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DISCUSSION SECTION

HISTORIC XXIInd CONFERENCE

The XXIInd Conference of the International Society for Fluoride Research, on August 24-27 at the Lakeside Inn, Bellingham, Washington, USA, was excellently organized by Ming-Ho and Ervena Yu and their team. Its 104 participants from 11 countries, were able to hear, view and discuss 35 papers and 29 posters. Some of the more significant presentations were the following.

Dr Phyllis Mullenix, a neurotoxicologist from the Boston Children's Hospital, Massachusetts, whose earlier work on the influence of fluoride on rat behaviour was commented on in our Editorial of May 1996, reported on a further experiment using the same methodology. She compared the effects of two steroids being used in the treatment of childhood leukemia, one of which had a fluorine atom in its structure. The results indicated that the fluorine-containing steroid caused behaviour patterns typical of hyperactivity. This steroid is currently being used, in preference to the other, because it is effective at much lower doses. A follow-up study of the children using this drug for two years showed a significant drop in their average intelligence score, compared with those using the non-fluorine drug. Possible explanations for this effect were discussed.

Dr Karl Jensen, a US Environmental Protection Agency neurotoxicologist, reported on details of his work with Professor Isaacson's team at the State University of New York at Binghamton. These long term (one year) studies compared animals fed aluminium fluoride (AlF_3) or sodium fluoride (NaF), both at very low concentrations (equal to the 1 ppm in fluoridated drinking water) and non-fluoride controls. They found there was an uptake of Al into the brains of both the AlF_3 and NaF groups (but greater in the former) and alterations to brain structures, compared with the controls. The increase in brain Al in those fed NaF must have come from Al in the animals' diet. Apparently fluoride facilitates the uptake of Al across the blood brain barrier. The kidneys were also damaged, which possibly affected the blood brain barrier.

Dr Jennifer Luke, from the University of Surrey in England, presented her work on the pineal gland, the small organ situated between the two brain hemispheres, and with functions which include the production of melatonin. Because it is outside the blood brain barrier, has a rich blood supply and is a calcifying tissue, it can accumulate fluoride. Enormous fluoride concentrations were found in the pineal glands of cadavers – higher than those found in the bones of skeletal fluorosis patients. Dr Luke reported on an experiment on two groups of animals (gerbils) – one on a low fluoride and the other on a high fluoride diet. A significant decrease in melatonin production occurred in the high fluoride group, with a corresponding earlier genital maturation. She postulated that higher blood fluoride levels in children could account for the recorded decline in the age of puberty.

Professor Roger Masters from Dartmouth College in New Hampshire and Myron Coplan, a chemical engineer from Natick, Massachusetts, reported a positive correlation between blood lead levels of 280,000 children in Massachusetts and the use of silicofluorides for water fluoridation (the commonest method). In that state and in Georgia behaviours associated with lead neurotoxicity (like violent crime) are more frequent in communities using silicofluorides than in areas not using them.

Other studies reported on new, hitherto unexamined, effects of fluoride, or confirmed and expanded on past findings of fluoride toxicity. At the meeting of the Society's members it was decided to hold the next conference in Szczecin, Poland, in the year 2000.

John Colquhoun

EDITORIAL CHANGES

At the XXIInd ISFR Conference, the meeting of Society members accepted the resignation of the journal's Editor for the past eight years, Dr John Colquhoun. Professor A W Burgstahler, of Lawrence, Kansas, who for the past year has shared the editorial work by acting as Scientific Editor while Dr Colquhoun remained Managing Editor, was appointed to the new post of Editor. Dr Colquhoun remains in the post of Treasurer, responsible for receiving subscriptions and printing and distributing the journal, and becomes a Co-editor. In future, therefore, as explained on the last page of this journal, manuscripts, letters and other information being submitted for publication should be sent to

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XXIIIrd Conference of the International Society for Fluoride Research
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FLUORIDE AND ALKALINE PHOSPHATASE

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SUMMARY: Since serum alkaline phosphatase increases in fluoride therapy for osteoporosis, it is generally accepted that fluoride stimulates bone formation. However, histochemical studies have shown that alkaline phosphatase is also increased in resorbing osteocytes. Fluoride is toxic to metabolically active bone cells, alkaline phosphatase is released, and serum alkaline phosphatase increases. We propose that the increased serum alkaline phosphatase following fluoride therapy may reflect a toxicity of fluoride for both osteoblasts (bone forming cells) and resorbing osteocytes.

When cells are injured their first response is to initiate repair processes and if this repair fails the cell dies. An increase of serum alkaline phosphatase and increased bone mass following fluoride therapy represent a failed repair response involving an initial increase in both bone formation and resorption. This repair response to cell injury results in pathological bone formation. Furthermore, as the repair process fails there is a toxic death of resorbing osteocytes and a decrease in bone resorption. Osteoclasia of fluorotic bone may result in secondary toxic effects of fluoride on osteoclasts, and contribute to decreased bone resorption.

The increased amount of trabecular bone in fluoride therapy is claimed to be the morphologic expression for fluoride as a stimulus for bone formation. We propose that the increased amount of trabecular bone results from pathological bone formation by injured osteoblasts and decreased bone resorption by resorbing osteocytes and osteoclasts. Both resorptive processes are required for the remodeling of trabecular bone into compact bone.

Fluoride has only negative effects on bone cell metabolism. Fluoride should be avoided, especially in osteoporosis.

Key words: Alkaline phosphatase; Bone; Cell injury; Fluoride therapy; Osteoporosis.

Fluoride therapy in osteoporosis has been shown to increase bone mass.[1] Increased bone mass can result from increased bone formation or from decreased resorption of bone. Since serum alkaline phosphatase (SAP) increases with fluoride therapy, it has been proposed that fluoride stimulates bone apposition. An example: "Fifty-six post menopausal women with overt osteoporosis were treated for up to three years with NaF, 33 subjects receiving 100 mg NaF/day and 23 subjects 50 mg NaF/day" and : "After eight months of NaF treatment, alkaline phosphatase reached a peak of +40% above base-line value in the 50 mg NaF treatment group ($P < 0.05$) and of +94% in the 100 mg NaF group ($P < 0.001$)".[1]

1. Alkaline phosphatase as an enzyme of bone apposition

In 1923 Robison[2] published his now classical paper: "The possible significance of hexosephosphoric esters in ossification." Alkaline phosphatase (AP) has ever since been considered an enzyme of bone apposition. SAP is higher in growing subjects and is greatly increased in osteosarcoma, a neoplastic proliferation of osteoblasts.

2. Alkaline phosphatase as an enzyme of bone resorption

In 1951, two extensive papers on AP as an enzyme of bone resorption were published.[3,4] The papers have, unfortunately, not received deserved attention. AP was studied by histochemical methods in bone of man and rabbits. One of the many illustrations[3] (reproduced in Figure 1) describes "... the life cycle of human bone cells

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concerning alkaline phosphatase." Osteoblasts and newly formed osteocytes are rich in AP. However, this enzyme disappears from older osteocytes, until deep seated bone resorption is initiated. At the beginning of osteocytic bone resorption the AP reappears in the osteocytes, reaches a maximum, and then disappears in the dying osteocytes (Figure 1). The process evolves in 18-36 hours in areas of rapid deep seated bone resorption.

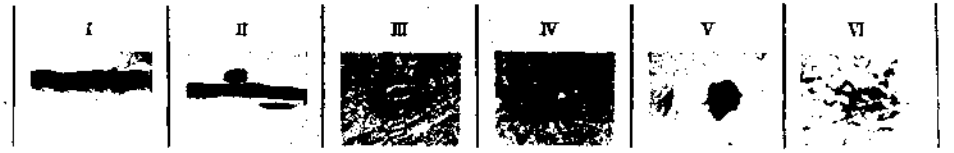

Figure 1. The life cycle of human bone cells concerning alkaline phosphatase. In Stage I, the osteoblasts show a rich concentration of alkaline phosphatase. In Stage II, the osteoblasts are rich in alkaline phosphatase and so is, to a lesser degree, the recently trapped osteocyte. The cell on top of the bone surface is a bone marrow cell. Stage III shows a more deep-seated, resting osteocyte with no alkaline phosphatase. In Stage IV, the beginning of osteocytic osteolysis, alkaline phosphatase reappears in the osteocyte and reaches maximum (Stage V). The alkaline phosphatase is now as prominent as in the osteoblasts (Stage I). At the end stage (VI), the dying osteocyte shows no alkaline phosphatase. (Reproduced from Reference 3.)

von Recklinghausen[5] used the term onchosis to refer to the "necrobiotic resorption of bone tissue." Mäjno and Rouiller[3] and Rutishauser and Mäjno[4] considered onchosis to be a regressive process (necrobiosis), as well as a form of bone resorption: pericytic osteolysis. This defines a deep-seated bone resorption mediated by osteocytes. This concept was brought to wide, if not general, acceptance by the contributions by Bélanger[6] who introduced the now more common term "osteocytic osteolysis". This is an active metabolic process that results in increased synthesis of alkaline phosphatase and metalloproteinases required for bone resorption, and culminates in apoptosis or "programmed cell death" of osteocytes. This activation, or increase in metabolic activity, of resorbing osteocytes may be initiated by any of the normal physiological, nutritional and/or endocrine mechanisms that regulate increases in bone turnover, and it may by more important than osteoclasia.[7,8] This activation can also be initiated by cell injury that initiates a repair response in both surface osteoblasts and deep seated osteocytes that have been activated metabolically. The repair response in both types of injured bone cells contributes to the increase in SAP. This increase in SAP is undoubtedly further augmented by the death of injured osteocytes when the fluoride toxicity overwhelms the repair process.

3. Alkaline phosphatase in nutritional secondary hyperparathyroidism

Young horses were fed a ration with normal calcium but with 3.6 times that high in phosphorus.[9] The high dietary phosphorus induced hyperphosphatemia which, in accord with the mass law equation, induced hypocalcemia, which is the stimulus for parathyroid hyperactivity. With the resulting increase in bone resorption, the hypocalcemia was corrected to nearly normal levels. SAP showed an inverse correlation to serum calcium: when serum calcium decreased, SAP increased and *vice versa*. In the second part of the experiment, weeks 23 through 42, dietary phosphorus was increased to 5.2 times dietary calcium. The sequences in serum calcium and in SAP were repeated. In similar studies in pigs,[10] serum calcium and SAP recordings were in

agreement with those in the horse study. The results can mean only one thing, *viz.* that SAP in nutritional secondary hyperparathyroidism reflects the fact that AP is also an enzyme of bone resorption.

4. Serum alkaline phosphatase in human populations

SAP was studied in 257 men and 195 women of various ages.[11] In the 20-30 age group, SAP was higher in men than in women. It increased in men in the 60-65 age group and was still higher in men older than 70 compared to the 20-30 age group. In women more than 70 years of age, SAP was higher than in ages 50-70. Similarly, in a series of 117 subjects, it was reported that SAP increased progressively after 50 years of age.[12] This rise in SAP at higher ages cannot be explained by increased bone formation. On the other hand, this will be the expected consequence of increased osteolysis, which results from the dietary calcium deficiency that is prevalent in most countries.[13]

5. Alkaline phosphatase in nutritional hypercalcitoninism

Bulls at an artificial insemination station (about 300 bulls) were fed calcium at 3.5 times recommended levels in young bulls and this was increased progressively to 5.9 times in older bulls.[14] The high dietary calcium induced hypercalcemia, a stimulus for the calcitonin producing C cells of the thyroid gland. Calcitonin is the antagonist of parathormone and decreases bone resorption. Serum calcium was reduced and reached hypocalcemic levels. High concentrations of calcium in the gastrointestinal tract stimulate the gastrin producing G cells of the gastrointestinal mucosa. Gastrin stimulates the C cells regardless of serum calcium.[15-17] The high dietary calcium eventually caused hypocalcemia and osteopetrosis, because of increased calcitonin production. Because of the resulting decrease in bone resorption SAP decreased and remained low in old bulls (more than 12 years of age). Thus, this osteopetrosis resulted from decreased bone resorption. If bone production had increased to produce the osteopetrosis, SAP would have increased.

In experimental hypercalcitoninism in growing dogs,[18] Great Dane puppies were fed *ad libitum* a diet high in protein, energy and calcium (2.05% compared to the recommended 1.0% on a dry matter basis). Control dogs of the same litter and sex were restricted to 2/3 of the amount of food consumed by the dogs fed *ad libitum*. In the *ad libitum* fed dogs an initial hypercalcemia was converted into isocalcemia after 30 weeks and at 60 weeks there was a pronounced hypocalcemia. Serum phosphorus went from hyperphosphatemia to hypophosphatemia, a parathormone effect. During the entire experiment, weekly recordings of SAP were consistently lower in *ad libitum* fed dogs. Electron microscopy studies of C cells[19] indicated that there was a marked stimulation of C cells which would be consistent with hypercalcitoninism. Radiography, microradiography, fluorescence microscopy and histopathology all indicated that bone formation was increased in *ad libitum* fed dogs, causing hypertrophic osteodystrophy and osteopetrosis, with delayed remodeling of lamellar bone into osteonic bone. Delayed bone resorption was further documented with analysis of fluorochrome incorporation into forming bone, and by the presence of failure of modeling of the femoral neck in the *ad libitum* fed dogs. We now have before us a condition of increased bone formation, which would be reflected in increased SAP and decreased bone resorption, which would be reflected in decreased SAP. Since SAP was always lower in *ad libitum* fed dogs, the decrease in SAP resulted from the decreased bone resorption.

DISCUSSION

Fluoride is a potent enzyme poison. The concept that fluoride is a specific stimulus for bone formation is preposterous. Instead, fluoride induced cell injury in both osteoblasts and osteocytes initiates a repair response and results in increased SAP production in both of these cell populations. The repair response in osteoblasts results in increased proliferation, matrix production and SAP production.[20,21] When the repair process in osteoblasts fails, the osteoblast undergoes either apoptosis or necrosis,[22,23] and is replaced by proliferation of osteoprogenitor cells. These new osteoblasts will then be injured and the cycle of increased repair and cell death would be repeated. This activation of a repair response in osteoblasts would contribute to increased SAP.

Activation of repair in resorptive osteocytes will increase their production of AP, collagenase and other degradative enzymes. Recent studies have shown that the collagenase-1 that is required for native collagen degradation is produced by osteoblasts/osteocytes,[24,25] while the gelatinase-B that degrades denatured collagen is produced by osteoclasts.[26] When the repair process in these activated osteocytes fails, osteonecrosis occurs and there is a decrease in osteocytic osteolysis and an increase in the release of AP from dying osteocytes. This decrease in bone turnover may be further enhanced by a secondary fluoride induced injury of osteoclasts that are formed to degrade the necrotic bone. If these injured osteoclasts die, new osteoclasts will form from monocytes, so secondary injury of osteoclasts will not be expected to result in a paucity of osteoclasts on the surface of fluorotic bone (Figure 2).[27] Furthermore, because fluoride injures all of the cells involved in bone formation and degradation, it is not the surprising that a poor quality of bone accumulates in patients treated with fluoride.[28]

Figure 2. Photomicrograph of mandibular cortex of a 3-year-old cow, in which triplicate assays showed that the fluoride content of bone ash was 4733 ± 67 ppm.. Many lacunae are empty because of death of osteocytes. Matrix is also being degraded, especially in the upper right. Osteoclasts and Howship's lacunae indicate that the surface of the devitalized bone is undergoing osteoclasia. Hematoxylin and eosin, x 120. (Reproduced from Reference 27.)

Figure 1 shows the changes in AP during bone formation and osteocytic osteolysis. Osteoblasts, immature osteocytes and resorbing osteocytes all produce alkaline phosphatase and are, thus, metabolically very active. As such, they are highly susceptible to a toxic agent, fluoride in this case. Fluoride injures all of the cells in bone, alkaline phosphatase is released and SAP rises. This increase in SAP is consistent with the increased bone formation and decreased bone turnover that results from the death of activated, resorbing osteocyte.

It is a consistent finding that fluoride treatment in osteoporosis results in greater amounts of trabecular bone and a decrease in compact bone.[1] This has been interpreted as evidence for enhanced bone formation, albeit abnormal bone.[28] We offer a fundamentally different explanation. Remodeling of trabecular bone into compact bone requires bone resorption, mainly osteocytic osteolysis. In addition to stimulating pathological bone formation by injured osteoblasts, fluoride reduces osteolysis by injured osteocytes and greater amounts of trabecular bone persist. The amounts are significantly increased by the failure of remodeling.

Bone is a dynamic tissue in a state of turnover: it is formed and resorbed, it is always being renewed. Resorption is a physiological event aimed at maintaining normal serum calcium levels and it is required for normal remodeling. Causing pathological bone formation and reducing bone resorption is the wrong way to treat osteoporosis. The right way is to promote physiological bone formation by increasing dietary calcium and vitamin D and by increasing physical activity.

Acceptance of the documentation that alkaline phosphatase is an enzyme of both osteoblasts and resorbing osteocytes, reveals the true nature of fluoride's effect of bone cells. It is poisonous to bone cells and every osteoporosis patient who is being treated with fluoride should know that fluoride is toxic for all of the cells in their bones. It is unfortunate that many physicians who treat osteoporosis with fluoride do not realize that they are prescribing a drug that is toxic for all metabolically active bone cells.

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ASSESSMENT OF THE EXPOSURE TO FLUORIDE FROM DRINKING WATER IN DURANGO, MEXICO, USING A GEOGRAPHIC INFORMATION SYSTEM

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Joel Milan,[b] and Fernando Díaz-Barriga[a]
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SUMMARY: A Geographic Information System (GIS) coupled with environmental data was used for the study of endemic fluorosis in the city of Durango, Mexico. The city was divided into four different risk areas. These areas were categorized according to fluoride levels in tap water. Mean fluoride levels ranged from 1.54 mg/L in area 1 to 4.70 mg/L in area 4. A level of 5.67 mg/L, the highest fluoride concentration in Durango, was found in area 4. Almost 95% of the 306,652 inhabitants living in this city were exposed to fluoride levels higher than 2.0 mg/L. Exposure doses to fluoride were calculated for all the areas. For example, the maximum estimated exposure doses were 1.86 mg/kg/day for infants, 0.28 mg/kg/day for children and 0.16 mg/kg/day for adults. Taking into account the minimal risk level of 0.05 mg/kg/day calculated by the Agency for Toxic Substances and Disease Registry, a health risk for the city of Durango became evident.

