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AUTHORITARIANISM VERSUS SCIENTIFIC EVIDENCE

Throughout history, officially-promoted viewpoints in science have often held sway for remarkably long periods of time. A familiar example is the Ptolemaic geocentric view of our solar system, which was shown to be incorrect but was nevertheless stoutly defended by ruling ecclesiastical authority.

Surprising as it may seem, a similar situation prevails in science policy even today. For over fifty years the United States Public Health Service, with the endorsement of leading national and international dental and other professional organizations, has insisted that the levels of fluoride ingested from water fluoridation not only significantly reduce tooth decay but are also virtually without any toxic effects other than mild dental mottling. This view, however, like that of Ptolemy, contradicts available scientific evidence.

As can be seen in the two unanswered letters to the presidents of the US National Academy of Sciences and the Institute of Medicine published herein on pages 153-157, officials of these two prestigious scientific bodies have ignored unrefuted evidence contradicting their proposed dietary reference standards for human exposure to fluoride. To anyone even slightly acquainted with the relevant peer-reviewed literature cited in the two letters, the suggested “tolerable upper level” fluoride intake of 10 mg/day for individuals aged nine or older clearly falls in a well-verified toxic range, not only for teeth and bones but also for various soft-tissue organs. Moreover, under scientific scrutiny, the major dental benefits claimed for ingested fluoride are illusory and are no longer widely accepted, at least in most non-English speaking countries (cf. letter to the editor, pages 171-174).

Further serious ethical aspects of this issue are highlighted by the manner in which administrators of the US Environmental Protection Agency in 1985 set 4 mg/L as the Recommended Maximum Contaminant Level for fluoride in drinking water. A recent paper delivered at the 23rd Annual Conference of the National Association of Environmental Professionals held June 20-22, 1998, in San Diego, California, dealt specifically with flagrant violations of this organization’s code of ethics by EPA management in setting the 4 mg/L RMCL for fluoride. In its report to the EPA, USPHS officials had altered the 2 mg/L RMCL of its scientific panel to 4 mg/L, and EPA management then ordered the support document prepared by EPA professionals to be altered in conflict with known facts.

Sadly, even today, authoritarianism can still overrule solid science.

AWB
XXXIInd CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH 
August 24-27, 1998
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Conference registration fee of $300 includes reception, lunches, coffee breaks, abstract book and guided bus tours.
Further information, and registration forms, can be obtained from Professor Ming-Ho Yu, Huxley College of Environmental Studies, Western Washington University, Bellingham WA 98225-9181, USA.
Phone (USA) 360 650 3676 Fax (USA) 360 650 7284, E-mail mhyu@titan.cc.wwu.edu

PAN – ASIA – PACIFIC CONFERENCE ON FLUORIDE AND ARSENIC RESEARCH
August 16-20, 1999
Shenyang City, Liaoning Province, China
Sponsored by the Fluoride and Arsenic Society of China (FASC)
In China at least 100 million people live in naturally high fluoride regions and two million live in arsenic poisoned ones, with most of the latter also affected by high fluoride. The Fluoride and Arsenic Society of China was founded in 1996 to study and control the serious situation. This first international conference aims to share the expertise of Chinese scientists with other experts from around the world, especially those from the Asian and Pacific region, with a view to working together to combat the situation where it occurs.
Shenyang is one of the most famous historical and cultural cities of China. Shenyang International Airport is about 45 minutes by air from Beijing, and 90 minutes from Shanghai.
Further information, and registration forms, can be obtained from Professor Sun Guifan, School of Public Health, China Medical University, 92 Beier Road, Shenyang 110001, China.
Phone/Fax 86 24 2387 1744 E-mail sungf@iris.cmu.edu.cn
SUMMARY: Fluoride and calcium concentrations in maternal plasma and in placental tissue were determined in 30 healthy women residing in an area with a relatively low water and air fluoride (fluorine) content. The mean fluoride concentrations in maternal plasma and in marginal and central parts of placenta were 4.27 μM/L, 42.1 μg/g of ash, and 33.7 μg/g of ash, respectively. The mean calcium concentrations in maternal plasma and in marginal and in central parts of placenta were 2.3 mM/L, 22.9 mg/g of ash, and 20.0 mg/g of ash, respectively. Fluoride contents of both parts of placental tissue differed significantly (p < 0.05). High positive correlations (p < 0.001) were found between maternal plasma fluoride concentration and the fluoride content of the marginal part of placenta as well as between maternal plasma calcium concentration and the calcium content of both parts of placental tissue. The same positive correlation was also found between fluoride and calcium contents of marginal part of placental tissue. Our data indicate that most placental fluoride is stored in the marginal part of the organ presumably as a result of the higher concentration of calcium found in that area.

INTRODUCTION

Recently published studies on placental transfer of fluoride clearly show that fluoride passes through the placenta. There is also evidence, however, that the placenta can accumulate fluoride and possibly play a regulatory role that helps protect the fetus from excessive amounts of fluoride, when maternal fluoride intake is high. The purpose of the present study was to investigate placental accumulation and distribution of fluoride and calcium in healthy women residing in an area with a relatively low water and air fluoride (fluorine) content.

MATERIAL AND METHODS

The studies were performed on 30 healthy women at term, aged from 19 to 40 years residing in an area with < 0.5 ppm of fluoride in the drinking water and < 1.5 μg/m³ of fluorine in the air. All women were hospitalized during the perinatal period and delivered by spontaneous labour. From each patient during the first period of normal delivery blood was drawn in tubes containing heparin as an anticoagulant. All blood samples were centrifuged for 10 minutes at 3000 rpm, and the plasma was stored at -20°C until fluoride and total calcium concentrations were determined. Placentas were obtained from all patients after birth and also stored at -20°C. Each placenta was divided into two parts: central and marginal. The fluoride and calcium content of both parts was determined by adding 10.0 mL of saturated solution of magnesium chloride, drying at 80°C for 48 hours and ashing at 600°C for 16 hours.

Ionic fluoride was determined in 1.0 mL of plasma with an equal volume of TISAB buffer, which adjusted the pH to 5.0. Total calcium was determined in 0.05 mL of plasma, which was diluted to 5.0 mL with distilled water.
To determine the fluoride content of the placenta samples, 30.0 mg of ash was first dissolved in 1.0 mL of 2.0 M HClO₄, then 0.2 mL of the resulting solution was neutralized with 0.8 mL of 1.0 M aqueous sodium citrate, and finally 1.0 mL of TISAB buffer was added.

To determine the calcium content of placenta samples, 30.0 mg of ash was dissolved in 1.0 mL of 14 M HNO₃ for 48 hours, then heated to 70°C, and finally diluted with distilled water.

The levels of fluoride were determined with the Orion-96-09 fluoride-ion-selective electrode (Orion), and the levels of calcium were determined with an atomic absorption spectrophotometer PU 9100 X (Philips).

The results were statistically analysed by the Student's t-test and by Pearson's correlation coefficient. A probability value of < 0.05 was taken as indicating significance.

## RESULTS

Concentrations of fluoride in maternal plasma, marginal part of placenta, and central part of placenta are presented in Table 1. Fluoride contents of both parts of placental tissue differed significantly (p<0.05). Calcium contents of maternal plasma, marginal part of placenta and central part of placenta are presented in Table 2.

A strong positive correlation (p<0.001) was found between fluoride concentrations in maternal plasma and marginal part of placenta (Figure 1). A strong positive correlation was also found between calcium concentrations in maternal plasma and marginal part of placenta (Figure 2) as well as between calcium concentrations in maternal plasma and central part of placenta (Figure 3). Figure 4 shows the high positive correlation between fluoride and calcium concentrations in marginal part of placenta (p<0.001).

### Table 1. Fluoride concentrations in maternal plasma, marginal part of placenta, and central part of placenta

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>maternal plasma</td>
<td>30</td>
<td>4.27</td>
<td>4.21</td>
<td>0.79</td>
<td>2.79</td>
<td>6.11</td>
<td>3.32</td>
</tr>
<tr>
<td>marginal part of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placenta [µg/g of ash]</td>
<td>30</td>
<td>42.1</td>
<td>39.5</td>
<td>11.4</td>
<td>19.7</td>
<td>76.4</td>
<td>56.7</td>
</tr>
<tr>
<td>central part of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placenta [µg/g of ash]</td>
<td>30</td>
<td>33.7</td>
<td>34.2</td>
<td>11.4</td>
<td>14.4</td>
<td>61.7</td>
<td>47.3</td>
</tr>
</tbody>
</table>

### Table 2. Total calcium concentrations in maternal plasma, marginal part of placenta and central part of placenta

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>maternal plasma</td>
<td>30</td>
<td>2.3</td>
<td>2.3</td>
<td>0.2</td>
<td>1.8</td>
<td>2.8</td>
<td>1.0</td>
</tr>
<tr>
<td>marginal part of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placenta [mg/g of ash]</td>
<td>30</td>
<td>22.9</td>
<td>21.5</td>
<td>7.5</td>
<td>12.2</td>
<td>49.0</td>
<td>36.8</td>
</tr>
<tr>
<td>central part of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placenta [mg/g of ash]</td>
<td>30</td>
<td>20.0</td>
<td>19.3</td>
<td>4.9</td>
<td>12.3</td>
<td>33.2</td>
<td>20.9</td>
</tr>
</tbody>
</table>
FIGURE 1. Correlation between fluoride concentrations in maternal plasma and marginal part of placenta

\[ y = 11.944x - 8.904 \]
\[ R^2 = 0.6767 \]

FIGURE 2. Correlation between calcium concentrations in maternal plasma and marginal part of placenta

\[ y = 24.111x - 32.858 \]
\[ R^2 = 0.5334 \]
FIGURE 3. Correlation between calcium concentrations in maternal plasma and central part of placenta

\[ y = 13.981x - 12.264 \]

\[ R^2 = 0.4125 \]

FIGURE 4. Correlation between fluoride and calcium concentrations in marginal part of placenta

\[ y = 1.4185x + 9.5864 \]

\[ R^2 = 0.8725 \]
DISCUSSION

In 1955, Feltman and Kosel observed much higher concentrations of fluoride in peripheral regions (in comparison with central ones) of two examined placentas. These authors suggested that this differentiation was closely related to the calcium content of these parts of tissue. These findings were discussed in more recent studies by Shen and Taves, who showed that fluoride accumulation in placenta may be connected with local focuses of calcification. Other studies by Zamorska and Niwelinski demonstrated that placentas from urban-industrial regions abounded in calcium fluoride deposits. These findings are supported by data of Jendryczko et al. and of Chlubek.

In recent Chinese investigations of fluoride exposure and intelligence in children the effect of fluoride appears to occur at an early stage of development of the embryo when the differentiation of brain nerve cells is taking place and development is most rapid. Furthermore, a higher concentration of fluoride has been found in embryonic brain tissue obtained from termination of pregnancy in areas where fluorosis due to coal burning was prevalent. These observations refute the view that the placenta protects the fetus from fluoride.

Based on our results, we conclude that placenta can accumulate fluoride in healthy women who are exposed in pregnancy to relatively low fluoride concentrations in water and in air. The greatest amount of placental fluoride is stored in the marginal part of the organ presumably as a result of the higher concentration of calcium found in that area.

The present paper was prepared in the framework of the KBN research project 4 S405 021 06 and was presented and discussed at the XXIst World Conference of the International Society for Fluoride Research in Budapest (25-29 August, 1996).

REFERENCES


PHOSPHATE FERTILIZER INDUSTRY IN JORDAN: ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO PHOSPHATE DUST AND FLUORIDE

Burhan A. AbuDhaisea and Naheid I AbuOmar

Aqaba, Jordan

SUMMARY: In order to assess environmental exposure, phosphate dust and gaseous fluoride concentrations were measured at various sites of a phosphate fertilizer complex in Jordan throughout the year 1993. Sixty six phosphate dust samples were collected using a high volume air sampler (Staplex, TFIA-2, USA) fitted with glass fiber filter media. Short-term drager tubes (Drager-Rohrchen, Germany) were used to measure fluorine concentrations in 300 air samples. The concentration of airborne phosphate dust varied widely throughout the year (0.3 - 379 mg/m³), and in one third of the samples it exceeded the ACGIH-TLV of 10 mg/m³ for total dust. The highest levels were encountered during truck unloading of the raw phosphate material, conveyor belt feeding, and in the export area during truck unloading of the finished product. Gaseous fluoride emissions from various processes ranged from 0.1 to 30 ppm. In at least 20% of the samples the concentration exceeded the ACGIH-TLV of 1 ppm for fluorine gas. The highest geometric mean concentrations were found in the reactor unit at the phosphoric acid plant (4 ± 3.02 ppm) followed by the crystallizer and calciner units at the aluminum fluoride plant (3 ± 2.4, 2.6 ± 3.4 ppm, respectively). It is concluded that phosphate fertilizer industry carries a hazard of exposure to gaseous and particulate fluoride, in addition to other phosphate dust components.

Key words: Environmental exposure; Fertilizer industry; Fluoride emissions; Jordan; Occupational hazard; Phosphate dust; Superphosphate.

INTRODUCTION

Phosphate is found in all igneous and sedimentary rocks but it is only mined when present in high concentrations. It represents an important source of fertilizer industry in the United States, Russia and North Africa. The world production of phosphate rock in 1977 was about 126 million tons; 47 million in the United States, 24 million in Russia, 18 million in Morocco and 4 million in China.1

Jordan has an enormous phosphate ore reserve that covers about 60% of its total area. A total of 7 million tons of different grades of phosphate rock is produced annually.2 The bulk is exported as a raw material, making Jordan probably the fourth largest exporter of phosphate rock in the world after USA, Russia and Morocco. About one million tons are utilized locally for the production of phosphoric acid and phosphate fertilizer (diammonium phosphate) by Jordan Phosphate Mines and Fertilizer Company (JPMFC). Extraction and processing of phosphate are not without hazards, however, as ores may contain, besides calcium phosphate, impurities such as uranium, silica and fluorides.1

It has been estimated that the fluoride content (as F-) of the rock phosphate mined annually in the USA alone is about 0.7 million tons.3 Phosphate fertilizer production emits fluoride at certain phases, particularly in gaseous and vaporized forms, that may exceed acceptable safety levels.4 Fluorosis has been reported in

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b Jordan Phosphate Mines and Fertilizer Complex, Aqaba, Jordan.
fluorspar processing and phosphate fertilizer workers indicating undue exposure to fluorine.\textsuperscript{5,6} However, studies investigating the morbidity of phosphate workers are scarce and still inconclusive. This part of the study aims at assessing environmental exposure to phosphate dust and fluoride at the JPMFC.

**MATERIAL AND METHODS**

**Study setting**

Jordan Phosphate Mines and Fertilizer Company (JPMFC) is one of the largest industries in Jordan employing about 5,500 persons. It comprises four phosphate production mines located in different parts of the country and an industrial complex at the port of Aqaba for the production of phosphate fertilizer from phosphate rock. The present study was done at the industrial complex and nearby export department. The complex is composed of four major plants. The first is a sulfuric acid plant that produces sulfuric acid from sulfur and water. The second is a phosphoric acid plant that produces phosphoric acid from phosphate rock and sulfuric acid, a reaction which also results in fluosilicic acid as a by-product. The third is a granulation plant that produces diammonium phosphate from the reaction of phosphoric acid and ammonia. The fourth plant has purposely been built to benefit from the hazardous by-product fluosilicic acid, formed in the production of aluminum fluoride by allowing it to react with aluminum hydroxide (see Figure).

Phosphate deposits are mined by the open cast method. The phosphate rock is then transported from the mines by trucks and is fed into the industrial complex from an intake station, where the trucks are unloaded and the rocks are carried by a conveyor belt (250 m long) to the ballmill for grinding. The product from the ballmill is conveyed to a reactor in the phosphoric acid plant where it reacts with sulfuric acid for the production of phosphoric acid as shown in the Figure. Treatment of fluosilicic acid with aluminum hydroxide trihydrate for the production of aluminum fluoride takes place in special reactors. The reaction product (slurry) is passed onto a belt filter to separate silica from the aluminum fluoride solution. The aluminum fluoride then enters a crystallizer to precipitate aluminum fluoride trihydrate crystals. The crystals are dried in a calciner to produce anhydrous aluminum fluoride which is cooled and conveyed to silos for bagging.

**Assessment of phosphate and fluoride exposure**

Three hundred and sixty six air samples were drawn throughout the year 1993 from various sites of production and processing, 66 samples for phosphate dust assessment and 300 for gaseous fluoride. The sites of sample collections are designated in Figure 1. For phosphate dust assessment, air samples were collected from same sites twice during the year, July and December. Fluoride levels were measured 6 times at each site throughout the year, and a total of 30 samples from each site were collected. The strategy was to calculate an annual average exposure, in order to allow for any seasonal variation and to draw a more consistent picture of exposure.

Total airborne phosphate dust concentration was measured with a high volume air sampler (Staplex, TFIA-2, USA) which uses glass fiber filter media that has 95% collection efficiency of particles >0.5 um in diameter. The sampler was placed 1.5 m above ground level to measure dusts in the respiratory zone.
The flow rate before and after sampling was recorded and the average rate was calculated. The duration of sampling was also recorded. The filter was weighed before and after sampling, and the difference was taken as a dust weight. After considering the duration of sampling and the average flow rate, the calculated dust weight was converted into airborne dust concentration in mg/m³. The concentration of fluoride gas was measured using short-term drager tubes (Drager-Rohrchen, Germany).

**FIGURE. Flow-chart of phosphate fertilizer and aluminum fluoride production at JPMFC, Jordan**

(*) Phosphate dust & (**) fluorine sampling sites
RESULTS

Two major environmental hazards encountered in a phosphate fertilizer plant in Jordan were assessed: phosphate dust and fluoride gas. Analysis of 366 air samples taken from various sites along the production lines and in the nearby area during one year gave the results described in Tables 1 and 2. The concentration of airborne phosphate dust varied widely throughout the year, ranging from 0.3 to 379 mg/m³ (Table 1). Out of the 66 dust samples collected from various sites, 22 samples exceeded the ACGIH-TLV \(^7\) (10 mg/m³ for total dust). The section of the industrial complex which receives phosphate raw material was found to have the highest average dust exposure (54.6 mg/m³) when compared with the production plant, silos, and export section. Within this section, the belt conveyor site was the main contributor to the elevated dust concentration (125.2 mg/m³) (Table 1). Along the production lines the overall average dust level was within the TLV, despite the high concentration observed at the phosphate gates (64.07 mg/m³).

<table>
<thead>
<tr>
<th>Section</th>
<th>No of samples*</th>
<th>Total Dust Concentration (mg/m³)</th>
<th>Samples &gt;TLV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Phosphate unloading</td>
<td></td>
<td>Range</td>
<td>Geometric mean ± SD</td>
</tr>
</tbody>
</table>
| a. resting area                | 2              | 3-5.5                           | 4.05±1.54       | 0
| b. Truck unloading             | 4              | 12-379                          | 101.5±4.46      | 100
| c. Belt conveyor               | 6              | 67.5-230                        | 125.2±1.86      | 100
| Total                          | 12             | 3-379                           | 54.6±4.64       | 83.3
| 2. Silos (7 in number)         | 14             | 1.7-4                           | 2.5±1.34        | 0
| 3. Phosphoric acid plant       |                |                                 |                 | 16.7
| a. Mill                        | 16             | 0.8-12.8                        | 4.26±2.44       | 12.5
| b. phosphate gates             | 2              | 69-69                           | 64.07±1.10      | 100
| c. 30% H₃PO₄                   | 2              | 0.8-1.2                         | 0.98±1.32       | 0
| d. Reactor                     | 2              | 0.3-0.8                         | 0.49±1.99       | 0
| e. Ground floor                | 2              | 0.4-1.2                         | 0.79±2.14       | 0
| Total                          | 24             | 0.3-69                          | 3.3±4.3         | 16.7
| 4. Export                      |                |                                 |                 | 50
| a. Truck unloading             | 4              | 81.6-229                        | 136.3±2.07      | 100
| b. train unloading             | 6              | 2.2-39.2                        | 8.85±4.22       | 33.3
| c. Store                       | 2              | 12.28.5                         | 18.54±1.85      | 100
| d. Background                  | 2              | 1.2-3.6                         | 2.08±2.18       | 0
| e. Ship loading                | 2              | 6.0-9.5                         | 7.61±1.38       | 0
| Total                          | 16             | 1.2-229                         | 15.18±6.0       | 50

* Samples were taken on two occasions during the year 1993, July and December

Gaseous fluoride emission from various processes within the complex varied widely (0.1-30 ppm) (Table 2). In about 20% of samples from each of the phosphoric acid and aluminum fluoride plants the TLV for fluoride was exceeded. Furthermore, the highest concentrations were measured from the reactor site at the phosphoric acid plant where almost all samples collected exceeded the TLV of 1 ppm for fluoride.\(^7\) Within the aluminum fluoride plant, the crystallizer and calciner units were associated with the highest fluoride concentrations, 3 and 2.6 ppm respectively.
Table 2. Environmental Fluoride gas concentration in various sections at JPFMC. Jordan, 1993

<table>
<thead>
<tr>
<th>Sampling site *</th>
<th>Fluorine concentration (ppm)</th>
<th>Samples &gt; TLV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Geometric M ± SD</td>
</tr>
<tr>
<td>1 H₃PO₄ Paint</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactor</td>
<td>1-30</td>
<td>4.3 ± 3.02</td>
</tr>
<tr>
<td>Filter</td>
<td>0.5-4</td>
<td>1.4 ± 1.71</td>
</tr>
<tr>
<td>30% H₃PO₄ tanks</td>
<td>0.1-1</td>
<td>0.5 ± 2.35</td>
</tr>
<tr>
<td>Concentration unit</td>
<td>0.5-2</td>
<td>1.3 ± 2.10</td>
</tr>
<tr>
<td>Cooling tower</td>
<td>0.1-0.5</td>
<td>0.3 ± 1.4</td>
</tr>
<tr>
<td>Ground floor</td>
<td>0.1-1</td>
<td>0.5 ± 2.2</td>
</tr>
<tr>
<td>Total</td>
<td>0.1-30</td>
<td>1.3 ± 1.4</td>
</tr>
<tr>
<td>2 AlF₃ Plant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactor</td>
<td>0.1-4</td>
<td>1.2 ± 2.7</td>
</tr>
<tr>
<td>Crystallizer</td>
<td>0.5-8</td>
<td>3 ± 2.4</td>
</tr>
<tr>
<td>Calciner</td>
<td>0.5-15</td>
<td>2.6 ± 3.4</td>
</tr>
<tr>
<td>Ground floor</td>
<td>0.1-3</td>
<td>1.5 ± 2.3</td>
</tr>
<tr>
<td>Total</td>
<td>0.1-15</td>
<td>2.1 ± 0.86</td>
</tr>
</tbody>
</table>

* 30 samples were taken from each site in the plant on more than one occasion throughout the year 1993

DISCUSSION

The phosphate fertilizer and aluminum fluoride industry in Jordan entails two main environmental work hazards, phosphate dust and fluoride gas exposures. The processes responsible for their emission and the amounts contributed by each process have been the subject of this article.

