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## QUARTERLY REPORTS

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# NATIONAL TOXICOLOGY PROGRAM – CRITIQUE OF PEER REVIEW DRAFT REPORT

by

Robert J. Carton
Washington, D.C., USA

## The Role of the Environmental Protection Agency Professional Union

I represent 1100 professionals in Washington, D.C., mostly lawyers, scientists, and engineers – people who provide the analysis of problems in our environment at the national level and recommend solutions. We decide on what problems should be addressed and how they are to be solved.

The ethical climate at EPA leaves something to be desired. The risk assessment of fluoride conducted in 1985, a case in point, far exceeds any previous unethical use of science at EPA. Failure to challenge this abuse would be shirking our duty as civil servants, defrauding the public (and harming the public health) and agreeing to become prostitutes for the aims of government politicians. To expose this abuse and establish mechanisms to insure that it does not happen again, NFFE Local 2050 is involved.

The recent cancer study by the National Toxicology Program (NTP) is vitally important to EPA since the Agency has decided to reevaluate its current fluoride in drinking water standards: the MCL (Maximum Contaminant Level) was set at 4 mg/L. EPA seems to have given the study inordinate importance by postponing its own review until the NTP study is finalized, and a new review of the benefits and risks of fluoride being conducted by the Department of Health and Human Services is released later this year. The reevaluation of the standard probably hinges on this study. Both I and Dr. William Marcus, Chief Toxicologist for the Office of Drinking Water, have asked permission of Mr. Reilly, EPA Administrator, to do our own independent analysis of the data. You will see why as we discuss the NTP report.

## Overview of NTP Report

The draft report of the National Toxicology Program (NTP) entitled the "Toxicology and Carcinogenesis Studies of Sodium Fluoride in F344/N Rats and B6C3F1 Mice" was released on April 2, 1990. On April 26, the report was "peer reviewed" at a public meeting in Research Triangle Park, North Carolina by the NTP Board of Scientific Counselors. The final report has not as yet been released.

The report concluded that there was "equivocal evidence of osteosarcoma" in male rats but not in female rats, or male or female mice. The term

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Given at the 18th Conference of the International Society for Fluoride Research, Humboldt State University, Arcata, California, August 4, 1990.

equivocal as it is used here means that the fluoride "may" cause cancer. This conclusion, which was concurred in with the peer review panel, represents a major change from the previous government positon that fluoride is perfectly safe. This report was prepared by the staff of NTP. The study itself, however, was actually done by Battelle Columbus Laboratories under contract to NTP. A quality assurance contractor audited the Battelle report, and a separate group of pathologists reviewed the slides. Apparently the Board of Scientific Counselors did not receive the Battelle report to review nor the deliberations of the pathology work group. The board members, toxicologists, pathologists, and statisicians, reviewed only what they had in front of them in the technical report. Apparently they were not familiar with the fluoride literature.

After reading this report and discussing it with such experts as Drs. John Lee, John Yiamouyiannis, Mel Reuber and Bill Marcus of EPA, I discerned that the report itself is a well-crafted piece designed to minimize the findings and thus the impact on previous positions of the medical community. Serious problems concerning the literature review, the study design and the use of historical controls in interpreting the data as well as serious questions regarding whether cancerous and precancerous lesions found in the rats and mice by the contractor were systematically downgraded in the draft report.

## Misleading Statements in the Report

The simplest facts in this report seem to have been targets for the "Spin Doctors", those scientists turned bureaucrats who know how to play with subtleties. On p. 19, the "Study Rationale" mentions that

> Concern over the possibilities that fluoride might have carcinogenic activity prompted the National Cancer Institute, the Environmental Protection Agency, and the National Institute for Dental Research to nominate fluoride for study by the National Toxicology Program.

The foregoing sounds reasonable. However, Congressional hearings in 1977 were required to force a reluctant government to conduct these studies.

At the outset I failed to realize that the title of the draft report itself was misleading. Called the "NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Fluoride in F344/N Rats and B6C3F1 Mice," the report actually contains the highly important results of a battery of genetic toxicology studies, as well as the studies on rats and mice but the title suggests that the report is concerned only with the health effects found during the 2 year animal studies. The findings of these genetic studies are minimized and obfuscated in the report. Although mentioned in the "Results" section on p. 79, their meaning is not elaborated upon in the "Discussion" section starting on p. 81. Finally, these studies are not mentioned in the "Conclusions" on p. 95, which is quite inexplainable because they show a positive relationship between fluoride exposure and genetic damage; they therefore lend support to the evidence for carcinogenicity. Dr. John Bucher, the study director himself, noted the significance of these genetic studies in a letter to the New York State Coalition Opposed to Fluoridation. His conclusions stated in the letter failed to appear in the NTP report.

The introduction is a marvel in its tiptoeing through the available literature. You would think that the fluoride literature was a mine field to be negotiated with all due haste and caution. Without going too deeply into it, I will mention just a few of the problems. The discussion of skeletal fluorosis fails to mention that crippling skeletal fluorosis is recorded to occur after 20 years from exposure of 20 mg/day (0.28 mg/kg/day). The discussion of levels of fluoride which cause various degrees of dental fluorosis is erroneous. EPA noted (Federal Register, May 13, 1985) that moderate and severe fluorosis have occurred in the USA at levels as low as 0.7 mg/L, not 4 mg/L as stated by this report.

## Flaws in Cancer Study

The cancer study on rats and mice was conducted with a fair number of animals: 100 animals of each sex in the control and high dose groups and about 70 animals of each sex in the low and mid-dose groups. Ten animals of each group were sacrificed at 24 and 66 weeks, the remainder to be sacrificed at the end of two years. In other words there were actually 50 animals in the low and mid-dose groups, 80 in the high dose and control groups. Considering the importance of this study – the exposure of the entire US population to this substance – this was a minimal effort which becomes even more evident when the doses are considered. Both rats and mice received 11, 45, and 79 ppm fluoride in drinking water. Male rats received 0.55, 2.2, and 3.6 mg/kg/day (low doses compared to most cancer studies). When it was determined that the study could only be run with low doses, the number of animals should have been increased.

The most important observation: the control animals were not fed fluoride-free diets. Also, the animals were four weeks old at the beginning of the study and obviously not fluoride free. Male rats were fed a diet containing 7.9 ppm or 0.2 mg/kg/day of fluoride, about six times the dose received by a human drinking 2 liters/day of water containing 1 ppm fluoride. Animal studies have been successfully completed with a diet containing 0.05 ppm fluoride. The historical controls had even more fluoride in the diet. NTP estimates that normally their standard feed has from 28 to 47 ppm, or about 0.7 to 1.2 mg/kg/day. The study had this to say about the dose concerning historical controls:

> Assuming a maximum bioavailability of 60%, the historical database animals actually constitute a group receiving sufficient fluoride to place them between the low- and mid-concentration groups in the current 2-year studies.

This is an excellent argument for excluding the use of historical controls as a comparison group anywhere in the report. Yet, time and time again, they are used to compare with the results of the study and often it is to downgrade the results.

The study found osteosarcomas in the bone of three male rats in the high-dose group and in one male rat in the mid-dose group. These four tumors were found to occur "with a statistically significant dose-response trend by the logistic regression test ($p = 0.027$). The three osteosarcomas in the high dose groups were also significantly higher than the rate of 0.6% of osteosarcomas and osteomas at all sites in control male rats in the historical database. Thus even though the historical controls were actually dosed with fluoride, the results at the high dose in this study were still significantly higher.

I should point out that another osteosarcoma was found in the subcutis of the flank of a male rat in the high dose group which was discounted because the radiographs showed no evidence of a primary neoplasm of the bone. Nevertheless one of the osteosarcomas which they did claim was not seen in the radiograph.

Still another soft tissue tumor described as ". . . collagenous connective tissue with well-defined islands of osteoid and/or woven bone" was ignored because they couldn't classify it.

Adding to the significance of this study is the level of fluoride found in the bone at the conclusion of the study. At the highest dose tested (79 ppm or 0.58 mg/kg/day as fluoride), the male rats had 5263 ppm of fluoride in bone ash. As noted in the report itself, this is within the range that has been found in humans exposed to less than the legal limit of 4 mg/L set by the EPA.

A number of other neoplasms were found to be elevated at various sites in the rats and mice. In the oral cavity, squamous cell papillomas and squamous cell carcinomas were found in the male rats as follows: 0/80 in the controls, 1/51 at the low dose, 2/50 at the mid-dose and 3/80 in the high dose. These results, according to NTP, were marginal and not significantly greater than the controls. A marginal increase in thyroid neoplasm in the high-dose group of male rats likewise was not considered significant. The incidence of malignant lymphomas in female mice was marginally increased relative to controls.