Key words: Durango, Mexico; Fluoride exposure; Fluoride from water; Geographic Information System

INTRODUCTION

As has been previously reported, endemic fluorosis may be a public health issue in Mexico.[1-5] Among the cities located in areas where drinking water contains excessive quantities of natural fluoride is Durango, in the northwest of Mexico. Preliminary data obtained in Durango showed that 96% of well water samples collected in the city had fluoride levels above the Mexican National Guideline of 1.5 mg/L.[6] However, this study failed to characterize the risk areas for fluoride exposure because of the lack of information regarding the areas of Durango which are fed by each well. The city has 70 municipal wells which are not interconnected. They are distributed all over the city and are the potable water source for a defined area.

Geographic information systems (GIS), coupled with environmental data, offer tools for the identification of populations at risk.[3] In the city of San Luis Potosi (SLP), our group used GIS to screen for fluoride exposure.[3] As a result of this work, SLP was divided into four different risk areas, where intervention programs were introduced. Considering the products that can be obtained with GIS, we used GIS for the identification of risk areas in Durango. For the present study, fluoride levels in tap water were analyzed.

METHODS

Tap water samples were collected from 212 homes distributed throughout the city of Durango (Figure 1). Samples were collected in polyethylene bottles. Fluoride levels were quantified by adding TISAB buffer to the samples just prior to the analysis with a sensitive specific ion electrode. As an internal quality control program, primary standard reference material was analyzed. Our fluoride recovery was 97.8%.

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A Geographic Information System provides a computational platform in which layered, spatially distributed databases can be manipulated easily and whereby selected topological attributes, which may not be known *a priori*, can be queried to obtain the spatial relationship between environmental/health parameters and demographic distributions. For this work, we used Arc-Info and Arc-View software, which operate in standard personal computer equipment.

The exposure doses were calculated by the following generic equation:

$$ED = \frac{C \times WI}{BW}$$

Where :

ED = exposure dose (mg/kg/day) C = fluoride concentration (mg/L)
WI = water intake (L/day) BW = body weight (kg)

The standard values that were used in estimating exposure are shown in Table 2. For the calculations, we assumed chronic exposure and total bioavailability of fluoride in water.

RESULTS

Four different risk areas were obtained using GIS coupled with fluoride levels in tap water (Figure 1, Table 1). A clear low risk area was detected in the south sector of the city (area 1) whereas a high risk area (area 4) was located both in the southeast and in the northeast sectors of the city. However, high levels of fluoride were also found in some locations in the rest of the city. Mean fluoride levels in tap water samples collected in each area are depicted in Table 1. Area 4 not only had the highest mean fluoride level but also had the highest percentage of samples with fluoride levels above 3.0 ppm. In contrast, area 1 had the lowest mean fluoride concentration and none of the samples collected in this area had fluoride levels above 3.0 ppm.

Figure 2 shows the distribution of population in each area. It is interesting to note that 95% of the inhabitants of Durango are heavily exposed to fluoride. For example, 17.5% of the population (54,186 inhabitants) live in area 4, which has a mean fluoride level in tap water of 4.70 mg/L. In order to give an idea of the magnitude of the exposure, in Table 2 we present the exposure doses. Minimum and maximum exposure doses were calculated, taking into account the minimum and the maximum level of fluoride in tap water found for the city of Durango. The minimum exposure dose for infants and the maximum exposure dose for infants, children and adults were higher than 0.05 mg/kg/day, which is the minimum risk level (MRL) estimated by the Agency for Toxic Substances and Disease Registry (ATSDR).[7]

Table 1. Fluoride levels in tap water samples collected in different risk areas of Durango

Area	n	MEAN	SD	RANGE	% of samples>3.0 ppm
1	9	1.54	0.55	1.0 - 2.7	0
2	49	2.63	0.42	1.0 - 3.9	14.3
3	111	3.57	0.46	2.0 - 4.5	90.1
4	43	4.70	0.36	3.6 - 5.6	100

Results in mg/L are shown for each area, as the arithmetical mean (mean) with the standard deviation (SD). Areas were defined according to Figure 1.

Figure 1. Risk areas according to fluoride levels in tap water.
In this map of the city of Durango, each risk area is represented with numbers and the approximate locations of the homes where the water samples were collected are shown with dots

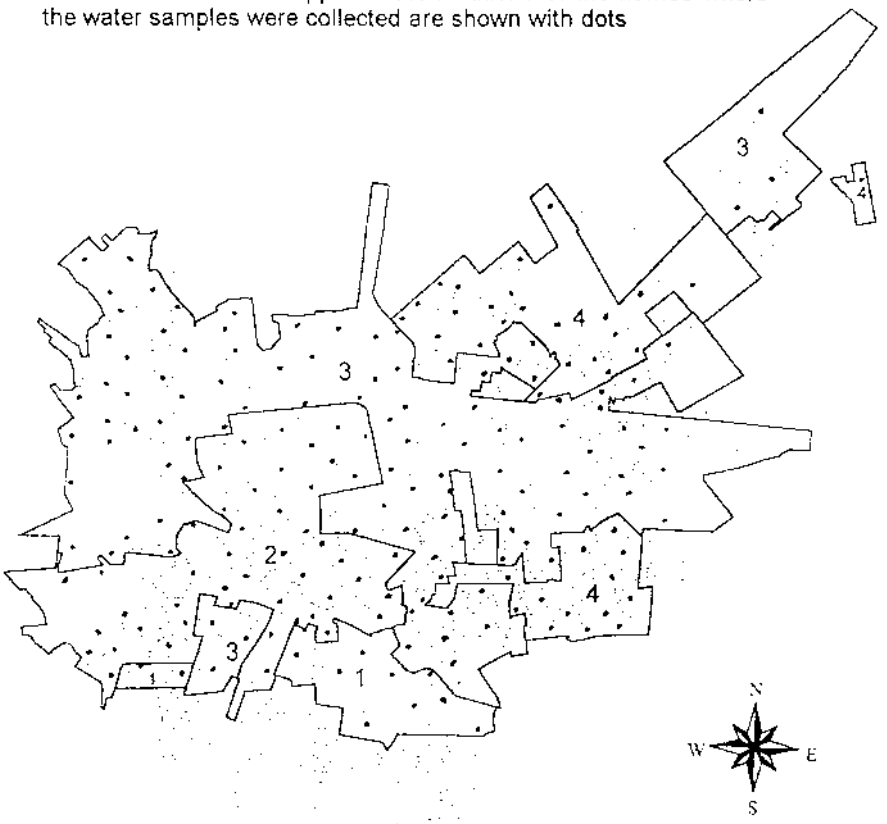

Figure 2. Population distribution in the risk areas
The bars represent the number of inhabitants in each of the risk areas for fluoride exposure in Durango

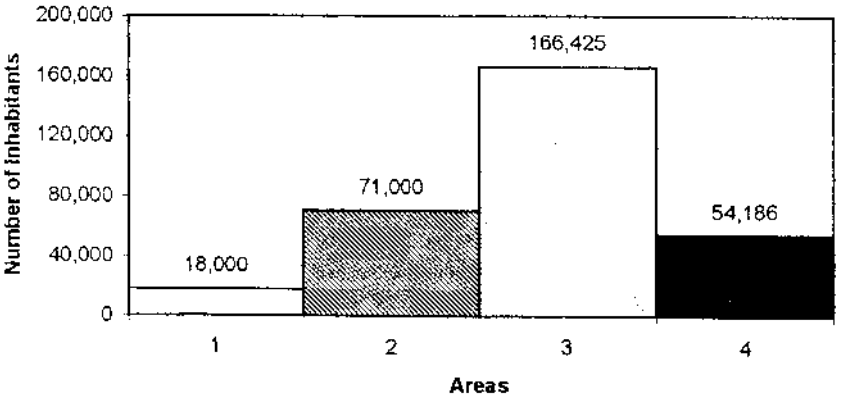

Table 2. Estimation of Exposure Doses for Fluoride in SLP

Example	Source of water	L/day	Fluoride (mg/L)	Fluoride intake (mg/kg/day) minimum	maximum
Infant (6 kg)	Boiled water[a]	1.00	2.00[b]	0.33	1.86
			11.2[b]		
Children (20 kg)	Tap water	1.00	1.0	0.05	0.28
			5.6		
Adult (70 kg)	Tap water	2.00	1.0	0.02	0.16
			5.6		

For the calculation of the fluoride intake we used the minimum fluoride level (1.0 mg/L) and the maximum fluoride level (5.6 mg/L) found in the city of Durango (Table 1).

[a] The source of boiled water for infants is the water used in the reconstitution of milk formulas.

[b] Considering that in boiled water, fluoride levels increase proportionally to the loss of volume,[1] the concentration of fluoride in tap water was doubled. This value represents the maximum range obtained after a survey done in San Luis Potosi.

DISCUSSION

A GIS coupled with environmental data has been shown to be useful for the study of human exposure to fluoride.[3] In Durango, 70 municipal wells, which are not interconnected, are the sources of the tap water. However a map of the area served by each well is lacking. Therefore, in this work, the study of fluoride levels in tap water was preferred to define the risk areas. Using GIS and mapping in it the fluoride levels in tap water, a division of the city into four different risk areas was obtained (Figure 1). The main result, however, is that almost 95% of Durango's population is exposed to high levels of fluoride. Almost 300,000 persons live in areas with fluoride levels higher than 2.0 mg/L. By calculating the exposure doses to fluoride, it can be concluded that a health risk exists for these individuals.

Exposure doses to fluoride from tap water were estimated for infants, children and adults (Table 2). For infants in their first semester of life (body weight 6 kg), we applied the risk factor of boiling the water,[1] since the main source of water for infants is that used in the reconstitution of milk formulas. The dose estimated for this group was between 0.33 mg/kg/day and 1.86 mg/kg/day. At these levels a clear risk for dental fluorosis is evident. For example, in San Luis Potosi, in an area where the exposure dose for infants is 1.1 mg/kg/day, a prevalence of 84% was found for moderate to severe dental fluorosis.[3]

Exposure doses from water were also calculated for children (20 kg body wt) and adults (70 kg body wt). In children, the doses were between 0.05 mg/kg/day and 0.28 mg/kg/day. Whereas in adults the doses ranged between 0.02 and 0.16 mg/kg/day. These doses were then compared with safety doses. For example, ATSDR has calculated a MRL of 0.05 mg/kg/day for chronic oral exposure, based on a lowest observed adverse effect level (LOAEL) of 0.48 mg/kg/day (at the high end of an uncertainty factor of 10) for increased nonvertebral fracture rate in osteoporotic women.[7] Therefore, the maximum exposure dose to fluoride for the adults in Durango living in area 4 (the area with the highest fluoride levels in water) is three times higher than the ATSDR's MRL. How serious a health risk this dose

represents is a question that deserves further research. However, our calculations did not take into account other sources of fluoride,[4] and therefore the real exposure doses in Durango are no doubt higher than the figures presented in Table 2.

In light of our results, a public health program is needed for the city of Durango. Epidemiological surveillance for dental and skeletal fluorosis will help determine the health risk in those areas of the city exposed to elevated fluoride levels. Furthermore, a source of potable water (low-fluoride or fluoride-free) has to be made available to the population. This requirement is especially important, since preliminary analysis of bottled water sold in Durango shows that it also has high levels of fluoride. The health program has to be designed taking into account all the fluoride sources, including diet.

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LEACHING CHARACTERISTICS OF FLUORIDE FROM COAL FLY ASH

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SUMMARY: Leaching of fluoride from fly ash has been studied at different ash/water ratios and temperatures and also over a broad range of pH. The efficiency of leaching was only slightly dependent on the ash/water ratio, thus revealing a solubility-controlled mechanism of the process. The efficiency was also independent of the temperature over the range 20 - 90°C.

Key words: Coal burning; Fluoride leaching; Fly ash.

INTRODUCTION

More than 150 million tons of fly ash are produced annually worldwide from the combustion of coal in power plants. At least a half of this amount is disposed of by landfill, thus contributing to environmental pollution due to leaching of its toxic constituents. One of the critical constituents is fluoride which may be toxic at elevated levels in water.

Disposal of huge amounts of fly ash in landfills and surface impoundments or its re-use in construction materials is of environmental concern. While much effort has been devoted to the problem of leaching of heavy metals from disposed fly ash,[1-8] the release of non-metals has attracted considerably less attention. Of these, arsenic,[9,10] selenium,[9,10] and boron[11] stand out as potentially harmful to both vegetation and animals.

The fluoride levels of coal fly ash vary within broad limits of 0.4 - 610 $\mu g/g$[12] and depend on the type of coal being burnt, the particle size of the ash, and the efficiency of electrostatic precipitators. Under natural leaching conditions, the fluoride levels may exceed legal standards for drinking water (1.4 - 2.4 mg/L)[7] attaining a level of 5.8,[13] or even as high as 18 mg/L.[14]

As the knowledge of the amount of leachable constituents of fly ash is important to estimate their availability for the biological systems, the primary objective of the present study was to investigate the release of fluoride from fly ash to water under a variety of conditions.

MATERIALS AND METHODS

Fly ash was collected directly at the electrostatic precipitators installed in two power-generating units of the Gdansk Thermoelectric Power Plant. One of the precipitators has three successive hoppers for collecting the ash and the other has four. The fluoride contents in the leachates obtained from the ash taken from the three successive hoppers were 0.37, 0.55 and 0.60 mg/L, whereas those from the ash taken from the 4-hopper precipitator were 0.56, 0.69, 0.72 and 0.75 mg/L after 3-hr leachings.

Further experiments were conducted with fly ash taken from the second hopper of the first-named precipitator. The sample represented dry materials collected at the station. The grain size of the ash ranged between 1 - 90 μm with a mean diameter of 20 - 30 μm. The results of its chemical analysis (main constituents) are as follows: SiO_2 52.7%; Al_2O_3 21.9%, Fe_2O_3 8.4%, and CaO 7.2% (a class F fly ash according to ASTM standards).

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All chemicals were of analytical reagent grade quality, and distilled water was used throughout.

Polyethylene labware was used for handling fluoride-containing solutions.

ANALYTICAL PROCEDURE

The total fluoride level in the leachates was determined potentiometrically by using a fluoride ion-selective electrode. As the electrode does not measure fluoride complexed with silicon, iron, aluminium and other polyvalent cations, the complexes had to be destroyed by adding a total ionic strength adjustment buffer (TISAB).[15] Further, an optimum pH of 5.2-5.5 was adjusted to prevent the formation of the HF_2^- ion emerging at a pH less than 5.

The buffer was prepared by dissolving 57 mL of glacial acetic acid, 58 g of NaCl, and 0.3 g of sodium citrate in distilled water and making the volume up to 500 mL in a volumetric flask. The pH of this solution was then adjusted to 5.2 with a 5 M NaOH solution.

Calibration graphs were constructed immediately before carrying out the measurements of the fluoride levels in the leachates for each series of measurements. The standard solution of fluoride was prepared by dissolving 0.2210 g of NaF (previously dried at 120°C), in 1 L of water. Its concentration was 100 mg F^-/L ($5.2.10^{-3}$ M). Solutions for constructing the calibration graphs containing 0.5, 1.0, 2.0, 5.0 and 10.0 μg F^-/8 mL were prepared by pipetting out 0.05, 0.10, 0.20, 0.50 and 1.00 mL of the standard solution, addition of 3.95, 3.90, 3.80, 3.50 and 3.00 mL of distilled water, respectively, and of 4 mL of the TISAB buffering system to each. During the measurements the solutions were stirred with a magnetic stirrer. Potential readings were recorded after fixed time intervals.

The calibration plot constructed in this way was linear for the concentration range 0.5 - 10.0 μg F^-/mL.

In a similar way, the fluoride concentration was measured in the fly ash leachates. Samples of the leachates (up to 4 mL, depending on the expected F^- concentration) were mixed with 4 mL of TISAB to ensure optimum pH of 5.2 for fluoride measurement. The results were taken from the calibration graph.

PREPARATION OF THE LEACHATES

During preliminary tests, the fly ash/water ratios used were 1g/100 mL water, 1 g/50 mL water, and 10 g/100 mL water. The slurries were placed on a mechanical shaker and shaken for 0.5, 1, 2, 3, 24, and 48 hrs, at 25°C. The results of the fluoride determinations in these slurries are shown in the Table.

TABLE. The kinetics of fluoride leaching at various fly ash/water ratios at 25°C (F^- concentration in mg/L)

Fly ash/water ratio	0.5 hr	1 hr	2 hrs	3 hrs	24 hrs	48 hrs
A: 1.0 g/100 mL	0.45	0.50	0.52	0.54	0.61	0.71
B: 1.0 g/50 mL	0.50	0.54	0.56	0.58	0.84	-
B/A ratio	1.11	1.08	1.08	1.07	1.38	-

Because the 1.0 g/50 mL slurry was too dense and difficult to handle, further experiments were conducted with the 1.0 g/100 mL water slurry only.

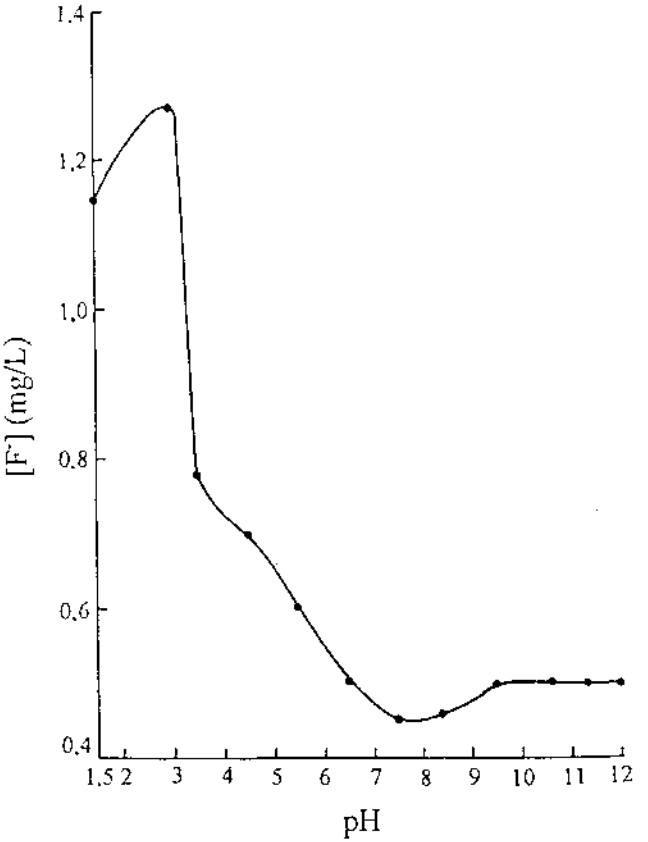
EFFECT OF TEMPERATURE

To check the influence of the temperature on the fluoride extraction, a series of measurements were run for 3 hrs at 20, 30, 50 and 90°C using the 1.0 g/100 mL water slurry. The concentrations of the fluoride released at these temperatures were 0.54, 0.58, 0.55 and 0.58 mg/L.