Phosphate dust was shown to be emitted from various processes of handling the raw material. The unloading station represented a source of exposure, during truck unloading and conveyor belt feeding. In both sites the mean concentration of phosphate dust greatly exceeded the acceptable safety level for total dust (10 mg/m³). This station was provided with a de-dusting ventilation system, but due to the intensity of work the system was not able to lower the airborne dust concentration to the acceptable safety level. Workers at this station, therefore, are expected to inhale considerable amounts of dust under such circumstances.

In the silos area, the mean dust concentration (2.5 ± 1.3 mg/m³) was within the TLV since each silo used a closed handling system. In the ballmill, the coarse phosphate particles are ground to sizes less than 80 mm in diameter and transported in a closed system to a silo and afterwards, to a reactor. The resulting dust due to grinding is collected via cyclones and dust bags. Dust is emitted to the working area only occasionally when there is some mechanical problem. This explains the low level of dust, in general, measured from the ballmill environment (4.3 ± 2.4 mg/m³).

Samples collected from the truck unloading bay and stores at the export section showed elevated concentrations of phosphate dust. The constituents of raw phosphate dust differ according to the country of origin. In Jordan, 80-85% of it is made of calcium compounds and the remaining part is present as impurities, mainly of fluoride (3-4%) and silica (3-6%). While inhalation of dust consisting of calcium compounds is harmless to the lung, chronic inhalation of phosphate dust was shown to produce lung fibrosis which was attributed to
high silica content of the dust. This finding, however, was not confirmed. Still, it is reasonable to assume that workers in the phosphate industry may be at risk of phosphate-induced lung effects, depending on the content of impurities and severity of exposure.

The hazard of fluoride exposure was also studied. Fluoride particulate exposure (fluorapatite) was indirectly assessed by measuring phosphate total dust concentration. Furthermore, the emission of gaseous fluoride was reported among various stages of aluminum fluoride production. The highest average concentrations were seen at the reactor of the phosphoric acid plant (4 ppm), crystallizer (3 ppm), and calciner (2.6 ppm) in the aluminum fluoride plant. About 20% of samples collected from each of the phosphoric acid and aluminum fluoride plants had fluoride concentration that exceeded the TLV of 1 ppm. Therefore, workers in the phosphate fertilizer and aluminum fluoride industries are expected to be at risk of exposure to fluoride gas or compounds. Studies have shown that such workers may develop fluorosis after prolonged exposure. Children living near an aluminum smelter were found to have marked abnormal pulmonary manifestations attributed to inhalation of airborne fluoride.

In conclusion: the phosphate fertilizer industry is an environmental source of gaseous and particulate fluoride, in addition to phosphate dust. Separate reports dealing with lung effects and osteofluorosis in phosphate fertilizer workers will be presented later.

REFERENCES
AMELIORATIVE ROLE OF AMINO ACIDS ON FLUORIDE-INDUCED ALTERATIONS IN MICE (PART II): OVARIAN AND UTERINE NUCLEIC ACID METABOLISM

Dhruva Patel and N J Chinoy
Ahmedabad, India

SUMMARY: Sodium fluoride (5 mg/kg body weight) was effective from the 45th day of treatment in causing a significant decline in DNA and RNA levels of mice ovary and uterus, indicating alterations in nucleic acid and protein metabolism in these organs. The oestrus cycle was irregular with prolonged duration of the diestrus stage which in turn severely affected the fertility rate in treated mice.

The administration of amino acids glycine and glutamine, individually and in combination along with NaF, helped in maintaining the status quo of all parameters as compared to control, thus elucidating their ameliorative role.

Key words: Amino acids; DNA; Fertility; Mice; RNA; Sodium fluoride.

INTRODUCTION

Tsutsui et al described genotoxic effects of fluoride.1 NaF has been reported to cause an increase in the frequency of Sister Chromatid Exchange (SCE) in human population of endemic areas of North Gujarat.2

Increased feeding of fluoride to animals and humans raises the fluoride concentration in maternal and fetal blood.3 Administration of 150-300 mg F-/kg body weight blocked gonadotropin stimulation of rabbit ovary.4 Hanley et al reported maternal weight loss and fetotoxic effects of fluoride in white rabbits and rats exposed to 225 ppm of fluoride.5 Messer et al have attempted to link infertility and fluorosis.6 Reports from our laboratory have clearly elucidated alterations in carbohydrate and oxidative metabolisms and altered functions of some vital and reproductive organs in fluoridated female mice.7-9 However, the effects in the ovary and uterus due to fluoride are not yet fully explored.

In the light of earlier data the purpose of the present investigation was to evaluate the effects of fluoride on nucleic acid and protein levels in the ovary and uterus and the fertility impairment in mice under experimental fluorosis. The possible role of amino acids (glycine and glutamine) in the amelioration of fluoride induced toxicity was also investigated.

MATERIALS AND METHODS

The animals used, exposures, protein levels and experimental protocol are the same as described earlier by Chinoy and Patel.9 The protein levels used in this study are also the same as given in Chinoy and Patel.9

Nucleic Acids:

The deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the ovary and uterus were first extracted in trichloroacetic acid (TCA) and the RNA levels were determined using orcinol as the colouring reagent by the method of Mejbor.10 The resulting colour was read at 670 nm on a Systronics Colorimeter 106 and expressed as μ moles/100 mg fresh tissue weight.
The DNA levels were estimated by the method of Giles and Meyer\textsuperscript{11} using diphenylamine as the colouring reagent and the OD of the resultant colour was read at 620 nm on a Systronics 106 Colorimeter and expressed as \( \mu \) moles/100 mg fresh tissue weight.

\textit{Fertility Test:} was assessed according to the WHO protocol MB-50.\textsuperscript{12}

\textit{Cyclicity:} The Oestrus cycle was recorded in normal and NaF treated mice, by observing the vaginal smear daily, for over a period of two months.

\textit{Statistics:} For each biochemical estimation, a minimum of 6 to 8 replicates were carried out and the data was analyzed using Analysis of variance (ANOVA) followed by Scheff’s test.

\section*{RESULTS}

\textit{Deoxyribonucleic acid (DNA):}

NaF treatment resulted in a significant suppression (\( P<0.001 \)) of ovarian and uterine DNA levels. However, after administration of glycine and/or glutamine (group III, IV, V) the DNA levels were almost similar to those of control (Table 1), which implies a significant increase (\( P<0.001 \)) as compared to the NaF treated group.

\textit{Ribonucleic acid (RNA):}

NaF treatment (45, 60 days) caused significant (\( P<0.001 \)) decline in ovarian and uterine RNA levels as compared to control, whereas, after administration of glycine and/or glutamine along with NaF (group III, IV, V) the ribonucleic acid levels were similar to those in the control mice (Table 2) which reveals a significant increase (\( P<0.001 \)) as compared to the treated group.

\textit{DNA/RNA ratio:}

The DNA/RNA ratio in uterus declined (\( P<0.05 \)) after 45 days and 60 days of treatment, whereas it increased (\( P<0.05 \)) in the ovary. NaF treatment along with glycine and/or glutamine resulted in increase (\( P<0.05 \)) in the DNA/RNA ratio in the uterus as compared to NaF treated group whereas in the ovary the ratio remained unaltered (Table 3).

\textit{RNA/Protein ratio:}

A significant decline (\( P<0.001 \)) in RNA/protein ratio occurred in the ovary after NaF treatment (45 and 60 days), while, no change was observed in uterus. After administration of glycine or glutamine along with NaF (group III and IV), the ratio was the same as in control (\( P<0.05 \)). The levels were almost the same as control in group V in which animals were fed glycine and glutamine in combination with NaF (Table 4), implying a significant increase as compared to the NaF treated group.

\textit{Fertility rate:}

Sodium fluoride treatment led to a significant decline (\( P<0.001 \)) in fertility rate after 45 and 60 days of treatment. The fertility rate was less affected after ingestion of glycine or glutamine alone and in combination along with NaF (Table 5) than in NaF treatment alone (group II).

\textit{Cyclicity:}

The cyclicity of NaF treated animals was disturbed. The cycles were irregular and prolonged. However, in groups III, IV and V regular cycles were observed but their duration was extended (Table 5).
### TABLE 1A. DNA levels (μ moles/100mg fresh tissue weight) in uterus and ovary of control and treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uterus 45 days</th>
<th>Uterus 60 days</th>
<th>Ovary 45 days</th>
<th>Ovary 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>886.34 ± 20.2</td>
<td>894.30 ± 18.1</td>
<td>1053 ± 36.3</td>
<td>1002.0 ± 21.2</td>
</tr>
<tr>
<td>NaF</td>
<td>484.79 ± 17.9*</td>
<td>408.26 ± 16.6*</td>
<td>658 ± 17.0*</td>
<td>599.0 ± 18.2*</td>
</tr>
<tr>
<td>NaF + Glycine</td>
<td>807.57 ± 12.2*</td>
<td>907.72 ± 18.2*</td>
<td>923 ± 16.3*</td>
<td>976.0 ± 19.1*</td>
</tr>
<tr>
<td>NaF + Glutamine</td>
<td>794.15 ± 19.9*</td>
<td>865.28 ± 15.1*</td>
<td>936 ± 19.9*</td>
<td>941.44 ± 16.6*</td>
</tr>
<tr>
<td>NaF + Glycine + Glutamine</td>
<td>907.12 ± 24.8*</td>
<td>974.56 ± 14.2*</td>
<td>1024 ± 32.6*</td>
<td>1056.0 ± 21.9*</td>
</tr>
</tbody>
</table>

Values are Mean ± Std Error * P < 0.001

### TABLE 1B. DNA ANOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MSS</th>
<th>f(cal)</th>
<th>f(tab)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus Groups</td>
<td>1104058.00</td>
<td>8</td>
<td>138007.00</td>
<td>119.80</td>
<td>3.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>20732.00</td>
<td>18</td>
<td>1151.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary Groups</td>
<td>849933.60</td>
<td>8</td>
<td>106241.00</td>
<td>46.10</td>
<td>3.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>41481.00</td>
<td>18</td>
<td>2304.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = Sum of squares df = Degrees of freedom MSS = Mean sum of squares

### TABLE 2A. RNA levels (μ moles/100 mg fresh tissue weight) in uterus and ovary of control and treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uterus 45 days</th>
<th>Uterus 60 days</th>
<th>Ovary 45 days</th>
<th>Ovary 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>151.52 ± 6.2</td>
<td>149 ± 7.6</td>
<td>221.51 ± 11.1</td>
<td>208 ± 19.6</td>
</tr>
<tr>
<td>NaF</td>
<td>112.51 ± 5.9**</td>
<td>99.21 ± 7.9***</td>
<td>104.19 ± 11.3***</td>
<td>98.85 ± 10.0***</td>
</tr>
<tr>
<td>NaF + Glycine</td>
<td>130.93 ± 6.7*</td>
<td>117.67 ± 8.2**</td>
<td>152.86 ± 8.7**</td>
<td>173.66 ± 12.1***</td>
</tr>
<tr>
<td>NaF + Glutamine</td>
<td>119.13 ± 2.3NS</td>
<td>113.48 ± 4.2**</td>
<td>142.97 ± 10.2**</td>
<td>168.71 ± 10.9***</td>
</tr>
<tr>
<td>NaF + Glycine + Glutamine</td>
<td>134.8 ± 9.2*</td>
<td>129.98 ± 7.6***</td>
<td>175 ± 8.6**</td>
<td>188.82 ± 11.3***</td>
</tr>
</tbody>
</table>

Values are Mean ± Std Error *** P < 0.001 ** P < 0.01 * P < 0.05

### TABLE 2B. RNA ANOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MSS</th>
<th>f(cal)</th>
<th>f(tab)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus Groups</td>
<td>8280.65</td>
<td>8</td>
<td>1035.00</td>
<td>5.57</td>
<td>3.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>3342.00</td>
<td>18</td>
<td>185.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary Groups</td>
<td>42369.30</td>
<td>8</td>
<td>5296.00</td>
<td>17.00</td>
<td>3.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>5604.60</td>
<td>18</td>
<td>311.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = Sum of squares df = Degrees of freedom MSS = Mean sum of squares

---

*Fluoride 31 (3) 1996*
TABLE 3A. DNA/RNA ratio in uterus and ovary of control and treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uterus</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 days</td>
<td>60 days</td>
</tr>
<tr>
<td>Control</td>
<td>5.76 ± 0.4</td>
<td>5.90 ± 0.3</td>
</tr>
<tr>
<td>NaF</td>
<td>4.29 ± 0.3*</td>
<td>4.17 ± 1.2*</td>
</tr>
<tr>
<td>NaF + Glycine</td>
<td>6.38 ± 0.9*</td>
<td>7.71 ± 0.6*</td>
</tr>
<tr>
<td>NaF + Glutamine</td>
<td>7.10 ± 0.6*</td>
<td>7.60 ± 0.1*</td>
</tr>
<tr>
<td>NaF + Glycine + Glutamine</td>
<td>6.93 ± 0.4*</td>
<td>7.35 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are Mean ± Std Error * P < 0.05

TABLE 3B. DNA/RNA Ratio ANOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MSS</th>
<th>f(cal)</th>
<th>f(tab)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus Groups</td>
<td>62.55</td>
<td>8</td>
<td>7.82</td>
<td>7.26</td>
<td>3.26</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>29.00</td>
<td>27</td>
<td>1.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary Groups</td>
<td>16.07</td>
<td>8</td>
<td>2.01</td>
<td>4.17</td>
<td>3.71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Residual</td>
<td>8.87</td>
<td>18</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = Sum of squares df = Degrees of freedom MSS = Mean sum of squares

TABLE 4A. RNA/Protein† ratio in uterus and ovary of control and treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uterus</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 days</td>
<td>60 days</td>
</tr>
<tr>
<td>Control</td>
<td>10.53 ± 0.32</td>
<td>10.41 ± 2.10 NS</td>
</tr>
<tr>
<td>NaF</td>
<td>11.49 ± 0.61 NS</td>
<td>10.88 ± 1.00 NS</td>
</tr>
<tr>
<td>NaF + Glycine</td>
<td>10.10 ± 0.77 NS</td>
<td>8.94 ± 0.96 NS</td>
</tr>
<tr>
<td>NaF + Glutamine</td>
<td>8.57 ± 0.31 NS</td>
<td>8.85 ± 0.62 NS</td>
</tr>
<tr>
<td>NaF + Glycine + Glutamine</td>
<td>9.25 ± 0.40 NS</td>
<td>9.05 ± 0.91 NS</td>
</tr>
</tbody>
</table>

Values are Mean ± Std Error * P < 0.05 ** P < 0.001
† For protein levels in ovary and uterus see Part I (reference no. 9)

TABLE 4B. RNA/Protein Ratio ANOVA

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MSS</th>
<th>f(cal)</th>
<th>f(tab)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus Groups</td>
<td>30.29</td>
<td>8</td>
<td>3.78</td>
<td>1.66</td>
<td>3.71</td>
<td>NS</td>
</tr>
<tr>
<td>Residual</td>
<td>36.47</td>
<td>18</td>
<td>2.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary Groups</td>
<td>143.37</td>
<td>8</td>
<td>17.92</td>
<td>7.57</td>
<td>3.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>42.57</td>
<td>18</td>
<td>2.38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = Sum of squares df = Degrees of freedom MSS = Mean sum of squares NS = Non significant

Fluoride 31 (3) 1998
TABLE 5. Fertility rate (%) and implantation sites of treated and control groups of mice

<table>
<thead>
<tr>
<th>No. of females mated/ No. of females pregnant</th>
<th>Fertility rate †† (%)</th>
<th>No. of implantation sites</th>
<th>Cyclicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 days 60 days</td>
<td>45 days 60 days</td>
<td>45 days 60 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45 days 60 days</td>
</tr>
<tr>
<td>Control</td>
<td>10/10 10/10</td>
<td>100 100</td>
<td>12.8±0.9 12.5±0.7</td>
</tr>
<tr>
<td>NaF</td>
<td>10/6 10/2</td>
<td>60 20</td>
<td>3.2±0.5** 1.5±0.5**</td>
</tr>
<tr>
<td>NaF+Glycine</td>
<td>10/9 10/9</td>
<td>90 90</td>
<td>8.5±1.4* 6.5±0.3</td>
</tr>
<tr>
<td>NaF+Glutamine</td>
<td>10/9 10/9</td>
<td>90 90</td>
<td>8.0±1.3* 7.0±1.1*</td>
</tr>
<tr>
<td>NaF+Glycine + Glutamine</td>
<td>10/10 10/10</td>
<td>100 100</td>
<td>9.0±1.5** 9.0±1.2**</td>
</tr>
</tbody>
</table>

† The oestrus cycle was found to be regular but duration of cycle was extended.
†† Females: Males used in ratio of 2:1.
** P<0.001; * P<0.01
At least 3 sets done for each group and mean values are presented.

DISCUSSION

Several human conditions including aging, cancer and arteriosclerosis have been associated with DNA damage and its misrepair.\textsuperscript{13} Fluoride has been reported to cause depression in DNA and RNA synthesis in cultured cells.\textsuperscript{14} In the present study, the DNA and RNA concentrations in the ovary and uterus were significantly decreased, which could be due to a decrease in their synthesis or an alteration in their metabolism. Earlier reports have also revealed that fluoride ingestion in rabbits resulted in a decrease in DNA and RNA levels in the ovary\textsuperscript{15} in corroboration with the present data. The inhibition of DNA and RNA synthesis may result in delayed mitotic and meiotic cycles including chromosomal breakages.\textsuperscript{16} Studies on fluorotic human population in endemic areas of North Gujarat have shown an increase in frequency of sister chromatid exchange as compared to the control indicating that fluoride might have a genotoxic effect.\textsuperscript{2}

The DNA/RNA ratio declined in the uterus, whereas it remained unaltered in the ovary. This decrease might be due to a significant decline in RNA concentration.

The DNA/Protein ratio was also significantly decreased in the ovary and uterus which could be related to the significant decline in protein levels. Thus it is likely that the process of transcription and translation would be affected in NaF treated mice. Further detailed studies on DNA repair mechanism on DNA polymerase activity as well as other enzymes involved in nucleic acid synthesis and metabolism are called for in the future.

In the present study, NaF treatment (60 days) induced complete loss of fertility and absence of implantation sites in NaF treated females when mated with control males. The loss of fertility might be due to irregularity in cyclicity related to altered hormone levels.

The present study thus elucidates that NaF brought about alterations in ovarian and uterine nucleic acid metabolism and had effects on reproduction. Since the structure and internal milieu of the uterus is maintained by priming of estrogen and action of progesterone, it is necessary to study the levels of these hormones and the ultrastructural changes in the ovary and uterus.
The present study also elucidates that supplementation of amino acids (glycine and/or glutamine) along with NaF manifested amelioration in all NaF induced effects, which was more pronounced with the combined administration.

Acknowledgement: The financial support provided by the Council of Scientific and Industrial Research (CSIR), New Delhi, to one of the authors (DP) is gratefully acknowledged.

REFERENCES

Published by the International Society for Fluoride Research. Editorial Office: 81A Landscape Road, Mount Eden, Auckland 1004, New Zealand

*Fluoride* 31(3) 1998
DRINKING WATER FLUORIDATION: BONE MINERAL DENSITY AND HIP FRACTURE INCIDENCE

R Lehmann, I M. Wapniarz, B Hofmann, B Pieper, I Haubitz, and E C Alloio
Warzburg, Germany

Abstract from Bone 22 (3) 273-278 1998

The role of drinking water fluoride content for prevention of osteoporosis remains controversial. Therefore, we analyzed the influence of drinking water fluoridation on the incidence of osteoporotic hip fractures and bone mineral density (BMD) in two different communities in eastern Germany: in Chemnitz, drinking water was fluoridated (1 mg/L) over a period of 30 years; in Halle, the water was not fluoridated. BMD was measured in healthy hospital employees aged 20-60 years (Halle: 214 women, 98 men; Chemnitz: 201 women, 43 men respectively) using dual-energy X-ray absorptiometry. Hip fractures in patients ≥ 35 years admitted to the local hospitals in the years 1987-1989 were collected from the clinic registers. There was no difference in age, anthropometric, hormonal, or lifestyle variables between the two groups. Mean fluoride exposure in Chemnitz was 25.2 ± 7.3 years. No correlation was found between fluoride exposure and age-adjusted BMD. We found no significant difference in spinal or femoral BMD between subjects living in Halle and Chemnitz (lumbar spine: 0.997 ± 0.129 (g/cm²) vs 1.045 ± 0.171 (g/cm²), p = 0.08, for men; 1.055 ± 0.112 (g/cm²) vs 1.046 ± 0.117 (g/cm²), p = 0.47, for women). The fracture incidence showed an exponential increase with aging in men and women with an incidence about 3.5 times higher for women. In Chemnitz, we calculated an age-adjusted annual incidence of 142.2 per 100,000 for women; and 72.5 per 100,000 for men, respectively. In Halle the incidences were 178.6 per 100,000 for women and 89.2 per 100,000 for men. There was a lower hip fracture incidence after the age of 85 in women in Chemnitz (1391 per 100,000 in Chemnitz vs. 1957 per 100,000 in Halle, p = 0.006). Using the age-adjusted incidences, significantly fewer hip fractures occurred in Chemnitz in both men and women. In conclusion our study suggests that optimal drinking water fluoridation (1 mg/L), which is advocated for prevention of dental caries, does not influence peak bone density but may reduce the incidence of osteoporotic hip fractures in the very old.

Key Words: Bone mineral density; Drinking water fluoridation; Hip fracture incidence.

Reprints: Dr B Allolio, Medizinische Klinik der Universitat Wurzburg, Schwerpunkt Endokrinologie, Josef-Schneider-Str 2, 97080 Wurzburg, Germany.

COMMENT

The defects in the study are many and include the following.

Due to random variation, any two communities may have different fracture rates that have nothing to do with the factor being studied. This problem can be partially addressed by knowing the fracture rates at different ages in both communities prior to fluoridation of one of them. It may be, for instance, that the fracture incidence among elderly women in Halle has, for many years, been greater than that of the elderly women in Chemnitz. If so, one cannot argue that the difference observed here is due to fluoridation.

Total fluoride intake was not measured or even estimated. This could have been accomplished by urine fluoride tests. It is entirely possible that the total fluoride intake was essentially the same in the women with fractures in both communities.

Past or current estrogen therapy (ERT or HRT) was mentioned as part of the questionnaire obtained but not cited in its Table 2, showing characteristics of the study population. Estrogen is well known to be an anti-resorptive agent and thus a bone factor of some significance.
Water supply source was not identified. The difference between well water and river water used for drinking might be significant. Well water provides not only important minerals but these same minerals would bind to fluoride to reduce F-absorption. Also, if both of these industrial cities were on the same river and river water was their drinking water source, it would be important to know which city was down-stream of the other. Industrial pollutants in the river greatly affect general health and can react synergistically with fluoride.

