):13-8.

ENDPOINT STUDIED:	Skeletal fluorosis (fluoride osteosclerosis)
TYPE OF STUDY:	Retrospective
POPULATION STUDIED:	23 patients, aged 44 to 85, primarily from Texas and Oklahoma and presenting with a diagnosis of fluoride osteosclerosis.
CONTROL POPULATION:	No control population was examined in this study.
EXPOSURE PERIOD:	Each of the 23 patients lived his entire life (44 to 85 years) in the same fluoride bearing area in which he was born, with drinking water containing 4 to 8 ppm fluoride.
EXPOSURE GROUPS:	Medical records from the Scott and White Clinic in Texas were evaluated between 1943 and 1953. A total of approximately 170,000 roentgen examinations of the spine and pelvis made on patients primarily from Texas and Oklahoma yielded 23 cases of fluoride osteosclerosis. Each of the 23 patients lived his entire life (44 to 85 years) in the same fluoride bearing area in which he was born, with drinking water containing 4 to 8 ppm fluoride. This group presented nothing in common except roentgenographic changes in their osseous systems. There was no unusual incidence of anemia, arteriosclerosis, arthritis, back stiffness, renal disease, or biliary calculi.
EXPOSURE ASSESSMENT:	Medical records, including roentgen examinations of spine and pelvis, were evaluated. Osseous changes and pelvic ligament calcification were graded on a 1 to 4 scale.
ANALYTICAL METHODS:	Data for measuring the fluoride concentrations in the drinking water supplies were not included in the study report. Water quality parameters were not measured.
STUDY DESIGN	Medical records from the Scott and White Clinic in Texas were evaluated for the eleven year period from 1943 to 1953. A roentgenologic diagnosis of fluoride osteosclerosis was recorded on 23 patients' records from a total of approximately 170,000 roentgen examinations of the spine and pelvis made on patients primarily from Texas and Oklahoma. No cases reported by the U.S. Department of Health, Education, and Welfare are included. All patients were given complete clinical examinations including serology, sedimentation rate, red and white blood cell counts, hemoglobin, blood urea, and routine urine studies. The physicians who examined the patients were familiar with dental fluorosis, but were unable to determine any relationship between the roentgenologic findings and the patients' disease processes or symptoms. There was no chemical analysis of bone for fluorine content. The diagnosis was based on bone changes as reported by Roholm, bone changes observed in cattle exposed to extremely high toxic doses of fluorides, and on bone changes noted in a few Bartlett, Texas (8 ppm fluoride drinking water) residents who were examined during Public Health Service surveys. The autopsy of a patient who had typical roentgenographic findings of fluoride osteosclerosis revealed bone fluoride content six times the normal amount expected. This information, plus the fact that this group of patients had been drinking water with high fluoride content for many years permitted the authors to assume that the diagnosis was correct, especially since these changes are not observed in individuals not exposed to fluorides.
PARAMETERS MONITORED:	Osseous changes were graded on a 1 to 4 scale, with grade 1 showing bone density of a very minimal degree, and grade 4 showing bone density of an extreme degree. Pelvic ligament calcification was graded with grade 1 representing minimal calcification in either the sacrotuberous or sacrospinous ligaments, and grade 4 showing calcification extending about 6 cm from the ischium towards the sacrum.

STATISTICAL METHODS:	Statistical analysis was not performed.
RESULTS:	
Fluoride osteosclerosis	Table 1 was copied directly from Stevenson and Watson (1957) and summarizes the age, fluoride content of the drinking water, degree of osseous changes and pelvic ligament calcification for all 23 patients with a diagnosis of fluoride osteosclerosis. Osseous changes were noted in these patients only when water contained fluoride at 4 to 8 ppm. Pelvic ligament calcification closely paralleled the degree of bone, with 15 of the 23 patients having calcification of the sacrospinous and sacrotuberous ligaments. The calcification began at the ligamentous attachment in the pelvis, was usually bilateral and extended towards the sacrum for distances up to 6 cm. The entire length of the ligaments did not calcify, and no instance of calcification at the sacral end of the ligaments was observed. Because of the nature of this type of sclerotic process with associated pelvic ligament involvement and the absence of any abnormal blood changes, the differential diagnosis of fluoride osteosclerosis from other diseases producing increased bone density was not difficult.