When the Board of Scientific Counselors met on April 26, 1990, their deliberations were preceded by public comments. A limited number of speakers were allowed a hearing. The testimony was never really examined by the Board, so that this exercise was merely window dressing.

## Possible Downgrading of Results by NTP

The most disturbing aspect of this study: At the April 26 public hearing, Dr. Yiamouyiannis and Dr. Mel Reuber pointed out that a number of cancerous and precancerous lesions, found by Battelle Columbus, were downgraded by a special Pathology Working Group hired by NTP to review the Battelle study. Dr. Reuber, a board certified pathologist and former employee of the National Cancer Institute, noted that hepatocholangiocarcinomas were downgraded to hepatoblastoma and finally to hepatocarcinoma. Upon reviewing the slides he stated that the pathology working group had erred.

Dr. Reuber's opinion is particularly significant because he was the one who first identified this lesion in the **Journal of the National Cancer Institute.** These rare liver tumors were found in mice, meaning that the study actually found cancer in both species which would have forced the NTP to change the equivocal rating of cancer to the next higher category — "some evidence." Other tumors were also downgraded: adrenal pheochronocytomas to hyperplasias and liver adenomas to eosinophilic foci. Dr. Marcus and I have recommended to the EPA Administrator that EPA conduct an independent analysis of the Battelle study.

## Summary

The possibility that fluoride causes cancer is much more likely than reported by NTP. The report itself seems to have been designed to downplay the results and therefore to mislead the public:

1. That all three genetic toxicology tests were positive adds support to the conclusion that fluoride may be carcinogenic.

2. The control groups actually received large amounts of fluoride in their feed, about 7.9 ppm, which caused the study to be less sensitive.

3. The historical controls, which were often used to downplay the results of this study, may have received 27 to 48 ppm in their feed. For this reason they should not have been used for comparison purposes.

4. There may have been a systematic downgrading of positive findings after Battelle Columbus reported the original data to NTP.

5. The public was excluded from any significant input into the review of this report.

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# HEALTH SURVEY OF WORKERS OF AN ALUMINUM PLANT IN CHINA

## III Respiratory Symptoms and Ventilatory Functions

by

Masanobu Tatsumi[1], Chuan-Jie Mu[2], Feng Liang[3], Humio Tsunoda[1], Shigenao Nakaya[1], Shiro Sakurai[1], Kazuyoshi Itai[1], Ming-Ho Yu[4], Fang-Ping Chen[3], Hui-Xian Ma[2], and Yu-Min Li[2]

SUMMARY: The respiratory symptoms and ventilatory lung functions of the production-line workers (F-exposed) at an aluminum plant in China have been studied. The data were compared with those obtained from the office workers (controls). F-exposed groups had a higher prevalence of respiratory symptoms than controls and their complaints of phlegm were significantly increased in the older subjects. Whereas several cases of chronic bronchitis were observed in the F-exposed groups, none could be found in the controls. The expiratory flow rate at 25% of the vital capacity/height ($\dot{V}25$/HT), which is sensitive in the detection of small airway obstruction, was decreased.

KEY WORDS: Aluminum refinery workers; Health survey; Respiratory symptoms; Ventilatory lung function.

### Introduction

Inhaled airborne fluorides, particularly gaseous fluorides such as HF, as they come in contact with the respiratory tract, may act as highly irritative and reactive materials, thus directly affecting the respiratory system. As a part of the health survey conducted on workers of an aluminum refinery in China reported previously (1), we carried out a study on the respiratory symptoms and ventilatory functions of the workers at the plant, where airborne F levels in the work environment were at or below the TLV of ACGIH. The data were then correlated with the history of exposure to fluoride.

### Materials and Methods

The participants in this study (99 F-exposed and 47 controls) were the same as reported previously (1). The F-exposed group was further divided into four age-groups, A, B, C, and D and age-matched controls were used for comparison. To obtain information concerning respiratory symptoms, interviews were conducted by participating Chinese physicians at the time of examination using the British Medical Research Council (BMRC)-approved questionnaire (2). Ventilatory function tests were carried out by following the flow-

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volume curves drawn on a spirometer, model Microspiro HI-229 (manufactured by Chest, Tokyo).

## Results

The prevalence of respiratory symptoms was generally higher in the F-exposed groups than in the controls (Table 1). The highest number of subjects with the symptoms was found in Group D, the oldest among the F-exposed groups. Significant differences were observed between Group D and its counterpart concerning phlegm: 11 workers in Group D were diagnosed as belonging to grade 2, compared to none in the control. In grade 2, phlegm is manifested in the morning, during daytime, and at night on most days, for as long as three months in the winter each year (3). Moreover, whereas none of the office workers had chronic bronchitis syndrome, seven of the F-exposed group were found to have this disorder.

The percent vital capacity (%VC) shown by the F-exposed group was high as a whole, but the expiratory flow rate at 25% of the vital capacity/height ($\dot{V}25/HT$) was found to be generally lowered. Whereas five cases of ventilatory lung disfunction, all of obstructive disorders, were identified in Group D, there were also five such cases in the control group, four with obstructive disorders and one with mixed disorders. Although the means of FEV1.0% and $\dot{V}25/HT$ were found to decrease with length of F-exposure, there were no significant differences between the F-exposed and the control group.

Table 1

Prevalence of Respiratory Symptoms

| Group | N | Age | Cough Grade 1 | Cough Grade 2 | Phlegm Grade 1 | Phlegm Grade 2 | Persistent cough and phlegm | Chronic bronchitis syndrome |
|---|---|---|---|---|---|---|---|---|
| A | 29 | 26.7 ±1.8 | 2[a] (6.9) | 3 (10.3) | 2 (6.9) | 3 (10.3) | 3 (10.3) | 1 (3.4) |
| Control | 14 | 26.5 ±2.1 | 2 (14.3) | 1 (7.1) | 1 (7.1) | 2 (14.3) | 3 (21.4) | 0 (0) |
| B | 22 | 32.0 ±1.4 | 0 (0) | 0 (0) | 1 (4.5) | 1 (4.5) | 0 (0) | 0 (0) |
| C | 19 | 36.9 ±1.3 | 2 (10.5) | 1 (5.3) | 2 (10.5) | 3 (15.8) | 2 (10.5) | 2 (10.5) |
| Control | 16 | 36.2 ±2.5 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| D | 29 | 50.4 ±3.0 | 3 (10.3) | 8 (27.6) | 1 (3.4) | 11 (37.9) | 9 (31.0) | 4 (13.8) |
| Control | 17 | 48.5 ±3.2 | 0 (0) | 2 (11.8) | 1 (5.9) | 0 (0) | 1 (5.9) | 0 (0) |

[a] Number of cases. Values in parentheses indicate prevalence rate.

Table 2

Ventilatory Lung Function

| Group | n | Age (yr) | Height (cm) | %VC (%) | FEV1.0% (%) | PF (L/s) | $\dot{V}50$ (L/s) | $\dot{V}25$ (L/s) | $\dot{V}25$/Ht |
|---|---|---|---|---|---|---|---|---|---|
| A | 29 | 26.7 ±1.8[a] | 162.8 ±6.2 | 106.9 ±12.1 | 85.1 ±7.6 | 7.99 ±1.40 | 3.97 ±1.30 | 1.82 ±0.69 | 1.12 ±0.42 |
| Control | 14 | 26.5 ±2.1 | 164.3 ±5.2 | 100.8 ±8.7 | 86.2 ±6.6 | 7.62 ±1.31 | 3.95 ±1.01 | 1.91 ±0.59 | 1.17 ±0.37 |
| B | 22 | 32.0 ±1.4 | 166.7 ±4.1 | 106.9 ±9.8 | 80.7 ±5.4 | 7.23 ±1.31 | 3.61 ±1.33 | 1.49 ±0.58 | 0.90 ±0.35 |
| C | 19 | 36.9 ±1.3 | 166.2 ±4.0 | 107.3 ±14.2 | 81.8 ±4.8 | 7.78 ±1.28 | 3.53 ±0.79 | 1.37 ±0.57 | 0.83 ±0.35 |
| Control | 16 | 36.2 ±2.5 | 162.2 ±4.6 | 111.8 ±3.5 | 82.0 ±3.7 | 7.39 ±0.76 | 3.82 ±1.03 | 1.36 ±0.39 | 0.84 ±0.24 |
| D | 29 | 50.4 ±3.0 | 162.8 ±6.0 | 106.9 ±11.6 | 76.3 ±10.1 | 6.28 ±1.71 | 2.42 ±0.95 | 0.96 ±0.46 | 0.59 ±0.28 |
| Control | 17 | 48.5 ±3.2 | 163.0 ±5.5 | 109.3 ±15.1 | 74.5 ±9.5 | 6.93 ±1.93 | 2.73 ±1.27 | 0.91 ±0.49 | 0.56 ±0.31 |

[a] Values are Mean ±S.D.