EFFECT OF pH

In order to estimate the influence of pH on leaching of the fluoride ion, a set of 3 M HCl, 6 M HCl and ammonia buffer solutions was prepared covering a pH range of 1 - 12. The fly ash/buffer solution ratio was 1g/100mL, and the temperature was held constant at 25°C. Three hours after preparation of the slurries, 4 mL aliquots were taken, filtered, 4 mL of TISAB buffer was added to the aliquot and the F^- level was determined potentiometrically. Following the addition of the TISAB, the pH of all aliquots fell within the range 4 - 5. The results are recorded in the Figure.

FIGURE. The effect of pH on leachability of fluoride from fly ash (for details, see text)



DISCUSSION

Fluoride in coal is typically associated with organic matter and with minerals of the apatite group, fluorite, clay minerals and phosphates.[16,17] Upon combustion, coal releases fluoride partly into the atmosphere and partly in the form of fly ash and bottom ash as well as in an organic phase adsorbed on unburnt coal particles.[13] According to Meij,[18] the mean fluorine concentration in coal combusted in the Netherlands is 80 ppm.

During combustion in dry-bottom boilers, 81±31% of F is vapourised, and its concentration in flue gases is 7000 $\mu g/m_n^3$. The relative enrichment factor in collected fly ash is 0.2 having an F concentration of 129 ppm, whereas, in emitted fly ash, respective values are 1.5 and 1089 ppm.[18]

Successive hoppers of the electrostatic precipitator collect fly ash particles of gradually decreasing grain size.[18] The accompanying surface area enlargement favours the leaching of fluoride as demonstrated by the results presented in the section Materials and Methods.

As far as the influence of the pH on the leaching of fluoride is concerned, there is a remarkable increase in the F^- level in strongly acidic medium below pH 2.5 (*cf* Figure). Additional thiocyanate tests carried out with the slurries revealed a striking red colour of a slurry with pH 1.5 and a fainter one of a slurry with pH 3.0, while at higher pH values the thiocyanate reaction was negative. Since it has long been known that the FeF^{2+} complex is destroyed at pH below 3.2, it is likely that in strongly acidic slurries the fluoride associated with iron(III) is released by the reaction:

$$2FeF^+ + H^+ \leftrightharpoons 2Fe^{3+} + HF_2^-$$

As shown recently by Reardon *et al* [6] by conducting fly ash leaching tests at two different water/ash ratios, it is possible to determine whether or not the concentration of an element in the leachate is controlled by mineral solubility.

If a mineral solubility control exists, the concentration of an elemental ion can be readily predicted with chemical equilibrium models.[6]

Our results presented in the Table show that the fluoride concentration is fairly constant with time, and that doubling the mass of solid relative to the mass of water does not double the F^- concentration in solution (B/A = 1.07 to 1.38; Table). Bearing Reardon's rule in mind, these findings indicate that F in ash occurs in the form of sparingly soluble salts embedded in surfaces of glass particles formed at low temperatures in the stack. This conclusion appears to be supported by the weak temperature response of F^- leaching over the range 20 - 90°C (see section on Effect of Temperature).

In this context it is interesting to note that, unlike fluoride, the chloride in ash occurs in the form of readily soluble salts as indicated by doubling its concentration upon doubling the fly ash/water ratio.[6]

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THE ROLE OF FLUORIDE IONS IN GLYCOSAMINOGLYCANS SULPHATION IN CULTURED FIBROBLASTS

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SUMMARY: The purpose of this study was to evaluate sulphur incorporation (by ^{35}S sulphate) into glycosaminoglycans (GAG) cultures of isolated cells, pericellular substance and medium, and into glycosaminoglycans present in sulphate fibroblasts with NaF added to the culture. In the study, primary cultures of fibroblasts were used, isolated by tissue trypsinization from mice livers. Fibroblasts were cultured with the addition of NaF ($[F^-] = 0.116 \cdot 10^{-3}$ M/dm^3) and the addition of NaF and [^{35}S]-Na_2SO_4 (activity ^{35}S = 30 μCi/cm^3). Simultaneously with the experimental cultures, control cultures were also examined. The effect of F^- ions on culture growth, protein content in fibroblasts, and their morphometric characteristics were evaluated. Three fractions were isolated from fibroblast cultures: cell, pericellular substance, and medium. From these fractions glycosaminoglycans were isolated. GAG obtained from fibroblasts were electrophoretically separated, resulting in heparan sulphate (HS), dermatan sulphate (DS), and chondroitin sulphates (CS). Even in low concentrations F^- ions have a toxic effect on fibroblast cultures. Growth inhibition and decrease in size, accompanied by a change in shape, were observed. Under the same conditions fluoride ions significantly modified incorporation of ^{35}S into fibroblasts and GAG from individual fractions of experimental cultures. Analysis of sulphated GAG content in the fibroblasts showed interference by F^- ions, both in their synthesis and metabolism, as well as their diffusion. The results suggest a significant increase in synthesis intensity and/or the degree of DS sulphation and a decrease in the intensity of the process in relation to CS and HS.

Key Words: Cultured fibroblasts; Fluoride ions; Glycosaminoglycans; Sulphation.

INTRODUCTION

Interest in the effect of fluoride compounds on the human body is, to a great extent, due to their use in osteoporosis treatment[1-3] and prevention of dental caries[4]. However, research should largely be focused on the effect of fluorides on connective tissue, where fluoride compounds cause changes in collagen[5-7] and the metabolism of glycosaminoglycans (GAG)[5,6,8], the main basic substance of connective tissue.

The aim of this study was the evaluation of the effects of fluoride ions on sulphur incorporation, in the form of SO_4^{2-} ions, into GAG in fibroblast cultures. The quantity of F^- ions did not exceed the value of the CaF_2 and MgF_2 solubility product. Qualitative and quantitative reciprocal relations among individual GAGs were examined. Fibroblast cultures were used in the study to evaluate F^- ion effects on culture growth, protein content in cells, and chosen morphometric parameters of fibroblasts.

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MATERIALS AND METHODS

The study was performed using primary fibroblast cultures, isolated by tissue trypsinization[9b] from the livers of 60 day old mice of both sexes of Balb c strain. Fibroblasts were cultured on Eagle's medium MEM 1959[9a] with fetal calf serum containing antibiotics (penicillin 100 U and streptomycin 20 $\mu g/cm^3$) in an atmosphere of 5% CO_2 and a temperature of 310°K. All isolation and fibroblast culture procedures were performed under asceptic conditions in a laminar chamber.

Cultures for evaluation of F^- ions effect on fibroblasts were carried out by addition of NaF in quantity $0.116 \cdot 10^{-3}$ M/dm^3; and cultures for evaluation of GAGs sulphation except NaF (in concentration as above) by addition of $[^{35}S]$-Na_2SO_4 with the activity ^{35}S 30 μ Ci/cm^3. Along with the examined cultures, control cultures were performed without NaF, with and without $[^{35}S]$-Na_2SO_4.

Culture growth was determined on days 2, 4, 7, 11 and 14 by counting cells in Bürker's chamber (with prior staining with trypane blue). For microscopic examination and morphometric analysis, the cells were trypsinated from the bottom of the culture vessel. Morphometric evaluation was performed with the VIDS computerized image analysis system (AMS, England).

The Lowry procedure was used to determine protein content in fibroblasts.[10,11] Fibroblasts for protein content determination were obtained without the use of trypsin.

Cultures to be examined provided 3 fractions (fibroblast, pericellular, and medium).[12] From each fraction GAGs were isolated by the method of Svajcar and Van Robertson [13] with Wosicki's modification.[14] Fibroblast cells were homogenized by use of a pressure press. Then the pericellular and medium cell homogenates were submitted to digestion by papain and afterward deproteinized by TCA solution. Finally, an ethanol solution of potassium acetate was used to precipitate GAG. GAGs obtained from fibroblasts were electrophoretically separated by Wessler's method,[15] consisting of cellulose acetate strips in barium acetate solution (in a Beckman apparatus and a current of 1 mA/cm^2 charge density) and after staining with altian blue and elution with potassium acetate solution. Separated GAG identification was performed on the basis of DS, HS, C4S and C6S patterns electrophoresis (Sigma prod.). In this way, heparan (HS) and dermatan sulphate (DS) were obtained along with the mixture of chondroitin sulphates (CS), namely, chondroitin-4-sulphate (C-4-S) and chondroitin-6-sulphate (C-6-S).

Radioactivity measurements of all samples containing ^{35}S, including also eluates from separated glycosaminoglycans, were performed with a Beckman scintillation counter after placing them on scintillation blotting-paper discs of the same type. Counting efficiency was 70-80%.

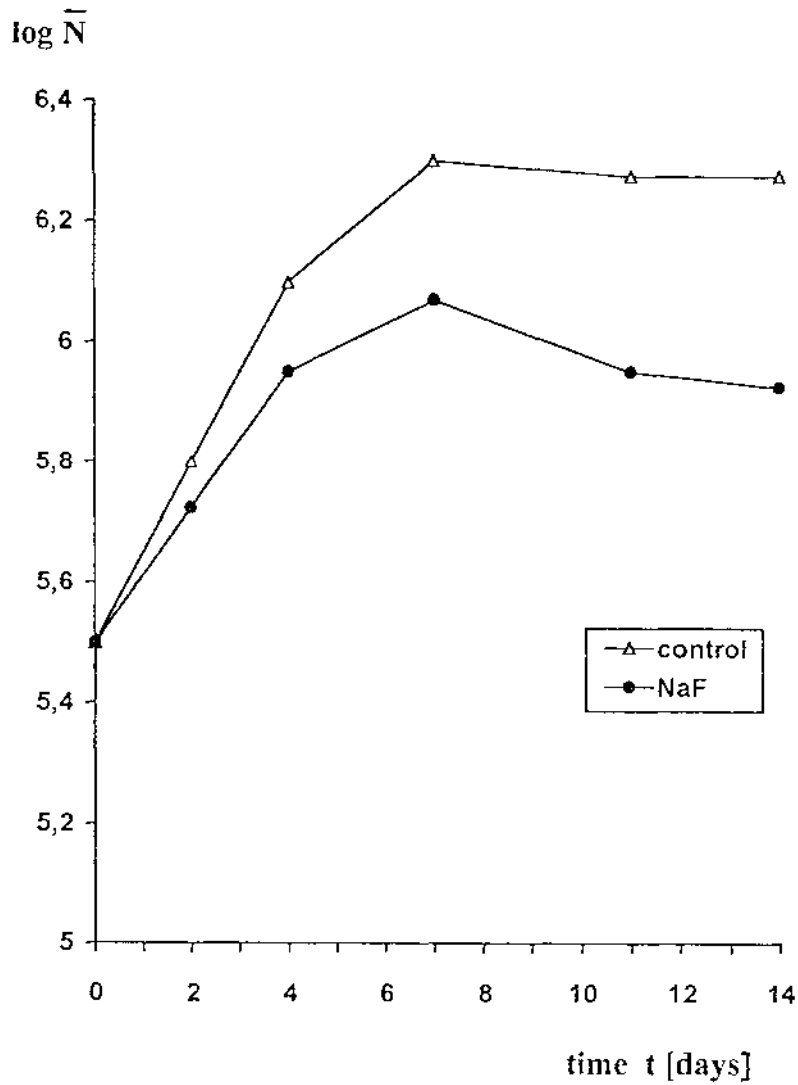
RESULTS

Due to the presence of calcium and magnesium ions in the culture medium, a low concentration of fluoride ions was used in the study, not exceeding the value of CaF_2 and MgF_2 solubility product. This means that the results obtained allow the assessment of fluoride ions effect in an invariable concentration of calcium and magnesium ions in fibroblast cultures, which was of great

significance in eliminating effects of a series of enzymes, particularly those dependent on Mg^{2+} ions presence.

Intensified fibroblasts growth inhibition caused by F^- ions was found during the culture period. Growth characteristics of the fibroblast cultures are shown in Figure 1 as growth curves, allowing determination of the logarithmic growth phase.

Figure 1. Growth curves logN = f(t) of fibroblast cultures with NaF and control cultures



The data in Table 1 indicate that culture growth index - N/N_0 (N = the number of cells per 1 cm^3 of the medium at the end of experiment, N_0 = the number of cells per 1 cm^3 at the beginning of the experiment) decreased in time (for 2-14 days of culturing) in the range 82% to 44% of the values of corresponding control cultures. As a result, in relation to duration, the value of the culture growth rate ($\mu = \frac{\ln N/No}{T}$) decreased from 74 to 54% with the increase in time value of cell doubling period ($T_d = \frac{0,6932}{\mu}$) from 145 to 185%.

Table 1. The effect of NaF on growth of fibroblast cultures

Time	Control					Fibroblasts cultured with NaF					Compared with control (%)		
(days)*	N/N†	$\bar{N}/\bar{N}o$	± S	$\bar{\mu}$	$\bar{T}_d$	N/N	$\bar{N}/\bar{N}o$	± S	$\bar{\mu}$	$\bar{T}_d$	$\bar{N}/\bar{N}o$	$\bar{\mu}$	$\bar{T}_d$
2	1.50-2.90	2.27	0.61	0.39	49	1.33-2.37	1.86	0.45	0.29	71	82	74	145
4	2.90-6.63	4.58	0.64	0.37	47	2.17-3.90	3.08	0.75	0.27	65	68	73	138
7	4.90-8.70	6.77	0.65	0.27	63	2.87-5.10	4.0S	0.76	0.20	87	60	74	138
11	4.63-8.37	6.52	0.62	0.17	101	2.47-3.83	3.09	0.59	0.10	171	47	59	169
14	4.40-8.03	6.29	0.57	0.13	130	2.37-3.20	2.76	0.36	0.07	241	44	54	185

* average of 9 measurements † defined in the text

At the same time, in the presence of F^- ions, alterations in the size and shape of cultured fibroblasts were observed, as shown in Tables 2 and 3. Fibroblast size decreased with the culture's duration, both in the experimental and control cultures, though fibroblasts cultured with NaF were from the onset (after 24 hours) smaller than those in control cultures (Table 2). Relatively greater changes concerned the fibroblasts area and spherical volume ($V = {}^2/_3 \cdot$area$\cdot$spherical diameter). In comparison with the control culture, the value of these two parameters decreased approximately 20 to 30%. The values of other parameters concerning the size of the fibroblasts, namely, their circumference and spherical diameter ($D = 2 \cdot \sqrt{\frac{Area}{\pi}}$) decreased only 10 to 20%. Alterations in fibroblasts shape parameters (Table 3), namely, their longest dimension and the greatest width seem to be correlated. This dependence is expressed as follows: maximal reduction of the longest dimension corresponds to minimal reduction of the greatest width and vice versa.

The effect of F^- ions on incorporation and ^{35}S content was studied in 3 systems: in fibroblasts, pericellular substance, and medium (3 culture fractions = system I, in GAGs isolated from these fractions - system II, in GAGs electrophoretically separated from sulphate fibroblasts = system III).

The results in the form of dpm (disintegrations per minute) were calculated per 1 mg of fibroblast cellular protein (protein assay average in corresponding fibroblast culture 2, 4 and 7 day old were considered) and also by percentages corresponding to individual components of the systems examined in relation to

TABLE 2. Size parameters of fibroblasts

Time (days)*	Control								Fibroblasts cultured with NaF								Compared with control (%)			
	P	±S	O	±S	D	±S	V	±S	P	±S	O	±S	D	±S	V	±S	P	O	D	V
2	127.50	51.91	48.38	13.27	12.47	2.73	1145	659	103.85	26.04	44.85	4.43	11.22	1.44	813	304	81 P < 0.01	93 P < 0.05	92 P < 0.05	71 P < 0.01
4	113.85	23.95	44.17	6.27	11.98	1.22	928	304	87.36	23.89	37.59	6.46	10.64	1.41	630	266	77 P < 0.01	85 P < 0.01	87 P < 0.01	68 P < 0.01
7	103.86	18.32	43.71	4.52	11.45	1.05	805	205	81.24	19.15	37.22	5.14	10.10	1.18	562	202	78 P < 0.01	85 P < 0.01	88 P < 0.01	70 P < 0.01

* Average of 9 measurements P = area (μm^2) O = circumference (μm) D = spherical diameter (μm) V = spherical volume (μm^3)

TABLE 3. Shape parameters of fibroblasts

Time (days)*	Control						Fibroblasts cultured with NaF						Compared with control (%)		
	L	±S	B	±S	F	±S	L	±S	B	±S	F	±S	L	B	F
2	17.08	4.40	11.53	3.40	0.69	0.14	16.68	2.52	9.37	1.67	0.64	0.10	98	81 P < 0.01	93 P < 0.05
4	16.47	2.32	10.38	1.83	0.74	0.11	13.59	2.53	9.81	1.62	0.77	0.08	83 P < 0.01	95 0.05 < P < 0.01	104 0.05 < P < 0.01
7	15.44	1.51	10.63	1.82	0.69	0.13	13.62	1.65	8.11	1.72	0.74	0.11	88 P < 0.01	86 P < 0.01	107 P < 0.05

* Average of 9 measurements L = longest dimension (μm) B = greatest width (μm) F = shape factor

total dpm value of a particular system set equal to 100%. The changes of ^{35}S percentages are shown in Tables 4 to 6.

Table 4. System I - Percentage of radioactive ^{35}S in fibroblast cultures

	Control	With NaF
Fibroblasts	0.4	0.4
Pericellulars	3.8	2.7
Medium fractions	95.8	96.9

Table 5. System II - % of ^{35}S in the GAG isolated from fibroblast cultures

	Control	With NaF	
Fibroblasts	9	17.0	(twice control)
Pericellulars	21	11.5	(half control)
Medium fractions	71	71.5	

Table 6. System III - % of ^{35}S in the GAG isolated from fibroblast cultures

	Control	With NaF	
Heparan Sulphate	40	37	(8% < control)
Dermatan Sulphate	26	42	(81% > control)
Chondroitin Sulphate	34	21	(38% < control)

The changes in system I seen in Table 4, caused by F^- ions acting on fibroblast cultures, are limited to pericellular substance. A slight decrease in ^{35}S content is seen in pericellular substance in relation to the control, expressed in a decrease of pericellular substance ^{35}S percentage from 3.8 to 2.7%. This change is explained in the light of further results, concerned directly with sulphated GAG. These results, obtained in system II, related to sulphated GAG movement among individual fractions of fibroblast cultures are shown in Table 5. In cultures with NaF, a nearly twofold increase (from 9 to 17%) of ^{35}S content in GAG isolated from pericellular substance was found. This is an obvious sign of F^- ions partly inhibiting sulphated GAG permeation from fibroblasts to pericellular substance. The observed phenomenon may also be the cause of the earlier noted decrease of ^{35}S content in fibroblast pericellular substance in system I (Table 4). The results concerning content alterations of sulphated GAG contained in fibroblasts in system III are shown in Table 6. As a result in GAG isolated from cells cultured with F^-, a 61% increase of ^{35}S content bound with DS and a 38.8% decrease, respectively, of ^{35}S content bound with CS and HS was found.