TABLE I

FLUORIDE OSTEOSCLEROSIS

Case	Age	Parts per Million Fluorides	Degree of Fluorosis	Pelvic Ligaments
1	83	8.0	4	0
2	50	8.0	1	1
3	85	8.0	1	0
4	55	8.0	1	1
5	66	7.6	2	2
6	67	7.6	4	4
7	72	7.6	3	2
8	73	5.4	4	1
9	66	5.4	4	0
10	67	5.4	4	0
11	57	5.2	3	2
12	50	5.2	4	1
13	62	5.0	4	4
14	46	5.0	1	0
15	79	4.0	4	4
16	50	C.T.*	1	1
17	58	C.T.*	4	1
18	48	C.T.*	1	0
19	44	C.T.*	1	0
20	80	C.T.*	4	4
21	44	W.T.†	1	2
22	63	Okla.‡	4	4
23	62	Kan.§	1	0

* Patients were central Texas farmers who moved several times, but drank water from shallow wells in known fluoride areas. Exact ppm. not known.
† Same as above, but lived in West Texas.
‡ Same, but lived in Oklahoma.
§ Same, but lived in Kansas.

	The earliest bone changes occurred in the pelvis and lumbar spine, consisting of slightly increased bone density, evenly distributed throughout the vertebral bodies and appearing bilaterally and symmetrically in the pelvis. There was a slight "ground glass" appearance. Advanced changes consisted of a chalky-white appearance of the vertebral column and pelvis plus a slightly increased density and coarse trabecular pattern in the ribs. The skull or extremities did not show changes except for slight periosteal roughening in the forearms or legs of a few patients. Involvement of the hands and feet was not noted. No cortical thickening or increased bone size was noted.
STUDY AUTHORS' CONCLUSIONS:	Twenty-three cases of fluoride osteosclerosis are presented, gathered from approximately 170,000 roentgenographic examinations of the spine and pelvis of patients living in Texas and Oklahoma where many communities have excessive fluoride in the drinking water. In each case, adequate clinical examination failed to establish any relationship between the roentgenologic findings and the clinical diagnosis of the patient's condition. Fluoride osteosclerosis causes no harmful changes, and develops in patients exposed to fluorides as high as 8 ppm over a period of several years, but is not evident roentgenographically in patients drinking water with fluoride content less than 4 ppm. In the patient presenting roentgen evidence of sclerosis of the bone, calcification of the sacrospinous and

		sacrotuberous ligaments is a distinct aid in the diagnosis of fluoride osteosclerosis.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)		None to report.
PROFILER'S REMARKS	***Initials/date SJG/ 11/14/07***	The study design does not aid in the development of a dose response to fluoride with respect to skeletal fluorosis. The objective of the study was to evaluate the systemic effects of excessive fluorides in drinking water by reviewing medical records on file at the Scott and White Clinic from 1943 to 1953 for cases of fluoride osteosclerosis. A very small number of cases were found, 23 from a total of approximately 170,000 x-ray examinations of the spine and pelvis. The authors state that "since no clinical correlation or significance could be attached to the roentgenologic findings, the validity of the roentgen diagnosis of fluoride osteosclerosis may be questioned." They base their diagnosis on bone changes reported by Roholm, bone changes observed in cattle exposed to high fluoride, and bone changes noted in some subjects exposed to up to 8 ppm fluoride in their drinking water. Fifteen of the 23 patients were exposed to 4 to 8 ppm fluoride in the drinking water, while exact fluoride exposure for 8 of the 23 was unknown, but they drank from shallow wells in known fluoride areas. The degree of skeletal fluorosis varied from minimally increased bone density (9 with grade 1 osseous change) to 'extreme' (11 with grade 4), with few having moderate changes (grade 2-3). The authors conclude that "calcification of the sacrospinous and sacrotuberous ligaments is a distinct aid in the diagnosis of fluoride osteosclerosis," but it should be noted that calcification of ligaments occurs in more advanced stages of skeletal fluorosis than increased bone density, so is not a diagnostic tool. Indeed, 8 of the 23 cases presented here did not have any detectable pelvic ligament calcification. Although physicians who examined these patients were stated to be familiar with dental fluorosis, it is not stated whether or not the patients also had mottled teeth, indicative of fluoride exposure and dental fluorosis.
PROFILER'S ESTIM. NOEL/NOAEL		Study design was not suitable for development of a NOAEL.
PROFILER'S ESTIM. LOEL/ LOAEL		Study design was not suitable for development of a LOAEL.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable (X), Poor (), Medium (), Strong () The study presented 23 cases of skeletal fluorosis, diagnosed as fluoride osteosclerosis and characterized by increased bone density with or without pelvic ligament calcification, in patients with prolonged exposure (44 to 85 years) to fluoride in the drinking water at 4 to 8 ppm.
CRITICAL EFFECT(S):		Skeletal fluorosis (fluoride osteosclerosis, including increased bone density and pelvic ligament calcification)

Susheela, A.K. and M. Bhatnagar. 2002. Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. Molec. Cell Biochem. 234/235: 335-340.