## Discussion

The present epidemiological study on the prevalence of respiratory disease was carried out according to the most widely and comprehensively validated MRC questionnaire. The questionnaire is characterized by its making group comparison of the prevalence of individual symptoms singly or in combination, instead of searching for names of specific diseases such as bronchitis. The results obtained from this study clearly indicate the effect of working environment on the respiratory system of workers (Tables 1 and 2) and confirm the earlier findings reported on Japanese aluminum refinery workers (4). A greater number of the pot-line workers had cough and phlegm than office workers at the refinery. This is particularly true in Group D with an average age of 50.4 years. Compared with the controls, a higher percentage of subjects in this group complained of cough and phlegm.

The means of $\dot{V}25/HT$ given by the F-exposed group (Table 2) were lower than that reported elsewhere (5). It is important that this criterion reflects the severity of the small airway obstruction resulting from F-exposure, an observation supported by the high prevalence of chronic bronchitis syndrome found in the F-exposed group (Table 1). Saric et al. (6) reported bronchoconstrictive symptoms among workers employed at an aluminum plant in Yugoslavia. Similarly, Wergeland et al. (7) observed persons working in the potrooms in a primary aluminum production plant in Norway to suffer from "potroom asthma." After cessation of exposure, these workers still had respiratory dysfunction such as morning cough, dyspnea on exertion, and wheezing. Further evidence of the linkage between fluoride air pollution and respiratory symptoms and lung function was shown in a study which surveyed North American Indian children living on a reservation adjacent to an aluminum smelter (8).

Results of the interviews in this study showed that about two-thirds of the subjects were cigarette smokers. Analysis of our data did not show that smoking itself contributed to the prevalence of respiratory symptoms or impairment of ventilatory function.

## Acknowledgement

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# HEALTH SURVEY OF WORKERS OF AN ALUMINUM PLANT IN CHINA

## IV. X-Ray Examinations of the Skeletal System


by

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SUMMARY: X-ray examinations of 98 potroom workers at an aluminum refinery plant in China have been carried out to study skeletal disorders that might be related to F-exposure. The examinations included the pelvis, lumbar vertebrae, radius, ulna, tibia, and fibula. Changes in skeletal system, such as bone density and trabeculae structure, the appearance of osteophytes and exostosis, and the calcification of interosseous membranes and ligaments were assessed by two orthopedic surgeons with the double blind test. No cases of typical skeletal fluorosis were found among the study group, but the appearance of lumbar vertebral osteophyte in the 45-54 year group, exposed to fluoride for more than 20 years, was significantly more frequent than that in the respective control groups. No significant differences in other aspects of osteosclerosis between the F-exposed and the control groups were observed.



KEY WORDS: Aluminum refinery; Potroom workers; Skeletal disorders; X-ray examination.


### Introduction

It is well known that workers, following prolonged exposure to fluoride in the work environment, can manifest osteosclerosis, a form of industrial fluorosis (1). Recent studies (2-4) have indicated that China, a country afflicted with a high prevalence of endemic fluorosis (5-6), also faces serious problems of industrial fluorosis. In 1983, the Chinese government established diagnostic criteria and principles of management of industrial fluorosis. According to these guidelines, a new employee to be assigned to work in a fluoride-contaminated work environment must receive a skeletal X-ray examination. In addition, a follow-up examination is required once every 3-5 years following the initial employment.


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Presented at the 17th ISFR Conference in Budapest, Hungary, June 22-25, 1989.

We carried out a health survey of the workers at an aluminum plant in China in 1987 and reported that the airborne F levels were higher than those found in many other industries (7). The serum and urinary F levels of the potroom workers were found to be higher than those of the office workers who served as controls (7). In an attempt to correlate the airborne F levels with changes in skeletal system, a radiological study on the F-exposed workers has been carried out and the results are reported in this paper.

## Materials and Methods

The participants in this study consisted of 98 potroom workers (F-exposed) and 46 office workers (controls) employed at an aluminum plant in southern China (7). The subjects in the F-exposed group were subdivided into four age groups, A through D (Table 1).

The subjects were examined for restriction in body motion and the presence of pains as they stretched their body, hands, and feet. X-ray examinations were performed on the pelvis, lumbar vertebrae, radius, ulna, tibia, and fibula. Changes in bone density, structure of trabeculae, appearance of osteophytes and exostosis, and calcification of interosseous membranes or ligaments were studied. Assessment of the fluoride-induced changes from X-ray results is often difficult, especially in early stages of fluorosis commonly encountered. Every radiograph was assessed by two orthopedic surgeons with the double blind test. The two specialists carried out a separate examination of each of the radiographs, without the knowledge of the worker's age or exposure history, and recorded the observed skeletal changes. An epidemiologist's determination based on these records would then follow. For each of the films under study, skeletal fluorosis was considered implicated if both doctors determined occurrence of a definite change, or if one of them observed a definite change whereas the other recorded possible. Determination of the severity of skeletal fluorosis was based on Roholm's classification (8).

## Results

A preliminary physical examination revealed a higher prevalence of restricted movement of the spine and/or many joints among the F-exposed workers. In addition, the prevalence increased with age and length of exposure

Table 1

Prevalence of Restricted Movement of the Spine and/or Joints Among Aluminum Plant Workers

| Group | Age | Exposure period (yr) | n | Observed no. of cases | % |
|---|---|---|---|---|---|
| Control | 25-54 | 0 | 46 | 17 | 37.0 |
| F-exposed, A | 25-34 | 5-9 | 29 | 22 | 75.9 |
| F-exposed, B | 25-34 | 10-14 | 22 | 20 | 90.9 |
| F-exposed, C | 35-44 | 15-19 | 19 | 18 | 94.7 |
| F-exposed, D | 45-54 | 20- | 28 | 27 | 96.4 |

to the pollutant (Table 1). While about a third of the control group showed some symptoms, 76-96% of the F-exposed groups manifested the disorders. However, limited movement of many joints was generally of small degree, and no extreme spinal ankylosis was observed.

No cases of skeletal fluorosis belonging to stages 1-3 in Roholm's classification were observed in any of the groups studied. However, several cases of slight osteosclerosis with ossification of obturator foramen or sacroiliac joint in pelvis were identified among the F-exposed and the control groups (Table 2). There were no significant differences in the incidence between these two groups. A mild lumbar vertebral osteophyte in some subjects was found in Groups B, C, and D and in the 45-54 age group of the controls (Table 3).

Table 2

Osteosclerosis of the Pelvis Among Aluminum Plant Workers

| Group | Age | Exposure period (yr) | n | Observed no. of cases | % |
|---|---|---|---|---|---|
| Control | 25-54 | 0 | 20 | 1 | 5.0 |
| A | | 5-9 | 29 | 1 | 3.4 |
| B | | 10-14 | 22 | 2 | 9.1 |
| Control | 35-44 | 0 | 13 | 0 | 0 |
| C | | 15-19 | 19 | 1 | 5.2 |
| Control | 45-54 | 0 | 14 | 0 | 0 |
| D | | 20- | 29 | 5 | 17.2 |

Table 3

Osteophyte of Lumbar Vetebrae Among Potmen Groups

| Group | Age | Exposure period (yr) | n | Observed no. of cases | % |
|---|---|---|---|---|---|
| Control | 25-34 | 0 | NA | — | — |
| A | | 5-9 | 17 | 0 | 0 |
| B | | 10-14 | 19 | 1 | 5.3 |
| Control | 35-44 | 0 | NA | — | — |
| C | | 15-19 | 19 | 1 | 5.3 |
| Control | 45-54 | 0 | 10 | 2 | 20.0 |
| D | | 20- | 25 | 16 | 64.0 |

NA: Data not available
* $p < 0.05$; ** $p < 0.01$

Although no significant differences in the prevalence of lumbar vertebral osteophyte were found among Groups A, B, and C (Table 3), there were significant differences between Group D and its counterpart. Whereas 64% of Group D were found to be with the disorder, only 20% of the control group were identified as symptomatic. Only one individual in Group B and three in Group D were found to have a slight pelvis and spine osteosclerosis, and none showed osteosclerosis in the forearm and lower leg.

## Discussion

Increases in bone density, coarsening and thickening of trabeculae, disorder in the arrangement of the structure, and periosseous hyperplastic calcification and ossification have been reported as the main skeletal changes in industrial fluorosis (3). Calcification and ossification at the sites of muscular attachment, on the interosseous membrane of the middle section of the radius and the upper middle part of the posterior margin of the tibia were considered to have important diagnostic value for industrial fluorosis (3).