DISCUSSION

Cell cultures are a primary research model replacing experimental animals in many areas of biological investigation. In studies concerning the effect of fluoride ions on animal organisms, cultures of different connective tissue cells are most often used - osteoblasts,[16-23] odontoblasts,[7] and fibroblasts.[24-29] In the present study fibroblast control cultures grew rapidly with 3 growth phases distinctly seen: the introductory phase (up to 24 hours), the logarithmic phase (from 2nd to 7th day), and stationary phase (from 7th day). The occurrence of a relatively long 5-day logarithmic phase during the growth of fibroblasts culture proved its usefulness in the studies performed here.

The observed inhibition of fibroblasts, intensifying with time, was due to the presence of F^- ions. Growth inhibition has its onset in the introductory phase and intensifies in the logarithmic phase, which consequently causes a significant decrease of fibroblasts in the stationary phase. This inhibition might indicate that the presence of F^- ions is favourable for lethal effects, occurring in each growth phase of the culture. A growth inhibition and lethal effects as the result of the action of F^- ions on fibroblast cultures were previously discussed by Sato *et al*,[27] Oguro *et al*,[30] and Veron *et al*[31]

Interesting results were obtained from the morphometric analysis of fibroblasts. Due to fluoride ions, fibroblasts decreased in area and spherical volume in relation to fibroblasts of control cultures. Simultaneously, smaller (in comparison with area and volume alterations) changes of fibroblasts shape (circumference and diameter) that were found might suggest folding of their surface (area). Similarly, Sato *et al*[27] observed folding of the surfaces of fibroblasts cultured with F^- ions.

Among numerous research papers on the effects of F^- ions on cell cultures, there are few reports[5,8,32-34] about the impact of these ions on sulphur metabolism and GAG sulphate. It is known that the contents of these latter compounds and the degree of their polymerization and sulphation change as the organism grows older; for instance in the skin, there is an increase of DS content and a decrease in HA content. An increase of DS content in dental tissues and in osseous tissue under the effect of F^- ions was observed by Susheela and Sharma.[32,33]

At the same time, these authors found a decrease in the molecular weight of GAG and more pronounced changes caused by F^- ions in relation to the age of experimental animals. Waddington *et al*[34] indicate F^- ions act on odontoblasts *in vitro* with a tendency to decrease GAG sulfate synthesis. Depending on the F^- ions concentration used in culture media, 3 or 6 mM/dm^3, there was an increase in CS or DS activity, respectively, as for the sulphate GAG content in cells. Simultaneously, they observed a decrease in the molecular weight of proteoglycans isolated from the odontoblasts. Investigating the segments of pig's skin, Ammitzboll *et al*[5] found no changes in sulphate GAG content in tissue, and only not totally unequivocal changes of CS, DS and HS content.

The study we performed using sulphur in fibroblast cultures introduced into a culture as SO_4^{2-} ions, focused on individual culture fractions (system I), in GAG isolated from these fractions (system II), and in some sulphated GAG isolated from fibroblasts (system III). These experimental systems are different in the possibilities and type of sulphate ions acceptors in them. In this way, the simplest is the system III in which all components belonging to sulphate GAG (DS, HS and CS), are potential acceptors of SO_4^{2-} ions. As for system II, apart from these acceptors, there may be, belonging to GAG, but not undergoing sulphation, hyaluronic acid - HA. In system I there is a possibility of occurrence other than GAG, of sulphate ions acceptors and their use in other processes, *i.e.* after reduction of these ions, that cannot be ruled out.

Changes in system I, as the result of F^- ions activity on cultured fibroblasts, are in fact limited only to pericellular substance fraction. The observed decrease in relation to control of ^{35}S content in pericellular substance, due mainly to sulphate GAG movement, without any increase in sulphur content of the fibroblasts, might suggest partial inhibition of GAG permeation from fibroblasts to pericellular substance.

The results in system II illustrated in Table 4 confirm this tendency. Changes concerning a decrease in ^{35}S content in sulphated GAG isolated from pericellular substance and a corresponding increase of its content in tested GAG isolated from fibroblasts, definitely indicate partial inhibition of sulphate GAG permeation from fibroblasts to pericellular substance caused by fluoride ions. The fact that in system I there was no corresponding balance of these changes may be due to the lack of technical potential, combined with an unsatisfactory quantity of stained ^{35}S content in the fibroblast fractions.

The results concerning GAG isolated from individual fractions of the fibroblast cultures were enlarged by qualitative and quantitative analysis of sulfated GAG present in them. The results obtained in system III, illustrated in Table 6, indicate fluoride ion interference, probably not only in the diffusion process of individual sulphated GAG synthetized in fibroblasts in pericellular substance, but also in synthesis and metabolism of the GAG. Changes in the range of ^{35}S content, incorporated into individual sulphate GAG of fibroblasts, caused by F^- ions, indicate a significant rise in the intensity of synthesis and/or the degree of DS sulphation and a decrease in the intensity of these processes in relation to CS and HS.

The results of this study concerning the effect of trace amounts of F^- ions on fibroblast cultures, indicate an influence on both the growth of these cultures and the size and shape of fibroblasts, as well as synthesis and/or sulphation of sulphated GAG and their diffusion from fibroblasts into pericellular substance.

CONCLUSIONS

1. It appears that even at low concentrations F^- ions may have a toxic effect on fibroblast cultures, as seen to their growth inhibition and a decrease in the size of fibroblasts, also accompanied by alterations in their shape.

2. F^- ions significantly modify sulphur incorporation, in the form of SO_4^{2-} ions, into glycosaminoglycans isolated from individual fractions of these cultures and into dermatan, heparan and chondroitin sulphates present in fibroblasts.

3. In relation to the above, F^- ions cause some changes in the biosynthesis and/or sulphation of glycosaminoglycans in fibroblasts, particularly in relation to an increase in the dermatan sulphate content in relation to heparan and chondroitin sulphates.

4. The observed changes in the intensity of biosynthesis and/or the degree of glycosaminoglycans sulphation in fibroblasts may be accompanied by partial limitation of GAG diffusion outside the cell to pericellular substance.

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AMELIORATION OF FLUORIDE TOXICITY BY VITAMINS E AND D IN REPRODUCTIVE FUNCTIONS OF MALE MICE

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SUMMARY: Studies on the beneficial effects of vitamins E and D supplementation on functions of caput and cauda epididymides, their spermatozoa, vas deferens and seminal vesicle of sodium fluoride (NaF) treated (10 mg/kg body weight) male mice *(Mus musculus)* were carried out. The NaF treatment resulted in significant decrease in the body and epididymis weight but those of vas deferens and seminal vesicle were not affected. NaF treatment brought about alterations in epididymal milieu as elucidated by the significant decrease in levels of sialic acid and protein as well as activity of ATPase in epididymides. As a result, the sperm maturation process was affected leading to a significant decline in cauda epididymal sperm motility and viability. This caused a significant reduction in fertility rate. The cauda epididymal sperm count was also significantly reduced. The data obtained suggest that fluoride treatment induced significant metabolic alterations in the epididymides, vas deferens and seminal vesicles of mice. The withdrawal of NaF treatment (30 days) produced incomplete recovery. On the other hand, sup-plementation of vitamins E or D during the withdrawal period of NaF treated mice was found to be very beneficial in recovery of all NaF induced effects, thus elucidating their ameliorative role in recovery from toxic effects of NaF on the reproductive functions and fertility. On the whole, a combination of vitamins E and D treatment was comparatively more effective than that with vitamin E or D alone. Therefore, vitamin therapy could be beneficial for the amelioration of fluoride induced changes in reproductive functions.

Key words. Epididymis; Fluoride toxicity; Reversibility; Seminal vesicle; Vas deferens; Vitamin D; Vitamin E.

INTRODUCTION

Fluoride is one of the potent toxicants to which humans are exposed. Extensive data on skeletal and dental fluorosis are available.[1] However, the effect of fluoride on the structure and metabolism of several soft tissues has been reported recently. Messer *et al*[2] reported that low levels of fluoride in food rendered mice infertile, while a high fluoride diet improved their fertility. These reports were contradicted by Tao and Suttie,[3] whose experiments showed that fluoride did not play any essential role in reproduction. Later Kour and Singh[4] reported that the testicular spermatogenic process was affected in mice administered fluoride at a dose of 500 and 1000 ppm in drinking water. Li *et al*[5] claimed that fluoride did not have adverse effects on spermatogenesis or sperm morphology. Earlier, our laboratory reported that ingestion of 10 or 20 mg sodium fluoride in mice caused alterations in the histology of reproductive organs, morphology of sperm and induced biochemical changes.[6,7]

Recent investigations[8,9] showed that fluoride interferes with the structural and functional integrity of testis, internal milieu of epididymis, vas deferens and also affected the metabolism and morphology of spermatozoa of mice, rats and rabbits and reduces fertility. The above results clearly indicate that some

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reproductive organs. The present study was undertaken because of the above conflicting reports and paucity of data on the toxic effects of fluoride on male reproductive system and its possible reversal by vitamins E and D.

MATERIALS AND METHODS

Healthy, adult male mice *(Mus musculus)* of Swiss strain, weighing between 25 to 30 g obtained from the National Institute of Occupational Health, Ahmedabad, were used for the experiments. They were housed in an air conditioned animal house at a temperature of 26±2°C and exposed to 10 to 12 hours of daylight.

The animals were divided into seven different groups (see protocol in Table 1) and caged separately. Group I (control) mice were maintained on standard diet and water *ad libitum*. Group II (control) mice were fed olive oil orally with vitamins E and D dissolved in the oil. Sodium fluoride (NaF) (Loba Chemie, Bombay, 99% purity) was administered orally to Group III-VII mice at a dose of 10 mg/kg body weight for 30 days using a feeding tube attached to a hypodermic syringe. The dose of NaF was based on the LD_{50} of male mice which is 54.4 mg F^- /kg body weight.[10] Group III mice were sacrificed on the 31st day. NaF treatment of Groups IV-VII animals was withdrawn after 30 days and the animals were maintained on standard diet and water *ad libitum* for a further 30 days to study any reversibility of the induced effects. Additionally, during this 30 day withdrawal period, Group V mice were administered vitamin E (Roche Products Ltd, Bombay), Group VI mice received vitamin D_3 (Teva Pharmaceuticals Ind. Ltd, Israel), and Group VII animals received a combination of vitamins D and E. The doses of vitamins D and E were 0.002 μg/day/animal and 2 μg/day/animal respectively. These doses were based on earlier studies.

Table 1. Experimental Protocol

Group	Treatment and dose	Duration (days)	Day of autopsy	No. of animals used
I	Untreated control	-	Sacrificed with treated	20
II	Vehicle (olive oil) treated control	-	Sacrificed with treated	20
III	Sodium fluoride (NaF, 10 mg/kg body weight)	30	31st	20
IV	NaF as in Group III, on 31st day NaF withdrawal for further 30 days	30 + 30	61st	20
V	NaF as in Group III, on 31st day NaF withdrawal + vit.E for further 30 days	30 + 30	61st	20
VI	NaF as in Group III, on 31st day NaF withdrawal + vit.D for further 30 days	30 + 30	61st	20
VII	NaF as in Group III, on 31st day NaF withdrawal + vit.E + vit.D for further 30 days	30 + 30	61st	20

The control and treated animals were sacrificed by cervical dislocation. The caput and cauda epididymides, vas deferens and seminal vesicle were carefully dissected out, blotted free of blood, weighed on a Roller Smith (USA) torsion balance and utilized for the various parameters as follows:

Biochemical studies

Sperm motility and count: The cauda epididymal sperm suspension was prepared in normal saline. The percent motility and count of cauda epididymal spermatozoa of normal and all treated groups of mice were determined by the method of Prasad *et al*[11] and expressed as percentage motility and millions/ml respectively.

Sperm viability: The ratio of live:dead spermatozoa of control and all treated groups of mice were determined using 1% trypan blue as described in the method of Talbot and Chacon.[12] The number of stained and unstained spermatozoa were scored in 10-20 separate fields and percentages (%) were calculated.

Fertility test: Conducted by cohabiting estrous or proestrous female animals with treated male animals in a ratio of 2:1 on the 31st day of treatment according to the WHO protocol MB50.[13] The vaginal smear was checked the next morning for the presence of sperm, which indicated that mating had occurred. This was day one of pregnancy. The animals were autopsied on day 16 of pregnancy and the implantation sites were counted and compared with the control.

Protein: The protein levels of caput and cauda epididymides and vas deferens of control and all treated animals were estimated by the method of Lowry *et al*[14] at 540 nm on a Spectronic-88 Bausch and Lomb spectrophotometer. Protein was expressed as mg/100 mg fresh tissue weight.

Adenosine Triphosphatase (ATPase) (E.C. 3.6.1.3): The ATPase activity in epididymides of control and all treated groups of mice were assayed following the method of Quinn and White[15]. The activity was expressed as μ moles of ip released/mg protein/30 minutes.

Sialic acid: Sialic acid in the epididymis of both control and treated animals was determined by the method of Jourdian *et al*[16] and was expressed as μg/mg fresh tissue weight.

Phosphorylase (E.C. 2.4.1.1): Phosphorylase activity in vas deferens of control and treated mice was determined by the method of Cori *et al*[17] and the enzyme activity was expressed as μg phosphorus released/mg protein/15 minutes.

Glycogen: The glycogen concentrations in vas deferens of control and treated mice were estimated by the method of Seifter *et al*[18] and expressed as μg glycogen/100 mg fresh tissue weight.

Fructose: Fructose concentration in the seminal vesicles was estimated by the method of Foreman *et al*[19] and was expressed as μg/mg tissue weight.

Statistics: For all biochemical estimations, a minimum of 8 to 10 replicates were done for each parameter. The data were statistically analysed using student's t test and Analysis of Variance (ANOVA) followed by Scheffe's test for least significance.

RESULTS

Body weight: The body weight of the NaF treated Group III animals decreased ($P<0.001$) after 30 days of treatment in comparison to both the control Groups I-II. After 30 days of NaF withdrawal, the body weight of Group IV mice did not recover as compared to control Group I. On administration of vitamins E or D alone and in combination, the body weights of Groups V-VII animals recovered significantly ($P<0.001$) compared to control Group II (Table 2).

Organ weight: The weights of caput and cauda epididymides in Group III mice declined significantly ($P<0.01$) compared to control Groups I-II. In Group IV (withdrawal) the recovery was less and the difference was not significant as compared to control, whereas significant recovery was obtained in Group V (vit E), VI (vit D) and VII (vit E + vit D). However, weights of seminal vesicle and vas deferens were not affected by different treatments (Table 2).

TABLE 2. Body (g) and organ weight (mg) of control and treated groups of mice

Groups	Body Weight (g)	Organ Weights (mg)			
		Caput Epididymis	Cauda Epididymis	Vas deferens	Seminal Vesicle
I	35.6 ± 0.19	14.0 ± 0.17	12.0 ± 0.4	11.6 ± 0.35	56.0 ± 0.37
II	36.5 ± 0.21	14.8 ± 0.33	12.6 ± 0.6	12.0 ± 0.20	56.9 ± 0.29
III	30.4 ± 0.24**	11.2 ± 0.30*	9.0 ± 0.5*	13.2 ± 0.33^{NS}	58.4 ± 0.16^{NS}
IV	33.5 ± 0.20^{NS}	13.0 ± 0.28^{NS}	10.6 ± 0.4^{NS}	12.4 ± 0.21^{NS}	57.2 ± 0.21^{NS}
V	37.6 ± 0.23**	15.0 ± 0.28*	13.8 ± 0.17*	12.8 ± 0.23^{NS}	57.0 ± 0.19^{NS}
VI	37.2 ± 0.20**	14.2 ± 0.20^{NS}	13.4 ± 0.21*	12.6 ± 0.19^{NS}	57.6 ± 0.22^{NS}
VII	37.8 ± 0.26**	15.2 ± 0.23*	13.9 ± 0.13*	11.9 ± 0.16*	57.9 ± 0.17^{NS}

Values are Mean ± S.E. * $P<0.01$ ** $P<0.001$ NS = Non Significant
Groups: I = Untreated Control; II = Vehicle (Olive oil) treated control; III = NaF treated; IV = NaF withdrawal for 30 days; V = NaF as in III, withdrawal on day 31 and vit E for further 30 days; VI = NaF as in III, withdrawal on day 31 and vit D for further 30 days; VII = NaF as in III, withdrawal on day 31 and vits E + D feeding for further 30 days

Sperm motility: The motility of cauda epididymal sperm of Group III NaF treated mice decreased significantly ($P<0.001$) as compared to control Groups I-II. At the end of the withdrawal phase, sperm motility recovered in Groups IV-VII, but was still lower than control Groups I-II. Group IV had significant but relatively low recovery ($P<0.01$). Sperm motility of Group V on vitamin E, and Group VII on vitamins E and D recovered comparatively better than Group VI on vitamin D but all three groups recovered significantly better ($P<0.001$) than Group IV (Tables 3A and 3B).

Sperm count: The sperm count in the cauda epididymis of Group III NaF treated mice declined significantly ($P<0.001$) compared to control Groups I-II. In Group IV (withdrawal) significant ($P<0.01$) recovery was obtained, whereas vitamin E or D treated animals showed very significant ($P<0.001$) recovery as compared to the NaF treated (Group III) mice which was again more with vitamin E. In Group VII the recovery was most significant ($P<0.001$) (Tables 3A and 3B).

Sperm viability: Cauda epididymal sperm viability (live:dead ratio) was significantly reduced ($P<0.001$) by NaF treatment as compared to control groups I-II. However, significant ($P<0.001$) recovery was obtained in Groups IV, V and VI (withdrawal, vit E, vit D). In the latter two groups, recovery was more than in Group IV. In Group VII almost complete recovery was noted and was comparable to normal values (Table 3A).