Both communities are described as industrial cities. If the industrial activity involved coal burning, atmospheric fluoride would significantly nullify any difference in water fluoride intake in these communities.

Bone health involves many other variables, so few of which are considered in this study that no meaningful conclusion can be drawn. This absence of considering other variables is common in the pro-fluoride dental literature.

As the authors admit, fracture incidence in both of these communities is lower than that found in former West Germany. They assert this is due to “unexplained regional differences” which is no explanation at all but allows the inference that communities only 100 Km apart can also have “unexplained regional differences.” It is likely, therefore, that other factors, such as diet, specific nutrients, environmental toxins, deaths from other causes, and other factors rather than water fluoridation affected the fracture incidence.

There is another curious thing about this study. Since the study was published in 1998 and the data were collected in 1989, one wonders why it took 9 years to write it? On the other hand, if the study was written in 1998, why choose only the 2-year time span of 1987-1989 instead of, say, a 5-year or 10-year time span for which data clearly were available. Given the known variability of fracture incidence, any given 2-year incidence can not be expected to be as representative as a 10-year mean incidence. Was there something the authors liked about this particular 2-year time span?

Finally, one must look at the one age period in which a statistically significant fracture incidence difference exists between the two communities. Why would women over age 85 experience a higher fracture incidence in Halle than in fluoridated Chemnitz? When fluoridation was started in Chemnitz, in 1959, the 85-year old women (in 1957-1989) were postmenopausal, i.e., when bone remodelling is much less active, weaker bones at age 85 suggest weaker bones prior to menopause. These women were in their late 30’s during the war. Was there something about the war experience or the post-war recovery period that adversely affected Halle women more than Chemnitz women? Is this the cause of their increased hip fracture incidence fifty years later? This study provides no information to answer this question. There exists no known mechanism by which fluoridated water could reduce fracture incidence in the very elderly without some sign of the same benefit in earlier age groups. Therefore, it is likely that this isolated divergence of fracture incidence has no relation to water fluoridation. The only finding of this study that seems sturdy is the fact that fluoridation produced no apparent gain in bone mineral density at any age, contrary to the claims of many fluoridation proponents.

The evidence of this study strongly supports the null hypothesis, namely, that water fluoridation had no effect on bone mineral density or fracture incidence.

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[Readers are invited to submit comments on papers published in the professional and scientific literature. Often, assessments of full papers are more helpful than the published abstracts. - Editors]
EFFECT OF CHRONIC FLUORIDE EXPOSURE IN UREMIC RATS

A Dunipace, E Brizendine, M Wilson, W Zhang, C Wilson, B Katz, A Kafrawy and G Stookey

Abstract from Nephron 78 (1) 96-103 1998

This study was conducted to test the hypothesis that the margin of safe fluoride exposure is narrowed in rats that are physiologically compromised by renal dysfunction. The study objective was to determine whether increases in fluoride retention and tissue fluoride levels in rats with surgically induced renal insufficiency result in toxic fluoride effects not ordinarily observed in healthy animals. Uremic and sham-operated control rats received 0 pg/ml, 5 (0.26 mmol/l), 15 (0.79), or 50 pg/ml (2.63 mmol/l) of fluoride in their drinking water for 3 or 6 months. Fluoride retention was monitored, and, following euthanasia, tissue fluoride and biochemical markers of tissue function were analyzed. Selected tissues were saved for histology, and bone marrow cells were harvested for determining the frequency of sister chromatid exchange, a marker of genetic damage. In spite of significantly higher levels of fluoride in the tissues of the animals with renal insufficiency, there were no clinically adverse, fluoride-induced, extraskeletal, physiological, biochemical, or genetic effects of chronic exposure to common levels of fluoride in these rats.

Key words: Fluoride metabolism; Fluoride toxicity; Rats; Renal insufficiency.

Reprints: A Dunipace, Oral Health Research Institute, 415 Lansing Street, Indianapolis, IN 45202, USA.

COMMENT

Many studies from the same source have supported the safety of fluoridated water. The authors’ position is made clear in the first sentence of their Introduction, which states: “Fluoride is widely used for its beneficial effect in reducing dental caries, . . .” and then states of 1 ppm fluoridated water, citing a 1993 review of the US National Research Council: “under normal conditions it does not cause adverse effects.” After reading the full study, I make the following observations:

1) The experiment occupied periods of 3 and 6 months, so is hardly relevant to human populations consuming fluoridated water, as the authors claim.

2) There was a high mortality rate (up to 45%) among the treated groups, clearly a result of the surgery to which the rats were subjected. Although the authors concluded that “there was no association between the level of fluoride exposure and mortality rate”, the possibility that the rats more susceptible to surgical trauma might also, had they survived, been more susceptible to non-skeletal effects of fluoride, does not appear to have been considered.

3) The brain tissue of the rats was not examined. Possible neurotoxic effects were not looked for.

It is interesting to compare these authors’ findings with those of other studies, e.g. the Brazilian one the abstract of which was published in the last issue of Fluoride (31 100-101 May 1998). Over 40 years ago both skeletal and non-skeletal adverse effects on rats fed low levels of sodium fluoride were reported (Ramseyer WF, Smith CAH, McCay CM. Effect of sodium fluoride administration on body changes in old rats. Journal of Gerontology 12 (1) 14-19 1957), but the experiment occupied a longer period of time (520 days) than the Dunipace et al study discussed above.

John Colquhoun
THE ROLE OF THE PUBLIC IN WATER FLUORIDATION: PUBLIC HEALTH CHAMPIONS OR ANTI-FLUORIDATION FREEDOM FIGHTERS?

G B Hastings, K Hughes, S Lawther and R J Lowry
Glasgow, Scotland and Newcastle upon Tyne, England.
Abstract from British Dental Journal 184 (1) 39-41 1998

Objective: Using the opportunity of pretesting a leaflet for the general public on water fluoridation, their views were also sought on the issue as a whole.
Design: Qualitative research using focus group discussions led by an experienced moderator.
Setting: Among the general public living in north east England.
Subjects: Members of the public living in both fluoridated and non-fluoridated areas in three age bands (20-35, 36-50 and 50+) and by social class.
Results: The study found: the low priority given to dental health; how emotive the subject of water is; the variable knowledge of fluoride in relation to dental and general health; and the desire for information if new water fluoridation schemes are planned.
Conclusions: The research confirmed public support for water fluoridation but highlighted the place of public health professionals in championing water fluoridation because of public apathy.

COMMENT

The British Dental Journal's acceptance of the above paper, which merely describes canvassing of opinions about artificial water fluoridation and pretesting of a propaganda poster, is surprising, particularly because the conclusions drawn are based on a non-random selected sample size of between 48 and 64 people. The use of the Social Marketing Department of the University of Strathclyde, where three of the authors work, is a novel approach to the controversial issue of water fluoridation, which one could reasonably expect to be settled by scientific discussion.

Bill Wilson
118 Forrest Hill Road
North Shore City, New Zealand

List of some other publications

Arnold CM, Bailey DA, Faulkner RA et al. The effect of water fluoridation on the bone mineral density of young women. Canadian Journal of Public Health (Revue Canadienne de Sante Publique) 88 (6) 388-391 1997. (A positive effect is suggested, from a comparison of small atypical samples from two communities.) Reprints: C M Arnold, University of Saskatchewan, School of Physiotherapy, Saskatoon, SK S7N 0W3 Canada.
Lane JM. Osteoporosis - Medical prevention and treatment. Spine 22 (24 Suppl) S32-37 1997. (Suggests fluoride therapy for low bone turnover cases.) Reprints: J M Lane, Hospital for Special Surgery, Osteoporosis Prevention Center, 535 E 70th St, New York, NY 10021, USA.
Lawrence HP, Sheiham A. Caries progression in 12- to 16-year-old schoolchildren in fluoridated and fluoride-deficient areas in Brazil. Community Dentistry & Oral Epidemiology 25 (6) 402-411 1997. (A non-blind study reporting slower progression in 183 from fluoridated central Rio de Janeiro than in 107 from other, “fluoride-deficient”, districts on the outskirts. In a further, similar, study on pp 412-418 of the same journal, the authors report radiographic detection of “subtle differences” in progression in the two areas.) Reprints: H P Lawrence, University of North Carolina School of Dentistry, CB 7450 Chapel Hill, NC 27599, USA.
TWO UNANSWERED LETTERS

The following letter received no reply or acknowledgment:

October 15, 1997
Dr. Bruce Alberts, President
National Academy of Sciences
2101 Constitution Avenue, NW
Washington, DC 20418

Dear Dr. Alberts:

As you may be aware, the Dietary Reference Intakes report on calcium, magnesium, phosphorus, vitamin D, and fluoride prepared by the Institute of Medicine of the National Academy of Sciences and scheduled for publication this month, contains a number of recommendations concerning fluoride that are cause for grave concern over their validity for setting public health policy. This concern has been heightened by statements made by speakers and panel members and their responses to queries at the recent September 23rd workshop on the report held at the National Academy of Sciences.

We, the undersigned, regard the problem as so serious that we are requesting you to take immediate steps to delete the fluoride section of the report and to have it re-addressed by a panel that includes members of the scientific community who are not committed to promoting or supporting fluoride use. What follows is a brief summary of the basis for our concern.

At the heart of the matter is whether fluorine, as fluoride (F\(^{-}\)), should be ranked with Ca, Mg, P, and vitamin D as an essential nutrient. In fact, there is no known essential biochemical role for fluoride in any animal, including humans. The formation of sound, decay-resistant and caries-free teeth as well as strong, sturdy bones, whether in animal or human populations, does not require fluoride, or at least not in more than minuscule, trace amounts. As acknowledged by sources cited in the report, even when a mother's fluoride intake is elevated, her milk is extremely low in fluoride, but owing to prenatal accumulation, her baby excretes more fluoride than it ingests from her milk. This fact clearly indicates that any natural physiological need for fluoride, if indeed any exists, must be exceedingly small and certainly far below that being recommended in the report.

At the September 23rd workshop, as recorded on videotape, fluoride was repeatedly regarded by speakers and panel members as an essential nutrient. But, toward the end, when challenged on this key issue, Dr. Vernon R. Young, Chair of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, bluntly stated that fluoride should not be considered as an “essential” component of the diet. Instead, without clarifying the distinction, he insisted that it should be viewed as only a “beneficial element.”

The fact that fluoride is incorporated into the mineral matrix of bones and teeth does not make it an essential nutrient. Other elements hardly considered essential, such as lead and cadmium, also accumulate in bones and teeth, and they are not regarded as beneficial. Obviously, if fluoride is not essential in human nutrition, any consideration of it in terms of an “adequate intake” is clearly not appropriate and should not be part of a “dietary reference intakes” report.

An association of fluoride with reduction in dental caries is cited as the basis for recommending the various intakes of fluoride. At the same time, the report acknowledges that most of the anti-caries effect attributed to fluoride occurs by topical exposure, not through systemic ingestion. Moreover, large, whole-population studies not cited in the report (e.g., in New Zealand) show that declines in tooth decay over the last 40-50 years
have been occurring independently of fluoride exposure and use, thereby further challenging current arguments for significant benefit from fluoride.

Without question, however, ingestion of even milligram amounts of fluoride during infancy and early childhood can produce the unmistakable toxic effects of dental fluorosis. This disruption of normal enamel formation is stated in the report not to be of "public health significance" if the fluoride concentration in the drinking water is below 2 mg/liter (2 ppm). But reports of disfiguring dental fluorosis with staining and pitting of the enamel in areas with 1-2 ppm fluoride in the drinking water were evidently overlooked, despite the claim at the workshop that the literature review was comprehensive and thorough.

Of even greater concern, in relation to public health, is the proposal in the report that only the early stages of skeletal fluorosis are the appropriate criteria for fluoride intoxication. For this purpose a tolerable upper level ingestion limit of 10 milligrams of fluoride per day for 10 or more years in persons age 9 or older is proposed. But this level of intake is not tolerable, and, according to the sources cited in the report, it can and does lead to crippling skeletal fluorosis (Hodge, 1979). For young adults, assuming 50% retention of ingested fluoride in hard tissues, as stated on page 8-2 of the prepublication copy of the report, an absorbed intake of 10 mg/day amounts to a yearly accumulation of 1.8 grams or over 50 grams after 30 years. At this level debilitating skeletal fluorosis was observed by Raj Roholm in his classic studies of cryolite workers. But before this condition is reached, there are various pre-skeletal phases of fluoride intoxication with serious health implications that arise from much lower levels of intake, especially when calcium and magnesium are marginal, an aspect not considered in the report. Among these manifestations are increased hip-fracture among the elderly from deterioration in bone strength and quality (in agreement with long-term laboratory animal studies), increased osteosarcoma in young males (also demonstrated in male rats), chronic gastrointestinal irritation (reversible with decreased exposure to fluoride), and various neuromuscular disorders whose connection with fluoride has been well confirmed in peer-reviewed publications without convincing refutation. Recent studies showing decreased IQ scores correlating with dental fluorosis (again backed up by laboratory animal research) were also omitted from consideration.

When questioned at the workshop about these omissions, the speakers and the members of the panel became defensive and were unwilling or unable to explain why such findings had been excluded in setting the upper tolerance level of fluoride at 10 mg/day. From the record of some of the committee members' past promotion or support of fluoride use, including slow-release fluoride for treatment of osteoporosis (known to produce abnormal bone of inferior strength), these responses, although disappointing, are perhaps not too surprising. But, in such an important matter, should not at least some balance of viewpoint have been represented? As seen in the videotape (a copy of which has been sent to the Academy) the attitude of some of the presenters and panelists toward those who cited contrary data and questioned why such findings were not discussed can only be described as condescending and demeaning.

Today, with so many additional sources of fluoride present in processed foods, commercial beverages, and dental care products that were not there when water fluoridation began, the total intake of fluoride, even among children, has increased to as much as 2-5 milligrams or more per day, well above the initially proposed optimum of 1 mg/day (from one liter of 1-ppm fluoridated water). With these higher levels of fluoride intake, dental fluorosis and other toxic effects noted above have also increased.

We are sure that you would agree that it is immensely important to both the national interest and the world of science that the publications of the National Academy of Sciences maintain the highest standards of competence, objectivity, and integrity. In our view, unless
Unanswered letter

the section on fluoride is withdrawn from this report on essential nutrients it could seriously threaten those standards. Therefore, we urge you to remove this section, and further request that should the fluoride issue be revisited by the Academy at some time in the future, that you should ensure that the investigating panel includes independent scientists who are fully conversant with the literature on the full range of fluoride’s harmful effects.

Sincerely yours,

ALBERT W. BURGSTAHLER, Ph.D. (Organic Chemistry and Environmental Fluoride), Professor of Chemistry, The University of Kansas*, Department of Chemistry, 4035 Malott Hall, Lawrence, Kansas 66045.

ROBERT J. CARTON, Ph.D. (Environmental Sciences and Risk Assessment), Former Risk Assessment Manager for the Office of Toxic Substances, U.S. Environmental Protection Agency. Mailing address: 2455 Ballenger Creek Pike, Adamstown, MD 21710.

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JOHN R. LEE, M.D. (Physician), 9620 Bodega Highway, Sebastopol, CA 95472.

WILLIAM MARCUS, Ph.D. (Toxicology).

GENE W. MILLER, Ph.D., (Biochemistry and Toxicology). Former Head of Biology, Associate Dean of Science and Dean of Environmental Science, Utah State University*, Emeritus, College of Science, Department of Biology, Logan, Utah 84322-5305.

PHYLLIS MULLENIX, Ph.D. (Pharmacology and Neurotoxicology). Former Head of the Department of Toxicology, Forsyth Dental Center*, Boston. Research Associate, Department of Psychiatry, Children’s Hospital*, Boston. Mailing address: P.O. Box 753, Andover, MA 01810.

ALBERT SCHATZ, Ph.D. (Microbiology). Former Professor of Science Education, Temple University*, Philadelphia, PA.

* These affiliations are listed for identification purposes only and do not imply endorsement of this letter by the institutions involved.
Sixteen weeks later the following letter was sent, also receiving no reply or acknowledgment:

February 4, 1998

Kenneth I. Shine, M.D.
President, Institute of Medicine
National Academy of Sciences
2101 Constitution Ave. NW
Washington, DC 20418

Dear Dr. Shine:

Last October my co-signers and I sent to Dr. Bruce Alberts the enclosed joint letter concerning the fluoride recommendations in the impending publication by the National Academy Press of the report on Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Although we do not understand why we have not received a reply, we trust that our letter was brought to the attention of the Food and Nutrition Board of the Institute of Medicine for appropriate action.

When we submitted our letter we were under the impression that the position of the Food and Nutrition Board on the questions we raised was not yet settled. We have since learned, however, through the publication of the full text of the Summary of the report in the September/October 1997 issue of Nutrition Today, pp. 182-188, that the upper level intake and other recommendations for fluoride had already been officially submitted for general distribution, even at the time the September 23rd workshop on the report was held at the Academy.

Does the appearance of the pre-publication version of the report Summary in that issue of Nutrition Today (which only reached our science library here on November 13) mean that the Food and Nutrition Board still considers an intake of 10 milligrams of fluoride per day (Table 6) to be a tolerable upper level for persons over age 9 without significant risk of serious adverse health effects?

What is especially troublesome about the Board’s position on this matter is that it explicitly and emphatically contradicts the recently published views of the most distinguished and long-time fluoride expert member of the Panel on Calcium and Related Nutrients - Professor Gary M. Whitford of the Medical College of Georgia. In the second, revised edition of his widely-cited monograph on The Metabolism and Toxicity of Fluoride (Karger, Basel, 1996), he states on page 138 (copy enclosed):

"Most estimates indicate that crippling skeletal fluorosis occurs when 10-20 mg of fluoride have been ingested on a daily basis for at least 10 years." With this clinical condition, he notes, "... bone ash fluoride concentrations generally exceed 9,000 ppm. Calcification of ligaments often precludes joint mobility and numerous exostoses may be present. These effects may be associated with muscle wasting and neurological complications due to spinal cord compression."

Why do the recommendations of the Food and Nutrition Board on this critical matter contradict these well-considered views of the leading fluoride expert on the Board’s Panel on Calcium and Related Nutrients? Clearly, a fluoride intake level that produces "crippling skeletal fluorosis" can hardly be regarded as tolerable and certainly should not remain uncorrected.

Although it is widely believed that "... crippling skeletal fluorosis has not been and is not a public health problem in the USA" (Whitford, op. cit., page 137), the same cannot be said.
of the situation in other parts of the world, e.g., in China, India, the Middle East, and Africa, where crippling skeletal fluorosis is still a serious endemic health problem—even at less than 10 mg/day fluoride intake. Moreover, in the absence of sufficient numbers of contemporary biopsy and necropsy bone fluoride analyses, it is very unwise to assume that little or none of the extensive middle and old-age osteoarthritis that plagues so many people in the United States is not an undiagnosed manifestation of various stages of skeletal fluorosis.

In this connection it is important to note that otherwise unexplained intermittent episodes of gastric pain and muscular weakness have been clinically linked in areas of endemic dental and skeletal fluorosis to fluoride intakes as low as 2 to 5 mg/day (ref. 1). These peer-reviewed reports fully validate earlier clinical findings of the occurrence of these very same and related effects in fluoridated communities in the United States and other countries which have been discounted or ignored without scientific refutation (ref. 2).

Today, when many officials at all levels in our government seem to have difficulty in being forthright and admitting what is true, it ill behooves us as scientists not to tell the public what is known to be true, whether or not it agrees with what is generally accepted as true. "For truth is truth, though never so old, and time cannot make that false which was once true." (ref. 3).

Because the recommendations of your report on Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride have been released to the public before correction of the serious errors and oversights pointed out here and in previous communications to the Food and Nutrition Board, I presume there is no objection to this letter being made part of the public record.

Yours sincerely,

Albert W. Burgstahler, Ph.D.
Professor of Chemistry

Enclosures: Copy of October 15, 1997 letter to Dr. Bruce Alberts
Page 138 of the 2nd, revised edition of The Metabolism and Toxicity of Fluoride
Copy: Bruce Alberts
Gary M. Whitford
Co-signers of October 15, 1997 letter to Dr. Alberts

References:
To the end of reckoning."
NEED FOR EVIDENCE ABOUT FLUORIDATION

I was disappointed to see that the recent article on fluoridation, which purports to look at new information published since 1989, references only articles that agree with the authors' viewpoint. As this is a supposedly contentious area, there must be some studies that do not support their viewpoint, and not all of these can have been published before 1989. To test this idea I did a simple search on Medline, using the search strategy fluoride (or fluoridation) and hip fractures, and fluoride (or fluoridation) and osteosarcoma, these being two of the areas mentioned. I restricted my search to articles published from 1989 onwards.

These simple searches turned up five articles on osteosarcoma and fluoride exposure and three on hip fractures and fluoride exposure that were not referenced by the authors. All eight of these found no association with fluoride. What is more, some of them were case-control studies and one was a cohort study, so should provide more reliable evidence than the ecological studies cited of whether or not there was an association. This is because both fluoride intake and the outcome were measured on individuals rather than populations. I am sure that more thorough searches would have found more articles, not all of which would agree with each other. Inclusion of these articles in the review could well have changed the conclusions.

It is well known that traditional reviews are subject to all sorts of biases. A recent series of articles in the BMJ points out the problems with traditional reviews, and looks at the advantages of systematic reviews that, despite some problems, present a more realistic view of a problem. The object of a systematic review is to specify a series of steps so that if other people do the same review they will get the same answer. This article seems to be loaded with more than the normal amount of bias, because the authors selected only articles that agree with their hypothesis, although they do not openly say this. It is a pity that the authors, in spite of saying that they 'hope that at least some kind of scholarly debate will ensue' could not provide a more solid foundation as a starting point.

Peter Herbison
Department of Preventive and Social Medicine
University of Otago, Dunedin, New Zealand

References
We read with some concern the article by Diesendorf et al., entitled 'New evidence on fluoridation'. The article purports to be a review of recent scientific literature and claims this 'reveals a consistent pattern of evidence' pointing to the existence of 'causal mechanisms by which fluoride damages bones'. The authors appear, however, to have considered only the literature that supports their point of view, and we are concerned that, as a result, the article does not present an accurate picture of this emotive issue to an uninitiated reader.

When an attempt is made to evaluate causality, a number of key issues have to be considered. The first, and possibly most crucial stage, is to ensure that such an evaluation takes into account all of the available scientific evidence. Any review must be comprehensive, with all of the literature on a given topic considered and evaluated with respect to the quality of the study and the validity of the results. There are then two main questions to consider. First, if there does appear to be an association between an exposure and ill-health in a particular

EVIDENCE ON FLUORIDATION

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study, is this association real? Or could it be an artefact, due to chance, bias or confounding? Certain types of study allow us to rule out the possibilities of confounding with a greater or lesser degree of certainty. In ecological studies, where comparisons are made between, for example, populations rather than between individuals, it is almost impossible to rule out confounding as a possible explanation for an observed association. If two areas differ in one particular aspect, with respect to the use of water fluoridation, for example, they are also likely to differ in other ways that could equally well explain any apparent health differences between the areas. It is also impossible to relate exposure and the occurrence of ill-health in the same individuals. Case-control studies overcome this latter problem but can still be affected by issues of bias and confounding. While, in the absence of intervention studies, prospective cohort studies offer the strongest evidence for or against an association.