Several authors have stressed a positive correlation between increased exposure period and the degree of fluorosis (3,4). Our study tends to support these observations. As shown previously, no significant osteosclerotic changes in the skeletal system could be demonstrated in Groups A, B, and C, and their respective control, all being less than 40 years of age and the exposure time relatively short. However, more than 60% of the subjects in Group D, ages 45-54 years and with an exposure time of 20 years or more, were found to suffer from mild osteophyte of lumbar vertebrae (Table 3). In contrast to the observations made by other workers (2,3), no cases of skeletal fluorosis were found in this study. It is possible that under the existing working conditions, where the airborne F levels were only slightly below the TLV, significant skeletal disorders may not result. It should be pointed out that the observed changes in the skeletal system shown previously were not found to be correlated with the levels of serum and urinary fluoride reported previously (7). This suggests the complexity with respect to factors that may cause changes in skeletal system of the F-exposed subjects. Variations in sensitivity to fluoride have been known in cases of industrial and neighborhood fluorosis (9). The importance of nutritional status of the subjects in relation to incidence and severity of bone disorders has been shown (10). In addition, the general health status including absorption capacity of fluoride through the gastrointestinal tract and renal function of the individuals may also be important (9,11). Guminska *et al.* (12) reported that the erythrocyte and urinary magnesium levels were lowered in human subjects chronically exposed to environmental fluorides and that administration of magnesium, even at doses as low as $^{1}/_{3}$ to $^{1}/_{5}$ of the daily recommended levels, was able to raise them.

## Acknowledgement

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# ARE THE FLUORIDE CONTENT AND THE PHYSICAL LOAD OF BONES IN MAN RELATED?

by

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SUMMARY: From a study of the mineral composition, including fluoride content of various bones from eight human skeletons, it is concluded that fluoride levels are related to bone function.

KEY WORDS: Bone fluoride; Human bones; Mineral composition.

## Introduction

The mineral composition of bone has been the subject of numerous investigations. Previously we have shown that the amounts of Ca, Mg, and P in bone are relatively constant (1). However, because F levels in individual bones exhibited considerable fluctuation, we undertook a study of the fluoride content of various bones in the human body in relation to their load-bearing functions.

## Materials and Methods

Eight skeletons of 60- to 80-year-old deceased homeless persons or residents of the Social Care Institution were provided by the Department of Anatomy. The surfaces of respective bones were spot drilled with a dental boring machine in the cortical part of the shaft. The collected powder, about 50 mg, was used for determination of fluorine, calcium, magnesium, and phosphorus. Fluoride was determined by dissolving the sample in 2N $HClO_4$ and implementing the ion-selective electrode (2). The resulting elements were analyzed in samples dissolved in $HNO_3$. Calcium and magnesium were determined by atomic absorption, and phosphorus by the colorimetric method on reaction with molybdate.

## Results and Discussion

From the Ca, Mg, and P determinations we were able to characterize the mineral compositon of the bones. Since the proportions of these elements were consistent with our expectations and data in the literature (3,4), they were not included in this work. Whereas their ratios were relatively constant, the F levels showed wide differences and in their standard deviations. The results of the fluoride determinations for various types of human bones are presented in Figure 1, arranged from highest to lowest values.

According to other research the mineral composition of cortical bone differs from that of the spongy part, owing to the slower transformation rate of the cortical layer (5). Moreover, the epiphyses and shaft part of bone also differ in fluoride content. To avoid such complications, the material

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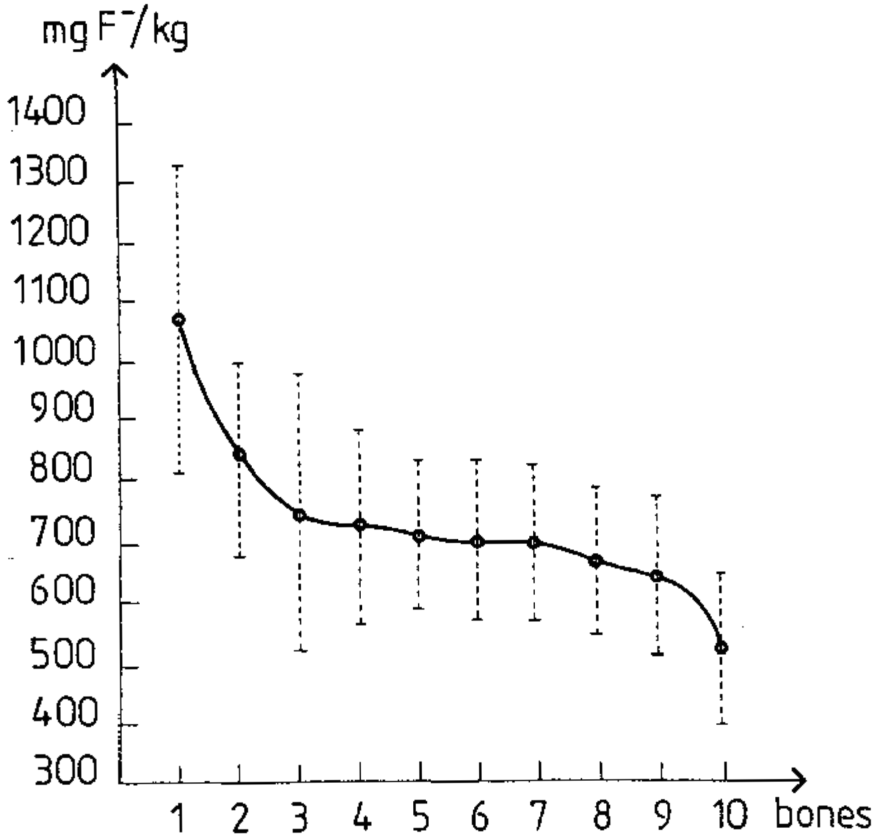
Figure 1

Mean Values (x) of Fluoride in Human Skeletal Bones, Expressed in mg $F^-$/kg and their Standard Deviation (SD).




No. 1 **Talus** (x = 1077; S.D. = 520), No. 2. **Heel Bone** (x = 841; S.D. = 318), No. 3. **Sternum** (x = 759; S.D. = 485), No. 4. **Lumbar Vertebra** (x = 745; S.D. = 321), No. 5. **Occiput** (x = 717; S.D. = 248), No. 6. **Iliac Bone** (x = 714; S.D. = 255), No. 7. **Pelvis** (x = 713; S.D. = 255), No. 8. **Rib** (x = 677; S.D. = 238), No. 9. **Shoulder Bone** (x = 649; S.D. = 256), No. 10. **Mandible** (x = 530; S.D. = 247).

used in this study was obtained from the cortical part of the bone shaft. Disregarding the changes which could have been caused by maceration of the skeletal bones, no correlation was observed between the fluoride level in bones and the remaining mineral elements.

The shape and construction of each bone are adapted to its load, which led us to assume that variable accumulation of fluoride in bones may depend on the function the bone performs, particularly upon the physical load it bears. In this way fluorine can influence the mechanical resistance of the bone. The heaviest load in man appears in the following bones: talus, calcaneus, and lumbar vertebra. Further changes in bone loads are shown successively in Figure 1. They may not all be arranged according to the views of orthopedists. A main difference is that the skeletons studied did not stem from fit, young subjects, but from infirm persons mostly edentulous and often bed-ridden for a considerable part of their lives. Therefore, the thigh bone, shoulder bone and toothless mandibles were probably not so intensively loaded as in healthy subjects. The mode of life, the habit and kind of nourishment as well as movement in these persons were different, and the metabolic processes of reconstruction and remodelling of their bones were also much slower.

Taking into account the cited differences in the mineral composition of the bone, it is reasonable to assume that there is a relationship between the physical load of the bone and its fluorine content. Fluorine is known to increase the physical resistance of the bone by forming fluoroapatites, which may therefore be of fundamental importance for correct functioning of the organ.

## Acknowledgement

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# INHIBITION OF SPONTANEOUS PRECIPITATION OF CALCIUM PHOSPHATE BY MONOFLUOROPHOSPHATE

by

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SUMMARY: To study the effect of MFP on spontaneous precipitation of calcium phosphate, solutions containing 0.5 to 6.0 mmol/L Ca, and P at a Ca/P ratio of 1.67, were incubated at 37°C for 24 h. The lowest concentration of Ca and P that formed a nodular precipitate on the tube wall was noted. Test series contained 4 mmol/L MFP. A control series (no F or MFP) required a relative degree of saturation (RDS) with respect to hydroxyapatite of 1.27 for consistent precipitation within the 24 h period. The RDS increased to 1.32 when commercial grade MFP was present and to 1.40 with purified MFP. Inhibition was not, therefore, due to polyphosphate impurities in MFP. Because of residual F in the purified MFP the experiment was repeated in the presence of excess F (250 μmol/L), when the RDS with respect to fluorapatite was 1.51 in the control and 1.87 and 1.95 with impure and purified MFP, respectively. Thus MFP stabilizes calcium phosphate solutions, an effect that is independent of F.