Table 3A. Cauda epididymal sperm motility, count, viability and fertility rate of control and treated groups of mice

Parameters	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Sperm motility (%)	75.28 ± 1.58	76.19 ± 1.34	26.31 ± 0.55**	43.73 ± 0.79*	67.42 ± 0.73**	52.46 ± 0.91**	68.66 ± 0.92**
Sperm count (millions/ml)	42.33 ± 0.45	43 ± 0.81	22 ± 0.81**	28.6 ± 0.55*	35.5 ± 1.70**	31.66 ± 1.37**	38.0 ± 0.59**
Sperm viability (%) Live : dead	72.15:27.85 ± 0.50	73.62:26.38 ± 0.54	13.89:86.11 ± 0.44**	39.67:60.33 ± 0.31**	66.52:33.48 ± 0.42**	59.23:40.77 ± 0.33**	69.98:30.0 ± 0.39**
Fertility Rate (%)	95 - 100 +ve	95 - 100 +ve	0**	27.97 ± 3.4** +ve	72.31 ± 0.77** +ve	65.47 ± 2.36** +ve	78.13 ± 0.93** +ve

Values are Mean ± S.E. * P<0.01 ** P<0.001 +ve = positive
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 3B. ANOVA of cauda epididymal sperm motility and count

Source of variation	df	SS	MSS	f(cal)	f(tab)
Sperm motility					
Groups	6	12629.82	2104.97	13.40	2.36
Residual	35	5495.46	157.01		
Sperm count					
Group	6	2048.41	341.40	279.83	2.26
Residual	35	42.74	1.22		

Significance at 5% level
df = Degree of Freedom SS = Sum of Squares MSS = Mean Sum of Square

Fertility rate: Fluoride treatment led to a significant ($P<0.001$) inhibition of fertility rate as compared to control Groups I-II. The fertility was restored significantly ($P<0.001$) upon withdrawal of treatment for 30 days as compared to NaF treated animals (Group III), but ingestion of vitamin E or D in Groups V and VI resulted in very significant ($P<0.001$) restoration of fertility which was more in Group V (vit E) and in Group VII (vit E + vit D) (Table 3A).

Protein: The protein levels in the caput and cauda epididymides and vas deferens decreased significantly ($P<0.001$) after 30 days of NaF treatment in Group III as compared to control Groups I-II. The protein levels in Group IV (withdrawal) showed insignificant recovery after 30 days except in cauda epididymis where recovery was comparatively more ($P<0.01$). However, administration of vitamins E or D (Groups V-VI) resulted in significant recovery in protein levels in caput and cauda epididymides ($P<0.001$) and vas deferens ($P<0.01$). In Group VII the protein levels recovered to almost the same levels as in control mice (Tables 4A, 4B).

ATPase: Activity of ATPase in caput and cauda epididymides of NaF treated mice showed a significant ($P<0.001$) decrease in comparison to both the control Groups I-II. The recovery was less in Group IV (withdrawal) but significant ($P<0.001$) recovery was obtained in caput and cauda epididymides in Groups V and VI (vitamins E and D treated). Combination of vitamin E + vitamin D treatment brought about more recovery than vitamin E or vitamin D alone (Tables 5A and 5B).

Sialic acid: Levels of sialic acid in caput and cauda epididymides declined significantly ($P<0.01$) after NaF treatment as compared to both the control Groups I and II. However, by withdrawal of treatment (Group IV) and subsequent administration of vitamin E or D (Groups V, VI), significant ($P<0.01$) recovery was noted as compared to control mice. The recovery was most significant ($P<0.001$) in Group VII where combination of vit E and vit D was given (Tables 6A and 6B).

Glycogen: A significant ($P<0.001$) accumulation of glycogen in the vas deferens of NaF treated mice was observed as compared to control Groups I-II. In Group IV the recovery was not significant. On the other hand, significant recovery was obtained after vitamin E ingestion (Group V) ($P<0.001$) and in Group VI ($P<0.01$) as compared to NaF treated mice. However, in Group VII (vit E and vit D) significant ($P<0.001$) recovery was observed (Tables 7A and 7B).

Phosphorylase: NaF treatment resulted in significant ($P<0.001$) suppression of vas deferens phosphorylase activity as compared to control Groups I-II. However, after withdrawal of treatment and administration of vitamins E or D (Groups V-VI), the activity of phosphorylase was restored back to normal (Groups IV, V, VI). The recovery was comparatively less in Group IV but was significant ($P<0.001$) with both vitamin treatments. Combined treatment of vit E and vit D was the most effective in restoring enzyme activity to almost normal levels (Tables 7A and 7B).

Fructose: Fructose levels in seminal vesicle increased significantly ($P<0.001$) after NaF treatment for 30 days as compared to control Groups I-II. The recovery was significant ($P<0.01$) by withdrawal (Group IV) of treatment. On administration of vitamins E or D (Groups V-VI) the levels of fructose recovered significantly ($P<0.001$). Moreover, the levels of fructose in vit E + vit D treated mice (Group VII) were also significantly ($P<0.001$) restored to normal as compared to NaF treated mice (Tables 7A and 7B).

TABLE 4A. Protein levels (mg/100 mg fresh tissue weight) in caput and cauda epididymides and vas deferens of control and treated groups of mice

Organs	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Caput Epididymis	11.43 ± 0.28	12.55 ± 0.14	8.47 ± 0.20**	9.25 ± 0.16^{NS}	11.00 ± 0.24**	10.61 ± 0.19**	12.0 ± 0.18**
Cauda Epididymis	14.10 ± 0.19	15.18 ± 0.37	8.52 ± 0.44**	9.89 ± 0.12*	12.92 ± 0.33**	11.48 ± 0.16**	13.62 ± 0.29**
Vas Deferens	14.09 ± 0.09	14.43 ± 0.21	8.87 ± 0.20**	9.82 ± 0.17^{NS}	11.79 ± 0.26*	11.77 ± 0.15*	12.98 ± 0.18**

Values are Mean ± S.E. * P<0.01 ** P<0.001 NS = Non Significant
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 4B. ANOVA of caput and cauda epididymis and vas deferens protein

Source of variation	df	SS	MSS	f(cal)	f(tab)
Caput epididymal protein					
Groups	6	81.75	13.62	38.91	2.36
Residual	35	10.86	0.35		
Cauda epididymal protein					
Groups	6	2047.43	341.23	494.53	2.36
Residual	35	24.49	0.69		
Vas deferens protein					
Group	6	135.94	22.65	266.47	2.44
Residual	28	2.38	0.085		

Significance at 5% level
df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

TABLE 5A. Adenosine triphosphatase activity (μ moles of ip released/mg protein/15 minutes) in caput and cauda epididymides of control and treated groups of mice

Organs	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Caput Epididymis	1.99 ± 0.04	2.01 ± 0.06	0.93 ± 0.07**	1.10 ± 0.03^{NS}	1.69 ± 0.08*	1.65 ± 0.06*	1.78 ± 0.05**
Cauda Epididymis	2.08 ± 0.07	2.12 ± 0.09	0.978 ± 0.03**	1.23 ± 0.05*	1.87 ± 0.08**	1.79 ± 0.10**	1.96 ± 0.08**

Values are Mean ± S.E. * P<0.01 ** P<0.001 NS = Non Significant
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 5B. ANOVA of caput and cauda epididymal ATPase

Source of variation	df	SS	MSS	f(cal)	f(tab)
Caput epididymal ATPase					
Groups	6	5.42	0.90	18.06	2.42
Residual	28	1.53	0.05		
Cauda epididymal ATPase					
Group	6	5.82	0.97	161.66	2.42
Residual	28	0.17	0.006		

Significance at 5% level
df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

TABLE 6A. Sialic acid levels (µg/mg tissue wt) in caput and cauda epididymides of control and treated groups of mice

Organs	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Caput Epididymis	4.72 ± 0.03	4.89 ± 0.02	3.76 ± 0.07*	4.14 ± 0.02^{NS}	4.45 ± 0.05*	4.34 ± 0.02*	4.60 ± 0.06**
Cauda Epididymis	5.56 ± 0.06	5.59 ± 0.09	4.48 ± 0.07*	5.05 ± 0.04*	5.37 ± 0.04*	5.18 ± 0.02*	5.47 ± 0.03**

Values are Mean ± S.E. * P< 0.01 ** P< 0.001 NS = Non Significant
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 6B. ANOVA of cauda and caput epididymal sialic acid

Source of variation	df	SS	MSS	f(cal)	f(tab)
Caput epididymis					
Groups	6	5.59	0.93	31.05	2.36
Residual	35	1.11	0.03		
Cauda epididymis					
Groups	6	6.32	1.05	87.77	2.36
Residual	35	0.45	0.012		

Significance at 5% level
df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

TABLE 7A. Showing glycogen concentration and phosphorylase activity in vas deferens and fructose level in seminal vesicle of control and treated groups of mice

Organs	Parameters	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Vas Deferens	Glycogen (µg/100mg fresh tissue wt)	776.54 ± 20.65	815.85 ± 19.76	1281.29 ± 15.36* *	1181.29 ± 5.36^{NS}	858.35 ± 11.56* *	1058.86 ± 19.92*	829.67 ± 13.63**
	Phosphorylase (µg phosphorus/mg protein/15 min)	53.17 ± 1.45	54.0 ± 1.32	36.76 ± 0.88**	42.30 ± 1.01*	51.07 ± 1.75**	45.22 ± 1.06**	52.01 ± 1.68**
Seminal Vesicle	Fructose (µg/mg tissue wt)	14.84 ± 1.11	14.98 ± 1.16	20.83 ± 1.20**	17.62 ± 0.99*	16.37± 0.90**	16.97 ± 1.01**	15.23 ± 0.93**

Values are Mean ± S.E. * P< 0.01 ** P< 0.001 NS = Non significant

TABLE 7B. ANOVA of glycogen, phosphorylase and fructose

Source of variation	df	SS	MSS	f(cal)	f(tab)
Glycogen (vas deferens)					
Groups	6	1293659.4	215609.9	8.20	2.44
Residual	28	735336.6	26262.02		
Phosphorylase (vas deferens)					
Groups	6	5697.17	949.52	5.96	2.42
Residual	28	4458.4	159.22		
Fructose (seminal vesicle)					
Group	6	123.12	20.52	18.48	2.42
Residual	28	31.19	1.11		

Significance at 5% level df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

DISCUSSION

The present investigation was carried out to explore the effects of fluoride (NaF) and the possible ameliorative role of vitamins E and D ingestion on epididymis, vas deferens and seminal vesicle of mice during withdrawal period.

The sodium fluoride treatment caused a decrease in the body weight. Similar results were reported by others in rats and mice fed with different concentrations of fluoride.[8,20] An insignificant reduction in weight of epididymis occurred but those of seminal vesicle and vas deferens were not affected.

In the present study the levels of protein in caput and cauda epididymides and vas deferens showed a significant decrease after 30 days of NaF treatment. This decrease might be due to impairment of protein metabolism/synthesis. Earlier studies carried out from our laboratory and elsewhere have also reported a dose dependent decrease in protein levels in the serum and reproductive organ of mice, rats and rabbits.[7,8,21]

Sialic acid is an important constituent of mucopolysaccharides and sialomucoproteins which are essential for the maturation of spermatozoa in epididymis and maintenance of the structural integrity of their membranes.[22] The levels of sialic acid in caput and cauda epididymides were decreased in the present study. Hence it is likely that the structural integrity of acrosomal membrane of the sperm might have been altered. A decline in sialic acid concentration of NaF treated mouse and rat epididymis has also been reported earlier.[8]

Adenosine triphosphatase activity in caput and cauda epididymides showed a decline with greater propensity in cauda epididymis. Alterations in the activity of ATPase in NaF treated mouse and rat epididymis and rabbit spermatozoa have been reported.[8,21] It was reported[23] that fluoride acts directly on the motile apparatus without substantially affecting other metabolic pathways as it inhibits the dynein ATPase in sperm. According to Hodge and Smith,[24] NaF toxicity involves inhibition of enzyme activity, particularly those in which divalent metal cations act as co-factors. In the present study too, the alterations in ATPase activity might be related to the fact that it is either a Mg^{2+} or a Ca^{2+} activated enzyme.

Reports from our laboratory have revealed that the glycogen concentrations were enhanced in vas deferens of fluoride treated rats and mice.[7,25] These results are in agreement with the observations of the present investigation, wherein glycogen was found to accumulate in vas deferens of NaF treated mice. The increase in glycogen could be correlated with the decrease in the activity of phosphorylase in the vas deferens by NaF ingestion. Fluoride has been reported to alter carbohydrate metabolism mainly by causing allosteric inhibition of some key enzymes in glycolysis and tricarboxylic acid (TCA) cycle.[26]

The increase in level of fructose in seminal vesicle after 30 days of NaF exposure further supports our observation on alteration in carbohydrate metabolism of fluorotic mice. As fructose has a vital role in providing energy to the sperm, it is evident that the increased fructose level might influence sperm metabolism.

Fluoride is known to inhibit sperm motility, glycolysis and respiration process. It has been demonstrated[27] that bovine sperm treated with 30 mM fluoride became immobile within two minutes. Human spermatozoa lost their motility *in vitro* in the presence of 250 mM NaF within 20 minute incubation.[28] In the present study too, a significantly low sperm motility was obtained by NaF ingestion.

The sperm density of cauda epididymis in NaF treated group of mice also declined significantly. The decrease could be correlated with the testicular spermatogenic arrest following fluoride ingestion in mice, rats and rabbits.[8,21]

The evaluation of NaF treated spermatozoa stained with trypan blue showed a large number of dead sperm, probably due to loss of membrane permeability, which might be another major factor in the decline in sperm motility.[25]

The above mentioned alterations in sperm motility, density and metabolism might be the outcome of the altered and hostile internal milieu of the epididymis of NaF treated mice since it is known that normal epididymal structure and its internal microenvironment are important for sperm maturation and for maintaining them in a viable, motile state.[22,29,30]

The reduction in sperm motility, count, viability and changes in their metabolism led to the significant decline in fertility of treated mice as also reported earlier in mice, rats and rabbits.[8,21,31]

In the present study, NaF treatment affected numerous androgen dependent parameters in the epididymis, vas deferens and seminal vesicle to a variable extent. Hence, it is evident that fluoride affects male reproductive organs and fertility.

In withdrawal group of animals (Group IV) the NaF induced effects were not restored completely to normal state after 30 days. However, in Groups V, VI, VII of animals treated with vitamin E or vitamin D alone and in combination in the withdrawal period, almost complete recovery from fluoride toxicity was obtained. The extent of recovery was more pronounced with vitamin E as compared to D and was most significant with the combined treatment.

In rats, the main symptoms of vitamin E deficiency are degeneration of the testis, abnormalities of gestation, regression in the ovary and changes in ovulation.[32] At the cellular-molecular level, vitamin E is believed to exert its protective effect primarily through destruction of cell-damaging free-radical oxygen species.[33] In the present study too, vitamin E ingestion was beneficial for recovery from fluoride toxicity.

According to earlier reports, elevated levels of vitamin D mitigated the symptoms of fluorosis. Studies carried out by Gupta *et al* 1996[34] have revealed that the treatment of vitamins C, D and calcium showed a significant improvement in the skeletal, clinical fluorosis and biochemical parameters in children consuming water containing 4.5 ppm of fluoride.

The above reports and earlier work carried out by Chinoy and associates have elucidated that therapeutic agents like amino acids, vitamin C and Ca^{2+} could mitigate fluoride induced effects.[8,35,36]

Thus, in conclusion, sodium fluoride has a definite effect on reproduction. However, the fluoride induced effects are reversible and transient and could be effectively reversed by withdrawal of treatment and subsequent supplementation of vitamins E and D. Thus vitamins E or D may be used as therapeutic agents for the mitigation of fluoride induced toxicity in endemic areas all over the world. Hence, these results have important implications for amelioration of fluorosis in endemic regions.

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FLUORINE AND BIOELEMENTS IN BIOLOGY AND MEDICINE
Report on the 8th Polish Fluorine Symposium
April 23-24,1998, Szczecin, Poland

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The 8th Fluorine Symposium was held in Szczecin on April 23-24, 1998 under the title "Fluorine and bioelements in biology and medicine". There were over 70 participants from academic centers in Poland. The program comprised six reports and 32 posters which were published in a book edition of 231 pages. Each Polish publication included titles and summaries in English. In his introductory address Professor Z Machoy explained that the program had been expanded to include problems concerning not only fluorine but other bioelements such as heavy metals. That extension allowed for wider discussion and exchange of viewpoints on the share of these elements in common metabolic exchanges.

The first report, by Dr Miklós Bély from the National Institute of Rheumatology, Budapest, Hungary, who is President of the International Society for Fluoride Research, was entitled " The structure and function of bone tissue and articular cartilage in osteofluorosis". Dr Bély had participated in an earlier fluorine symposium in Szczecin in 1988.

The first Polish lecturer was Prof Z Zablocki of the Agricultural Academy in Szczecin, who presented a paper, "Changes in the fluorine content of some components of the environment in the area affected by the 'Police' Chemical Works emissions in 1977-1996", which described how emissions from this biggest chemical plant caused fluorine contamination of the natural environment - air, water, soil as well as various species of plants - over the last 20 years.

An interesting report was presented by W Czarnowski, K Stolarska and K Krechniak of Gdansk, entitled "Exposure of hair to hydrogen fluoride *in vitro*". It dealt with the kinetics of fluorine accumulation in hair and described the degree to which fluorine is built into hair structure. For the study they utilized special equipment for determining fluorine in the hair of people professionally exposed to HF (hydrogen fluorine).

Extensive investigations were reported by a team of stomatologists from Poznan, directed by Prof M Borysewicz-Lewicka, in a paper entitled "Fluoride levels in infants and small children fed on different diets". The authors demonstrated that children fed naturally with mother's milk had lower levels of urinary fluoride than children fed with unnatural mixtures. However, fluoride supplements to children for stomatological reasons must also contribute.

Workers from the State Veterinary Institute in Pulawy have for some years studied prenatal fluorine toxicology in animals. In her report "Prenatal toxicity of fluorine: summary of results from studies on rodents", Dr Minta described studies conducted on rats and hamsters. At F concentrations higher than 20 mg/L greater teratogenic sensitivity was shown by rats than by hamsters.

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The last of the reports, entitled "Effects of sodium fluoride on bone turnover in growing rats", was presented by Dr A Bohatyrewicz from the Orthopedics and Traumatology Clinic in Szczecin. He described the parameters of bone reconstruction in female rats after administering sodium fluoride in drinking water at various concentrations. He reported a beneficial influence during the period of genital maturation of 6 to 12 weeks.

The poster presentations may be divided into subject groups involving problems associated with: fluorine analysis, enzymology, molecular biology, industrial toxicology, cell culture, and dynamics of fluorine cumulation in living organisms. There were 14 studies on humans, 14 on animals, and 4 on plants. Each presentation was followed by discussion. At the closing of the symposium, participants were informed that the next meeting would take place along with the World Conference of the International Society for Fluoride Research on June 11-14 in the year 2000, in Szczecin.