Only if we are fairly confident that a real association does exist can we then go on to consider whether or not this association is likely to be causal. Factors that can influence an evaluation of causality include: the strength of the association (a strong association is much less likely to be due to bias or confounding than a weak one); its consistency across a range of different studies in different populations; evidence that exposure really did precede the development of disease; and an increasing risk of disease with increasing level of exposure. A biologically plausible mechanism whereby, the exposure could cause disease, and supporting data from experimental systems, including animal studies, can also influence the conclusion.

Our major concern about the article by Diesendorf et al. is that it appears to discuss only those studies that have reported a positive association between water fluoridation and ill-health. Studies that found no association are summarily dismissed because of either limited sample size or because the women studied were not exposed to fluoride before the menopause.

The authors cite five ecological studies that report higher rates of hip fracture in areas with higher levels of fluoride in the drinking water and a single prospective study. This latter study followed only 827 women, who experienced a total of 86 fractures, and compared an area with high fluoride (4 parts per million (ppm): that is, more than four times the levels recommended for fluoridation) and low calcium levels with a control area with fluoride levels compatible with fluoridation (1 ppm) and normal calcium levels.

In addition to several earlier ecological studies that reported either no association or a negative association between fluoride levels in water and fracture, three recent ecological studies have also reported no association as have another two prospective studies. The majority of these studies are more recent and each had a larger sample size than one of the studies cited above. In addition, two of the three ecological studies included younger subjects (aged 45 or 50 upwards, compared with a minimum age of 65 in most of the studies above), who would have been more likely to have been exposed to fluoride premenopausally. Thus, the evidence for an association is clearly not consistent
and the newest data do not appear to support the view that water fluoridation increases the risk of fracture.

A similarly one-sided view is presented of the potential association between fluoridation and osteosarcoma. Diesendorf et al. cite two reports, not peer-reviewed articles, of ecological studies that show an increased risk of osteosarcoma in young men. At least another four ecological studies and three case-control studies have, however, been published since 1990, and all of these show either no association or a potential protective effect of fluoridation on osteosarcoma.

Diesendorf et al. strengthen their argument by reference to the data from the United States National Toxicology Program, which showed an increase in osteosarcoma in male rats exposed to high levels of fluoride in their drinking water but not in female rats or mice. The second paper that they cite does not, however, support their argument, concluding that 'results from this study indicate that NaF is not carcinogenic in Sprague-Dawley rats'. The results of the National Toxicology Program study have not been confirmed by other studies and, although they cannot be completely discounted, they cannot be used to prove carcinogenicity.

In summary, therefore, the authors have not met the prime criterion for a valid review because they have not considered all of the available scientific evidence. When this is done it becomes clear that there is little reliable evidence on which to base an evaluation of causality because many of the available data come from ecological studies. These can provide only very limited evidence for or against causality, because it is impossible to rule out confounding in this type of study. Furthermore, what evidence there is does not meet any of the Bradford Hill criteria for strength of association, consistency or dose-response.

We agree that, at high levels, fluoride does cause damage to bones, but maintain that there is no good scientific evidence to suggest that it does so at the levels to which people are exposed when drinking fluoridated water. Many other chemicals are beneficial at low concentrations but harmful when taken in excess. We wholeheartedly support the authors' desire for scholarly debate on the issue but request that such debate should indeed be scholarly and, as such, should consider the whole picture.

Penelope M Webb and Ken Donald
Department of Social and Preventive Medicine
University of Queensland, Brisbane

References


Freni SC. Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. *J Toxicol Environ Health* 42 109-121 1994.


The Australian Dental Association Queensland Branch is deeply concerned about your decision to publish the paper written by Drs Diesendorf, Colquhoun, Spittle, Clutterbuck and Everingham.1

Diesendorf et al. are noted anti-fluoridationists whose research work was severely criticised by Australia’s National Health and Medical Research Council, in their report, *The effectiveness of water fluoridation.*2

The publication of such a mischievous article, which we are confident will draw criticism from the academic community, was irresponsible because an opportunity was not provided to balance the controversy and give the full facts on fluoridation of public water supplies. News stories in the popular press show how much damage is being caused by publication of the article in your journal.

Our branch is currently campaigning to introduce the well-proven health measure of fluoridation to the Queensland community. Besides the proven health benefits of water fluoridation as a safe, equitable and cost-effective public health intervention, the branch estimates that Queenslanders could save more than $20 million in unnecessary, dental treatment if this measure is introduced.

It would be our hope and expectation that you will counter the effect of the article with responsible refutation of the article and publication of articles supportive of water fluoridation.

Pat Jackman
President, Australian Dental Association
Queensland Branch, Brisbane,

References

**REVIEW OF EVIDENCE ON FLUORIDATION**

I refer to the recent article on water fluoridation, ‘New evidence on fluoridation’.1 I am surprised that a reputable scientific journal has not checked the veracity of statements made in articles it publishes.

The article appears to be a mix of review of the scientific literature and commentary on water fluoridation. The review is selective and presents more of the scientific literature that suggests adverse effects of fluoride on health than that indicating inconclusive or positive findings.
In examining whether this article adds any new information to the accumulated research on water fluoridation, I have considered the report by the Public Health Commission, *Water fluoridation in New Zealand* (PHC report) which is referenced by the authors. This is a technical report, published in 1994, which analysed the published literature on fluoride, water fluoridation and fluoride's effect on oral health status.

The Jacqmin-Gadda *et al.* reference is a letter to the *Journal of the American Dental Association*. The letter reported a comparison of hip fracture rates in areas with water fluoride levels above and below 0.11 mg/L. It is the only study referred to by the authors, and published since the PHC report, that shows higher rates of hip fracture with higher water fluoride levels. A critical appraisal of the content of this letter shows dubious internal validity, as confounding variables known to affect osteoporosis and hip fractures were not considered. The results may be due to these other factors, such as dietary calcium intake, ethnicity, bone age, rather than fluoride intake. Daily individual water intake was not assessed, and data on water fluoridation levels were available only from 1991. Fracture history was self-reported. I wrote to the authors requesting a copy of the report to enable complete critical appraisal and peer review to ascertain the significance of the findings. They replied that no report was available because the study was still in progress.

I am uncertain of the validity of the assertion, used to reject the study of post-menopausal women, that 'fluoride would be expected to affect bone most before menopause'. In general, fluoride accumulates in the skeleton with age. I would also dispute the assertion that 'low levels of fluoride ingested for several decades can cause . . . skeletal fluorosis. It is very unlikely that in developed countries skeletal fluorosis would be associated with exposure to 1 part per million of fluoride in the absence of high long-term intake and/or metabolic susceptibility.'

Diesendorf *et al.* assert that:

> In three to four decades when people in areas where water is artificially fluoridated have accumulated fluoride in their bones from birth to old age, the increase in skeletal fluorosis will be larger.

If this is true, then the rates of hip fracture should be higher already in older people in naturally fluoridated communities than in unfluoridated communities. The authors present no information on this.

The PHC report comments on studies indicating that there is an association between water fluoridation and osteosarcoma. The toxicological evidence is referred to in the PHC report as 'weak and inconclusive'. Diesendorf *et al.* have no evidence that alters this summary.

There is also no mention of studies published since 1994 that have inconclusive findings or do not support the evidence cited of an association between fluoride and adverse effects on bone.

New Zealand and Australian references in the PHC report that show a difference in prevalence of dental caries associated with fluoridation are not
mentioned by the authors. The greater benefits of fluoridation for lower socio-economic groups are also not acknowledged.

Health agencies and professionals within New Zealand believe, on the basis of present evidence, that water fluoridation is a safe and effective strategy for protecting and improving health status and, in particular, in reaching those groups most at risk of dental decay.

It is not practical to respond to all the claims of the authors within the scope of a letter; these have been amply detailed elsewhere. Reports of independent experts in relevant fields of medicine, epidemiology, oral health and water engineering have been unanimous that benefits of water fluoridation outweigh any (very small) potential risks. Research studies on the safety of water fluoridation have been reviewed repeatedly by international and Australasian experts, including World Health Organization expert group. The conclusion of all these reports is uniform. There are no significant health risks associated with water fluoridation at optimal levels. Mortality rates and health statistics (other than for oral health) in fluoridated and unfluoridated communities are similar.

The New Zealand Ministry of Health has data on dental decay rates that show a real difference between fluoridated and unfluoridated areas, with children who have access to fluoridated water having lower rates of dental decay. There is no evidence of significant adverse effects on health from water fluoridation at the level recommended for New Zealand water supplies (0.7 to 1.9 mg/L).

Water fluoridation has been shown to provide significant benefits to oral health, particularly for disadvantaged people. Latest research shows it is effective in reducing root caries. Water fluoridation is of benefit to everyone with natural teeth.

Gillian Durham
Director of Public Health
Ministry of Health
Wellington, New Zealand

References
FLUORIDATION AND BONES: AUTHORS' RESPONSE TO CRITICS

In our article 'New Evidence on Fluoridation' we referred to recently published comprehensive data which indicate an association between fluoridation and damage to bone (hip fracture, skeletal fluorosis and possibly osteosarcoma). We pointed out the limitations of some other studies which reported no such association. We then drew attention to other relevant studies which support a causal explanation for the association.

We reject the charge levelled at us in the June issue by Herbison, and by Webb and Donald, that we cited only studies which support our view. That charge is more fairly directed at the extensive pro-fluoridation literature. For example, Webb and Donald in their submission supporting fluoridation of Brisbane's water supply, excluded any mention, in either their text or their reference list, of the fact that fluoride accumulates in bones and reaches levels consistent with widely recognised adverse effects - an important aspect of causality which we note they also omit in their attempt to criticize our article. They likewise excluded all studies reporting harm from fluoride published in Fluoride, journal of the International Society for Fluoride Research. They also did not cite a single original paper reporting skeletal fluorosis in areas naturally fluoridated at concentrations between 0.7 and 2.5 parts per million. A 1990 review by one of us (MD) examined nine such papers from five countries. That information is very relevant to the issue of causality.

Our lengthy reference list could not possibly include all published studies, but cited representative ones, which is the usual convention. We also did not cite a recent paper reporting a positive correlation between fluoridation and hip fractures, which supported our assessment.

Our critics seem to hold a naive belief that conclusions can be based on the quantity, rather than the quality, of published papers on controversial issues. They list studies that, in their view, counterbalance the comprehensive data on which we based our conclusion that fluoridation should be discontinued. Such publications do not nullify the compelling evidence of harm represented by the comprehensive data we reviewed. In any case, even if the evidence is conflicting, so that conclusions remain in dispute, the precautionary principle is itself grounds for discontinuing the mass uncontrolled fluoride dosing of entire populations.

We wonder why our critics do not apply the same stringent requirements for proof of causality that they seem to apply to evidence of harm from fluoride to the many flawed studies claiming a fluoride dental benefit. At the Brisbane Lord Mayor's Taskforce on Fluoridation, Professor Donald was asked that question and replied that he had not examined the fluoridation studies because he had never been asked to. We wonder how many other professors at medical schools continue to advocate fluoridation without examining the evidence for it.

An examination of the quality of some of the studies our critics cite to support their case is revealing. They include two studies, which their authors claim suggest that fluoride may be protective against the rare bone cancer, osteosarcoma (which we pointed out has increased among young males aged 9 to 19 years in fluoridated areas of America, but not in unfluoridated areas).
One of these studies, from the dental literature, was based on only two cases, of unstated age and sex, who spent more than a third of their life or childhood in a fluoridated area, and seven cases, also of unstated age and sex, who spent less than a third of their life or childhood in a fluoridated area. In the other study, which appeared to suggest a protective effect from fluoride, the study design was based on an assumption that osteosarcoma victims would require (if ingested fluoride was the cause) higher fluoride exposure than those without the disease. The possibility that such victims might be more susceptible to equal or smaller fluoride exposures was not considered. A critical review of that report was not cited by our critics.

Other studies they cited do not, on close examination, support their claim of no fluoride/osteosarcoma link. For example, Hrudey et al. admitted that their data from small populations do not allow any definitive conclusions about the role of fluoridation as a risk factor for osteosarcoma in humans. The claim of Mahoney et al., of no difference in bone cancer incidence between fluoridated and unfluoridated areas of New York State, can be disputed on the grounds that the authors failed to consider male rates separately. The Moss et al. study did not calculate the water fluoride-osteosarcoma association for 10- to 19-year-old males, the age-sex group for which the association has been reported. It combined both sexes for its two age groupings (under and over 45 years) and combined all ages for its female and male calculations.

Two studies that they described as 'ecological' were claimed by Webb and Donald to show no osteosarcoma-fluoride association. The first, a letter to editor by Cook-Mozaffari et al., simply expressed the same opinion of our critics, and presented no new evidence. The other does not deal with osteosarcoma at all, but reported instead that water fluoride reduces human male fertility.

Another study, claimed by Herbison to counteract our observations on a possible fluoride/osteosarcoma link, is a very good study by an eminent researcher and his associates, but is irrelevant to this discussion because it deals with male and female adult workers exposed to fluoride, not with young males growing up in fluoridated areas.

Other studies discounting an association between fluoride and hip fracture (for example, that of Jacobsen et al. 1993) are of doubtful value because, as we pointed out in our article (citing a review), many are of small samples or the women were not exposed to fluoride before menopause. The same applies to the more recent Cauley et al. study: of the 41 hip fracture cases aged 65 or over, only four had lived more than 10 years in a fluoridated area. The study by Suarez-Almazor et al. compared one fluoridated and one unfluoridated Canadian city. It is true that this study did not find more hip fractures in women in the fluoridated area, as other studies have done. However, it did find significantly more hip fractures in men in the fluoridated city. The authors of another recent study cited by our critics (Karagas et al.), stated: 'Our findings with respect to water fluoridation have important limitations, however.' These limitations included: 'Fluoride exposure was assessed at the time of fracture and thus does not necessarily reflect exposure during what may have been a more relevant time period (for example, during peak bone formation).
The recent Finnish study which our critics cited reported no differences in the prevalence of female fractures in its fluoridated and nonfluoridated groups, and slightly higher (1 to 2.6 per cent) bone mineral density in women exposed to fluoride for more than 25 years. However, the fluoride-exposed women were younger, more physically active, and more likely to use hormone replacement therapy.

Our other critics, Pat Jackman of the Dental Association and Gillian Durham of the New Zealand Ministry of Health, objected to our views even being published, but added nothing of substance to the debate. However, we thank our critics for contributing and hope the debate continues in a scholarly manner.

Mark Diesendorf
Institute for Sustainable Futures
University of Technology, Sydney

John Colquhoun
Honorary Research Fellow, School of Education
University of Auckland

Bruce Spittle
Department of Psychological Medicine
University of Otago Medical School, Dunedin

References
4 Webb PM, Donald K. A report to the Brisbane City Council taskforce on fluoridation on the non-dental human health effects of water fluoridation. Department of Social and Preventive Medicine, University of Queensland, Brisbane 1997.
NEW EVIDENCE ON FLUORIDATION - Authors' response


23 Freni SC. Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. *Journal of Toxicology and Environmental Health* 42:109-121 1994.


PUBLIC HEALTH GOAL FOR INGESTED FLUORIDE

David Kennedy and William Hirzy

On June 19-21, 1998, the International Academy of Oral Medicine and Toxicology (IAOMT) co-hosted a symposium as a final step in developing a public health goal (PHG) for ingested fluoride. We firmly agree with the findings of the November 1997 Canadian Dental Association conference on fluoride drops and tablets which found "no reliable scientific evidence of significant dental benefit from ingested fluoride." The IAOMT has received scientific input for this PHG from more than a dozen sources. Adverse health effects demonstrated were: fluorosis; cancers; genetic damage; bone pathology; trans placental and brain transport; histological brain, artery, and kidney damage; and neurological impairment.

The Environmental Protection Agency (EPA) Guidelines for Carcinogen Risk Assessment recommend using a multistage model procedure and linearized upper bounds for low-dose extrapolation in developing quantitative dose-response assessments. The degree of multistage polynomial fitted to the data was selected by the GLOBAL 86 program. Reference doses (RfD) are calculated from animal experiments (cow, rat) and a human study from the peer reviewed literature. Modifying factors of 10 for each condition of uncertainty factor were used for animal to human conversion, human variations in sensitivity, conversion from lowest-observed-adverse-effect level (LOAEL) to no-observed-adverse-effect level (NOAEL), and severity of effect (diminished IQ, brain damage, abortion rate and calf death).

The resulting PHG is calculated at $1 \times 10^{-4}$ mg/kg-day (human) and $1.5 \times 10^{-4}$ mg/kg-day (cow) and $7 \times 10^{-6}$ mg/kg-day (rat). Current human exposure in fluoridated communities may exceed $7 \times 10^{-3}$ mg/kg-day. This risk assessment raises serious concerns about the pervasive over-exposure to fluoridated drinking water, fluorine-containing foods, beverages, pharmaceuticals, time-release dental cements and fillings and oral care products.

CORRECTION

On page 116 of the last issue of Fluoride (Vol 31 No.2 1998), in the reference list to the article "Why I changed my mind . . .", reference no. 17 had wrong page numbers ("301-308" instead of the correct 59-66. Thus the correct reference is:

FLUORIDATION IN EUROPE

I have been engaged in scientific and political work on water fluoridation for 30 years. During this time I have analysed many fluoridation studies, published scientific papers, lectured on this subject at international conferences and congresses, was nominated as an expert in official hearings, and have discussed the problem in panels, in newspapers, on radio, and on television.

I would now like to inform your readers about the present stage of development of water fluoridation mainly in Europe since the resolution of the WHO in 1969:

1. The World Health Assembly (WHA) adopted Resolutions in 1969 (WHA22.30), in 1975 (WHA28.64), and in 1978 (WHA31.50) which recommended that Member States introduce community water fluoridation as a safe, inexpensive and effective measure, and urged Member States to consider fluoridation of public water supplies as part of their national plans for the prevention and control of oral disease; and it suggested that, where community water fluoridation is not feasible, alternative methods of achieving optimum daily intake or application of fluorides should be envisaged.

These Resolutions are promoted by the International Dental Federation (FDI),\(^1\) which prepared the Report by the Director General of the WHO, and backed up by Public Health Officials with their National Dental Organization\(^2\).\(^3\)

2. Scientific analyses of water fluoridation studies and experiments show that none of them can actually prove any caries prophylactic effect of fluoride.\(^5\)\(^-\)\(^7\) The "caries reductions" reported by dentists were undoubtedly constructed by dentists (e.g. in the 21-cities-study by H T Dean \textit{et al} (1942) or in the Grand Rapids/Muskegon Study by F A Arnold Jr and J W Knutson \textit{et al} (1950-1962)) or are the result of influences other than those of fluorides. The dentist J W Knutson, Assistant Surgeon General, Chief Dental Officer, US Public Health Service, was engaged as an expert of the WHO in the expert committee 1957\(^48\) and an Adviser Member of the Health Assembly in 1969.\(^3\) In my opinion the dentists in the WHO who recommended water fluoridation as safe, inexpensive, and effective, are not competent in this field of science because the problems are statistical and epidemiological and not problems of dentistry. The dentists in the WHO defend dogmatism instead of relying on facts. It is impossible to prevent and control oral disease by water fluoridation.

3. Whereas the WHO and WHA recommended the introduction of community water fluoridation in 1969, 1975, 1978, water fluoridation was stopped in some of the European Member States of the WHO.\(^48\) The reason for these cessations of water fluoridation was not a political one, but the consequence of scientific discussion of its effectiveness and side effects. Water fluoridation was stopped in the following States: Federal Republic of Germany\(^50\) (introduced 1952, stopped 1971); Sweden (introduced 1952, stopped 1971); Netherlands\(^51\)\(^-\)\(^53\) (introduced 1953, stopped 1976); Czechoslovakia\(^49\) (introduced 1958, stopped 1988/90); German Democratic Republic\(^49\) (introduced 1959, stopped 1990 (Spremberg 1993)); Union of Soviet Socialist Republics\(^49\) (introduced 1960, stopped 1990);
Finland\textsuperscript{49} (introduced 1959, stopped 1993); outside Europe: Japan\textsuperscript{49} (introduced 1952, stopped 1972).

In Europe more than 53 million people who had water fluoridation for many years are now free from it.

4. Dentists and WHO experts have predicted a very large caries increase ("a tide of caries") after termination of fluoridation.\textsuperscript{49} Analyses of the data, however, reveal a significant decrease in dental caries (caries decline) after suspension of water fluoridation in Japan,\textsuperscript{49,54} in the Netherlands,\textsuperscript{55} in Prague,\textsuperscript{43,58} in the German Democratic Republic,\textsuperscript{49} and elsewhere. Never has any real increase in dental caries been observed after water fluoridation was discontinued.

Furthermore, many fluoride tablet measures were stopped also. In Graz\textsuperscript{23} (Austria), for instance, the dental caries of children had increased during the fluoride tablet actions in schools since 1956 and decreased after the stop in 1973.

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References
4 "Dr. Rowlett, who is the secretary of the International Dental Federation, is a very persistent fellow. He began beating a path to the doorstep of WHO two years ago in Rome. He found it a bit hard to get the doors open more than just a little crack, but he was persistent..." Leonard A Scheele, US Surgeon General, at the 1951 WHA conference. See Reference 2, p 2.
5 Ziegelbecker R. Gesetzmäßigkeiten im Verlauf der Zahnkaries. Prophylaxe 8 73-83 1969
8 Ziegelbecker R. Acerca de la demostración de una acelerada presentación de caries y una retrasada erupción de la piezas dentarias permanentes por el aporte incrementado de fluor. Folia Clinica Internacional 20 332-350 1970.
ENVIRONMENTAL FLUORIDE POLLUTION:

HEALTH EFFECTS OF FLUORIDE POLLUTION CAUSED BY COAL BURNING IN CHINA. 1. FLUORIDE EXPOSURE OF RESIDENTS RESULTING FROM INDOOR COAL BURNING

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The emission from coal combustion is one of the major indoor air pollution sources in China exhibiting serious toxicological effects on the residents. In some rural areas in southern China, for example, a large number of fluorosis patients are found as a result of exposure to fluoride (F) emissions from combustion of high F-containing coal. These residents burn coal as a source of energy for heating and cooking and drying food. In these residents, therefore, F exposure occurs not only through inhalation of F-contaminated air, but also through ingestion of F-contaminated food. An international cooperative study has been conducted to assess the health effects of such exposure on those residents.