KEY WORDS: Calcium phosphate; Monofluorophosphate; precipitation.

## Introduction

Sodium monofluorophosphate (MFP) is commonly used as the active anti-caries agent in toothpaste. While it is often referred to as a "fluoride" compound, and the toothpastes as "fluoride toothpastes," the MFP anion is quite different from the fluoride ion, $F^-$, in that it is a divalent ion, $PO_3F^{2-}$, with the fluorine atom covalently bound to the phosphorus atom. Properties of fluoride (F) might, then, be expected to be quite different from those of MFP. For example, F enhances the growth of hydroxyapatite seed crystals (1) while MFP has the opposite effect (2).

We have observed that a solution containing $CaCl_2$ (20 mmol/L) and $NaH_2PO_4$ (12 mmol/L), pH 5.0, is stable for many weeks when it contains commercial grade sodium monofluorophosphate at a total F level of 5 mmol/L, but rapidly forms a precipitate if fluoride alone is added to the solution at the same concentration, 0.3 mmol/L, present as a impurity in the 5 mmol/L technical grade MFP. The solution is equally highly supersaturated with respect to fluorapatite in both cases, but precipitation appears to be inhibited in the presence of MFP. It is known that commercial grade MFP also contains various polyphosphate impurities such as pyrophosphate (3), which may be responsible for inhibiting precipitation (4).

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The object of the present study was to examine the effect of MFP on spontaneous precipitation of calcium phosphate from supersaturated solutions.

## Materials and Methods

A series of 11 solutions, each containing calcium (Ca) and orthophosphate (P) in increasing concentrations, was prepared by mixing stock solutions containing Ca or P or neither. Each stock solution was adjusted to the same ionic strength (100 mmol/L) with KCl and the same pH, 7.0 (buffered with imidazole, 10 mmol/L), and was prepared with distilled and deionized water. The lowest Ca concentration in the series was 0.5 mmol/L and increased by 0.5 mmol/L steps to a maximum of 5.5 mmol/L. P was included at a Ca/P ratio of 1.67 (see Table).

One test series of 11 solutions contained 4 mmol/L commercial grade MFP, another 4 mmol/L purified MFP, while in the control series the MFP was omitted. A second test and control series duplicated the first, but also included 250 μmol/L NaF in all solutions. The solutions were mixed in 10 mL quantities, adding the Ca stock solution last, and were incubated without agitation in glass-stoppered tubes at 37°C for 24 h, after which the solutions were examined under uniform lighting conditions for the formation of a precipitate visible to the naked eye. All series were run in triplicate, except those containing purified MFP which were run in duplicate. Glassware was cleaned in detergent, soaked in acid solution, and rinsed 3 times in deionized water.

Commercial MFP was purified by a silver monofluorophosphate precipitation technique (5). Reconversion to the sodium form was achieved by the addition of the minimum quantity of NaCl to precipitate all AgCl. The resulting MFP solution contained 0.02 to 0.03 $F^-$ as a percentage of the total F, and were stored frozen until required (6).

Degrees of supersaturation (DS) with respect to hydroxyapatite (HA), brushite (BSH) and fluorapatite (FA) were calculated using the method of Carey et al. (8). Ion activity products were calculated by a method described previously (9). Logistic regression was used to model the probability of precipitation, given Ca and P concentration levels and group (test/control) membership.

## Results

As expected, precipitation was clearly related to Ca and P concentration levels ($p < 0.001$ for both experiments) although the lowest concentration to show a precipitate was not always the same in each replicate (Table). MFP inhibited the spontaneous precipitation of calcium phosphate in the first experiment with no added fluoride, precipitation occurring at significantly higher concentrations of Ca and P when both commercial MFP ($p = 0.013$) and purified MFP ($p = 0.003$) were present. However, no significant difference could be demonstrated between these two grades of MFP.

The commercial grade MFP contributed 250 μmol $F^-$/L to the solution, and since $F^-$ decreases Ca and P concentrations necessary for spontaneous precipitation (10), this would be expected to obscure the MFP effect to some extent. Even purified MFP contributed a small amount of $F^-$, 1.6 μmol/L, Table

Effect of MFP on Precipitation (X) in Solutions with Increasing Calcium and Phosphate Concentration.

| Calcium (mmol/L)<br>Phosphate (mmol/L) | Replecate | 0.5<br>0.3 | 1.0<br>0.6 | 1.5<br>0.9 | 2.0<br>1.2 | 2.5<br>1.5 | 3.0<br>1.8 | 3.5<br>2.1 | 4.0<br>2.4 | 4.5<br>2.7 | 5.0<br>3.0 | 5.5<br>3.3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MFP (Commercial) | 1 | . | . | . | . | . | . | . | X | X | X | X |
| (4 mmol/L) | 2 | . | . | . | . | . | . | . | X | X | X | X |
| | 3 | . | . | . | . | . | . | . | X | X | X | X |
| MFP (Purified) | 1 | . | . | . | . | . | . | . | . | . | X | X |
| (4 mmol/L) | 2 | . | . | . | . | . | . | . | . | X | X | X |
| Control | 1 | . | . | . | X | X | X | X | X | X | X | X |
| (No MFP) | 2 | . | . | . | . | . | . | X | X | X | X | X |
| | 3 | . | . | . | . | . | . | X | X | X | X | X |
| DS HA[a] | | 0.54 | 0.80 | 0.95 | 1.06 | 1.14 | 1.21 | 1.27 | 1.32 | 1.36 | 1.40 | 1.43 |
| DS BSH[b] | | -0.72 | -0.42 | -0.25 | -0.13 | -0.04 | 0.04 | 0.10 | 0.16 | 0.20 | 0.25 | 0.29 |
| With Added Fluoride (250 μmol/L) | | | | | | | | | | | | |
| MFP (Commercial) | 1 | . | . | . | . | . | . | . | X | X | X | X |
| (4 mmol/L) | 2 | . | . | . | . | . | . | X | X | X | X | X |
| | 3 | . | . | . | . | X | X | X | X | X | X | X |
| MFP (Purified) | 1 | . | . | . | . | . | . | . | X | X | X | X |
| (4 mmol/L) | 2 | . | . | . | . | . | . | . | . | . | X | X |
| Control | 1 | . | . | X | X | X | X | X | X | X | X | X |
| (No MFP) | 2 | . | . | X | X | X | X | X | X | X | X | X |
| | 3 | X | . | X | X | X | X | X | X | X | X | X |
| DS FA[c] | | 1.09 | 1.35 | 1.51 | 1.62 | 1.70 | 1.77 | 1.82 | 1.87 | 1.91 | 1.95 | 1.99 |

All solutions are at pH 7. Degree of supersaturation with respect to [a]HA and [b]BSH. [c]Degree of supersaturation of the MFP (purified) and control solutions only with respect to FA. Additional fluoride in the MFP (commercial) sample raised DS FA values by 0.04.

hence a second experiment was carried out in the presence of excess fluoride, 250 µmol/L. In this case the inhibitory effect of purified MFP was more pronounced (Table). Again, precipitation occurred at higher Ca and P concentrations in the presence of commercial MFP ($p = 0.001$) and purified MFP ($p < 0.001$), but in this case the purified MFP was significantly more effective ($p = 0.023$).

## Discussion

Inhibition of spontaneous precipitation by purified MFP can not have been due to polyphosphate impurities such as pyrophosphate as the silver precipitation technique removes nearly all this material (11). It is thus concluded that the MFP anion, at a level of 4 mmol/L, is able to stabilize solutions that might otherwise form a spontaneous precipitate of calcium phosphate, an effect that is the reverse of the F effect (10).

The small amount of precipitate precluded X-ray diffraction analysis of its nature. Precipitates formed in the absence of F arose from solutions highly supersaturated with respect to HA, and this or its amorphous calcium phosphate precursor was the likely solid phase. Solutions containing added F were highly supersaturated with respect to FA and this mineral, or an even less soluble fluorhydroxyapatite salt (8), may then have formed the solid phase. Solutions with a Ca concentration < 3 mmol/L were unsaturated with respect to BSH and this salt could not form part of the precipitate here. However, at higher concentrations where the solutions were even slightly supersaturated with respect to brushite, formation of this salt cannot be excluded as it tends to precipitate more readily than apatite at low levels of supersaturation (12).