ENDPOINT STUDIED:	Dental and skeletal fluorosis; fluoride in serum, urine, and drinking water, and health symptoms of people with fluorosis.
TYPE OF STUDY:	Prospective cohort
POPULATION STUDIED:	India/New Delhi and neighboring states: 10 people (6 males, 4 females, aged 8-60) with clinical manifestations of fluorosis, who lived in rural areas.
CONTROL POPULATION:	None
EXPOSURE PERIOD:	Unknown
EXPOSURE GROUPS:	10 people who were exposed to excessively high levels of fluoride in their drinking water and/or in their food, which resulted in their clinical diagnosis of fluorosis.
EXPOSURE ASSESSMENT:	Fluoride levels in the blood, urine, and drinking water were measured using an ion selective electrode. Exposure prior to the study initiation was not quantified, but was confirmed by establishing that the subjects' drinking water had high fluoride levels, and by evaluating tooth discoloration in children of the family, joint stiffness, and finding a family history of gastrointestinal (GI) complaints that would disappear 10-15 days after switching to safe low-fluoride water. During the one-year intervention program, the subject's clinical symptoms and the fluoride levels in the drinking water, blood, and urine were monitored and reported at 1-3 unspecified time points (impact assessments). The only information provided regarding other possible sources of fluoride exposure was that three of the patients (who had relatively low fluoride in their drinking water) ingested food contaminated with fluoride.
ANALYTICAL METHODS:	Fluoride levels in the serum, urine, and drinking water were measured using ion selective electrode technology.
STUDY DESIGN:	Ten subjects with clinical manifestations of fluorosis were referred to the study investigators by clinicians from hospitals in New Delhi, India, and from neighboring states. The clinical diagnosis of fluorosis was made in hospitals on the basis of the people's case histories, clinical complaints, forearm X-rays, and by testing fluoride levels in their blood, urine, and drinking water. In rural areas without diagnostic facilities, fluorosis was diagnosed after first determining that the drinking water had high fluoride levels. Then the following were evaluated: tooth discoloration of children in the family, joint stiffness by three physical tests in the subject (ability to bend over and touch the toes without bending the knees; to touch the chest with the chin; and to touch the back of the head with the hands), and a family history of GI complaints, which would disappear 10-15 days after switching to safe water. Once fluorosis was confirmed, the subjects participated in an intervention protocol, which consisted of drinking safe defluoridated water from village sources or home filtration with activated alumina, and nutritional counseling to avoid high-fluoride foods and to consume adequate vitamins C, E, and other antioxidants. Subjects were monitored for up to a year afterwards at three unspecified intervals (i.e., impact assessments), at which time their serum, urine, and health status were assessed. Evaluated health manifestations included GI complaints, muscular weakness, polyurea, polydypsea, and pain and rigidity in the joints). A single value was provided for the water fluoride concentration during

	intervention, with no description of how/when the value was obtained.
PARAMETERS MONITORED:	Subjects were monitored for levels of fluoride in serum, urine, and drinking water, and health symptoms on 1-3 occasions for up to a year after the beginning of fluoride intervention.
STATISTICAL METHODS:	No statistical analysis was conducted.
RESULTS:	
Fluoride levels in the drinking water, serum, and urine of fluorosis patients	In all subjects, serum and urine fluoride levels progressively decreased over the course of the one-year intervention period, as shown in Table 1. For 2/10 of the subjects (1 and 2), serum fluoride was reduced to levels considered normal (0.02 mg/L). Urinary fluoride levels were still above those considered normal (0.1 mg/L) for all subjects by the third (last) impact assessment. Water fluoride concentration during the intervention period was significantly lower than prior to intervention for 7 of the 10 subjects, and was unchanged for the remaining three subjects, who ate food contaminated with fluoride.