The study included determination of total exposure to F by rural residents through inhalation and food ingestion. High concentrations of the pollutant were found in the indoor air of coal burning families. The indoor airborne F is also absorbed into stored food, such as corn and chili. Urinary F levels of the residents were much higher than those of the residents in non-polluted areas in China and in Japan. The prevalence of fluorosis among the residents was found to be extremely high. In the residents living in the study areas, urinary deoxypyridinoline, an important biochemical marker of bone resorption, was much higher than that in the healthy residents in Japan. Therefore, it is suggested that bone resorption was markedly stimulated in the residents under study and that F may stimulate both bone resorption and bone formation.

Since indoor airborne F caused by combustion of coal is easily absorbed into stored food, and ingestion of F-contaminated food is a main source of fluoride exposure, development of technological measures to reduce airborne F is essential for the prevention of serious fluorosis in China.
CHRONIC FLUORIDE POISONING IN RURAL AREAS OF JIANGXI PROVINCE, CHINA. 1. FLUORIDE CONCENTRATIONS IN FOODS AND INDOOR AIR

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In neighboring rural areas of Pingxiang City in Jiangxi Province, China, indoor air was polluted by fluoride (F) caused by combustion of coal in the kitchen room. A high prevalence of dental fluorosis was reported. To investigate the relationship between the residents’ F body burden and dental fluorosis, F concentrations of drinking water, food, indoor air, urine, and scalp hair were determined. The prevalence of dental fluorosis was also studied.

Nine sites were selected to sample food and indoor air. Staple foods of these areas were collected. Fluorides in the air were separated into particulate and gaseous forms. The food samples and filters were sealed in polyethylene bags and brought back to our laboratory in Japan. All samples were measured by an apparatus developed in our laboratory. Particulate soluble F concentrations in the indoor air of kitchen rooms ranged from 1.3 μg/m³ to 6.1 μg/m³, and gaseous F concentrations ranged from 0.33 μg/m³ to 8.8 μg/m³. The percentage of the particulate soluble F was 31.4% - 80.9%, whereas gaseous F was 19.1% - 80.9% of total F, respectively. Fluoride concentrations of food were almost always below 10 μg/g.

FLUORIDE TRANSFER CONTRIBUTING TO FLUOROSIS IN PINGXIANG REGION IN JIANGXI, CHINA

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In order to find out how to prevent air pollution-dependent fluorosis in Pingxiang Region, Jiangxi Province, China, we studied the environmental geology and determined the levels of fluoride (F) in soil and water.

The results showed that the region is characterized by semi-moist aluminum and ferrum-rich geochemical environment, where F is abundant in neutral and acidic soils. This F-rich soil is the residents’ main source material for mixing with coal and making bricks and tiles. Large amounts of toxic substances including F, sulfur dioxide, and polycyclic aryl compounds are emitted as the coal-soil mixture is burned or the F-rich soils are heated at high temperature. These poisonous gases are transferred to humans through different pathways as shown in the diagram below:

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Air

<table>
<thead>
<tr>
<th>Stored food</th>
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</thead>
<tbody>
<tr>
<td>Coal and Soil — Poisonous gas — Drinking water — Human body</td>
</tr>
<tr>
<td>Soil — Plants</td>
</tr>
<tr>
<td>Plants</td>
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</tbody>
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FLUORIDE PROBLEM IN THE MOLDOVA REPUBLIC

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Fluoride (F) investigation in Moldova has been carried out for more than 20 years. Moldova is a region where high concentrations of F are mainly contained in natural objects. Phosphorites and other F-containing minerals enrich the water-bearing horizons of the Moldovan artesian drainage basin with F. The range of the total F in soils depends on their granulometric composition. Thus the supersandy soils contain from 64-269 ppm and hard ones, for example, sodic soils - from 542 to 794 ppm. Flood soils are characterized by a high content of F - from 500 to 1120 ppm. Maps have been prepared containing the average F content in soils and soil forming rocks.

Subsurface waters are used in the Republic as a water source. The cretaceous water-bearing horizon is saturated with F. High concentrations of this element in mixed waters containing chalk and torton, and the waters of lower sarmation deposits showing low concentrations of F (up to 0.5 mg/L) constitute a major part of the territory. Deficiency of F is known to create preconditions to the development of dental caries and its surplus leads to intoxication.

The anthropogenic contribution of F to total soil content was studied. The F content has also been studied in plants, and the reaction of soils and plants to different doses of phosphoric fertilizers was investigated. Fluoride research was also carried out in stockbreeding. Fluorosis in animals can occur subsequent to absorption of F from potable water. Assimilation of F in organisms depends on ingested material. Three degrees of animal fluorosis have been established: spotted cretaceous-pigmented, speckled, and deforming.

Under conditions of high F pollution, it is important to minimize exposure, and study its environmental behavior and ecological consequences.

FLUORIDE DISTRIBUTION IN BUROZEMS OF MOLDOVA

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The investigations were carried out in the central part of Moldova on two different subtypes of burozems. The average fluorine content in the humic horizon (A2) of forest podzolic loamy burozem was 244 ppm. A decrease in fluoride (F) concentrations was noticed in the podzolic horizon (A2) (176 ppm) mainly due to weathering and removal of clay minerals. In the alluvial horizon (B2) the F level was 303 ppm. The increasing F content in the B2 horizon reflected the response to the high content of clay minerals, and the formation of insoluble complexes of F with iron, aluminum, and silica. In mother rock (C) the concentration of F was lower compared with the alluvial and humic horizons (203 ppm). Compounds containing F were leached from this sandy rock.

The behavior of F in forest modal burozem of fine textured soils differed from that of the previous subtype. Fluoride distribution in the soil profile was weakly differentiated. The highest concentrations of F were observed in the humic horizon (509 ppm), and an increased level of F was found in the transitional horizon (BC). This is correlated with the increased carbonate content and can be explained mainly by the formation of slightly soluble fluorite.

The results obtained showed that the mobility of F in burozems is a function of soil-forming processes and that the main factors affecting the concentration of F are the amount of clay minerals, levels of calcium, iron, aluminum, and silica in soils, and soil pH.
FLUORIDE IN SOILS AND PLANTS

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We studied the fluoride (F) levels in various types of soils and vegetation in the Moldova Republic. For this purpose, soil samples were collected from 72 soil profiles. The levels of F in wheat and corn were studied.

Results showed that the levels of F in soils depended on the origin of the soils. The lowest F level was found in soils with high leaching capacity. The average concentration of F in humoziems and grey forest soil, ordinary and calcareous chernozems, and podzolic, leached and modal chernozems was 343, 386 and 476 ppm, respectively. F accumulation was observed in river valley and sodic soils (648-732 ppm).

The effects of applying high-phosphorus (P) fertilizers on F accumulation in wheat and corn were studied. Application of the P fertilizers to soil resulted in doubling the biomass of wheat. In all plant tissues except the roots, F concentrations increased 2-3 times compared to the control. This may be due to a synergistic effect induced by P following its increased uptake.

Despite an excessive accumulation of F in the leaf tissues of wheat and corn (20.9 mg/100g d.w.) the concentration of F in the grain was low (4.7 mg/100g d.w.). This indicates that in the growing plants protective mechanisms may function to inhibit F translocation into developing grains. No visible symptoms of F toxicity for wheat were observed. However, corn was susceptible to high F levels, as manifested by chlorosis, impaired growth, and reduced yield.

FLUORIDE CONCENTRATIONS IN VOG (volcanic-derived fog)
FROM HAWAII VOLCANOES NATIONAL PARK

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Volcanic surface condensate samples were collected from 27 locations in the Hawaii Volcanoes National Park, USA, during December and January 1995/96 and March 1996. The samples were collected with a passive cloud water collector commonly used in terrestrial cloud water chemistry studies. Samples were analyzed for H⁺, NH₄⁺, Na⁺, K⁺, Ca²⁺, Fe²⁺, Mg²⁺, Si⁴⁺, Al³⁺, NO₃⁻, SO₄²⁻, Cl⁻, and F. The first collection period was characterized by low surface lava flow activity, and increased activity during the March sampling. The average fluoride (F) concentrations in the condensate from the upland areas in the park were relatively low (0.06-2.5 ppm). Average condensate F concentrations from the area known as the point of entry, where lava currently flows into the sea, were higher (10-32 ppm). Molten lava (1105°C) entering the ocean causes sea water to dissociate and form hydrofluoric acid and other products. Higher F levels and acidity (pH 0.75-1.5) generally occurred at the point of entry during periods of high volcanic activities. Fluoride levels and acid content of the condensate were good predictors ($r^2 = 0.65$) for metal concentrations at this location.
DIETARY FLUORIDE:

A STUDY ON SAFE DIETARY FLUORIDE LEVEL

V K Desai and B B Desai

Although food has been accepted as the second most important source of fluoride (F), studies on food F in relation to fluorosis are scanty. Such studies are important particularly in endemic fluorosis areas where both water and food contain high levels of F. This study was conducted to investigate the dietary F intakes of children from two villages in the Surat District in India: one with high water F levels (range: 1.07-3.12 ppm), and another (control) with low water F levels (range: 0.40-0.57 ppm). Children between 10 and 15 years were selected and their daily food and water intake was studied using the standard inventory method. A fluoride ion specific electrode was used to determine the F content in water and food (Villas known addition method). This information was translated into the children’s daily F intake from food and water. Dental fluorosis (DF) and grade of DF of each child were recorded. Since cereals and pulses consist of more than 70% of total food intake by the villagers and are predominantly grown locally, they are major contributors to dietary F. Fluoride from cereals, pulses, and water are, therefore, considered indicators of dietary F intake.

The results showed that the mean dietary F intake of children with and without DF was 1.80±0.581 and 0.46±0.054 mg/day, respectively. When the mean dietary F intake was analyzed according to Dean’s DF classification, grade 0.5 was at mean dietary F level of 1.42±0.404 mg/day. A consistent increase in DF severity (grade) was observed with increase in mean dietary F intake. For children with DF grade 3, the mean dietary F intake was found to be 2.26±0.534 mg/day. This study indicates that a dietary F intake below 1.0 mg/day is safe and does not lead to DF in the study area.

ROLE OF FOOD IN FLUORIDE TOXICITY IN ENDEMIC AREA OF MAHESANA DISTRICT, GUJARAT, INDIA

B B Desai and V K Desai

Water is the main cause of fluorosis in endemic areas. Studies have shown that, apart from water, food may be another major source of fluoride (F) in endemic areas. This study was conducted to assess the contribution of food-borne F in F-induced toxicity.

The study was carried out in 25 villages of five blocks (talukas) of Mahesana District located in north Gujarat of Western India. From each taluka 5 villages with different F contents in water supply were randomly selected. A total of 1067 subjects from 489 families were studied for 1) dental fluorosis; 2) total food intake (inventory method questionnaire method) and nutrient intake, 3) levels of F in water, and 4) intake of food-borne F (from staples only). Fluoride content in the water supply was determined using Orion ion specific electrode 94-09 with a model 720 A ion analyzer. Since cereals contributed 47% of the total food intake, they were used as a representative of food F intake. Food F intake was classified into three categories on percentile basis for further analysis and interpretation.

Results revealed that there was a consistent increase in DF prevalence rate with increase in food F intake. However this positive association was not statistically significant. DF prevalence rate was also assessed in relation to food F intake and water F content. At water F levels >2 ppm, a statistically significant positive association was observed, i.e., there was a consistent increase in DF prevalence with increased intake of food-borne F.

Nutritional status of an individual influences F toxicity, also. This was assessed using RDI (Recommended Dietary Intakes), established by ICMR (Indian Council of Medical Research), as a cut-off point. It was observed that when calorie and calcium intakes were less than RDI, DF prevalence rate was significantly high. No similar strong associations were observed with protein and vitamin C. Our study indicates that food F plays a contributory role in endemic fluorosis while nutritional status plays a prophylactic role in F toxicity.

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OFFICIAL LEVELS OF FLUORIDE FOR DAIRY CATTLE ARE TOXIC

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The official tolerance levels of fluoride (F) for dairy cattle are 30 ppm in feed for heifers and 40 ppm for mature cows. The suggested levels in water are from 2.5 to 4 ppm and from 3 to 6 ppm, respectively.

The economically most important effect of F ingestion is decreased milk production. Milk calcium (Ca) is derived in equal parts from food and bone tissue. Fluoride is toxic to bone resorbing cells and with decreased resorption the cow does not produce Ca deficient milk but less milk in proportion to the F burden.

Tolerance levels of F are from old studies in cows with a milk production (14 Kg/day) of about half of today's standard (28 Kg/day).

For maintenance, a 550 Kg cow needs 10.5 Kg dry matter (DM) and 0.5 Kg DM/Kg milk. At 14 Kg milk production DM requirement is 17.4 and at 28 Kg is 24.2. At 40 ppm F in DM, daily F intake is 420 (dry cow), 660 and 968 mg, respectively. Bone Ca requirements for milk production is 0 (dry cow), 8.26 g for 14 Kg and 16.5 for 28 Kg. The tolerance levels thus propose that with greater demand on bone Ca, greater levels of F would be tolerated.

Suggested tolerance levels of F in water fail to observe that increased water consumption raises the F burden to toxic levels. Water requirement is from 3.1 to 5.2 Kg/DM and additionally from 2 to 3.8 Kg/Kg milk: values increase linearly with increasing ambient air temperature. A high producing cow in a warm climate would require up to 200 Kg of water. The F burden would be from 600 to 1200 mg/day by suggested tolerance levels.

It has been reported that in a dairy farm with forage F levels at 19 ppm, decreasing milk production caused the farmer's bankruptcy.

The ideal F ingestion is zero. Tolerance levels should be reduced to levels that protect cattle and farmers.

HEALTH/BIOLOGICAL EFFECTS:

TEA-DEPENDENT FLUOROSIS IN CHINA
A REVIEW

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This paper is concerned with a review of the status of tea-dependent fluorosis in China. The study includes assessment of major sources of fluoride (F) and total F intake; F levels in various varieties of Chinese tea; effects of tea-borne F on human health, and the prevalence of teen-agers' dental and adults' skeletal fluorosis, etc.

The highest incidence of tea-dependent fluorosis is found in the minority regions where the residents customarily drink brick teas. A critical factor involved in tea-dependent fluorosis is the quality of raw tea leaves. The latter, in turn, is affected by (a) the levels of soluble F in soils; (b) the F content of fertilizers applied to soils; (c) the variety of tea plants; and (d) the time at, and the manner in which, tea leaves are plucked. Several measures that may be taken for the control of tea-dependent fluorosis are suggested.
HEALTH SURVEY OF WORKERS OF AN ALUMINUM PLANT IN JIANGXI PROVINCE, CHINA. 1. ON RESPIRATORY SYMPTOMS AND VENTILATORY FUNCTIONS

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When inhaled airborne fluoride (F), particularly gaseous F such as hydrogen fluoride, comes in contact with the respiratory tract, it may act as a highly irritable and reactive material, thus directly affecting the respiratory system.

The symptoms and respiratory functions of workers (154 males and 9 females) at an aluminum plant in Jiangxi Province, China, have been examined. Average F levels in the air at the workplace were 0.55 mg/m³, with a range of 0.089-2.30 mg/m³. Gaseous F accounted for 0.38 mg/m³ (69%).

The prevalence of respiratory symptoms was surveyed by means of an arranged British Medical Research Council's Questionnaire. The ventilatory function tests were carried out by the flow-volume curves drawn on a spirometer. The examined indices were: relative vital capacity (%VC), percentage forced expiratory volume in one second (FEV1.0%), peak flow speed (PF), flow velocity at half of vital capacity (V50), flow velocity at a quarter of vital capacity (V25), and V25 divided by stature (V25/height). Workers (N=112) from the electrolysis melting department were also examined for urinary F levels.

The urinary F levels of workers exposed for long periods were more than 1.5 times higher than those of the control (1.04 mg/L). The prevalence of a persistent cough with phlegm was found to be high in over 7% of the male workers. There was no significant correlation between %VC and F exposure period, but between FEV1.0% and exposure period a significant correlation was observed (p<0.05). The V50, V25, and V25/height were also significantly lower in exposed workers compared to the control group.

A STUDY ON THE CAUSE OF CHILDHOOD FLUOROSIS IN GUIZHOU, CHINA

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We investigated the causes of childhood fluorosis in Guizhou Province, China. The study included analyses of fluoride (F) contents in biological samples, staples and vegetables, and environmental samples. The results indicated that the main factors contributing to childhood fluorosis include: (a) high intakes of F from various sources - for example, total F intake was 56.22 mg/day; staples, 25.82 mg/day; and vegetables, 30.12 mg/day; (b) malnutrition, manifested by low intakes of animal protein, fat, iron, calcium; and (c) F-Al combined toxicosis. It is concluded that reduction of F intake is the most effective means of fluorosis prevention.
TOXIC EFFECTS OF WATER FLUORIDATION ON CROCODILIANS AND RODENTS IN CAPTIVITY

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For 20 years prior to the spring of 1981, caimans, alligators, and rats were successfully bred and raised at Parrot Hill Croc Farm in Kansas City, Missouri. During these years, the rare reproduction of caimans in captivity occurred, e.g., in June 1978. Among the more than two dozen crocodilians at the farm there were only two deaths. Many of the rats lived for three years or more, and only four tumors were found among the hundreds of rats in the colonies.

On 9 April 1981, fluoridation of city water with hydrofluosilicic acid (industrial phosphate fertilizer by-product) began. Within three days the eye membranes of the caimans and alligators started to swell and later became discolored and ulcerated. The animals also began to avoid being in the water, preferring to remain on deck more than normal and going from tank to tank, evidently seeking water less toxic to their eyes. These eye conditions, diagnosed by a veterinarian, have continued and worsened.

By 1983 bloated bellies, gastric distress, and spinal deformities were also noted. During the next 15 years, 21 caimans and 3 alligators died, often in apparent agony. Many were less than 10 years old (natural life span 35 years or more). Autopsies showed severe disintegration of the GI tract as well as other gross abnormalities. None of the eggs laid since 1981 have hatched, and all were infertile, even though matings had occurred.

During the first six months after fluoridation began, the health of the rats declined dramatically. Over 200 tumors were counted, with as many as 6 per rat. Beginning on 1 October 1981, the rats were given only distilled water to drink. Their condition quickly improved, and no new tumors were detected.

These findings clearly indicate that water fluoridation as practiced in Kansas City, Missouri, has induced severe adverse health effects in reptiles and rodents raised in captivity. As observed previously with chinchillas in captivity, these effects can be prevented in rodents by changing to distilled water.

EFFECTS OF INHALED FLUORIDE ON MICE

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The effects of inhaled fluoride (F) on mice were studied under laboratory conditions. Five-week-old male ICR mice were exposed to airborne F (13.3 ± 1.3 mg F/m\textsuperscript{3}) in an inhalation exposure chamber for 4 hours/day for 10, 20, or 30 days. Aerosol F was generated by atomizing 0.1 mol/L NaF solution followed by dehydrating the aerosol to form submicron particles. The aerosol F in the chamber was monitored using a portable cascade impactor. Body weights of mice were measured during the exposure. Weights of kidney, liver and lung were also measured after 10, 20, and 30 days of exposure. Fluoride concentrations in the chamber, urine, and bone were determined by F ion-specific electrode.

Significant differences in relative lung weight (mg lung weight/kg body weight) were observed in all exposed mice, although no significant differences were found in body weight and the weights of kidney or liver in exposed mice. Urinary F excretion in the exposed mice was significantly increased, with increases depending on exposure time and F concentrations in the exposure chamber. A linear correlation was found between dose of inhaled F and urinary F excretion (r=0.936, p<0.01; n=10). For example, in animals exposed for 3 days, the urinary F excretion was twice as high as that in control mice. The F content in bone exceeded 650 mg/kg in mice exposed for 10-days, which was 60% higher than that of the control. In mice exposed for 30 days, the bone F exceeded 1200 mg/kg, which was approximately twice as high as the control value. These and our previous results suggest that airborne F causes pulmonary edema in mice.
DOSE RESPONSE RELATIONSHIP BETWEEN INTRAVENOUS ADMINISTRATION OF SODIUM FLUORIDE AND ACUTE RENAL DAMAGE IN RATS
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Fluoride (F) excretion mainly depends on kidney function. Urinary F concentration is measured to monitor the health of workers exposed to F. Acute exposure to high doses of F damages renal tissue and causes renal dysfunction. In this study, the relationship between F doses and acute kidney damage in laboratory rats was investigated using urinary parameters.

Twenty-eight 11-week-old SPF Wistar male rats were divided into four groups. Group 1 (control) received 16 ml saline intravenously. The other three groups were given saline, and then saline solution containing NaF (F concentration 1,000 µg/ml) intravenously. Group 2 was administered 15 ml saline and then 1 ml NaF solution (1 mg as F). Group 3 was administered 14 ml saline and then 2 ml NaF solution (2 mg as F). Group 4 was administered 13 ml saline followed by 3 ml NaF (3 mg as F). Following the intravenous drip injection, urine samples were collected from the bladder every 2 hours a total of 3 times. The urinary parameters studied included urine volume, and the excretion of F, creatinine (Cr), α-glutathione S-transferase (α-GST), and N-acetyl-β-D-glucosaminidase (NAG).

Urine volume increased in Group 2 and decreased in Groups 3 and 4 compared with the control. No significant increases of F excretion were observed in Group 4 compared with Group 1. The excretion of Cr decreased, whereas that of α-GST and NAG increased in Group 4 compared with the control. F-induced polyuria was observed in Group 2. The decreased Cr excretion observed in Group 4 rats was caused by dysfunction of glomeruli, whereas the increases in α-GST and NAG levels indicate acute proximal tubular injuries. These results showed that an intravenous dose of 3 mg F to rats caused acute renal dysfunction. A dose-response relationship was recognized between intravenous administration of F and kidney damage in laboratory rats.

URINARY BIOMARKERS FOR MONITORING KIDNEY INTOXICATION IN RATS EXPOSED TO FLUORIDE
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The objective of this research was to study urinary biomarkers that may be used for monitoring acute kidney intoxication in rats exposed to fluoride (F). For this purpose, Wistar rats were given an excessive dose of sodium fluoride (NaF, 135 mg/kg body weight), and the time-dependent variations of urine volume and the excretion of F, creatinine (Cr), α-glutathione S-transferase (α-GST), and N-acetyl-β-D-glucosaminidase (NAG) were followed.

The results showed that F administration markedly affected these urinary biochemical indices. In particular, urinary excretion of α-GST, which is a useful marker of tubular damage, particularly in the S3 segment of the proximal tubule, increased about 100-fold one day after F administration, and this increase continued for a week. The change of α-GST in response to F exposure was the most sensitive and prolonged one among the observed indices.