A concentration of 4 mmol/L MFP was tested because this was approximately the level used in a supersaturated mouthrinse solution studied previously in our laboratory (13). Since the effect of phosphate inhibitors on the level of supersaturation necessary for spontaneous precipitation is a direct function of their concentration in solution (14) it seems likely that lower concentrations of MFP would have less effect. If so, MFP is a far less sensitive inhibitor of spontaneous precipitation than diphosphonate or pyrophosphate, compounds which are effective at a level of 1 to 10 µmol/L (4,14).

True spontaneous precipitation is, for all practical purposes, an unachievable aim in this type of study. Submicroscopic debris in solution and even the walls of the vessel are likely seeding sites for non-specific heterogeneous nucleation (15). Thus, where onset of precipitation did not agree between replicates the higher values probably approach those required for true homogenous nucleation most closely.

It is generally agreed that F enhances the spontaneous precipitation of apatite from solution because it increases the driving force for precipitation of fluorapatite, or fluorhydroxyapatite (16,17). However, there is no evidence that MFP can substitute in the apatite lattice as does F, and therefore its presence in solution would not, on theoretical grounds, be expected to enhance precipitation. On the other hand, phosphate compounds in which the P group is covalently bound to another group often inhibit calcium phosphate precipitation (2), and it appears that the MFP action demonstrated here is simply another example of this phenomenon. Whether the inhibitory action results from a complexing of Ca and thus a lowering of the driving force for HA precipitation, or from adsorption of MFP on crystal nuclei, preventing further growth, is not known.

The results of the present study show that MFP has the ability to stabilize supersaturated calcium phosphate solutions. This property could possibly be exploited in a pharmaceutical application where it is desired to increase the range of metastability of a calcium phosphate mouthrinse solution for the remineralization of incipient dental caries lesions.

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# FLUORIDE UPTAKE BY DUCK-WEED SPIRODELA POLYRRHIZA

by

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SUMMARY: Fluoride uptake by duck-weed Spirodela polyrrhiza studied in field and under laboratory conditions showed substantial uptake by fronds from 5 ppm upwards under laboratory conditions. The low threshold value shown by S. polyrrhiza is significant in view of the fact that earlier reports had indicated a higher threshhold for aquatic plants rendering them unsuitable in defluoridation of natural waters with a lower fluoride concentrations. Consequently, the present study indicates the promising role of S. polyrrhiza in defluoridation even at lower concentration.

KEY WORDS: Aquatic uptake; Duckweed; Fluoride; Spirodela polyrrhiza.

## Introduction

Fluoride contamination of water resources is a serious problem prevailing in India. Of 6 Lakh villages over 50% contain fluoride in drinking water ranging from 0.5 to 50 ppm. It is estimated that more than one million people have developed skeletal fluorosis due to the high level of fluoride contamination (1).

Aquatic macrophytes are reported to absorb nutrients and heavy metals from polluted water (2-6). Use of plants, reported to accumulate fluoride, as defluoridating agents has also been suggested (7-10). Although water hyacinth has been reported to accumulate substantial amounts of fluoride (10) because of its high threshold level it has not been found suitable for defluoridation purposes.

The present study was undertaken to assess the threshold of Spirodela polyrrhiza L., a profusely occurring duck-weed. The plant grows luxuriantly in four ponds around Lucknow, (U.P.), India, which contain high levels of fluoride. In view of the plant tolerance toward fluorides it was considered worth while to determine its threshold level under laboratory conditions and to assess the feasiblity of its use in defluoridation of natural waters.

## Materials and Methods

Water and plant samples from four ponds (Sites I-IV) around Lucknow were collected and fluoride content was estimated in each.

The laboratory experiments were carried out on healthy fonds of S. polyrrhiza collected from unpolluted bodies of water and acclimatized for six weeks in 20% Hoagland solution under 16 h white fluorescent light/114 $\mu$ moles/s at 25 ±2°C.

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Acclimatized fronds (1.5 g) were exposed to varying concentrations (1, 5, 10, 15, 20,, and 25 ppm) of fluoride. Fluoride ions were provided by NaF, and fronds kept in nutrient solution without fluoride served as controls. Fronds were harvested after 24, 72, 120, 168, and 360 h.

The method suggested by Hall (11,12) was used for the estimation of fluoride. The treated fonds were ashed in a platinum crucible, and, after developing color by Lanthanum Alizarin complexan O.D. was determined at 570 nm. Chlorophyll and protein were estimated following the procedures given by Arnon (13) and Lowry et al. (14), respectively. The optical densities were determined on a Milton Roy Spectronic 1201 spectrophotometer.

The level of significance within concentration and exposure was determined with respect to fluoride uptake and effect of fluoride on protein by subjecting the data to an analysis of variance. Coefficient of variation in chlorophyll content was determined with respect to concentration (15).

## Results and Discussion

Fluoride concentration in the bodies of water (Site I-IV) and its uptake by Spirodela fronds are shown in Table 1. The uptake of fluoride by the fronds varied from site to site depending upon the concentration of fluoride in the water.

Table 2 shows uptake of fluoride by the fronds under laboratory conditions. Although the uptake is negligible at 1 ppm F, it is quite substantial from 5 ppm and upwards. The fronds showed a concentration and duration dependent uptake. The protein content of the fronds varied in the different concentrations of fluoride (Table 3) and the percent reduction in protein content increased with the increase of fluoride concentration in the nutrient solution.

The effect of different concentrations of fluoride on chlorophyll content was found up to 25 ppm.

The results indicate that the fronds of S. polyrrhiza are capable of accumulating fluoride both in field as well as under laboratory conditions. However, accumulation was substantial only at 5 ppm and higher concentrations

Table 1

Fluoride Content of Ponds (Site I-IV) and in S. polyrrhiza fronds

| Site | Fluoride Content in water (ppm) | Fluoride Content in fronds (μg/g dry wt.) |
|---|---|---|
| Gomti Nagar (I) | 0.60 ±0.03 | 32.82 ±3.92 |
| Tiwariganj (II) | 0.89 ±0.11 | 39.30 ±1.82 |
| Ismailganj (III) | 0.76 ±0.12 | 29.47 ±4.74 |
| Mehmoodpur (IV) | 0.59 ±0.12 | 29.47 ±4.74 |

(± Standard Deviation)

Table 2

Fluoride Uptake by S. polyrrhiza (ug/g dry wt.)
With Different Concentrations of Fluoride

| Conc. (ppm) | 24 h | 72 h | 120 h | 168 h | 360 h |
|---|---|---|---|---|---|
| 0 | 36.8 ±4.8 | 34.76 ±2.26 | 38.4 ±2.82 | 35.4 ±2.12 | 34.0 ±2.54 |
| 1 | 40.92 ±2.03 | 41.76 ±1.97 | 45.8 ±0.28 | 49.0 ±7.91 | 49.2 ±3.95 |
| 5 | 119.6 ±21.49 | 134.76 ±2.54 | 230.0 ±10.74 | 236.6 ±27.15 | 248.8 ±28.84 |
| 10 | 401.2 ±35.63 | 411.96 ±7.07 | 421.6 ±11.87 | 429.0 ±66.75 | 450.0 ±48.84 |
| 15 | 634.0 ±61.65 | 644.36 ±69.57 | 658.0 ±45.82 | 667.4 ±89.94 | 672.4 ±7.35 |
| 20 | 680.0 ±65.78 | 765.16 ±13.57 | 844.4 ±3.95 | 918.6 ±85.41 | 922.0 ±40.16 |
| 25 | 1028.4 ±39.59 | 1033.96 ±114.14 | 1039.2 ±0.56 | 1058.2 ±146.51 | 1065.6 ±78.06 |

± Standard Deviation; $p < 0.01$

Table 3

Effect of Different Concentrations of Fluoride on Soluble Protein
in S. polyrrhiza (µg/g fresh wt.)

| Conc. (ppm) | 24 h | 72 h | 120 h | 168 h | 360 h |
|---|---|---|---|---|---|
| 0 | 55.69 ±0.88 | 57.54 ±2.76 | 51.94 ±2.16 | 54.7 ±2.25 | 57.0 ±1.61 |
| 1 | 54.87 ±1.79 (1.47) | 56.41 ±2.48 (1.96) | 50.49 ±0.68 (2.79) | 52.5 ±0.5 (4.02) | 53.2 ±0.12 (6.66) |
| 5 | 53.82 ±0.01 (3.35) | 54.14 ±1.54 (5.90) | 47.51 ±2.15 (8.52) | 49.5 ±1.12 (9.50) | 50.5 ±0.61 (11.40) |
| 10 | 53.62 ±0.27 (3.71) | 53.6 ±1.65 (6.84) | 46.07 ±1.28 (11.30) | 48.2 ±2.25 (11.88) | 48.5 ±0.38 (14.91) |
| 15 | 51.74 ±1.67 (7.09) | 53.4 ±1.75 (7.19) | 45.47 ±0.24 (12.45) | 47.8 ±2.0 (12.61) | 44.7 ±0.43 (21.57) |
| 20 | 50.03 ±1.19 (10.16) | 50.0 ±1.7 (13.10) | 43.3 ±1.91 (16.63) | 44.5 ±1.5 (18.64) | 40.4 ±1.32 (29.82) |
| 25 | 49.85 ±1.2 (10.48) | 48.11 ±2.55 (16.38) | 40.14 ±1.9 (22.71) | 42.2 ±2.25 (22.85) | 37.2 ±2.33 34.73) |

Values in parentheses represent percent reduction; $p < 0.01$

of fluoride. These levels are much lower than the threshold level (10 ppm) reported for water hyacinth (10). In view of the high tolerance and lower threshold value shown, the use of S. polyrrhiza fronds is suggested for reducing the level of fluoride in natural waters.