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FLUORIDATION AND CHILD DENTAL HEALTH IN NEW ZEALAND - AN UPDATE

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SUMMARY: The most recent available statistics indicate that child dental health in New Zealand is still not significantly better in fluoridated areas.

Key words: Child dental health; Dental caries; DMFT; Fluoridation; New Zealand.

New Zealand is unique in that dental health statistics are available for almost the entire child population. These statistics are collected annually for all 12- or 13-year-olds as they leave the care of school dental clinics. The two key pieces of information from each health authority's area are: the average percentage of the children who are free of dental caries; and the average number of decayed, filled and missing teeth, or "DMFT".

More than a decade has passed since studies using these annual surveys compared the state of children's teeth in fluoridated and non-fluoridated areas.[1,2] These studies revealed that, when similar kinds of communities were compared, child dental health (in terms of dental caries prevalence) was slightly better in the nonfluoridated areas. If one considered also the prevalences of dental fluorosis,[3,4] child dental health was substantially better in the non-fluoridated areas.

Being curious to know the present situation, I obtained the Ministry of Health's most recent available (1995) child dental health statistics for my own region (Northland) where I practise dentistry. The results suggest that the situation has not changed:

TABLE 1. Dental caries prevalences in Northland, New Zealand, in 1995

	No. of children	% caries-free	DMFT
Fluoridated	113	46.02	1.04
Non-fluoridated	2106	46.58	1.60

Only one town (Kaitaia) in Northland is fluoridated. The non-fluoridated area comprises other towns and large rural areas which, according to our Official Census, are of low average income. Northland is, in fact, the most poverty-stricken area in New Zealand. Lower-income areas have always had higher tooth decay prevalences. So the small (half-tooth) difference in DMFT between the fluoridated and nonfluoridated parts of the province, and higher decay-free rate in the nonfluoridated part, do not support the claimed benefits of fluoridation

The same information supplied from the central region of New Zealand, which includes the capital city, Wellington (a much more affluent region than Northland) is equally revealing:

TABLE 2. Dental caries prevalences in central region, New Zealand, in 1995

	No. of children	% caries-free	DMFT
Fluoridated	6469	49.73	1.24
Non-fluoridated	5601	49.83	1.39

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The nonfluoridated area in this region contains small-town and rural areas of lower income level than the fluoridated larger towns and cities. Yet there is a slightly higher decay-free percentage in the nonfluoridated area (as in Northland) and only 0.15 of a tooth difference in DMFT.

DISCUSSION AND COMMENT

It is clear from this information that water fluoridation not only does not provide the traditionally claimed "40-60%" reduction in tooth decay, but is of doubtful if any benefit at all. Despite the availability of the above statistics, they receive no publicity in our media. Instead, the public is continually presented with assertions from our health "authorities" that fluoridation is effective and safe. The New Zealand Public Health Commission report in 1994 claimed that immense savings in expenditure on dental treatment resulted from fluoridation.[5] Close examination of its references for that assertion reveal that the claim was based, not on New Zealand statistics, but on a review in 1989 of various pro-fluoridation studies around the world, by a prominent US fluoridation proponent.[6] That review was written before many of the comprehensive studies discrediting fluoridation,[7-11] which were available to the Public Health Commission by 1994, had been published. Also, the author of the 1989 review had omitted the comprehensive studies from New Zealand[1,2] which had by then been published.

The reason why our public health officials and academics cling to their orthodoxy is difficult to find. Could it be because they cannot face the reality that they have for decades been promoting a procedure which is ineffective as well as, from recent evidence,[12] probably unsafe?

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RISK FACTORS FOR FRACTURES IN THE ELDERLY

H Jacqmingadda, A Fourrier, D Commenges and J F Dartigues
Bordeaux, France

Abstract from *Epidemiology* 9 (4) 417-423 1998

We report the results of a 5-year prospective cohort study of risk factors for fractures, including drinking fluoridated water, in a cohort of 3,216 men and women age 65 years and older. We studied risk factors for hip fracture and fractures at other locations separately. We found a higher risk of hip fractures for subjects exposed to fluorine concentrations over 0.11 mg per liter but without a dose-effect relation [odds ratio (OR) = 3.25 for a concentration of 0.11-0.25 mg per liter; OR = 2.43 for greater than or equal to 0.25 mg per liter]. For higher thresholds (0.7 and 1 mg per liter), however, the OR was less than 1. We found no association between fluorine and non-hip fractures. Non-hip fractures were associated with polymedication rather than with specific drug use, whereas fracture was associated with polymedication and use of anxiolytic and antidepressive drugs. Subjects drinking spirits every day were more likely to have hip fractures. Tobacco consumption increased the risk for non-hip fractures.

Key words: Elderly; Fluoridated water; Fracture; Hip Fracture; Osteoporosis; Psychotropic drugs.

Reprints: H Jacqmingadda, 146 Rue Leo Saignat, F-33076 Bordeaux, France.

USE OF TOENAIL FLUORIDE LEVELS AS AN INDICATOR FOR THE RISK OF HIP AND FOREARM FRACTURES IN WOMEN

D Feskanich, W Owusu, D J Hunter, W Willett, A Ascherio,
D Siegelman, S Morris, V L Spate and G Colditz
Boston, Massachusetts, USA

Abstract from *Epidemiology* 9 (4) 412-416 1998

The relation between fluoride intake and risk of osteoporotic fractures remains unclear. The lack of individual measures of long-term fluoride intake has limited epidemiologic studies. We used toenail fluoride in this study as a measure of long-term intake to evaluate the relation between fluoride intake and subsequent risk of hip and distal forearm fractures. Between 1982 and 1984, we collected toenail clippings from 62,641 women in the Nurses' Health Study who were free from cancer, heart disease, stroke, and previous hip or forearm fracture. We identified fracture cases (53 proximal femur and 188 distal forearm) through subsequent biennial mailed questionnaires and matched controls to cases on year of birth. The odds ratio of hip fracture among women in the highest quartile of toenail fluoride (>5.50 parts per million), compared with those in the lowest quartile (<2.00 ppm) was 0.8 (95% confidence interval = 0.2-4.0), with adjustment for menopausal status, postmenopausal hormone use, caffeine intake, and alcohol consumption. The corresponding adjusted odds ratio for forearm fracture was 1.6 (95% confidence interval = 0.8-3.1). Further adjustment for body mass index, smoking status, and calcium and vitamin D intake did not alter these results.

Key words: Bone; Femur; Hip fractures; Osteoporosis; Radius fractures; Water fluoride.

Reprints: D Feskanich, Channing Laboratories, 181 Longwood Ave, Boston, MA 02115, USA.

NEW, OR BIASED, EVIDENCE ON WATER FLUORIDATION

A J Spencer, Adelaide, Australia

Abstract from *Australian and New Zealand Journal of Public Health 22* (1) 149-154 1998

The recent review, 'New evidence on fluoridation', by Diesendorf, Colquhoun, Spittle, Everingham and Clutterbuck (*Australian & New Zealand Journal of Public Health* 1997 21 187-90) claims a consistent pattern of evidence pointing to fluoride damaging bone, a negligible benefit in dental caries reduction from ingested fluoride, and any small benefit from fluoride coming from the action of fluoride at the tooth surface. Public health authorities are allegedly reluctant to pursue such evidence. In the interest of scholarly debate, invited by Diesendorf *et al*, this reaction paper examines six separate areas raised in the original paper: fluoridation and hip fracture; fluoridation and osteosarcomas; pre-eruptive and post-eruptive benefits in dental caries reduction; fluoride ingestion; benefit in dental caries reduction for contemporary Australian children; and bias of health authorities and responsible science. Numerous examples of bias in the identification, selection and appraisal of the evidence on water fluoridation presented by Diesendorf *et al* are developed. Further, this reaction paper puts forward both studies and appraisal indicating that water fluoridation should continue to be regarded as a safe and effective public health measure.

Key words: Dental caries; Fluoridation; Fluorosis; Hip fracture; Osteosarcoma.

Reprints: A J Spencer, University of Adelaide, Department of Dentistry, Adelaide, SA 5005, Australia.

COMMENTS

The arguments advanced in Dr Spencer's lengthy critique were mostly answered in our reply to earlier critics (see *Fluoride 31* (3) pages 166-169 August 1998). In that reply we commented: "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 then dealt in detail with the various small-scale and inadequate studies which our critics, and now Dr Spencer, claimed were adequate rebuttals of our review. In his concluding section Spencer alleges bias because of our "rejecting the findings of groups tasked with reviewing and evaluating the evidence on the safety, effectiveness and efficiency of water fluoridation" – such groups being pro-fluoridation "health authorities" whose reviews he describes as "responsible science".

John Colquhoun

More needs to be said about the so-called "experts" who are alleged to do "responsible science". Perhaps readers could be reminded that earlier this century such experts in the field of medicine

- treated pregnant women with stilboestrol which led to the development of vaginal cancers in their daughters;
- prescribed large oral or intravenous doses of radium salts for circulatory, nervous, endocrine and psychiatric disorders;
- encouraged the over-use of aspirin resulting in outbreaks of kidney disease and gastric ulcers;
- x-rayed pregnant women routinely, resulting in an increase in childhood cancers.

Mark Diesendorf

AGRONOMIC IMPACT OF TEPHRA FALLOUT FROM THE 1995 AND 1996 RUAPEHU VOLCANO ERUPTIONS, NEW ZEALAND

S J Cronin, M J Hedley, V E Neall and R G Smith
Palmerston North, New Zealand

Abstract from *Environmental Geology 34* (1) 21-30 1998

Eruptions from Ruapehu Volcano on 11 and 14 October 1995 and 17 June 1996 distributed at least 36 x 10^6 m^3 of sulphur(S)-rich tephra over the central and eastern North Island of New Zealand. The tephras added between 30-1500 kg ha^{-1} S to at least 25,000 km^2 of land in primary production. Smaller but beneficial amounts of selenium (Se) and in some areas potassium and magnesium were also supplied. Addition of S to the soils in the form of sulphate and elemental S resulted in a drop in soil pH and an increase in pasture S contents within seven weeks of the eruptions. The soils affected by the tephra are naturally low in S and Se, but following the eruptions S was not required in fertilizer applications in many areas. The strongest and longest lasting effects of S and Se deposition were in high anion-retention soils particularly Hapludands (moist, moderately weathered soils, derived from volcanic ash). Soluble fluorine concentrations within the tephras were low compared to historic Icelandic and Chilean examples. However, pastoral livestock deaths were apparently caused by fluorosis in addition to starvation when tephra covered feed. The Ruapehu tephra contained very low concentrations of other soluble toxic elements.

Key words: Fluorosis; Ruapehu volcano; Selenium; Sulphur; Tephra; Volcanic hazards.
Reprints: S J Cronin, Massey University, Department of Soil Science, Palmerston North, New Zealand.

COMMENT

There have been other reports of fluorosis in animals resulting from this eruption (*e.g.* see abstract in *Fluoride 31* (1) pages 51-52 February 1998). Strangely, the medical literature mentions no effects of fluoride on humans inhabiting the same area. There were, however, complaints of distress, as the following abstract shows.

John Colquhoun

THE EFFECTS OF A SERIES OF VOLCANIC ERUPTIONS ON EMOTIONAL AND BEHAVIOURAL FUNCTIONING IN CHILDREN WITH ASTHMA

Kevin R Ronan
Palmerston North, New Zealand

Abstract from *New Zealand Medical Journal* 25 April 1997

Aims: To determine whether children with asthma experienced disruptions in emotional and behavioural functioning following a series of volcanic eruptions.
Methods. Multitrait, multimethod assessment was carried out with children living in the volcanic area. Self reports, teacher reports, and parent reports were collected on 118 children and addressed issues related to psychiatric disruptions resulting from the eruptions.
Results. Asthma was reported by 30% of the sample and this figure compares favourably with previous findings with other New Zealand samples. These asthmatic children were compared with a group of nonasthmatic children on a

range of psychiatric symptoms following the volcanic eruptions. Asthmatic children reported, and were observed by parents and teachers, to manifest greater levels of eruption related distress when compared to a group of nonasthmatic children. Children with asthma were found to have significantly higher symptom scores on several indices including those related to eruption-related general distress and context-specific problems (eg, upset at home, upset when eruptions were discussed). Additionally, these children perceived their parents to be significantly more upset than the parents themselves reported. It is important to note that asthmatic children, while clearly more distressed, did not as a group evidence clinical levels of posttraumatic stress disorder symptomology. Conclusions. Children with asthma were more psychologically vulnerable to the volcanic eruptions than children without asthma. These findings have implications for the behavioural management of asthma in children. Discussion integrates current findings with other recent data in highlighting the potential in supplementing traditional asthma management techniques.

Key words: Asthma; Behaviour; Children; Volcanic eruptions.
Reprints: K R Ronan, Psychology Department, Massey University, Palmerston North, New Zealand.

COMMENT

Although fluoride air pollution is a well-known result of volcanic eruptions, the word "fluoride" does not appear in the above study. Respiratory distress resulting from fluoride air pollution was described by Roholm in his classic work (*Fluorine Intoxication. A Clinical-Hygienic Study.* H K Lewis, London 1937, pp 201-202). Respiratory symptoms, including asthmatic wheezing, were reported by subsequent investigators, cited by Waldbott *et al* (*Fluoridation: the Great Dilemma*, Coronado, Lawrence KS 1978 pp 132, 299).

John Colquhoun

HAEMATOLOGICAL CHARACTERISTICS AND BONE FLUORIDE CONTENT IN *BUFO MELANOSTICTUS* FROM AN ALUMINIUM INDUSTRIAL SITE

P C Mishra and A K Mohapatra
Jyoti Vihar, Orissa, India

Abstract from *Environmental Pollution* 99 (3) 421-423 1998

Fluoride concentration in bones and differential haemotological characteristics (RBC, haemoglobin, haematocrit, mean corpuscular haemoglobin and mean corpuscular volume) were measured in amphibians, *Bufo melanostictus*, collected from fluoride-contaminated and uncontaminated areas. The average haemoglobin content, total RBC count and haematocrit (%) in blood samples were found to be significantly reduced, while mean corpuscular concentration and volume were significantly elevated in individuals from the contaminated area in comparison to those from the uncontaminated area. Fluoride concentration was approximately 11 times greater in the bones of toads from the contaminated area.

Key words: Aluminium industry; Bone fluoride; Haematology; *Bufo melanostictus*.
Reprints: P C Mishra. Sambalpur University, Department of Environmental Science, Jyoti Vihar 769019, Orissa, India.

FLUORIDE INTOXICATION IN BOVINES DUE TO INDUSTRIAL POLLUTION

D Swarup, S K Dwivedi, S Dey and S K Ray
Uttar Pradesh, India

Abstract from *Indian Journal of Animal Sciences 68* (7) 605-608 1998

A clinical survey conducted in the vicinity of an aluminium smelter revealed occurrence of fluoride intoxication in cattle population. Affected animals exhibited lameness, reluctance to move, thickening of metatarsal, metacarpal rib and mandibular bones with the presence of palpable bony exostoses. Moderate to severe dental lesions were also observed in the majority of animals. Overall incidence of disease was 42.31%. The highest incidence (58.27%) was within 3 km distance which declined exponentially with the distance from the smelter. Biochemical examination of serum revealed significantly higher levels of alkaline phosphatase (22.08 ± 2.12 KA unit/dl), inorganic phosphorus (5.15 ± 0.24 mg/dl) and creatinine (1.88 ± 0.26 mg/dl) and decreased level of triiodothyronine (0.59 ± 0.14 ng/ml) in the affected animals than normal animals. Fluoride level in urine of affected cattle averaged 26.45 ± 3.28 ppm in the close vicinity of the smelter. Contamination of pasture from smelter smoke was considered to be the potential source of the fluoride intoxication.

Key words: Aluminium Smelter; Bovines; Fluoride intoxication; Industrial pollution.
Reprints: D Swarup, Indian Veterinary Research Institute, Izatnagar 243122, Uttar Pradesh, India.

EXPOSURES IN THE ALUMINA AND PRIMARY ALUMINIUM INDUSTRY AN HISTORICAL REVIEW

G Benke, M Abramson and M Sim
Prahan, Victoria, Australia

Abstract from *Annals of Occupational Hygiene 42* (3) 173-189 1998

We reviewed specific chemical exposures and exposure assessment methods relating to published and unpublished epidemiological studies in the alumina and primary aluminium industry. Our focus was to review limitations in the current literature and make recommendations for future research. Although some of the exposures in the smelting of aluminium have been well characterised, particularly in potrooms, little has been published regarding the exposures in bauxite mining and alumina refining. Past epidemiological studies in the industry have concentrated on the smelting of aluminium, with many limitations in the methodology used in their exposure assessment. We found that in aluminium smelting, exposures to fluorides, coal tar pitch volatiles (CTPV) and sulfur dioxide (SO_2) have tended to decrease in recent years, but insufficient information exists for the other known exposures. Although excess cancers have been found among workers in the smelting of aluminium, the exposure assessment methods in future studies need to be improved to better characterise possible causative agents. The small number of cohort studies has been a factor in the failure to identify clear exposure-response relationships for respiratory diseases. A dose-response relationship has been recently described for fluoride exposure and bronchial hyper-responsiveness, but whether fluorides are the causative agent, co-agent or simply markers for the causative agent(s) for potroom asthma, remains to be determined. Published epidemiological studies

and quantitative exposure data for bauxite mining and alumina refining are virtually non-existent. Determination of possible exposure-response relationships for this part of the industry through improved exposure assessment methods should be the focus of future studies.

Key words: Aluminium industry; Industrial fluorosis; Lung-cancer mortality; Potroom workers; Respiratory symptoms.
Reprints: G Benke, Alfred Hospital, Monash Medical School, Department of Epidemiological and Preventive Medicine, Prahran, VIC 3181, Australia.

INVESTIGATION OF FLUORIDE ELIMINATION DURING A DIALYSIS SESSION

A Nicolay, P Bertocchio, E Bargas, F Coudore, G Alchahin and J P Reynier
Marseille, France

Abstract from *Clinica Chimica Acta* 275 (1) 19-26 1998

We have conducted a study of the elimination kinetics of fluoride ions by a log-linear regression analysis of plasma levels obtained during a bicarbonate hemodialysis session, with a dialyzer in polymercaprin for six patients with chronic renal failure. Using plasma fluoride levels of 35 patients studied for 20 months, we have validated these kinetics for hemodialysis with sodium bicarbonate, acetate-free biofiltration, hemodiafiltration with low flow rate and other dialyzers. Our results show that the decrease in plasma fluoride levels is statistically significant only after the first hour, and the fall reaches approximately 30% after a 4 h dialysis session. We propose that post-dialysis measurements of plasma fluoride are now not necessary if levels before dialysis are known.