Table 1. Fluoride level in patients with Fluorosis before and during intervention

Patient no.	Fluoride in drinking water (mg/l)		Fluoride in serum (mg/l)				Fluoride in urine (mg/l)			
	Before intervention	During intervention	Before intervention	During intervention			Before intervention	During intervention		
				1st IA	2nd IA	3rd IA		1st IA	2nd IA	3rd IA
1.	3.00	0.27	0.08	0.03	0.03	0.02	8.00	4.50	1.60	0.60
2.	5.80	0.90	0.12	0.10	0.08	0.02	9.00	1.80	1.00	0.21
3.	26.07	0.55	0.22	0.13	0.09	0.05	24.10	15.00	6.00	0.58
4.	1.74	0.55	0.08	0.04	0.03	0.03	2.21	1.16	0.80	0.31
5.	29.00	0.80	0.63	0.40	0.10	0.08	5.00	4.11	1.00	0.50
6.*	1.06	1.06	0.20	0.16	0.11	0.03	2.50	1.46	1.00	0.70
7.*	0.38	0.38	0.09	0.04	–	–	1.00	0.90	–	–
8.	2.00	0.38	0.04	0.04	–	–	2.00	0.80	–	–
9.*	0.14	0.14	0.09	0.04	–	–	0.70	0.51	–	–
10.	0.90	0.52	0.09	0.04	–	–	1.27	1.00	–	–

Permissible limit of fluoride in drinking water: 1.0 mg / L or less. Normal upper limit of fluoride in serum: 0.02mg/L [53]. Normal upper limit of fluoride in urine: 0.1 mg/L [53]. IA – Impact assessment. *Food contaminated with fluoride.

Health symptoms of fluorosis patients	All 10 patients had complete recovery of their health symptoms by the end of the third impact assessment, as shown in Table 2. Recovery was the quickest for GI complaints, with 70% of the participants reporting a recovery at the first impact assessment. Symptoms were ameliorated more quickly in subjects who drank low-fluoride water and had nutritional supplements, as compared to those who only drank the low-fluoride water.

Table 2. Health improvements: expressed by the patients (n = 10)

Manifestations	Percent affliction before intervention	Percentage recovery during intervention		
		1st impact assessment	2nd impact assessment	3rd impact assessment
Gastro-intestinal complaints	100	70	100	–
Muscular Weakness	60	40	50	Complete recovery
Polyurea	30	20	30	Complete recovery
Polydypsea	50	20	40	Complete recovery
Pain and rigidity in the joints	90	30	60	Complete recovery

STUDY AUTHORS' CONCLUSIONS:	Susheela and Bhatnagar (2002) concluded that fluorosis can be reversed. Removing fluoride sources and a diet containing essential nutrients and antioxidants can significantly improve health (i.e. reduce fluoride toxicity) and reduce fluoride in the urine and serum of fluorosis patients. This was shown in 10 patients who had complete recovery of a variety of clinical symptoms and lower urine and serum fluorine after reducing their intake of fluoride in the drinking water.
DEFINITIONS AND REFERENCES CITED IN PROFILE THAT ARE NOT FOUND IN NRC (2006)	
PROFILER'S REMARKS ***Initials/date SM/1/10/07***	The study unambiguously showed that reducing water fluoride intake led to decreased fluoride levels in the serum and urine of fluorosis patients, as well as recovery from a number of health symptoms that appeared to be fluorosis-induced.

		The data may be useful for estimating the levels of serum fluoride associated with adverse health effects. Insufficient data were provided, however, for a quantitative dose-response assessment of water fluoride levels and fluorosis in the subjects, or of the decrease of urinary and serum fluoride with time. For example, there were no quantitative estimates of the cumulative fluoride intake of the 10 subjects, and the time at which the serum and urine were collected were not provided. Also, the study had no reference control group.
PROFILER'S ESTIM. NOEL/NOAEL		Cannot be determined from this study.
PROFILER'S ESTIM. LOEL/ LOAEL		Cannot be determined from this study.
POTENTIAL SUITABILITY FOR DOSE-RESPONSE MODELING:		Not suitable (x), Poor ( ), Medium ( ), Strong ( ) Data were insufficient for a quantitative dose-response assessment of water fluoride levels and fluorosis, or for the decrease of urinary and serum fluoride with time. No reference control group was provided.
CRITICAL EFFECT(S):		Increased serum and urinary fluoride levels, associated with adverse health symptoms (GI complaints, muscular weakness, polyurea, polydypsea, and pain and rigidity in the joints).