In conclusion, the toxic effect of F on the kidney of rats is more serious in the proximal tubule than in the glomerular region, and the disorder of the proximal tubule is more prolonged in the S3 segment than in the S1 or S2 segment. α-GST appears to be a useful marker for early detection and long term observation of proximal renal tubular injury induced by F. This model should help to establish guidelines for treating F-induced acute renal failure in laboratory animals or industrial workers suffering from occupational accidents.
DISTRIBUTION OF FLUORIDE IN ALVEOLI AND MANDIBLES OF EUROPEAN DEER

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The physical strain applied to bone causes bone mineral reconstruction. This was proven in laboratory studies by the assay of fluoride (F) content in various human bones. The aim of this study was to determine F content in various parts of lower jaw of deer. The lower jaw of ruminants differs from that in humans. The process of chewing involves some forces which depend on the age of the individual, dentition, kind of occlusion, and the animal's general condition. In this study 62 jaws of deer, obtained from Polish hunters in the years 1996 and 1997 were studied. Bone samples were taken from the cleaned and degreased jaws. The bone powder was stored in plastic containers. An aliquot was dissolved in 2N HClO4 and F content was determined with a potentiometer.

The jawbones were divided into two groups. The first stemmed from animals of industrial areas, the second, from areas not contaminated by industrial emissions, was the control (19 jaws). Within each group a subsample of jaws, which came from young deer (two years old), an other – from older animals (8 years old), was taken. From each jaw, samples were taken from 7 places, these being: alveoli of molars, alveoli of the incisors, lobe projectile, beak projectile, mandible shank (around the molars), and the toothless part and chin (protuberance). The results show that, (i) in the younger animals of both groups (2 years old), the fluoride content in the various sampling sites of the bone did not very much, (ii) in the jaws which belonged to the older animals from industrial areas the largest differences of the fluoride contents were found in the molars alveoli (765 ppm), whereas jaws of the control animals contained 290, 235, 293 ppm respectively, (iii) in the bones of older deer from industrial areas the overall mean fluoride content was higher (664 ppm), than of deer from the control group (229 ppm). The most marked differences in F content were found in spots most associated with chewing. This must affect the physical, chemical and functional properties of the bone tissue.

BONE MINERAL DENSITY AND FLUOROSIS IN RESIDENTS OF TIANJIN, CHINA

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We examined the bone mineral density (BMD) data of the residents of Tianjin, China, for use as a diagnostic standard of fluorosis.

We used an XR-series X-ray Bone Densitometer (part No. 388D621) for BMD and BMC examination of lumbar vertebrae for 1419 persons. We examined the height, body weight, serum calcium, sugar, phosphorus, serum and urinary fluoride. X-rays of the forearms were taken to identify the calcification of interosseus membrane. The history and habits of drinking water consumption were also investigated.

There were 103 cases with higher BMD levels, and 54 of these are fluorosis patients. We have BMD and BMC data, as well as a diagnostic standard for these people. The BMD and BMC are valuable for the diagnosis of fluorosis.

There are two kinds of diagnosis of fluorosis, one is vertebral stenosis due to fluorosis and the other is bone alteration due to fluorosis.
EPIDEMIOLOGICAL ANALYSIS OF ENDEMIC FLUOROSIS IN HEBEI PROVINCE, CHINA

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Endemic fluorosis is prevalent in many areas of Hebei Province, China, and represents a considerable health hazard for the local population. An investigation was accordingly conducted from 1980 to 1982 to determine the fluoride concentration in the drinking water, soil, crops and atmosphere, as well as the fluoride level in human urine and the prevalence of mottled enamel and osteosclerosis.

Patients with endemic fluorosis numbered 5,672,539 persons living in 126 cities, 1,200 communes and 10,727 villages throughout the province. These patients represent 10% of the total resident population. The high incidence areas divide geographically into plateaus, mountains, basins, plains and coastal regions.

Most of the sources of drinking water showing elevated fluoride levels were shallow wells, although some deep wells, springs and rivers were similarly contaminated. Fluoride concentrations were generally higher in the northern part of the province and lower in the south.

No significant relation was observed between the prevalence of fluorosis and the fluoride levels in grain, vegetable crops or the atmosphere. Although atmospheric fluoride levels were higher in areas with a prevalence of fluorosis, its source was attributed to smoke from coal burned as fuel.

The prevalence of mottled teeth was found to increase sharply when the fluoride level in the drinking water rose above 1.0 mg/L and accounted for 100% of this prevalence when the fluoride level was greater than 4.0 mg/L. In patients from areas of relatively low concentrations, the tooth enamel showed opaque whiteness. Patients from areas of high concentrations exhibited pitted enamel. Osteosclerosis was also found to increase in severity with higher concentrations of F in the drinking water.

As a result of this investigation, we conclude that endemic fluorosis in Hebei Province is caused by high fluoride in drinking water. Accordingly, lowering the fluoride concentration in drinking water is the primary measure for preventing this condition.

EPIDEMIOLOGICAL SURVEILLANCE OF ENDEMIC FLUOROSIS IN JAINGSU PROVINCE

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This study investigated the endemic fluorosis epidemic from the 1980s to the beginning of the 1990s in Jaingsu Province, China. Endemic fluorosis in Jaingsu is mainly caused by drinking contaminated phreatic water. In the 1980s there were a total of 2026 villages with endemic fluorosis. Of these, 236 were severe, 992 were moderate and 798 were not severe. A total of 3,660,000 people were surveyed and 320,000 of these exhibited dental fluorosis yielding a 67.8% incidence rate. Groups of subjects were sampled according to fluoride concentrations in the water supply and checked by X-ray providing an estimate of at least 300,000 cases of skeletal fluorosis. Methods of control and prevention include using currently available low-fluoride sources of water, fully utilizing the resources of surface water, and rationally exploiting other resources of groundwater only when the above measures are not available.

Since 1983, effective measures have been taken in 9 counties with severe endemic fluorosis. Through 1995 over 1 million people have benefited from such improvements. This is about 25% of the people living in endemic fluorosis areas. From 1996 to 1998, the Jaingsu provincial government allocated nearly three thousand million RMB to lower fluoride concentrations in drinking water. This would benefit about 900,000 people per year. In 1998, all levels of the provincial government will make great efforts to complete the last part of this 3 year plan.
ASSOCIATION OF FLUORIDATION WITH CANCER PROMOTION
BASED ON ANALYSIS OF CANCER REGISTRY STATISTICS
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The incidence of registered cancers in communities in USA (WHO, 1987) and the Fluoridation Census 1985 by the U.S. Department of Health and Human Services have enabled us to conduct epidemiological analysis of the correlation between cancer incidence and water fluoridation in the United States. The State of Utah, supplying fluoridated water to only 2% of its residents, was selected as the control.

A cancer incidence ratio was calculated for each community and tested for significance by the T-test. In both sexes the incidence ratios of cancers in the digestive system (tongue, mouth, pharynx, esophagus, stomach, colon, rectum and pancreas), the respiratory system (larynx, bronchus, and lung), and the renal system were significant (p<0.05).

In the sexual organs contradictions in males and females were observed. In females, cancers of the breast, cervix uteri, corpus uteri, and ovary were increased in fluoridated areas whereas in males, those of the prostate, testis, and penis were inhibited. This indicates the possible feminizing hormonal action of fluoride.

The dose response relationship between the selected incidence of bone cancer in male teenagers and the percentage of fluoridation of the areas was statistically significant. The significant relationships were at the maximum 4.80 (95% lower confidence limit 2.60) indicating that fluoride may not be an initiator but a promoter of cancer. The different fluoride effects on male and female sexual organs suggest a possible mode of action of fluoride as an environmental hormone.

The low incidence of lip cancers and of skin melanomas in most U.S. states except Utah and New Mexico suggest these two types of cancer may be independent of fluoride. More exposure to sun due to high altitude may likely be the cause.

The variety of ethnic differences in cancer promotion remains a difficult problem in this type of analysis. Further analysis of data from other countries may be processed when data on the concentrations of fluoride in drinking water become available.

A STUDY OF FLUOROSIS CAUSED BY WATER-BORNE FLUORIDE IN TIANJIN, CHINA
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In Tianjin, China, endemic fluorosis is caused by consumption of high fluoride (F)-containing water from deep wells over an extended period of time. In this study, we investigated dental fluorosis and skeletal fluorosis in residents who had been exposed to water containing high levels of F for 30 years. The study area was divided into three zones: Moderate (1.1-2.0 mg F/L), Medium (2.1-4.0 mg F/L), and Serious (> 4.1 mg F/L).

Among the 17,242 subjects (8 to 15 years old) studied, 13,963 had dental fluorosis, a prevalence rate of about 90%. For those subjects at and above 16 years the prevalence rate was 34.9%. X-ray examinations on one out of every 20 subjects showed that 66% exhibited skeletal fluorosis.

Our data indicate that the prevalence of dental fluorosis is positively correlated with elevated fluoride concentrations in drinking water. In the study areas, the occurrence of skeletal fluorosis in Moderate, Medium, and Serious zones was 8.8%, 36.8%, and 41.1%, respectively, whereas that of dental fluorosis was 63.3%, 73.3%, and 91.1%, respectively. The effects of consuming the high fluoride containing water are correlated with duration of exposure: longer exposure led to more severe effects.

Methods of defluoridation, such as physical or chemical removal or the use of alternate water sources would greatly reduce the fluorosis problem in Tianjin.
EVALUATION OF FLUOROSIS PREVENTION BY IMPROVEMENT OF WATER QUALITY IN JILIN PROVINCE, CHINA

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Endemic fluorosis in Jilin Province, China is caused by consumption of high fluoride (F)-containing drinking water. In the early 1960s water-improvement projects were initiated to reduce F concentrations in drinking water, working from most severe areas to less severe areas. This study is an evaluation of the social and economic benefits of the water improving project over the last 30 years.

Of the most severe endemic villages 60.6% had water improvement implemented, however, the F content of the water-improving project as a whole was still much higher than the national standard.

Over the last 30 years water improvement has brought substantial health, economic, and social benefits. Three investigations (1980, 1985 and 1991) revealed that the occurrence of bone and dental fluorosis has dropped markedly since the water improvement implementation. The total grain product of the endemic area has increased by 39.6% per person and annual income has increased by 43.5% per person. After water improvement 46.3% of all fluorosis patients investigated recovered their ability to care for themselves. Domestic animals have also been benefited.

While the benefits are great, the water improving task is heavy, the resources are few and the investment insufficient. There are still 2110 endemic villages without water improvement and 177 of these are severe. Fund availability is still the largest obstacle.

INTELLIGENCE AND FLUORIDE EXPOSURE IN NEW ZEALAND CHILDREN

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A birth cohort of 1265 children born in Christchurch in 1977 was studied longitudinally including assessment measures of cognitive ability at ages 8 and 9 years using the Wechsler Intelligence Scale for Children (WISC-R). It was possible to study the association between intelligence and fluoride exposure as approximately half of the sample resided in Waimairi County, fluoridated with 1 ppm of fluoride, while the other half resided in non-fluoridated Christchurch with <0.1 ppm. The children were grouped according to the duration of fluoridation exposure to age 7 years with the groups being exposed for 0, 1-3, 4-6 and 7 years. The mean IQs (WISC-R) at 8 years for these groups were 100.0, 99.04, 99.40 and 100.5 (p>0.30). The values at 9 years were 99.9, 99.3, 98.7 and 101.2 (p>0.70). It was evident that there was no trend for IQ to decline with increasing exposure to fluoridated water. In fact those who lived for 7 years in an area with fluoridated water had the highest mean IQ scores, although this difference was not statistically significant. The results can be seen as being consistent with other studies. In the study by Zhao et al on Chinese children intelligence was impaired with drinking water with 4.12 ppm of fluoride but not with 0.91 ppm. In the rat study by Mullenix et al neurotoxicity was present with plasma fluoride levels of 0.059-0.640 ppm which were seen to be similar to those found in humans of 0.076-0.25 ppm drinking water with 5-10 ppm of fluoride. Thus a threshold effect for fluoride toxicity may be present with demonstrable effects being present with water containing 4-10 ppm but not 1 ppm of fluoride. However, recent work by Varner, Isaacson et al suggests that fluoride may have the potential to cause neurotoxicity at a level of 1 ppm if present with trivalent aluminium ions at a concentration of 0.5 ppm.
DENTAL COSTS NOT AFFECTED BY FLUORIDATION – LARGE-SCALE TOOTH DECAY STUDIES SHOW LACK OF ‘BENEFICIAL’ EFFECTS

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Dental care costs were determined for California residents in 1994 and 1995 among counties with various degrees of fluoridation as tabulated by the California State Department of Health. Of 58 counties reporting, cost data were not available for 3.

Since the average dental costs in counties with large populations are more reliable and less variable, and since reliability varies linearly as the square root of the population considered, dental costs were weighted by the square root of the number of eligible recipients of the county.

The average annual Medi-Cal costs for dental work per eligible recipient was $108.48 in 1994 and $110.06 in 1995 in 33 counties with no fluoridation, $113.97 in 1994 and $107.26 in 1995 for 9 counties (one not reporting) with 0.5-10% fluoridation, $125.50 in 1994 and $123.70 in 1995 for 9 counties with 11-40% fluoridation, and $120.01 in 1994 and $125.27 in 1995 for 3 counties with 91-100% fluoridation. Of the three counties with 41-90% fluoridation, only one reported with the necessary data: $96.43 in 1994 and $97.32 in 1995.

Unweighted data showed relatively lower dental costs in nonfluoridated areas. This is due to the generally lower dental costs in counties of low population, which are predominately nonfluoridated. Thus, the 10 large comparably sized counties with 0-10% fluoridation had dental costs of $123.46 in 1994 and $124.45 in 1995 compared to dental costs of $120.01 in 1994 and $125.27 in 1995 for three counties with 90-100% fluoridation.

These data were evaluated in comparison with other large-scale studies. The study done by Yiamouyiannis using 1986/87 data from the U.S. Public Health Service showed a tooth decay rate of 2.06 DMFT and this study showed a 1994/95 dental cost figure of $141.40 for Los Angeles (5.3% fluoridated) compared to a 1986/87 tooth decay rate of 1.91 DMFT and a 1994/95 dental cost figure of $144.41 for San Francisco (100% fluoridated).

CHRONIC FLUORIDE POISONING IN RURAL AREAS OF JIAINGXI PROVINCE, CHINA. 2. PREVALENCE OF DENTAL FLUOROSIS

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In this study, the relationship between fluoride (F) concentrations in drinking water and prevalence of dental fluorosis was investigated in the chronic fluoride endemic areas of Jiangxi Province, China.

Two hundred and two school children, age 8 to 13 (mean age: 10.6), born and raised in these areas, were selected for dental examinations. Prevalence of dental fluorosis was examined according to the Dean's classification. Community fluorosis index (CFI) for dental fluorosis was calculated. The F concentrations in drinking water were determined using an apparatus of flow-injection analysis with a fluoride ion-selective electrode as a detector.

The caries prevalence of the subjects was found to be 51.0% and extremely low compared with that of Japanese school children. The prevalence of dental fluorosis was found to be 73%. The CFI of the area was 1.87. The mean and standard deviation of F concentrations in drinking water were 0.85±0.84 mg/L, ranging from 0.08 to 4.20 mg/L.

A correlation was confirmed between the F levels in drinking water and the severity of dental fluorosis. This suggests that F in drinking water could contribute to dental fluorosis in these areas.
HEALTH EFFECTS OF FLUORIDE POLLUTION CAUSED BY COAL BURNING IN CHINA. 2. EXPOSURE TO FLUORIDE OBSERVED IN TERMS OF DENTAL FLUOROSIS

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Absorption of fluoride (F) into the human body is known to be an inhibitor of tooth formation. Dental examinations on patients were performed deep in the mountains of the southwestern Chinese province of Guizhou to examine the degrees to which schoolchildren aged 8-15 yr and their parents had been exposed to F during the phase of tooth formation. The findings show that about 30% of the children suffered from severe dental fluorosis with a defective tooth structure. In sharp contrast, children with a normal tooth structure accounted for about 10%.

Almost every child did not have dental caries. It is evident, therefore, that the degree of exposure differs markedly, depending on the individual response even in one and the same area. A comparison between children and their parents reveals that the prevalence of dental fluorosis was 50% greater in the children than in the parents. From this finding, it is clear that the degree of the children’s exposure to environmental F during tooth eruption was greater than that of the parents.

HEALTH SURVEY OF WORKERS OF AN ALUMINUM PLANT IN JIANGXI PROVINCE, CHINA. 2. BONE MINERAL DENSITY

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The effect of airborne fluoride (F) on bone mineral density of workers of an aluminum plant in Jiangxi Province, China, was investigated. The subjects were 154 male workers aged 21 to 66 (mean 35.7 years).

Bone mineral density values of the left radial distal part were measured using a DXA apparatus (DTX-200, Osteometer Company).

The time workers spent doing electrolysis work was estimated. By summing these time periods, a F exposure period for each individual was made. The relationship between exposure period to airborne F and the bone density value of workers was examined. For determination of airborne F levels, 24 air samples were taken from different sites of the potroom. The average total F level in the air of the workplace was 0.55 mg/m3.

A significant negative correlation was observed ($\gamma = -0.196$) between disclosure period and radial bone density as well as between age and bone density ($\gamma = -0.202$).

The relationship between disclosure period and radial bone density is considered to depend on age as older workers experience longer refinery exposure. It is concluded that at the aluminum plant airborne F does not exert an influence on bone density of the workers.
HOW FAST DOES FLUORIDE ACCUMULATE IN HUMAN BONES?
A REVIEW AND NEW EVIDENCE FROM THE UK

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No official monitoring of fluoride (F) intake has been undertaken in the UK, despite repeated authoritative recommendations to do so. Templegarth Trust therefore in 1990 funded modern laboratory equipment for use by Good HealthKeeping in the largely unfluoridated East Midlands. Urinary samples from all clients were assayed for F concentration. A proportion were asked to provide 24-hour urine samples for repeat examination.

The results from 648 individuals whose water F status was known indicated sizeable minorities receiving more than 3 mg F daily – 14% in non-fluoridated and 17% in fluoridated areas. These findings prompted us to solicit urine samples from the largest and longest-fluoridated region of the UK (the West Midlands). Of the 151 samples analyzed to date, 60% indicated consumption of 3 mg F daily or more, in a bimodal distribution.

A literature review included the principal references cited by the British Fluoridation Society Ltd in support of the safety of fluoridation. Discrepancies came to light between the findings of Roholm and their interpretation by Hodge up to 1979 and thereafter, which have resulted in errors 2-3 times greater in estimates of the level of F tolerable by humans.

The most important issue, however, concerns the criterion of safety. Industrial fluorides, far more toxic than calcium fluoride salts found naturally, have not yet been regularly consumed for longer than 50 years, anywhere in the world. Since fluorides accumulate lifelong, we have yet to learn the effects of consuming artificial fluorides continuously for an entire lifetime. The danger depends on the rate of accumulation and therefore of consumption. Calculations will be presented that suggest 3 mg F/day is a figure that should never be exceeded, since it virtually guarantees (on present knowledge) a high prevalence of skeletal pathology by age 70.

If artificial fluoridation results in skeletal F accumulation sufficient to damage skeletal health within a normal lifetime, as these considerations suggest, then it should be stopped.

THE IMPACT OF WATER-BORNE FLUORIDE ON BONE DENSITY

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The aim of this study was: (1) to determine the fluoride (F) levels in urine and hair of persons with known water F intake and documented bone mineral density, and (2) to assess whether a correlation exists between bone density and the levels of F in drinking water, urine, and hair.

For this purpose, 282 patients, mostly females, age 24-77 (mean 50), from the Gdansk region were selected. The mineral bone density of left femur bone and vertebra L₂ – L₄ was measured with a Lunar Expert densitometer. Urine and hair samples from the occipital part of the scalp as well as samples of drinking water were collected from these subjects. Fluoride contents in drinking water and urine samples were determined directly after dilution with equal volumes of TISAB. The hair samples were washed and digested with NaOH solution. Fluoride concentrations were measured potentiometrically using a F-specific electrode.

The mean F concentration in water, urine, and hair samples was 1.94 mg/L (range: 0.325-3.07), 2.43 mg/L (range: 0.77-5.35), and 11.41 µg/g (range: 4.13-14.51), respectively. The bone mineral density of vertebra and femur bone was 1.16 g/m³ (range: 0.97-1.39) and 1.01 g/m³ (range: 0.86-1.14), respectively. Positive correlations were found between water F content and bone density (r = 0.366), urinary F content and bone density (r = 0.300), water and urinary F content (r = 0.739), and water and hair F content (r = 0.510), respectively. A negative correlation was found between age and bone density (r = -0.293).

The results of this study indicate that F contents in drinking water as well as in urine and hair have an impact on bone density.
COMPLEX DAMAGE OF JOINTS IN EXPERIMENTAL OSTEO- AND ARTHROFLUOROSIS OF RATS (SPONDYLARTHROSIS AND GONARTHROSIS FLUOROTICA)

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The objective of this study was to examine the changes in the bone, articular cartilage and joint capsules, and intervertebral discs in rats administered with sodium fluoride. The joint was evaluated as a functional unit of articular cartilage, capsule, and subchondral bone tissue and the changes were interpreted from this complex functional point of view. Methods, results, conclusions and interpretation based on previously published works (1-9) are summarized and documented in detail.

Diarthrodial joints have to be considered as functional units of synovial membrane, articular cartilage and subchondral bone tissue. Any changes, or pathological processes in one of these structures may affect consecutive alterations in other tissue components of the involved joints. For example, fragments of articular cartilage cause reactive, secondary synovitis. During the inflammatory process cytokines and enzymes (matrix metalloproteinases - MMPs) are released which further increase the enzymatic destruction of cartilage. The action of tissue inhibitors of MMPs (TIMPs), and the balance between MMPs and TIMPs should also be considered. The damaged articular surface becomes overloaded (unchanged load acting on a smaller intact, congruent surface); the subchondral bone trabeculae may collapse, with rupture of perforating arteries, accompanied by secondary osteonecrosis in small areas. The damaged blood supply in the articular cartilage may cause focal, scattered necrosis of chondrocytes, etc. These vicious circles lead to progressive destruction of the affected joint with the driving circle depending on the primary joint disease. These interrelated processes are schematically summarized in Figure 1.

The morphological changes and destruction of the joint, the speed of progression, the clinical signs and limitation in movement, the complaints and tolerance of pain are also variable and show great individual differences.

**FIGURE 1**

- Synovitis
- MMPs & TIMPs
- Incongruence
- Relative Overload
- Cartilage Destruction
- Debris & Fragments
- Enzymatic Autolysis
- Reduced Synthesis & Repair
- Chondrocyte Necrosis
- Relative Overload
- Microfractures of the subchondral bone tissue
- Damage of blood supply
- Osteonecrosis
HEALTH EFFECTS OF FLUORIDE POLLUTION CAUSED BY COAL BURNING IN CHINA. 3. SKELETAL FLUOROSIS

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The effects of airborne fluoride (F) pollution on the bone health of residents from three coal burning areas in China were studied. For this purpose, the residents from three rural areas with varying degrees of fluoride pollution were selected. The study areas included Xaochang Village in Sichuan Province, known as one of the most polluted areas in China; Minzhu Village in Guizhou Province, a moderately polluted area; and Wanli Village in Jiangxi Province (control), which has the least air pollution problems among the three areas studied. Most of the subjects were in their 40s and 50s and had lived in the same areas since childhood.