Key words: Hemodialysis; Kinetics; Plasma fluoride level; Vichy St-Yorre water.
Reprints: A Nicolay, Hopital St Marguerite, F-13009 Marseille, France.

pH-ALTERED INTERACTION OF ALUMINIUM AND FLUORIDE ON NUTRIENT UPTAKE, PHOTOSYNTHESIS AND OTHER VARIABLES OF *CHLORELLA VULGARIS*

L C Rai, Y Husaini and N Mallick
Varanasi, Uttar Pradesh, India

Abstracted from *Aquatic Toxicology* 42 (1) 67-84 1998

This study presents information on the pH-induced toxicity of $AlCl_3$, AlF_3, NaF and $AlCl_3$ + NaF on growth, nutrient uptake, photosynthesis, photosynthetic electron transport, enzymes of nitrogen and phosphorus metabolism and ATPase activity of *Chlorella vulgaris*. The interaction of $AlCl_3$ and NaF produced additive effect at pH 6.8, and synergistic at pH 6.0 and 4.5. It is suggested that phosphate restricts the entry of Al into the cell, while fluoride promotes it. AlF toxicity could be due to its interference with phosphate binding site of ATPase thereby arresting the release of energy.

Key words: Aluminum; *Chlorella vulgaris*; Fluoroaluminate; Infra-red spectroscopy.
Reprints: L C Rai, Banaras Hindu University Department of Botany, Varanasi 221005, Uttar Pradesh, India.

ANOMALOUS FLUORIDE IN GROUNDWATER FROM WESTERN PART OF SIROHI DISTRICT, RAJASTHAN AND ITS CRIPPLING EFFECTS ON HUMAN HEALTH

P B Maithani, R Gurjar, R Banerjee, B K Balaji, S Ramachandran and R Singh
Hyderabad, Andhra Pradesh, India

Abstract from *Current Science 74* (9) 773-777 1998

Anomalously high concentration of fluoride (up to 16 ppm) has been observed in dug/tube well water, which is being used for drinking and irrigation purposes, around Palri, Andor and Wan villages, in western part of Sirohi district, Rajasthan. Fluoride concentration in groundwater is much higher than the permissible limit of 0.6-1.5 ppm of fluoride recommended for potable purposes. Water samples with more than 5 ppm fluoride are confined to Andor and Wan villages. Mottling is commonly observed in people of this area with a few cases of crippling fluorosis. Areas with such a high fluoride content require serious attention and remedial measures like setting up of large-scale defluoridation plant, use of simple domestic defluoridation methods and public awareness for preventing harmful diseases like fluorosis.

Key words: Fluorosis; Groundwater fluoride; Rajasthan, India.
Reprints: P B Maithani, AMD Complex, Department of Atomic Energy, Hyderabad 500016, Andhra Pradesh, India.

DEFLUORIDATION OF SEPTENTRIONAL SAHARA WATER OF NORTH AFRICA BY ELECTROCOAGULATION PROCESS USING BIPOLAR ALUMINIUM ELECTRODES

N Mameri, A R Yeddou, H Lounici, D Belhocine, H Grib and B Bariou
Algiers, Algeria

Abstract from *Water Research 32* (5) 1604-1612 1998

The purpose of this paper is to suggest an efficient defluorination process which does not require a big investment. For this, the electrocoagulation process with aluminium bipolar electrodes was used. In the first step, the influence of parameters such as inter-electrode distance, fluoride concentration, temperature and the pH of the solution, were investigated and optimized with synthetic water in batch mode. In the second step, the optimization process was continued with Oued Souf water (South Algeria) where the influence of the current density and the area/volume ratio on the defluorination process was evaluated. The electrocoagulation process with aluminium bipolar electrodes permitted the defluorination of Sahara water without adding soluble salts to the treated water. The aluminium-fluoride weight ratio attained 17/1.

Key words: Aluminium electrodes; Defluoridation; Drinking water; Electrocoagulation.
Reprints: N Mameri, Ecole Nationale Polytech Algeria, Department of Environmental Engineering, Biotechnical Laboratory, 10 Aver Pasteur, Algiers, Algeria.

PARENTS SATISFACTION WITH CHILDRENS TOOTH COLOR – FLUOROSIS AS A CONTRIBUTING FACTOR

J A Lalumandier and G Rozier
Cleveland, USA

Abstract from *Journal Of The American Dental Association 129* (7) 1000-1006 1998

The authors surveyed parents of 708 patients in a pediatric dental practice about their satisfaction with the color of their children's teeth and factors associated with their level of satisfaction. Overall, 43 per cent of parents were dissatisfied with their children's tooth color, and 78 per cent of children had a Tooth Surface Index of Fluorosis, or TSIF, score greater than 0. The worst TSIF score was the only factor associated with parent satisfaction.

Key words: Dental fluorosis; Parent satisfaction; Tooth color; Tooth Surface Index.
Reprints: J A Lalumandier, Case Western Reserve University, Department of Community Dentistry, School of Dentistry, 10900 Euclid Ave, Cleveland, OH 44106, USA.

CROSS-CULTURAL COMPARISON OF ATTITUDES AND OPINIONS ON FLUORIDES AND FLUORIDATION BETWEEN AUSTRALIA AND JAPAN

A Tsurumoto, F A C Wright, T Kitamura, M Fukushima, A C Campain and M V Morgan
Yokohama, Japan

Abstract from *Community Dentistry And Oral Epidemiology 26* (3) 182-193 1998

This paper reports on two studies exploring similarities and contrasts in knowledge, attitudes and opinions on fluorides and fluoridation of two culturally different population groups. The first study compares the attitudes and opinions of parents of primary (elementary) schoolchildren in Melbourne, Australia, and Yokohama, Japan, and the second study compares the attitudes and opinions of dentists drawn from the same geographic areas. A self-administered questionnaire collected data on 517 parents and 629 dentists. The questionnaires were of similar design and content for both parents and dentists. They included a series of knowledge and attitudinal statements on preventive dentistry and use of fluorides. Attitudinal responses were measured on a 5-point agree-disagree Likert scale. Data were analyzed using both bivariate and multivariate techniques. Australian parents appeared better informed on the benefits of water fluoridation and held more favorable opinions on fluorides and fluoridation than their Japanese counterparts. Similarly, Australian dentists held more positive attitudes toward the use of fluorides and fluoridation than their Japanese peers. Cultural norms and experiences appear to shape parental attitudes, whereas the focus of dental education and dental practice on restorative treatments in Japan appears to be a substantial influence on the attitudes and opinions held by Japanese dentists.

Key words: Australia; Dental profession; Fluoridation; Fluoride use; Japan; Parents; Preventive Dentistry.
Reprints: A Tsurumoto, Tsurumi University, Department of Preventive Dentistry, Tsurumi Ku, 2-1-3 Tsurumi, Yokohama, Kanagawa 230, Japan.

APPLYING THE NATIONAL ASSOCIATION OF ENVIRONMENTAL PROFESSIONALS CODE OF ETHICS TO THE ENVIRONMENTAL PROTECTION AGENCY AND THE FLUORIDE IN DRINKING WATER STANDARD

Robert J Carton and J William Hirzy
(National Federation of Federal Employees, Local 2050)*
Washington DC, USA

Abstract of paper presented at the National Association of Environmental Professionals 23rd Annual Conference, San Diego, California, June 20-26, 1998

As stated in the NAEP (National Association of Environmental Professionals) Code of Ethics and Standards of Practice for Environmental Professionals, the "keystone of professional conduct is integrity." This means, among other things, that professionals must be responsible for the validity of their work. This work must be conducted without "dishonesty, fraud, deceit or misrepresentation or discrimination." They must not put professional judgment aside in order to twist facts and/or conclusions to give a client, or a superior, a desired outcome. Further, professional integrity does not stop when a report is signed. There is a continuing responsibility for seeing that a report is not misrepresented by others, or altered to change its data or conclusions.

The National Federation of Federal Employees, Local 2050, representing all 1200 non-management professionals at the headquarters of the US Environmental Protection Agency (EPA), has attempted to incorporate a modified version of the NAEP code of ethics into their collective bargaining agreement with EPA. This paper explains the content of this proposal and the event that galvanized this effort - the November, 14, 1985 Federal Register notice setting a health-based standard for fluoride in drinking water.

The NAEP code required some modification to better clarify the role of professionals who provide analyses of issues in a regulatory context, in an agency run by politicians. Regulations require specific scientific endpoints to be defined. Politicians often require analyses that support politically acceptable solutions. This presents a serious dilemma in that professional ethics often take a back seat to political expediency. An enforceable code of ethics is needed to permit honest analysis for decision-making to surface from the professional staff without fear of intimidation or reprisal.

The need for a code of ethics at the Agency has been emphasized time after time since its inception in 1970. This need became critical, in the opinion of the leadership of the professionals' union, when EPA published the fluoride in drinking water standard in 1985. An investigation by the union revealed that scientific support documents for the health-based standard were invalid. Political decisions were found to have influenced and altered scientific conclusions.

Key words: Code; Environmental Protection Agency; Ethics; Fluoride in drinking water standard; National Association of Environmental Professionals.

Reprints: Dr Robert J Carton, 2455 Ballenger Creek Pike, Adamstown, MD 21710, USA.

* As of April 20, 1998, EPA professionals are represented by the National Treasury Employees Union (NTEU), Chapter 280.

UNANSWERED LETTER

The following letter, sent in September 1996 to the International Labour Office, Case Postale 500, 1211 Geneva 22, Switzerland, has received no acknowledgement or reply.

Dear Colleagues

We have received no reply to our letter of May 25, enquiring why your bulletin never reports on fluoride as an industrial pollutant or health hazard to workers. Although for many years we have sent you our journal, and received in return your ILO-CIS Bulletin *Safety and Health at Work*, our published articles and abstracts on occupational fluorosis and industrial fluoride pollution, as well as those on the same subjects from other journals, have not been reported in your Bulletin. However, other industrial pollutants and health hazards are regularly reported on. Please advise why this is your policy.

The following is a list of reports in our journal which have not been noted in your Bulletin.

Original research reports:

Fluoride Vol. 29 No. 3 pp 163-165 1996. Fluoride in urine, hair and nails of phosphate fertilizer workers, by W Czarnowski, K Stolarska, B Brzezinska and J Krechniak, Poland.

Fluoride Vol. 28 No. 4 pp 203-208 1995. Endemic fluorosis in San Luis Potosi, Mexico. II. Identification of risk factors associated with occupational exposure to fluoride, by Jaqueline Calderón, Isabelle Romieu, Magdalena Grimaldo, Héctor Hernandez and Fernando Diaz-Barriga, San Luis Potosi, Mexico.

Fluoride Vol. 27 No. 4 pp 194-200 1994. A method for estimating individual predisposition to occupational fluorosis, by E V Polzik, V E Zinger, G A Valova, V S Kazantsev and M Y Yakusheva, Ekaterinburg, Russia.

Fluoride Vol. 26 No. 4 257-262 1993. Factors of individual predisposition to occupational fluorosis, by E V Polzik, G A Valova, S V Shcherbakov and M Y Yakusheva, Ekaterinburg, Russia.

Abstracts of papers presented at XXth Conference of the International Society for Fluoride Research, Beijing, China, September 5-9 1994:

Fluoride Vol. 28 No. 1 p 40 1995. Serum and urinary fluoride concentration in fluoride-exposed workers of an aluminium smelting factory in China, by K Kono, Y Yoshida, M Watanabe, K Usuda, A Harada, G Sun, G Ding, and Y Hu, Osaka, Japan and Shenyang and Fushun, China.

Fluoride Vol. 28 No. 1 p 40 1995. Fluoride metabolism and kidney function: health care of fluoride exposed workers, by K Kono, Y Yoshida, M Watanabe, K Usuda, M Shimahara, A Harada, K Doi, Japan.

Abstracts of papers presented at XIXth Conference of the International Society for Fluoride Research, Kyoto, Japan, September 8-11 1992:

Fluoride Vol. 26 No. 3 p 223 1993. Research on chronic effects of occupational exposure to organic fluorine, by G Z Zhang, Z Y Zhang, B C Xu, G F Sun and J K Guan, Fuxin and Shenyang, China.

Fluoride Vol. 26 No. 3 pp 223-224 1993. Effects of organic fluorine exposure on the reproductive function of female workers and the development of their offspring, by Z Y Zhang, G Z Zhang, X J Liu et al, China.

Fluoride Vol. 26 No. 3 pp 224-225 1993. Effects of food intake on serum and urinary fluoride concentrations as an indicator of occupational fluoride exposure, by H Nagaie, Y Yoshida, K Kono, M Watanabe, Y Tanioka, Y Orita, T Dote, Y Takahashi, K Umebayashi and A Takasu, Osaka, Japan.

Fluoride Vol. 26 No. 2 p 140 1993. Effects of fluoride exposure on the health of workers in an aluminium smelter, by G F Sun and Y L Hu, Shenyang and Fushun, China.

Fluoride Vol. 26 No. 2 p 141 1993. Study of the effect on pulmonary function in workers with chronic exposure to organic fluoride, by G F Sun, G F Yang, Q K Meng, Z Y Zhang and G Z Zhang, Shenyang and Fuxin, China.

(continued next page)

Abstracts from other journals:

Fluoride Vol. 29 No. 2 p.103 1996. Historical cohort study of spontaneous abortion among fabrication workers in the semiconductor health study - agent-level analysis, by S H Swan, J J Beaumont, S K Hammond *et al*, USA. Abstract from *American Journal of Industrial Medicine 28* (6) 751-769 1995

Fluoride Vol. 29 No. 2 p.103-104 1996. Hydrofluoric acid-induced skin necrosis, by V Saada, M Patarin, S Sans and P Saiag, France. Abstract from *Annales de Dermatologie et de Venereologie 122* (8) 512-513 1995

Fluoride Vol. 29 No 2 p.104-105 1996. Bronchial responsiveness, eosinophilia, and short term exposure to air pollution by V Søyseth, J Kongerud, P Broen *et al*, Norway. Abstract from *Archives of Disease in Childhood 73* (5) 418-422 1995

Fluoride Vol. 29 No. 2 p 109 1996. Generalized osteopathy with pathological fractures in a patient with longterm exposure to fluorine-containing plastics, by W Rhomberg, F Bohler, A Vith and G Breitfellner, Austria. Abstract from *Schweizerische Medizinische Wochenschrift; Journal Suisse de Medecine 125* (48) 2330-2337 1995.

Fluoride Vol. 28 No. 4 p 218 1995. Endemic fluorosis in San Luis Potosi, Mexico I. Identification of risk factors associated with human exposure to fluoride, by M Grimaldo, V H Borja-Aburtom, A L Ramirez *et al*, Mexico. Abstract from *Environmental Research 68* (1) 25-30 1995.

Fluoride Vol. 28 No. 4 p 223 1995. Relation of exposure to airway irritants in infancy to prevalence of bronchial hyper-responsiveness in schoolchildren, by V Søyseth, J Kongerud, D Haarr *et al*, Norway. Abstract from *Lancet 345* (8944) 217-220 1995.

Fluoride Vol. 28 No. 4 p 223 1995. Asthma and respiratory problems - A Review, by T V O'Donnell, Wellington, New Zealand. Abstract from *Science of the Total Environment 163* (1-3) 137-145 1995.

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Sincerely
John Colquhoun
Editor

THE LORD MAYOR'S TASKFORCE ON FLUORIDATION BRISBANE CITY, AUSTRALIA. FINAL REPORT

TASKFORCE CONCLUSIONS

1. This report has been structured to reflect the sequence in which the Taskforce tackled the many issues arising from the fluoridation debate, and also attempts to capture the dynamic nature of Taskforce discussions and deliberations. As the report shows, Taskforce members were able to reach a consensus on a broad range of the less contentious issues. However, in relation to fundamental questions concerning the efficacy, effectiveness and safety of water fluoridation, the Taskforce was deeply divided between those who were strongly committed to water fluoridation as a public health measure, and those who remained unconvinced by the arguments that fluoridation was necessary, effective and safe.

A small majority of Taskforce members (52%) stated that they were opposed to the fluoridation of Brisbane's water supply. A significant proportion of members (23%) who had initially been supportive of fluoridation had changed their opinion to opposition by the end of the Taskforce process.

2. The Taskforce was satisfied that the weight of scientific evidence overwhelmingly supported the decay-reduction effect of water fluoridation. However, there was considerable disagreement about the extent of the benefits and the use of percentages to express reductions in dental decay.

3. Many Taskforce members were unconvinced by assurances that serious risks to health were negligible or non-existent. In particular, there was concern about ambiguous scientific evidence of an association between water fluoridation and higher levels of hip fracture.

The Taskforce noted that the National Health and Medical Research Council (NHMRC) Working Group, which had supported fluoridation, had expressed considerable concern about the fact that it could not point to a single Australian study which had monitored adequately the impact of possible adverse consequences of fluoridation (NHMRC) 1991, Section 8). The majority of the Taskforce was concerned that these inadequacies have still not been addressed. Many Taskforce members were also concerned that the pro-fluoridation case had relied heavily on studies from abroad which do not take account of aspects particular to Brisbane *e.g.* its sub-tropical climate.

4. There was also concern about the lack of scientific research on the lifetime effects of an accumulation of fluoride in the body, in spite of the 1991 NHMRC Working Group statement that 'it was imperative that public health recommendations in the future be based on accurate knowledge of the total fluoride intake of Australians (NHMRC 1991, Section 8.3). This aspect was highlighted by most Taskforce members as an area which required further scientific investigation.

5. Many Taskforce members had doubts that the available evidence proved that the dental decay problem in Brisbane was serious enough to warrant water fluoridation:

For copies of full report, send to: Jim Soorley, Lord Mayor of Brisbane, Box 2287 GPO, Brisbane 4000, Australia.

- dental decay rates have been falling for three decades in both fluoridated and unfluoridated communities.
- there was a lack of contemporary dental evidence on the scale of the problem in Brisbane, particularly in relation to adults.
- research evidence showed the complexity of trying to separately identify the benefits of water fluoridation alone, as illustrated by the recent comparison study of children in Brisbane and Townsville (University of Adelaide).
- dental decay rates (DMFT for 12 year olds) amongst children in Queensland (1.37 in 1995) appear to be similar to the Australian average (1.01 in 1995), as illustrated in the tables below:

DMFT rates (National Oral Health Survey, 1987 p.45 Table 20)

Capital city	Brisbane	Sydney	Canberra	Hobart	Perth	Adelaide	Melbourne
Age 10-14	2.3	1.4	1.1	1.0	1.8	2.4	2.1
Age 15-19	5.3	2.8	3.2	3.4	4.4	4.8	5.0

DMFT rates (School of Dentistry, University of Adelaide, 1995)

State or Territory	New South Wales	Victoria	Queens land	South Australia	West Australia	Tasmania	Northern Territory	Australian Common-wealth Territory
12 yr-olds	0.93	1.02	1.37	0.64	1.04	0.86	0.82	0.61

National Average: 1.01

- the widespread availability and use of other sources of fluoride.