## Acknowledgement

Gratitude is extended to Dr. P.V. Sane, Director, National Botanical Research Institute, for providing the necessary facilities and for his keen interest in this work.

Table 4

Effect of Different Concentrations of Fluoride on Chlorophyll in S. polyrrhriza (mg/g fresh wt.)

| Conc. (ppm) | 24 h | 72 h | 120 h | 168 h | 360 h |
|---|---|---|---|---|---|
| 0 | 1.14 ±0.11 | 1.18 ±0.14 | 1.23 ±0.02 | 1.25 ±0.01 | 1.26 ±0.00 |
| 1 | 1.15 ±0.11 | 1.20 ±0.00 | 1.26 ±0.00 | 1.27 ±0.06 | 1.29 ±0.00 |
| 5 | 1.16 ±0.11 | 1.22 ±0.01 | 1.29 ±0.00 | 1.29 ±0.02 | 1.29 ±0.04 |
| 10 | 1.18 ±0.00 | 1.23 ±0.04 | 1.29 ±0.06 | 1.30 ±0.02 | 1.30 ±0.01 |
| 15 | 1.19 ±0.02 | 1.24 ±0.07 | 1.32 ±0.03 | 1.32 ±0.06 | 1.33 ±0.01 |
| 20 | 1.14 ±0.06 | 1.18 ±0.08 | 1.26 ±0.00 | 1.30 ±0.02 | 1.29 ±0.00 |
| 25 | 1.15 ±0.11 | 1.17 ±0.01 | 1.23 ±0.02 | 1.26 ±0.05 | 1.24 ±0.01 |
| Mean ± S.D. | 1.15 ±0.019 | 1.20 ±0.027 | 1.26 ±0.033 | 1.28 ±0.025 | 1.28 ±0.028 |
| C.V.(%) | (1.65) | (2.25) | (2.61) | (1.95) | (2.18) |

± Standard Deviation

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# $F^-$ CONTENT OF INDIAN TOOTHPASTES AND SELECTED FOOD ITEMS

by

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SUMMARY: Fourteen brands of toothpastes, nine brands of tea leaves, five brands of table salt, and three types of green leafy vegetables were analyzed for $F^-$ content. The range of $F^-$ levels in all the items analyzed was wide. The amount of $F^-$ which may be ingested from a 2-g brushing of toothpaste and from consumption of 4 cups of tea has been calculated. The difference in the $F^-$ extracted from tea leaves boiled in distilled water and then in tap water was significant, and also varied from brand to brand. Of three green leafy vegetables, Chulai, an uncommon green leafy vegetable, had an exorbitantly higher $F^-$ than the two common green leafy vegetables, cabbage and spinach.

KEY WORDS: Cooking salt $F^-$; Fluoride; Green leafy vegetable $F^-$; Tea leaves $F^-$; Toothpaste.

## Introduction

Fluorosis, an important public health problem in India, is endemic in states like Andhra Pradesh, Tamil Nadu, Karnataka, Gujarat, Rajasthan, Utter Pradesh, peripheral parts of Delhi, and Haryana (1). The primary cause of endemic fluorosis is excessive intake of the trace element fluoride ($F^-$). Food items are now believed to contribute much to the $F^-$ intake; high concentration of $F^-$ in drinking water and the length of period over which it is consumed are critical for development of fluorosis. Poor nutritional status, and consumption of some staples like jowar with which $F^-$ retention and toxicity is greater than with wheat- or rice-based diets also make the poorer segments of the population more prone to fluorosis (2,3). Foods such as green leafy vegetables, tea leaves, and salt contribute appreciable amounts of $F^-$ to the daily intake. Tea leaves accumulath more $F^-$ than any other edible plant. Toothpastes and mouth washes used personally as well as solutions applied in dental clinics may contribute significant amounts. The majority of people in rural, urban, and semi-urban areas, falling in fluorotic zones of India, use underground water for cooking and drinking purposes. Although the Water Technology Mission of India is spending large sums of money for removal of excess $F^-$ and for development of defluoridation plants so that not more than 1 ppm of fluoride remains in drinking water, toothpaste manufacturers continue to add $F^-$ to toothpastes and mouth washes.

Some 10% or less of the Indian population are known to use toothpastes. Of the total toothpaste production, about 9.8 percent are fluoridated by the manufacturers. Fluoridated toothpastes are advertized heavily on TV, and

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the written media highlight the role of fluoride in promoting tooth health. The public is unaware of the hazards of $F^-$ toxicity as well as the role played by indiscriminate fluoridation of consumer goods.

Irrespective of the socio-economic status, caste, creed, or age group, the people of India are known to consume daily a few cups of tea infused excessively with sugar and milk. Since tea leaves are known to contain concentrated amounts of $F^-$, tea drinking could be a potential fluorosis aggravating factor in this country. The table salt is also known to contain high $F^-$ levels. In India urban consumers use powdered packaged brands of iodized and un-iodized salt. A majority of the rural and a significant proportion of the urban population still purchase and use the raw, unrefined salt after grinding it. In addition to table salt, two other rock salts are used for flavoring special dishes like pickles and other savories.

Thus the main purpose of this study was to analyze the fluoride levels of these Indian products as well as a few green leafy vegetables.

## Materials and Methods

Fourteen brands of toothpaste including one tooth powder, nine brands of tea leaves including four samples of tea dust, six kinds of cooking salt, and three green leafy vegetables (cabbage, spinach, Chulai) were obtained from the Hisar local market. $F^-$ in green leafy vegetables and toothpastes was determined by the method of Villa (4) using $F^-$ specific ion electrode 9040900 and reference electrode 900100. $F^-$ standard contained 2000 ppm $F^-$. Dried and ground (60 mesh) samples were treated with 0.1 N perchloric acid. Perchloric acid was used as blank, the reading of which was read on Systronic's Digital pH meter. $F^-$ standard (0.2 mL) was added and reading noted. Similarly the reading of samples was read and the concentration calculated using the equation of Villa. Tea leaves and salt were analyzed for $F^-$ by the spectrophotometric method using Zirconium-Eriochrome Cyanine R Lake (5). For analysis tea leaves were prepared according to the typical Indian tea process, namely one g of leaves were boiled uncovered in 100 mL water in a 250-mL Corning beaker for a period of 4 min. The tea leaves were allowed to settle to the bottom of the beaker and the tea infusion poured out into another beaker. One and a half teaspoon of activated charcoal was added and the mixture allowed to stand before filtering for further analysis. The infusion was prepared in both distilled and tap water to determine the difference of $F^-$ extraction. A suitable volume of these aliquots was treated with 7:3 $HCl:H_2O$ after addition of p-nitrophenol indicator. Eriochrome Cyanine R solution was added to this zirconium oxychloride solution. Finally the above procedure was repeated substituting the zirconium oxychloride with 7:3 $HCl:H_2O$. A standard curve was prepared.