All the subjects received x-ray examinations of the skeletal system, including the forearms and lower legs. In addition, the subjects from Xaochang and Minzhu villages received x-ray examinations of the lumbar spine and pelvic bone.

Forty-five out of 49 subjects from Xaochang Village, and 25 out of 49 subjects from Minzhu Village were classified into Stage 3, according to Singh and Jolly’s classification. None of the 47 subjects in the control area were classified into Stage 2 or 3. Among the subjects from Xaochang Village, prevalence of dental and skeletal fluorosis was well correlated: 37 out of 41 subjects exhibiting Grades 3 and 4 dental fluorosis showed Stage 3 skeletal fluorosis. In contrast, the results from Minzhu Village residents showed only a weak correlation (5/17). These observations suggest that most of the residents in Xaochang Village may have been exposed to airborne fluoride pollution since childhood, whereas the residents in Minzhu Village may not have been.

PREVENTION OF IODINE DEFICIENCY IN HIGH FLUORIDE AREAS IN TIANJIN CITY, CHINA

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In this paper we studied the efficiency of supplying iodized salt as measured by the incidence of goiter in high-fluoride areas of Tianjin, China. Data before (1994) and after (1997) the provision of iodized salt was compared. The study began in 1994 by dividing the city into 5 areas (north, south, east, west and central). A total of 5281 school children, 8 to 10 years of age, from 50 primary schools were examined for goiter and urinary iodine (I2) content. Levels of I2 in the supplied salt was also determined. The study was repeated in 1997, two years after iodized salt had been supplemented. Occurrence of goiter dropped from 28.8% in 1994 to 19.1% in 1997. The levels of I2 in urine doubled from 128 mg/L in 1994 to 268 mg/L in 1997. Of the salt samples taken from the pupil’s homes, 90.8% met the national standard for iodine content with the average content being 50.4±24.7 mg/L. The results indicate that the levels of I2 in salt and urine are up to national standards for normal metabolism and that these increased levels are causing a decrease in the occurrence of goiter. However, the goiter incidence rate is still higher than the 5% standard, but this could be time related as goiter responds slowly to treatment. Our primary conclusion is that I2 absorption by the thyroid is competitively inhibited by F, especially in high-fluoride areas, and is alleviated by the provision of iodized salt.
INFLUENCE OF TIME AND SOIL ENVIRONMENT ON FLUORINE ACCUMULATION IN BONES FROM ARCHEOLOGICAL EXCAVATIONS IN POLAND

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One of the basic factors influencing fluorine (F) accumulation in human bones is the length of time the bones rest in the ground. The second important factor may be the type of soil, particularly the content of F compounds that can penetrate the bone tissue. The purpose of this paper was to determine the influence of soil type on F accumulation in human skulls stemming from archeological excavations in Poland, over various time durations. The studies were performed on 188 human skulls obtained from collections owned by Polish Academy of Sciences and the Department of Anthropology at the University of Wroclaw (Poland).

Soil samples were taken from localities where the skulls had been found for determination of F content. In this way two groups of soil were isolated and classified. In the first soil group the F content was about 150 mg/kg of dry soil, while in the second soil group the F content was on the average 420 mg/kg. In each of these soil groups the F content was analyzed in the excavated human skulls according to the time they were buried in the soil. Thus, the possibility was created to determine two parameters of F accumulation simultaneously, the length of time deposited in the soil as well as the F content of the soil.

In group I where the F content in soil was 150 mg F/kg, there were 4 localities in abbreviation marked as Lu200, Sy700, Sa1000, ZI4500 (the numeric index denotes the time of skulls having remained in the soil). In group II with soil F content of 420 mg F/kg there were 3 localities being marked as W500, C700, G800. In two (Sy700, C700) of the 7 analyzed localities, the deposition time of skulls in the ground was equal (700 years), but the soils differed with regard to F content, being 150 mg/kg and 420 mg/kg respectively. In this situation a larger amount of F in the skulls was found where the amount of F in the soil was higher. The time parameter behaves in another way. The skulls buried a longer time in soil with lower content of F (150 mg/kg) displayed more F than the skulls resting shorter in the soil with greater amount of F (420 mg F/kg). In conclusion it may be ascertained that the main parameter influencing F accumulation in bones stemming from archeological excavations is the duration of deposition in the soil. The second parameter regarding the significance in F accumulation in bones is F content in the soil.

REVIEW OF OSTEOPOROSIS AND THE ROLES OF FLUORIDE, SEX HORMONES AND BISPHOSPHONATES

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Osteoporosis is a common and important cause of morbidity and mortality among postmenopausal women, particularly in industrialized countries. It occurs as a consequence of progressive loss of bone and results in increased risk of fracture. Present conventional therapies are primarily directed at inhibition of bone resorption. They cannot, however, reverse or repair bone loss. At the present time, research continues concerning the mechanisms by which bone mass and trabecular microarchitecture can be restored. In this context, the roles of F, sex hormones and bisphosphonates will be reviewed and evaluated. In addition, the various diagnostic techniques of early bone loss will be reviewed, as well as the various factors that impact on this multifactorial metabolic bone disorder.
FLUORIDE CONCENTRATION IN BONE INFLUENCES
PERIPROSTHETIC BONE MINERAL LOSS AFTER
UNCEMENTED TOTAL HIP ARTHROPLASTY

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Factors influencing bone remodeling after cementless total hip arthroplasty (THA) with Parhofer prosthesis (Aesculap, Tuttlingen, Germany) were evaluated in a longitudinal study of 18 hips in 18 patients (14 females and 4 males, aged 42-76 years) who had undergone uncemented THA due to osteoarthritis.

Bone mineral density (BMD) in the femoral neck was determined by dual-energy X-ray absorptiometry (DXA) with a Lunar DPX-L densitometer (Lunar Co, Wisconsin, USA) preoperatively. DXA measurements in seven regions of interests (Gruen zones) were performed prospectively 2 weeks, 3, 6, 12 and 24 months after operative treatment.

The concentrations of calcium, magnesium and fluoride were measured in cortical and trabecular bone samples taken intraoperatively from the resected femoral head and neck. The fluoride concentration was measured with “Orion” fluoride ion-selective electrode after dissolving the prepared bone pieces in perchloric acid; calcium and magnesium concentrations were measured by mass absorption spectrometry.

Neither the bone mineral density nor bone fluoride, calcium and magnesium concentrations correlated with patient’s age.

At 12 months after the operation, the regional BMD in all seven zones showed a maximal significant decrease ranging from 7.3 to 38.8% of BMD present at 2 weeks postoperatively. Thereafter the bone density appeared to be stabilized. The most significant postoperative bone loss (12.1-38.8%) was found in the calcar area. The cortical zone below the prosthesis showed lower but still significant decreases (7.3-18.1%).

The analysis of preoperative femoral neck BMD and fluoride content in trabecular bone proved that osteopenia and lower fluoride concentrations correlated significantly with more bone density reduction after THA. No other factors (age, weight, sex, calcium and magnesium concentrations in bone and fluoride concentration in cortical bone) showed significant associations.
RELATIONSHIP OF BONE DENSITY OF THIRD METACARPAL AND PROXIMAL PHALANX TO MENOPAUSE IN POPULATIONS WITH AND WITHOUT ENDEMIC FLUOROSIS

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The present study was conducted to examine the differences in bone mass between urban and suburban women before and after menopause by measuring fluoride (F) levels in urine, bone morphometry, and bone mineral content of the third metacarpal and proximal phalanx. The subjects under study were 186 women age 40 to 59 in the urban and suburban communities near Beijing, China. The urban cohort consisted of 56 premenopausal women (mean age 44.5±4.3) and 35 postmenopausal women (mean age 53.3±3.2), while the suburban cohort consisted of 49 premenopausal women (mean age 45.1±3.4) and 46 postmenopausal women (mean age 52.9±3.6). The suburban residents had been exposed to excessive levels of F in the drinking water for 10-15 years. Quantitative analyses of the midshaft of the third metacarpal and proximal phalanx were made with a DIP method.

The urinary F levels were 0.92-1.16 mg/L higher in the suburban subjects than in the urban subjects, irrespective of menstrual history. There was no evidence of F involvement in the third metacarpal of the suburban subjects. Morphometry revealed marked changes in bone morphometry between pre- and postmenopausal women in the urban cohort. In either community, the values for bone mineral content and bone density were significantly larger in the premenopausal women than in the postmenopausal women, and there was little difference between the two communities. The effects of F on the third proximal phalanx were clearly reflected in the combined cortical bone thickness and metacarpal index, but not in the bone mineral contents.

Fluoride accumulated in the skeleton is released into urine over a long period of time. Our findings suggest that F at 1-2 mg/L affected the bone mineral content and bone density in the hand differently.
INCIDENCE OF CARDIOVASCULAR ABNORMALITIES IN ENDEMIC SKELETAL FLUOROSIS

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Approximately 20 million people in India are afflicted with endemic skeletal fluorosis (ESF). ESF is often associated with secondary hyperparathyroidism. Primary and secondary hyperparathyroidism have been implicated for increased cardiovascular mortality in western population. This prospective study was designed to detect abnormalities in cardiac muscle, valves, and myocardial function. Complete, two dimensional transthoracic echocardiography (by 2 independent observers in a blinded manner) was used to evaluate myocardial mechanical performance, thickness of left ventricular wall, and myocardial calcification in 37 consecutive patients, age 14-56 (16 males, 21 females), with moderate to severe ESF. Twenty age-matched controls from non-endemic area were similarly studied simultaneously. Baseline screening included complete history, physical examination, skeletal radiology, QCT spine, sonography of the abdomen, 12 leads electrocardiogram and echocardiography. At least three consecutive blood pressure readings were taken. Baseline biochemical studies included serum total and ionized calcium, fluoride, phosphate, creatinine, alkaline phosphatase, iPTH, urine 24 h-calcium, phosphate, creatinine, CAMP and drinking water fluoride. None had associated diseases, such as diabetes, affecting cardiovascular functions.

ESF patients had higher systolic blood pressure and increased left atrial diameter than controls (P<0.05). Mitral valve calcification (38%) and aortic valve calcification (14%) were detected in ESF group while none of the controls had these abnormalities. Myocardial calcific deposits were not recorded in any subjects. No significant differences in left ventricular ejection fraction, end diastolic left ventricular posterior wall, interventricular septal thickness, or QTc alterations were noted. Significant reduction in systolic blood pressure was recorded with daily intake of calcium (1 g calcium carbonate) and vitamin-D (0.25 mg 1oD₃, Panacea) supplementation in ESF (p<0.01) after 4-6 months follow up. Our study suggests that patients with ESF should be regularly assessed for cardiovascular abnormalities.

FAST RESPONSE TO FLUORIDE IN HUMANS

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Since 1969 we have been studying the effect of fluoride (F) on osteoporotic patients treated with sodium fluoride (NaF) and on patients suffering from industrial fluorosis. We have reported our results in several ISFR conferences.

In two prospective studies in 1990 and 1992, we treated 82 and 102 osteoporotic patients with 60-80 mg NaF/d and 50-70 mg NaF/d, respectively. In both studies we found a positive effect of the treatment (subjectively, clinically, radiologically, fracture rate) in 85% and 82% of the two studies, respectively. The effective treatment time was 26.5 months on the average, but we also found fast responses in 20% of the first study and 15% of the second study after the treatment of 13 months. We confirmed this fast response by X-ray findings (reossification), a relatively subjective method.

Since 1990 we have used DEXA (Dual Energy X-ray Absorptiometry) for monitoring the fluoride therapy of osteoporosis. Generally, the aim of the F therapy is to increase bone mineral density (BMD) 8-10% per year. In the last 4 years in 7 patients we observed a strong increase of BMD of 24.4 to 43 per cent after 12 to 17 months of treatment.

This fast response was combined with some adverse effects (femoral neck fracture, stress fractures, lower leg pain syndrome) and high fasting F levels in the serum. This fast response adverse effects could be avoided by careful monitoring of the F therapy by DEXA measuring every 12 months and the control of the fasting serum F level every 4 months.

Also in cases of industrial fluorosis we observed a so-called fast responder, i.e., a stage I fluorosis developed after 9 years exposure to F in comparison with the average exposure time of 15 years for stage I. In this case as fast as the bone mineral density (osteosclerosis) increased (9 years) it decreased (6½ years) rapidly after cessation of the F exposure. This fast response is the result of an individual's reaction to F.
NEUROLOGICAL EFFECTS:

FLUORIDE AND THE BRAIN: HIDDEN "HALO" EFFECTS

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Our laboratory studies linking fluoride (F) with alterations in behavior and other studies spotlight the impact of F and/or fluorite on central nervous system (CNS) function. The F-CNS link is a concern for certain clinical therapies and environmental exposures (i.e., those involving anticholinesterases). Risks emerge whenever 1) multiple sources are allowed to contribute to the body burden of F; 2) fluorination is used to "enhance" biological activity, and 3) side effects are induced such that a predisposition to F toxicity is common.

Long-term administration of adrenocortical steroids, such as dexamethasone in the treatment of childhood leukemia, is a therapy where the F-CNS link may apply. The treated population is susceptible to dental problems and frequently given F. Use of dexamethasone instead of prednisolone is increasing because of its greater cytotoxic effects and penetration into the brain. Both steroids interfere with calcium (Ca) uptake in the gut and increase Ca excretion by the kidney. Decreased total body Ca stores are known to alter susceptibility to the effects of F. Furthermore, the chemical structure of dexamethasone differs from that of prednisolone by a fluorine in the 9a position of the ring B and a methyl group at C16 on ring D. In humans, the behavioral effects of such fluorination are unknown, as is whether or not F ions are released when dexamethasone is metabolized. In contrast, rats have multiple pathways for microsomal NADPH-dependent biodehalogenation, any of which can free F ions.

With respect to behavior, we found that the fluorinated and nonfluorinated steroids were not equal. Behavioral outcome was measured in 6-week-old male rats given either prednisolone (18 mg/kg, s.c.) or dexamethasone (1 mg/kg, s.c.) on postnatal days 17 and 18. Dexamethasone resulted in 23 significant changes in motor act initiations, total time, and time structures, whereas prednisolone changed only 7. Thus, attempts to minimize behavioral problems in this therapy may require further investigation into the role of F.

ALTERATIONS IN NEURONAL AND CEREBROVASCULAR INTEGRITY IN RATS CHRONICALLY ADMINISTERED ALUMINUM-FLUORIDE OR SODIUM-FLUORIDE

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Long-Evans rats were administered one of three treatments for 52 weeks: the control group was administered distilled deionized drinking water (ddw); the AlF3 group received ddw with 0.5 ppm AlF3; and the NaF group received ddw with 2.1 ppm NaF containing the equivalent amount of fluoride as in the AlF3 water. Tissue aluminum levels of brain, liver and kidney were assessed with the direct current plasma technique and its relative distribution in brain and kidney was assessed with histochemistry. In addition to routine histological assessments of brain, kidney, liver, and spleen, sections of brain were immunostained for β-amyloid, amyloid A, and IgM to assess the integrity of the cerebrovasculature. The aluminum levels in brain and kidney were higher in both the AlF3 and NaF groups relative to controls. The extent of alterations in neuronal density and the cerebrovasculature were greater in animals in the AlF3 group than in the NaF group, and greater in the NaF group than in controls.
NEUROTOXICITY OF FLUORIDE IN RATS - NEUROPATHOLOGICAL STUDIES

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The purpose of this study was to investigate the neuropathological changes in rats administered sodium fluoride (NaF) in drinking water. Sixty male rats of Wistar strain were divided into 4 groups. Animals in Group 1 (control) were given ordinary drinking water, whereas those in Groups 2, 3, and 4 were given 60 ppm NaF in drinking water for 21, 42, and 63 days, respectively. At the end of the experiment, the animals were sacrificed under anesthetic ether, and the brains were separated for F determination and neuropathological studies. Tissue F was determined chromatographically, and the results were given as μM F/g protein. Tissue protein content was determined by Lowry's method.

The levels of F in Group 2 rats were significantly higher than in other groups (p<0.05), but there were no significant differences between Groups 3 and 4 and between these two groups and the control. A neuropathological study and computerized morphometric analyses revealed a marked shrinkage of cerebellar granular and Purkinje cells, perivascular myelin swelling, and astroglia reaction, especially in the white matter of brains in the NaF-treated animals. Neuronal and myelin changes appeared to be more pronounced in Group 2 animals than in others (p<0.01), but astroglia reaction was similar to each other in all groups.

This investigation shows that F neurotoxicity in rats may be greater in the early stage of intoxication.

EFFECTS OF FLUORIDE ON THE PHYSIOLOGY OF THE PINEAL GLAND IN THE MONGOLIAN GERBIL MERIONES UNGUICULATUS

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The human pineal gland avidly attracts fluoride (F) from the bloodstream because the gland calcifies physiologically (even in childhood) as hydroxyapatite. For example, hydroxyapatite in the aged human pineal gland contains 8900±7700 mg F/kg. The child's pineal gland is exposed to F at an earlier age and at higher levels following the prophylactic use of fluorides in pediatric dentistry. Does this affect metabolism? Melatonin, the main pineal hormone, has several putative functions including suppression of the reproductive axis. Infants and prepubescent children have the highest nocturnal plasma melatonin concentrations. The aims of this study were to determine whether F alters (i) pineal secretion of melatonin during puberty, and (ii) the age of sexual maturation in experimental animals.

To accomplish the aims, the gerbil Meriones unguiculatus was used as the animal model.

Animals were divided into two groups, low-fluoride (LF) and high-fluoride (HF), (12 females, 12 males/group), received food containing 7 and 37 mg F/kg, respectively, and distilled water ad libitum, after weaning at 24 days. In addition, the HF pups received 2.3 μg F/kg BW/day orally at 1-23 days. At 7, 9, 11.5, and 16 weeks (at prepubescence, through reproductive maturity, 9-12 weeks, to young adulthood), the urinary excretion of the major Melatonin metabolite, 6-sulphatoxymelatonin (αMT6s), was measured at 3-hourly intervals over 48 hours using radioimmunoassay. Body weight, age at vaginal opening and area of the ventral gland were used as indices of pubertal development. Lighting was 12L:12D. The HF group excreted significantly less urinary αMT6s than the LF group (p<0.01): males at 7, 9, and 11.5 weeks, females at 7 and 9 weeks. Compared to the LF females, HF females were heavier at 7 weeks (p<0.004), ventral glands differentiated earlier (p<0.004), age of vaginal opening occurred earlier (p<0.01). In conclusion, fluoride inhibits pineal gland melatonin synthesis in the immature gerbil. This is associated with an accelerated onset of pubertal development on the female gerbil. If these results can be extrapolated to humans, high plasma-fluoride levels during early childhood may be a contributory factor in the current decline in the age of puberty.
WATER TREATMENT WITH SILICOFLUORIDES AND ENHANCED LEAD UPTAKE

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Toxic metals like lead, manganese, copper and cadmium damage neurons and deregulate neurotransmitters like serotonin and dopamine, which are essential to normal impulse control and learning. Exposure and uptake of lead has been associated with industrial pollution, leaded paint, plumbing systems in old housing, lead residues in soil, diet, and demographic factors. Here, we report on an additional “risk co-factor” making lead and other toxic metals in the environment more dangerous to local residents: the use of silicofluorides as agents in water treatment.

The two chemicals in question - fluosilicic acid and sodium silicofluoride - are toxins that, despite claims to the contrary, under normal conditions of usage change water chemistry and do not dissociate completely. As a result, water treatment with silicofluorides apparently functions to increase the cellular uptake of lead. Data from lead screening of over 280,000 children in Massachusetts indicates that silicofluoride usage is associated with significant increases in average lead in children's blood as well as percentage of children with blood lead in excess of 10 µg/dL. Consistent with the hypothesized effect of enhanced lead uptake, in communities with more old housing or lead in excess of 15 ppb in first draw water samples, and where silicofluorides are also in use, children are especially at risk of higher blood lead levels. Preliminary findings from county-level data in Georgia confirm that silicofluoride usage is associated with higher levels of lead in children's blood. In both Massachusetts and Georgia, moreover, behaviors associated with lead neurotoxicity are more frequent in communities using silicofluorides than in comparable localities that do not use these chemicals. Because there is no record of animal or human testing of silicofluoride treated water, further study of the effect of silicofluorides is needed to clarify the extent to which these chemicals are risk co-factors for lead uptake and the hazardous effects it produces.

BIOCHEMICAL EFFECTS:

SERUM BILIRUBIN AND FLUORIDE WITH CHANGES IN FLUORIDE INTAKE OVER A DECADE

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Gilbert’s syndrome is characterized by an increase in serum bilirubin concentrations due to fasting or certain other stresses. It has been reported that bilirubin concentrations in this syndrome increased with an increase in fluoride (F) intake. This study was a 12-year attempt to reproduce these findings in an individual with Gilbert’s syndrome. In addition to a 12-hr fast, the study was refined by taking samples after a 36-hr fast (300 Calorie diet). Further, larger shifts in F intake, longer periods of exposure, and F analyses of serum, food, and urine were added.

The 36-hr fast doubled the 12-hr fasting serum bilirubin concentration. The mean values (SD, n = 7-9) for low-F periods were 1.87 ± 0.59 and 4.17 ± 0.63 mg/dL. For the high-F periods the values were 1.86 ± 0.48 and 3.66 ± 0.65 mg/dL. The serum F levels after 12-hr and 36-hr fasting periods (0.1-0.2 mg F intake) were 0.60 ± 0.19 µM and 0.57 ± 0.15 µM, respectively. Supplementing F intake by 1-2 mg per day without fasting gave serum levels of 2.01 ± 0.75 µM (n = 4). Collection of timed urine samples before and after shifting to non-F toothpaste showed a drop of 0.3 mg of fluoride output per day. With one exception the serum F concentrations roughly corresponded with the long-term intake of F.

These results failed to confirm an association between F intake and serum bilirubin concentrations in Gilbert’s syndrome. Repetition of the earlier findings with 36-hr fasts are needed before giving them much credence. The stability of serum F concentrations after 12- and 36-hr periods of fasting and only rough correspondence with long-term F intake raise several questions of interest for future studies.
ROLE OF FREE RADICALS IN FLUORIDE-INDUCED TOXICITY IN LIVER AND KIDNEY OF MICE AND ITS REVERSAL

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The present study was designed to explore the effects of sodium fluoride (NaF) (10 mg F/kg body weight) for 30 days on the metabolism and structure of the liver and kidney of mice. The reversibility of the induced effects by withdrawal of NaF and by administering vitamins E and D was also studied.

The results revealed structural alterations in the liver revealing fatty deposition and necrosis, while in the kidneys marked necrosis and atrophy of the glomeruli occurred. Fluoride treatment decreased the activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase and the concentrations of reduced glutathione, total ascorbic acid, and reduced ascorbic acid. On the other hand, the levels of dehydroascorbic acid and lipid peroxidation were elevated significantly in F-fed mice. The levels of F were found to be increased in the liver, kidney, serum, and urine, indicating accumulation of F which could be correlated with altered structure and metabolism of the liver and kidney.

The administration of vitamins E and/or D to NaF-treated mice revealed significant recovery from F toxicity in all the above parameters. The recovery was more pronounced in the animals treated with vitamins E and D in combination. These studies suggest that vitamins E and D produced significant recovery in GSH redox system, which helps in the prevention of F induced cell damage. The recovery might be related to the potent biological antioxidant nature of vitamin E and influence of vitamin D in promoting the absorption of calcium and phosphorus, thus maintaining their optimal concentrations in the blood for metabolic activity in various tissues. The above report elucidates that F affects hepatic and renal functions. Therefore, vitamins E and D may be useful for amelioration of F toxicity in endemic areas throughout the world.

REVERSAL OF FLUORIDE-INDUCED ALTERATIONS IN CAUDA EPIDIDYMAL SPERMATOZOA AND FERTILITY IMPAIRMENT IN MALE MICE

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The effects of NaF ingestion (10 mg/kg body weight) and possible therapeutic effects of ascorbic acid (15 mg/animal/day) and/or calcium (25 mg/animal/day) were investigated to evaluate the reproductive functions and fertility of male mice. NaF ingestion brought about significant decline in the sperm acrosomal acrosin and hyaluronidase. Cauda epididymal sperm stained with acidic alcoholic silver nitrate revealed acrosomal damage and deflagellation. However, sperm nuclear integrity was not affected by the treatment. The reduced activity of the enzymes, and structural and metabolic alterations in the sperm led to a significant decrease in sperm motility, count and live:dead ratio. An increase in abnormal sperms was observed, which finally led to poor fertility rate. The administration of ascorbic acid and/or calcium to NaF treated mice revealed significant recovery from fluoride toxicity in all above parameters. The recovery was more pronounced in the animal group treated with both ascorbic acid and calcium, thus elucidating their synergistic or additive action.

Fluoride has definite effects on male reproduction and fertility. Hence, AA and Ca are proposed as therapeutic agents in endemic populations for amelioration of effects of fluoride on reproductive functions.
INFLUENCE OF FLUORIDE ON BIOLOGICAL FREE RADICAL REACTIONS IN OVARY OF MICE AND ITS REVERSAL

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The effects of sodium fluoride (NaF) ingestion (5 mg/kg body weight) and possible therapeutic effects of ascorbic acid (AA) (15 mg/animal/day) and/or calcium (Ca) (25 mg/animal/day) were investigated on ovarian metabolism in mice. Fluoride (F) impaired the production of free radical scavengers such as glutathione and functioning of the protective enzymes (glutathione peroxidase, superoxide dismutase, and catalase), thereby increasing ovarian lipid peroxidation which might render the tissue susceptible to injury. Furthermore, the analysis of F levels in serum, urine, and ovary of NaF-treated mice revealed an enhancement, indicating that F accumulates in the tissues and would affect their metabolism. However, withdrawal of treatment and administration of AA and/or Ca to NaF-treated mice revealed significant recovery from fluoride toxicity in all the parameters studied. The recovery was more pronounced on administration of both AA and Ca. Since AA acts as an antioxidant and both AA and calcium are known inhibitors of phosphodiesterase and would thereby increase the levels of C-AMP. Thus the studies suggest that AA and Ca help in the amelioration of F-induced cell damage in the ovary of mice.

ULTRASTRUCTURAL AND HISTOPATHOLOGICAL CHANGES IN OVARY AND UTERUS OF FLUOROTIC MICE AND REVERSAL BY SOME ANTIDOTES

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The present study was designed to explore the effects of sodium fluoride (NaF) on the structure and metabolism of ovary and uterus of mice (Mus musculus). The reversibility of the induced effects by withdrawal of NaF and by administration of ascorbic acid (AA) and/or calcium (Ca) were also studied.

Exposure to NaF (5 mg/kg body weight) for 45 days induced structural alterations in the ovary with degeneration and dense vacuolization in the stromal tissue, follicular atresia and pyknosis in follicular cells. Ultrastructural alterations revealed distortion of mitochondrial shape and size with disorganised cristae. The nuclear membrane was found to be indented and the cytoplasm was packed with lipid inclusions and vacuoles. Changes were observed in the uterus with significant reduction in the extent of serosa, myometrium and endometrium. Vacuolization in the myometrium and endometrium, pyknosis in the endometrium and reduction in the number of endometrial glands were also observed. NaF caused ultrastructural alterations in the cytoplasm of the epithelial cells and the nucleus showed indentations. The cytoplasm was packed with vacuoles. The extent of rough endoplasmic reticulum and Golgi complex, otherwise found in abundance, was reduced. NaF thus caused significant alterations in the structure of ovary and uterus. However, withdrawal of NaF for 45 days produced partial recovery which was more pronounced by AA and Ca treatment alone than by withdrawal of treatment. Their combined treatments manifested an additive/synergistic effect for recovery of ovarian and uterine structure almost to the control state. Thus the effects of F are transient and reversible.
THE INFLUENCE OF SODIUM FLUORIDE ON CLONOGENICITY OF HUMAN HEMATOPOIETIC PROGENITOR CELLS DERIVED FROM CORD BLOOD

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Hematopoiesis takes place in bone marrow cavities. Since fluoride (F) accumulates in the bones, it could directly influence the characteristics of the hematopoietic cells at the site of their active proliferation. The objective of this study was to evaluate the potential toxicity of sodium fluoride (NaF) to early human myeloid (CFU-GM) and erythroid (BFU-E) progenitors.

CD34⁺ cells isolated from cord blood (CB) samples were exposed to 0–50 mM NaF at 37°C or 4°C for 30–120 min. Low concentrations of NaF (1 mM) did not influence the clonogenicity of CB CD34⁺ cells; but at higher concentrations (10 and 50 mM) were toxic to CB myeloid (CFU-GM) and erythroid (BFU-E) progenitors. This suggests that NaF may be potentially toxic to early human hematopoietic cells and that further studies are required into the influence of NaF on human hematopoiesis.

BIOCHEMICAL STUDIES OF RESIDENTS IN FLUORITE MINE AREAS

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This research aims at providing scientific support for early diagnosis and pathological mechanisms of endemic fluorosis. Seven villages located in fluorite mine areas were selected for this study. They were divided into three groups according to fluoride (F) levels in drinking water: Group 1: 1.1 to 2.0 ppm; Group 2: 0.5 to 1.0 ppm; and Group 3: <0.5 ppm. The dental fluorosis incidence rates in children aged 8-15 in Groups 1, 2, and 3 were 75.3%, 27.8%, and 20.5%, respectively. The mean F levels in food and air among the villages were within normal levels.

The subjects for biochemical studies consisted of 100 residents aged 30-70 and were randomly selected from the three groups. Urine, saliva, and blood samples were collected for F determination, and analysis of serum biochemical indexes including the activities of phosphatase (ALP), glutamic oxalacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), lactic dehydrogenase (LDH), creatine phosphokinase (CPK), glutamyl transpeptidase (GT), and hydroxybutyrate dehydrogenase (HBDH), and blood urea nitrogen (BUN), total protein (TP), and albumin (ALB) levels. The results show that the F levels of saliva, urine, and serum all increased slightly with increase in F concentrations of the drinking water. These increases, however, were not statistically significant. The activities of ALP, LDH, and HBDH in Group 1 were significantly lower than those in Groups 2 and 3. No significant differences in other biochemical indexes were observed among the three groups.

This research concluded that the levels of ALP, LDH, and HBDH were more sensitive and easier to measure than the actual concentrations of F in urine, saliva, and serum. Because of this, we consider it reasonable to use ALP, LDH, and HBDH as indicators for early diagnosis of fluorosis. Furthermore, in areas with slight fluorosis (Group 1) fluoride did not cause any injury to vital organs including the heart, liver, kidney, and brain.
EFFECTS OF $\beta$-CAROTENE AND SOD ON LIPID PEROXIDATION INDUCED BY FLUORIDE: AN EXPERIMENTAL STUDY

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The effects of $\beta$-carotene and superoxide dismutase (SOD) on lipid peroxidation in rats exposed to fluoride (F) in vitro and in vivo were studied using the rat liver microsomal system. Fluoride was administered to the animals by means of F-containing drinking water at 150 mg F/L. The experimental results showed that: (1) in vitro F administration significantly increased the level of lipid peroxide (LPO) in rat liver microsomal system, and the presence of added $\beta$-carotene or SOD to the microsomal system decreased lipid peroxidation; (2) In vivo experiments showed that $\beta$-carotene and SOD improved the impaired growth of the rats caused by F; the growth rate of rats in $\beta$-carotene- and SOD-treated groups was higher than that of rats treated with F alone; (3) $\beta$-carotene and SOD reduced blood F and enhanced F excretion in urine and feces; (4) In the F-treated animals the levels of LPO in the serum, liver, kidney, and heart were increased, whereas the activities of SOD and GSH-Px and the levels of GSH were decreased; and (5) orally administered $\beta$-carotene and SOD improved the poor antioxidant state caused by F. These results suggest that F could cause lipid peroxidation and weaken the anti-oxidation system in rats, and that administration of $\beta$-carotene and SOD could counteract F action through lowering LPO levels and raising SOD.

DEFLUORIDATION:

A REVIEW ON FLUORIDE LEVELS IN HEALTH AND METABOLIC DISEASES AND THEIR IMPORTANCE ON FLUORIDE PHENOMENON

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The purpose of this study was to review the low concentration fluoride (F) levels that are widely distributed throughout the human body and the considerable increase in metabolic diseases which accompany such levels. An example is pathological tissues in which hardening of the arteries has been observed, namely in arteriosclerotic aortas which cause thickening and narrowing of arteries. This is owing to the atheromatous plaque whereby F levels are greatly increased more than ten-fold, probably by exchange processes. The reasoning behind this accumulation of F still needs to be explored.

Fluoride is widely distributed in our daily diets in low concentrations. The major contributor to dietary intake is tea, fish and red meat. For example, an infusion of Indian tea contains 1.60 ppm of F whereas German herbal tea (Brustee) contains 0.10 ppm. The study carried out by Stookey and Muller (1970) reported that 28.3% of the ingested F is retained in the bone, which is in fact an adequate amount, and is beneficial to normal tissue metabolism.

Further studies of water analysis showed that 0.15 ppm to 1.0 ppm of F is present in our water system (U.K.). It is now widely accepted that added F up to 1.00 ppm is beneficial to our health, particularly in preventing tooth decay, as major brands of toothpaste have added F. However, when the mechanism fails, F accumulates in the tissues resulting in dental fluorosis and osteoporosis.
HETEROGENEOUS FLUORIDATED APATITES SYNTHESIZED
WITH STEP-FLUORIDE SUPPLY SYSTEMS

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Biological apatites are sometimes formed heterogeneously. To investigate such complex phenomena in biological systems, we studied heterogeneous apatite formation. Since it is well known that fluoride (F) contributes to caries prevention, we focused on fluoridated apatites. A two-step fluoride supply system was adopted to simplify the phenomena and two different types of heterogeneous fluoridated apatites were formed: hydroxyapatite covered with fluorapatite (H-F type apatite), and fluorapatite covered with hydroxyapatite (F-H type apatite). Fluoridated hydroxyapatite was synthesized at 80±1°C and pH 7.4±0.2 using a 5 step fluoride supply system. While mechanically stirred, 0.1 L of 60 mmol/L NH4H2PO4 solution containing 0, 5, 10, 15, and 20 mmol/L HF (in this order) were added to 0.5 L of 1000 mmol/L calcium acetate (Ca(CH3C0O)2·H2O) solution containing 1.3 mol/L acetate buffer.

X-ray diffraction analyses showed a typically apatitic pattern, although the reflection (200) was broader than that of homogeneous fluorapatite. Scanning electron micrograph observation indicated that the apatite was composed of needle-like crystals similar to fluorapatite. High-resolution transmission electron microscopy showed a slender hexagonal crystal shape similar to homogeneous hydroxyapatite in sectional area perpendicular to the c-axis and an electron damage in the core of the crystal. Electron spectroscopy for chemical analysis of the apatite pellet showed a slight decrease of fluoride concentration from the surface toward the core with depth in the crystal, although a clear gradual decrease was not observed. The apparent solubility of the apatite in 0.5 mol/L acetate buffer solution (37°C and pH 4.0) was 12.1±0.2 mmol/L, much less than that of homogeneous hydroxy apatite (32.3±1.9 mmol/L). We speculate that a multilayer fluoridated apatite may have formed.

FLUORIDE SORPTION ON CLAY AND CLAY MINERALS: AN ATTEMPT TO SEARCH FOR VIABLE DEFLUORIDATING AGENT

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Fluoride (F) sorption studies were carried out on two clay minerals, montmorillonite KSF and kaolin, and a silty clay sediment series (SCSS, used in earthenware making). The function of F concentration, clay concentration, and pH in clay-water suspensions was studied. Kaolinite, a dioctahedral two layered (silica + alumina) silicate (1:2 type), exhibited very little tendency for F sorption while montmorillonite, a 2:1 type material characterized by an octahedral sheet of alumina sandwiched between two tetrahedral sheets of silica, showed significant F sorption. The F sorption on montmorillonite KSF was found to be greatest at pH 1.9±0.3, the natural pH of montmorillonite-water suspension. At pH 4.0±0.36 the percentage F sorption on montmorillonite decreased, followed by an increase around pH 5-6, after which the percentage decreased with increasing pH. The applicability of the Freundlich isotherm was also verified in case of montmorillonite KSF at low F concentrations. As a result of F sorption, increased release of Fe2+, Cl-, and NO3 ions from the montmorillonite matrix was observed. There was no effect on SO4 or PO4 solubility. Fluoride sorption on SCSS was also significant and decreased regularly with increasing pH.

On the basis of experimental data a plausible mechanism of F sorption by clay minerals is suggested. Based on the results of F sorption mentioned above, a pilot study on defluoridation of water employing clay (SCSS) as an adsorbent was also undertaken which yielded promising results.
FLUORIDE DIFFUSION IN SOIL: EFFECT OF ALUMINIUM AMENDMENTS

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One dimensional diffusive transport of the fluoride (F) ion in alluvial soil (Entisol) and in soil amended with aluminium has been investigated under the following experimental conditions: bulk density = 1.56 g/cm³, soil water content = 0.25 cm³/cm³, pH = 7.3±0.5, and temperature = 290°K. Experiments were performed by joining both the open ends of the fluoride-loaded soil core (termed as a source section: length 5 cm, diameter 2.13 cm) with the soil core (termed as soil section: length 6 cm, diameter 2.13 cm). After 4 to 16 days of incubation, with proper sealing of all the joints to avoid moisture loss, water soluble F was measured in each 1 cm segment of the system. The transient state computations of the porous diffusion coefficient (DP) employing the mass balance principle and Crank equation, produced data that indicates less F mobility in Al³⁺-amended soil compared to non-amended soil. A tentative mechanism for Al³⁺- inhibition of F mobility has been suggested. The obtained results may, in the future, help in understanding the fluorine cycle in soil with respect to its plant availability. Work in this direction is being pursued.

CONTROL OF FLUOROSIS IN CHINA

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The incidence of endemic fluorosis is very high in China. It exists in almost all the provinces, municipalities and autonomous regions. According to the 1997 statistics, the population in the disease stricken areas is around 100 million, with more than 2.7 million bone fluorosis patients. There are two types of endemic fluorosis in China, that caused by drinking water contamination and the other caused by coal burning.

The Ministry of Health is in charge of the planning and policy making. In the provincial level, task forces are set up for the implementation. The task requires joint efforts from a number of authorities related to water resources, agriculture, geology and minerals, and environmental protection.

The control of fluorosis is conducted in the following aspects,
1) education of the people in disease stricken areas
2) setting up comprehensive and specialized technical teams for water and stove improvement;
3) the government issuance of a series of provisions and regulations governing disease control, duty allocation, monitoring plans and safe environmental standards, etc.;
4) setting up the National Fluorosis and Arsenic Committee for technical guidance;
5) since 1991, there is a nationwide monitor network established which covers 24 counties in 21 provinces,
6) epidemiology, clinical diagnosis, geology, basic and preventative researches have been actively conducted since the 1970s
7) Chinese and western medicine combined therapy has immediate efficiency to more than 70% of the clinical patients

Through the ‘decades’ effort in water and stove improvement, fluorosis has been visibly controlled in China. However, the control of fluorosis is a long-term arduous task. Presently, 80% of the stoves and 60% of the water supply still need improvement. The future water and stove improvement will be financed through multiple channels in the current marketing system. The management will be emphasized simultaneously with construction.
**FLUORIDE ANALYSIS:**

**CHRONIC FLUORIDE POISONING IN RURAL AREAS OF JIANGXI PROVINCE, CHINA. 3. FLUORIDE LEVELS IN HAIR AND URINE**

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The fluoride (F) concentrations of hair and urine have been used as indicators of F exposure. The aim of this study was to evaluate the F body burden of school children in the chronic F poisoning areas of Jiangxi Province, China, by analyzing F concentrations in hair and urine.

Subjects were 199 school children (age 8-13, mean age: 10.6) who were born and raised in these areas. The concentrations of F in drinking water ranged from 0.08 to 4.20 mg/L. Fluoride concentrations in urine were diluted with pure water 5-10 times and determined using our apparatus of flow-injection analysis with a fluoride ion-selective electrode. Hair samples were washed with 0.1% TritonX-100 for 3 minutes and then determined by the method described at the XXth ISFR Conference in Beijing in 1994.

The differences in hair F concentrations between male and female were significant. The geometric mean (geometric SD) of hair F concentrations of males and females was 3.35 (2.33) µg/g and 11.8 (2.25) µg/g, respectively. The mean of F concentrations in all urine samples was 2.51 ±1.56 mg/L. There was no significant difference between age and female. The F concentrations in both hair and urine of the subjects were much higher than those of the Japanese subjects. The F concentration of water supply in Japanese area was below 0.025 mg/L. The results indicated that the F body burden of individuals in these areas of Jiangxi Province is considerably high.

**EFFECT OF FLUORIDE-CONTAINING GLASS IONOMER CEMENT ON FLUORIDE CONCENTRATION IN SURFACE ENAMEL**

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Fluoride (F) has been used for caries prevention in various ways, but it should be applied in an adequate and effective amount to the teeth before the caries risk reaches a high level. The purpose of this study was to investigate the potential of glass ionomer cement as a means of local F application. Fluoride uptake by the enamel surface was measured in vitro.

Thirty-two extracted caries-free human maxillary first molars were used for analysis. Windows of enamel of about 3 mm diameter were left uncovered, and the other surfaces were masked with nail varnish. The glass ionomer cement containing F was then applied on the two different sites (mesio-buccal, disto-buccal). After 1 day, 1 week, 1 month, or 3 months, the glass ionomer cement was removed mechanically, and the exposed enamel was sampled by dissolving it in 2 µl of 0.5 M perchloric acid for 60 seconds. The amount of F in the sampled solution was determined with a fluoride ion specific electrode.

The results showed that F concentration was higher in the enamel that had been in contact with the glass ionomer cement than in the enamel without the contact. These data suggest that F containing glass ionomer cement may contribute to caries prevention when used as a local F application.
EXAMINATION OF TOOTH MOTTLING WITH A MICRO-COMPUTER
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We present a method based on image processing that enabled us to extract white area on tooth surface and measure opacity area.

In Japan, the legal upper limit of natural fluoride (F) in drinking water is set at below 0.8 ppm. However, our recent survey suggests that F levels in drinking water below the legal threshold can still cause mottling. Out of 32 children consuming village natural water containing about 0.6 to 0.7 ppm F, we found six children classified as “Questionable”, nine as “Very Mild” and one as “Mild”. This clinical classification of tooth mottling is based on the ratio of the mottled area to the total surface area on the labial portion of surface area, and we applied an area curve meter to determine the ratio on photographs.

In assessing opaque areas with diffused border on photographs, it is often difficult to detect the edge of gradient affected areas due to lack of precision and objectivity. To resolve this problem, we discuss the usability of two image processing filters, one called Order-N filter and the other called entropy filter, to obtain a smoothed image on the 256-value grey level which was extracted from full color images. We used a flat bed scanner to input the non-reflective photography that we shot by using two dichroic polarizers. Then we converted an RGB images to a hue plane image. Using a structure element of 3x3 or 5x5 Order-N filter and 2x2 entropy filter, we then processed images to average hue value on diffused opacities and threshold to get the necessary 2-value image for measuring the area ratio with this value.

An Order-N filter is an extension of a Median filter, and the value N arrows cause blur effects on the image. Opacity area fluctuated about 26% on assays using an Order-N filter. This area fluctuation results from grey level dilation caused by surrounding white dots at diffused area. As we were able to measure the requested area with an entropy filter, we believe that an entropy filter is suitable for large images because there is no need for adjustment and denoise image.

IONIC SERUM FLUORIDE CONCENTRATIONS IN JAPANESE WOMEN
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We reported the method for the determination of ionic serum fluoride (F) at the XXIst ISFR Conference held in Budapest in 1996. We have been using this method to study ionic serum F levels. In this report, we present ionic serum F concentrations in women in their twenties to seventies, living in neighboring rural areas of Morioka City, Japan. Fluoride concentrations of tap water in these areas were below 0.05 mg/L and ambient air F concentrations were below 0.05 μg/m^3. Serum samples were separated by centrifugation within 24 hours after taking blood and stored in a refrigerator at -80°C until analysis. When blood samples were collected, inquiries about the time of taking meals and each individual’s favorite foods were made. Serum samples collected from subjects 5 hours and longer after taking meals and favorite foods were selected and determined for F.

Ionic serum F concentrations were determined by an apparatus developed in our laboratory of flow-injection analysis with a fluoride ion-selective electrode as a detector. Before determination, serum was diluted by 5 -10 times with pure water and the pH value was adjusted to 6-7. Over 100 serum samples were determined. Normal ionic serum levels in the 10-year-old group were examined and the relationship between the F levels and age was studied.
BEHAVIOR OF ORGANIC FLUORIDE IN WHOLE BLOOD AND SERUM AFTER DRIP INFUSION OF FLUORIDE-CONTAINING DRUGS IN HUMANS

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The measurement of drug concentration in blood is important in the evaluation of various pharmacokinetic parameters such as the degree of absorption into blood, clearance, effecting time, and effective drug concentrations. Several methods have been developed for the determination of drug concentrations in serum. Radioactivity measurements, HPLC, and immunoassay have been the primary methods employed for the study of pharmacokinetics. Although these methods have made measurements of drug concentrations in serum easier, it is still difficult to measure the concentrations in whole blood because of the interference from various coexisting substances. On the other hand, we have established a method called “Low-temperature Oxygen Plasma Ashing (LOPA)” for an accurate fluoride (F) determination in whole blood and reported at the 19th Conference of ISFR. In this report, after the drip infusion of Floxofex sodium to human subjects, changes of organic F concentrations in serum and whole blood were monitored. Organic F was converted into ionic F by LOPA and the concentration of ionic F was then determined by gas chromatography after trimethylsilylation of F. The organic F showed different behavior of absorption into and clearance from blood, as well as the distribution between serum and clot.
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