## Results and Discussion

The $F^-$ content of toothpastes (Table 1) range from a low of 17.96 to a high of 3169 ppm on a dry matter basis. However, calculations were also made to get the quantity of $F^-$ present in 2 g of moist paste, on an average, for each brushing. Fluoridated toothpaste contained more $F^-$, compared to its non-fluoridated counterpart of the same brand. The amount of $F^-$ in 2 g of moist paste varied from 0.02 to 4.25 mg. According to Eichler *et al.* (6), 20% of $F^-$ in a single brushing is retained. The amounts of $F^-$ likely to be retained from the 14 different brands (Table I) are important in view of the following fluorosis aggravating conditions: high water $F^-$ levels, poor nutritional status and toxicity conducive staple diets, etc. of the people of various states of India. Toothpastes which provide about 1 to 2 mg $F^-$ per brushing may be adding significant amounts of $F^-$ to the already critically high intake of this element by the people

$F^-$ content of tea leaves from five popular brands sold in cartons and four types of tea dust sold openly by shopkeepers in quantities desired by consumers is given in Table 2. Leaf size of tea dust is much smaller than the popular brands sold in cartons. Tea dust is also much cheaper than the popular brands in cartons and is favored by a majority of the population. In distilled water infusions $F^-$ ranged from 10 to 100 ppm. Tap water infusions contained 3.12 to 50 percent more $F^-$. The four tea dust samples had the $F^-$ range in distilled water boiled samples between 5 to 60 ppm. The one with 5 ppm $F^-$ showed a phenomenally higher (650%) extraction compared to that infused in tap water. In the other three dust samples the increase was 8.26, 25 and 50 percent, indicating that tap water extracts more $F^-$ from tea leaves than distilled water. Calculations made from the amount of $F^-$ that might be ingested by a person consuming four cups (200 mL each) of tea daily ranged widely from 160 µg to 1320 µg.

$F^-$ content in cabbage, spinach, and Chulai were found to be 1.64, 4.6, and 17.02 ppm, respectively. The Kala Namak and Dendha Namak used in

Table 1

$F^-$ Content of Toothpastes

| Toothpaste | Moisture (%) | ppm $F^-$ (moisture free basis) | $F^-$/2 g of paste (mg) | mg $F^-$ retained on brushing |
|---|---|---|---|---|
| A | 41.00 | 17.96 | 0.02 | 0.004 |
| B | 21.25 | 20.56 | 0.03 | 0.006 |
| C | 43.80 | 489.00 | 0.55 | 0.11 |
| D | 26.08 | 448.00 | 0.66 | 0.13 |
| E | 39.40 | 2328.00 | 1.99 | 0.39 |
| F | 18.59 | 1488.00 | 2.42 | 0.48 |
| G | 32.87 | 1824.00 | 2.44 | 0.49 |
| H | 25.65 | 1824.00 | 2.74 | 0.53 |
| I | 13.04 | 1488.00 | 2.88 | 0.57 |
| J | 33.22 | 2327.00 | 3.10 | 0.62 |
| K | 27.54 | 2328.00 | 3.77 | 0.75 |
| L | 31.60 | 2328.00 | 3.18 | 0.63 |
| M | 39.00 | 3169.00 | 3.87 | 0.77 |
| N | 31.23 | 3169.00 | 4.25 | 0.85 |

Table 2

Fluoride Content in Different Tea Brands

| Sample | $F^-$ ppm | | | $F^-$ in Four cups of tea (μg) |
|---|---|---|---|---|
| | Infused in Distilled Water | Infused in Tap Water | % Difference | |
| **Carton Packed** | | | | |
| Brand I | 20 | 25 | 25 | 200 |
| Brand II | 10 | 20 | 50 | 160 |
| Brand III | 30 | 40 | 25 | 320 |
| Brand IV | 60 | 67.5 | 12.5 | 567 |
| Brand V | 160 | 165 | 3.12 | 1320 |
| **Tea Dust** | | | | |
| 36/- Rs/kg | 20 | 40 | 50 | 320 |
| 24/- Rs/kg | 60 | 65 | 8.26 | 520 |
| 28/- Rs/kg | 40 | 50 | 25 | 400 |
| 32/- Rs/kg | 5 | 37.5 | 650 | 300 |

* 1 g tea boiled in 100 mL of tap/distilled water for 4 minutes.

specifically flavored spicy dishes like pickles, chat (fruit and legume concoction) contained 2.75 and 2.50 ppm of $F^-$, respectively. The two kinds of salts which are used for every day cooking, whole (unground salt) and polypacked powdered salt, contained 3 ppm $F^-$. A popular band of iodized polypacked salt contained 1 ppm; non-iodized contained only 0.25 ppm of $F^-$. Variation in farm products may be observed from field to field, and season to season, necessitating $F^-$ level analysis of all lots.

## Conclusion

In endemically fluorotic zones of developing countries, recommendations of the low $F^-$ products may not be very helpful due to non-patented sale of most consumer goods. However, it can be concluded that national governments need to scrutinize carefully the policy of allowing indiscriminate toothpaste fluoridation in developing countries. In addition to underground drinking water, tea may also contribute the next major amount of $F^-$ intake for the people in endemic zones.

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## ENDEMIC FLUOROSIS WITH SPINAL CORD COMPRESSION
### A Case Report and Review

by

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(Abstracted from **Arch. Intern Med.** 149:697-700, 1989)

A case of spinal cord compression in a Mexican immigrant due to vertebral osteosclerosis from chronic fluoride intoxication is reported. Endemic fluorosis is acquired through drinking water. Groundwater sources with high fluoride content occur worldwide. Epidemiology, metabolism, and clinical features of fluorosis are reviewed. Greater physician awareness of this entity is important to identify correctly patients with this unusual and potentially devastating clinical disorder.

KEY WORDS: Endemic fluorosis; Osteosclerosis; Spinal cord compression.

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## FLUORIDE ACTIVATION OF NEUTROPHILS: SIMILARITIES TO FORMYLMETHIONYL-LEUCYL-PHENYLALANINE

by

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(Abstracted from **Inflammation**, 13:47-58, 1989)

Fluoride induced degranulation of both primary and specific granules from-neutrophils pretreated with cytochalasim B. A similarity in dependency on extracellular $Ca^{2+}$ for fluoride- and for FMLP-stimulated $O_2$-generation and degeneration was observed. Pertussis toxin, but not cholera toxin, inhibited FMLP and fluoride activation of neutrophils, whereas neither toxin affected PMA activation of these cells. Results suggest that fluoride and FMLP activate neutrophils through a common $Ca^{2+}$-dependent and pertussis toxin-sensitive pathways.

KEY WORDS: Degranulation; Fluoride activation; Neurophils activation; Pertussis toxin.

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## ANTAGONISM OF FLUORIDE TOXICITY BY HIGH LEVELS OF CALCIUM BUT NOT OF INORGANIC PHOSPHATE DURING SECRETORY AMELOGENESIS IN THE HAMSTER TOOTH GERM IN VITRO

by

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(Abstracted from **Arch. Oral Biol.**, 34:625-636, 1989)

To determine whether the interference by fluoride ($F^-$) with secretory amelogenesis in vitro could be modulated by altering the levels of calcium (Ca) and inorganic phosphate (P) in the medium, hamster first upper molar tooth germs in the secretory phase of amelogenesis, were exposed to 10 µM-1.31 mM (0.2-25 part/$10^6$) of $F^-$ in vitro for 2 days in the presence of either low (1.2 mM), moderate (2.1 mM) or high (4.1 mM) levels of Ca, or moderate (1.6 mM) and high (3.6 mM) levels of P. $F^-$ increased the uptake of $^{45}Ca$ and $^{32}P$ in a dose-dependent manner; $F^-$ failed, however, to inhibit the synthesis of matrix proteins but, to a moderate extent, impaired their secretion.

In explants grown in the presence of 52 µM of $F^-$ the superficial layers of enamel matrix deposited in vitro (fluorotic matrix) failed to materialize. Increasing P levels in the medium had no clear histological effect; lowering Ca levels, however, sometimes seemed to aggravate the $F^-$ effect. Raising Ca levels improved the histological pattern. In spite of the presence of $F^-$, high Ca levels allowed a limited mineralization of the superficial layer of fluorotic matrix along with a strong rise in mineralization of the deeper layers of pre-exposure enamel. High Ca levels also considerably reduced the cellular changes in secretory ameloblasts induced by 52 µM of $F^-$ and slightly counteracted the inhibition of matrix secretion, as measured biochemically.

Some of the effects of $F^-$ on secretory amelogenesis in vitro can thus be reversed by raising Ca levels in the medium. Therefore, the effect of $F^-$ on secretory amelogenesis in vitro seems to be primarily interference with the enamel mineralization process per se and, secondarily, an impairment of matrix secretion.

KEY WORDS: Amelogenesis; Calcium levels; Fluoride levels; Hamster tooth buds.

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# INSTRUCTIONS TO AUTHORS

**Fluoride**, the official journal of the International Society for Fluoride Research (ISFR) publishes quarterly (Winter, Spring, Summer, Autumn) reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference appear in **Fluoride**. Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

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1. **General** – No precise limit is given on the length of the paper. However it should be written concisely in English, submitted with a copy, doublespaced with generous margins. Measures are given in metric system (SI).

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