6. The Taskforce accepted that the effectiveness of water fluoridation in reducing dental decay has declined in the last 20 years as a result of the advent of other sources of fluoride, as well as other factors. However, the Taskforce was sharply divided on the current level of effectiveness of water fluoridation, in the light of falling decay rates.

The effectiveness of water fluoridation varies with the concentration of fluoride in the water supply. At the optimal concentration for a temperate climate of 1 ppm, effectiveness in decreasing dental decay would be greater than at the 0.7 ppm level recommended by USPHS for a sub-tropical climate.

The majority of Taskforce members, however, agreed with the WHO recommendation that 0.5 ppm would be the appropriate level for a sub-tropical climate. This would further reduce the effectiveness of fluoride while also reducing the risk of dental fluorosis.

7. There was considerable concern amongst many Taskforce members that water fluoridation could increase the total intake of fluoride in excess of a safe level for babies and young children.

8. The evidence relating to what constituted a safe or a toxic dose of fluoride was uncertain and confusing. A majority of Taskforce members were concerned that the margin of safety between a safe and toxic dose may not be sufficiently wide.

9. The majority of the Taskforce accepted the findings of Dr Miller's limited study of the environmental impact of a fluoridated water supply on the Brisbane area. The majority agreed that the study had raised concerns about the possibility of adverse effects on some sensitive plant and marine species, and that further experimental studies and other biological assessments would be required to reach more definite conclusions. The majority of the Taskforce accepted that there had been little examination of the environmental effects of water fluoridation world-wide.

10. The Taskforce agreed that dental decay is not a disease which is spread equally throughout the population, and that there are clearly many individuals and groups who are more susceptible and at more risk. Water fluoridation is particularly aimed at those who do not or are unable to look after their teeth, for example, young children and those in lower socio economic groups. Although the Taskforce did not discuss the options for tackling the problem in detail, there seemed to be scope for more effective targeting of those at risk, and for obtaining the benefits of using different fluoride treatments in combination.

11. The Taskforce concluded from the evidence, and from correspondence with the Australian Dental Association and NHMRC, that the recommendations of the 1991 NHMRC Working Group for an immediate increase in Australian dental public health research, and for improved dental health monitoring, have not been implemented.

The majority of Taskforce members would not support the introduction of water fluoridation to Brisbane until the recommended Australian research has been carried out. However, if the required data gathering and research were carried out, the Taskforce could be reconvened to consider any new evidence.

Background note: The above Taskforce was established in January 1997 in response to debate in the media and the political arena about whether Brisbane's water supply should be fluoridated. Brisbane is the only State capital in Australia to remain unfluoridated. In his Foreword to the Final Report, the Lord Mayor stated: "The Taskforce was structured to provide a forum to hear both sides of the argument. The 17 members included experts from professional organisations which support fluoridation, such as the Australian Dental Association and the Australian Medical Association, and also representatives from other professional bodies with opposing views. I considered that it was particularly important that the Taskforce include representatives of the public and also those other local governments which share Brisbane's water supply. Both of these groups came to the Taskforce without pre-determined or fixed views and, as such, were able to consider the issue from a more neutral perspective. .... Throughout this six month process, the representatives of the community listened to the experts argue the case. These community representatives came down clearly against the fluoridation of Brisbane's water at this time."

CHANGING ONE'S MIND: AN EXAMINATION OF EVIDENCE FROM BOTH SIDES OF THE FLUORIDATION DEBATE

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Publication of John Colquhoun's article on why he changed his mind about fluoridation[1] along with a critique by Howard Pollick[2] and Colquhoun's reply[3] provides an opportunity to re-examine the issues raised by this debate on fluoridation. The problem is to determine what evidence is sound and what is not. This analysis concerns the sixteen points raised by Pollick.

1. *Printing error.* In the interchange of views it was clarified that this first point was a non-issue and arose from a printing error.

2. *Conspiracy to withhold information.* There does not appear to be any matter of scientific evidence in dispute in this point.

3. *Bias in the use of titles.* Again no matter of scientific evidence appears to be under dispute on this point, but the published record speaks for itself.

4. *Water fluoride levels.* This point is the first involving the examination of scientific data. The point raised by Pollick was that "the work of Teotia[4] in India" concerned areas with a very high fluoride beyond the recommended concentrations for water fluoridation. Colquhoun countered that the work had included both low and high fluoride areas.

Reference to the original article indicates clearly that the studies by Teotia and Teotia[4] involving 400,300 children were done in areas including both endemic fluorosis with fluoride levels greater than 1 ppm and non-endemic fluorosis areas with fluoride levels less than or equal to 1 ppm. The total population of children surveyed was 800,750 of whom 400,300 volunteered for complete examination and inclusion in the study. In the nonendemic area the mean water fluoride level was 0.5 ± 0.24 ppm and contained 200,000 children while the endemic area had 200,300 children and a fluoride level of 4.19 ± 2.03 ppm. A subgroup of 23,270 children were studied involving 12,150 who lived in a low-fluoride area with a mean water fluoride level of 0.70 ± 0.25 ppm and 11,120 who lived in a high fluoride area with a mean water fluoride of 2.85 ± 0.75 ppm.

Since 1962, the "optimal" concentration of fluoride in drinking water for the United States has been set at 0.7-1.2 ppm depending on the mean temperature of the locality.[5] Assessing the evidence suggests that it is incorrect to dismiss the work of Teotia and Teotia[4] on the grounds that only areas with very high fluoride levels were involved.

5. *Effects of nutrition on tooth decay.* There does not appear to be any scientific data in dispute in this point with both Pollick and Colquhoun agreeing that nutrition may have a role in tooth decay. Pollick points to the place of sugar while Colquhoun says he does "not know the answer for sure" and wonders about fresh fruit, vegetables and cheese. However, the observation that influenced Colquhoun towards changing his mind, involving the decline in tooth decay commencing before the availability of fluoride and continuing even when fluoridation was fully implemented, is the central part of Colquhoun's argument at this stage. This point is not addressed by Pollick.

6. *Benefits of fluoridation.* Pollick states that Colquhoun asserts that in all of the studies published there is bias in population selection and examiner diagnosis and that "most of the examiners were keen fluoridationists". Colquhoun has replied that a study has not been produced which counters the statement that "It is just not possible to find a blind fluoridation study in which the fluoridated and nonfluoridated populations were similar and chosen randomly". In his paper Colquhoun indicates that he considered most, rather than all, pro-fluoridation studies were not blind and that the examiners knew which children received fluoride.

The Hardwick *et al* study,[6] mentioned by Pollick, does not contradict Colquhoun's position that although the examiners were blind as to where the children came from, no evidence is given to show that, of the two areas which were compared, the choice as to which one would be fluoridated was made randomly. Whether this study is the best one to illustrate a beneficial effect for fluoridation is open to debate. It is not mentioned in the review by Newbrun[7] in 1989 on the effectiveness of water fluoridation but is referred to in reviews by Beltran and Burt,[8] 1988, and Ripa,[9] 1993, as evidence of the benefit fluoride may give to teeth already erupted. Hardwick *et al*[6] indicate that the primary aim was to test the null hypothesis that there would be no post-eruptive effect of artificial water fluoridation on the caries increments of 12-year old children over the following three years. A difficulty in the study was that of the 305 children in the fluoride group at the baseline examination only 47% or 144 were able to attend the fourth annual re-inspection. Another difficulty for the study, in demonstrating a beneficial effect for fluoride in reducing dental decay, was that although the control and fluoride groups of children showed "close agreement" in "mean initial DMFS and DMFT rates", at the end of the study the figures given, in Table 1, show that the fluoride group did not have lower rates for either the DMFS or the DMFT (Mean ± SD; Control group DMFS 6.60 ± 4.80; Fluoride group DMFS 7.33 ± 6.47; Control group DMFT 4.27 ± 2.82; Fluoride group DMFT 4.58 ± 3.49). It is not clear that the Hardwick *et al*[6] study shows that fluoridation of water supplies exerts a benefit systemically as well as topically. The authors saw "substantial topical effects on teeth already erupted at the start of fluoridation", during the study period "fluoride dentifrices were used extensively", and at the end of the four years the DMFS and DMFT rates were no better in the fluoride group than in the control group.

As has been shown elsewhere[10] there is negligible benefit to be gained from fluoride ingestion and also evidence that fluoride acts topically. The mean level of fluoride in the saliva, after ingesting fluoridated water at a concentration of 1.2 ppm, is only 0.87 ± 0.047 μmol/L or 0.017 ± 0.001 ppm.[11] Burt[12] has noted

tablets and that it is "generally accepted" that water fluoridation is not harmful in this way. No references are given whereas there are 22 references in the review article cited by Colquhoun.[13] Colquhoun includes a case history of a patient developing dental fluorosis with access to fluoridated water who did not use fluoridated toothpaste or tablets. In the absence of data, Pollick has not established a case to refute the evidence linking, in 11 communities, higher dental fluorosis rates in the range of 16-51% with water fluoride levels of 0.4-1.4 ppm, in contrast to, in 9 communities, dental fluorosis rates of 2-15% with water fluoride levels of 0.0-0.4 ppm.[13]

8. *Effects of fluoride on bone.* Pollick indicates that studies, such as those reviewed by the National Research Council,[14] have found an association between bone fractures and fluoride. Colquhoun, in his paper,[1] notes that he expressed concern that this might arise back in 1984 at a time when his colleagues dismissed his need to worry. He related how his views on fluoridation were affected by the report[15] of hip fractures being associated with higher fluoride levels six years later. There appears to be a basic agreement that the effect of water fluoridation on hip fracture rates needs to be considered.

Whether one can accept the conclusion of the National Research Council "that there is no basis at this time to recommend EPA (the Environmental Protection Agency) lower the current standard for fluoride in drinking water" [14] (a maximum contaminant level of 4 ppm) would require a more detailed analysis of the evidence than is possible to provide here. Pollick suggests five of the ten studies looked at by the National Research Council[14] showed a positive association between fluoride and hip fracture. In reviewing the report Lee[16] found that seven of the ten showed a positive correlation and that there were reasons why the other three studies were not of value, such as lack of exposure to fluoride prior to the menopause and an insufficient number of subjects.

Pollick appears to be in agreement with Colquhoun that when fluoride is used in high doses to treat osteoporosis it has led to an increase in hip fractures. Thus on this point there appears to be a basic agreement between Pollick and Colquhoun that in some circumstances fluoride can contribute to hip fractures.

Pollick observes that the Subcommittee on Health Effects of Ingested Fluoride[17] "concluded that the weight of evidence indicates that bone strength is not adversely affected in animals that are fed a nutritionally adequate diet unless there is long-term ingestion of fluoride at concentrations of at least 50 mg/L in the drinking water or 50 mg/kg in the diet". However the exact quotation[17] qualifies the conclusion by referring to the "evidence currently available" and notes that recent reports from epidemiological studies of human populations have provided conflicting evidence on this subject and indicate the need for additional research. Some reservations were held about the appropriateness of the methods used to cause bone fractures. Of the 15 animal studies reviewed, reduced bone strength was found in some of the experimental conditions in seven studies.[17] Higher bone fluoride levels occurred when the diet was low in calcium. The bone-ash fluoride levels reported, associated with reduced bone strength, were 640 ppm, 7,398 ppm, 12,600 ppm, 7,393 ppm, 5000 ppm, and 11,000 ppm. The presence of adequate amounts of calcium in the diet may reduce the rate of absorption of fluoride, but if the calcium intake is inadequate or the intake of fluoride is continued for a longer

period, the potential for fluoride to accumulate in bone and be associated with reduced bone strength has not been excluded by the animal studies. Arnala *et al*[18] found mean ashed trabecular bone fluoride level of 3.72 ± 2.39 mg/g (3720 ± 2390 ppm) in 25 patients with hip fracture from a high fluoride area with a fluoride level in the water greater than 1.5 ppm.

Pollick notes that Colquhoun omitted mention in his 1997 paper[1] of the successful results described in 1994 by Pak *et al*[19] with low-dose, slow-release sodium fluoride in the treatment of osteoporosis. This first report of the study gave the results of the use of intermittent slow-release sodium fluoride plus continuous calcium citrate administered for about 2.5 years. Colquhoun notes that the opinion that this is helpful is not shared by many other clinicians. Lee[20] expresses such a viewpoint: that the fluoride treatment recommended by Pak *et a* should be regarded as controlled osteofluorosis and that the 3.5 year trial described in the final report[21] was insufficient to evaluate the potential increased risk of hip fracture.

A recent review[22] noted that while the study by Pak *et al*[21] found a significant reduction in the rate of vertebral fracture, in three other studies[23-25] the effect was small. In a randomized, placebo-controlled, two-year trial of sodium fluoride (50 mg a day) and monofluorophosphate (two doses) in 354 women with osteoporosis, fluoride therapy, as compared to placebo, had a large effect on bone mineral density in the lumbar spine (increase, 10.8% vs. 2.4%), but no effect on the rate of vertebral fractures[26] Thus, even at relatively low doses, fluoride had little beneficial effect on fracture rates. It was noted that sodium fluoride causes stress fractures.[23,26] In discussing the therapeutic choices for women most at risk for fractures no mention is made of a role for fluoride[22] The hope offered by fluoride in 1994 for the treatment of osteoporosis appears to have passed.

9. *Omission of paper with negative evidence on hip fractures.* Pollick argues that while Colquhoun referred to two papers by the Finnish researchers Alhava *et al*[27] and Arnala *et al*[28] which noted fluoride accumulation in bones with fluoridation he omitted reference to a later paper by Arnala *et al*[18] in which no difference was found in the incidence of hip fractures between low-fluoride, fluoridated and high-fluoride areas.

No question involving the interpretation of scientific data appears to be at issue in this point. Colquhoun was recounting the factors which led him to change his mind about fluoridation, and it is understandable that studies not showing a relationship between fluoride and adverse effects would be less likely to have an influence.

He notes that, at the time, the later paper by Arnala *et al*[18] had to be seen alongside a Finnish paper by Simonen and Laitinen[29] suggesting fluoridation was associated with fewer hip fractures. His point that later studies on hip fractures with larger numbers of subjects were more relevant is reasonable. The 1986 paper by Arnala *et al*[18] had 461 subjects with hip fracture. This is fewer than the 651 patients with hip fracture in the 1993 study by Jacobsen *et al*[30] which Lee[16] found to have an insufficient sample size to be able to detect important medical effects. In contrast the studies finding significant effects involved 541985 patients with hip fracture (Jacobsen *et al* 1990[15]) and 20393 patients with hip fracture (Cooper *et al* 1991[31]). Colquhoun has previously referred[32] to the 1986 paper by

Arnala *et al*[18]. Later studies with a much greater data base made it less important to cite it in his new paper under discussion. Hence there is no significance to the fact that it was omitted.

10. *Fluoride and hip fractures.* There does not appear to be any new point being made different from those raised in point 8.

11. *Effects of fluoride on bone.* Again no new issues are raised.

12. *Association of fluoride and osteosarcoma.* Pollick and Colquhoun agree that the appropriate reference for the study in which osteosarcomas were found in rats given fluoride was one referring to the National Toxicology Program study,[33] rather than to the Procter and Gamble study[34] in which the osteosarcomas found were not at a statistically significant level.

There does not appear to be any dispute about the term "equivocal evidence" of carcinogenicity. The Peer Review Panel[35] indicated that equivocal evidence is a category for uncertain findings demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

Although Colquhoun indicates that he was influenced by the finding of 3-7 fold increases in osteosarcoma rates in young males in fluoridated areas of New Jersey compared to non-fluoridated areas,[36] there does not appear to be any basic difference in the interpretation of the findings. Pollick notes that Cohn[37] stated there was insufficient basis to draw conclusions about whether osteosarcoma incidence and fluoridation were causally linked and, in the reference cited by Colquhoun, Cohn[36] notes that "Because of the limitations of the study design and the small numbers of cases that occurred, this analysis does not imply a causal connection between fluoridation and osteosarcoma". Colquhoun includes a question mark after the sub-title of Bone Cancer for this section of his paper indicating questions about the nature of the link still exist.

Questions about the sample studied by Gelberg *et al*[38] are raised by Lee[39] because 67% of the patients were female whereas the majority of patients with osteosarcoma are usually male. Colquhoun notes that there is disagreement about whether it is safe to continue with water fluoridation while the nature of the associations between fluoridation and osteosarcoma are considered.

13. *Effect of fluoride on testosterone synthesis.* Pollick states that only high levels of fluoride have been associated with reductions in testosterone levels in contrast to Colquhoun's assertion that very low levels can interfere with the male hormone testosterone. In the reference given of Kanwar *et al*[40] it is noted that from 1 ppm to 200 ppm of fluoride, the degree of inhibition in testosterone synthesis seems to be dependent on fluoride concentration. In an *in vitro* assay there was a noticeable, though marginal, inhibition even at 10 ppm, a significant marked fall at 100 ppm and maximal inhibition at 200 ppm. The highest reported levels of fluoride in soft tissues[41] have been given as: brain 6.1 ppm, heart 8.1 ppm, pancreas 8.2 ppm, lung 17 ppm, thyroid 23.5 ppm, liver 61 ppm, lens 77.3 ppm, prostate 86 ppm, fat 145 ppm, hair 171 ppm, kidney 181 ppm, bladder 185 ppm, nails 186 ppm, skin 290 ppm and aorta 8400 ppm. The evidence suggests that inhibition of testosterone synthesis begins at 1 ppm of fluoride and a noticeable effect occurs with a level of 10 ppm.[40] Although a reference to fluoride levels in the testes has not been found, the levels which may occur in other soft tissues

suggest that levels affecting testosterone production might occur. It thus appears to be fair comment that very low as well as high levels of fluoride might affect testosterone levels.

14. *Effect of fluoride on intelligence.* On this point Pollick states that Colquhoun misstates the facts of the research from China[42,43] linking dental fluorosis in children with lower intelligence and that to suggest that children with dental fluorosis have on average lower intelligence scores is a gross misstatement of the facts.

Colquhoun in his paper said "Even more chilling is the evidence from China that children with dental fluorosis have on average lower intelligence scores". In the first reference given by Colquhoun, the paper by Li, Zhi and Gao[42] a significantly lower Intelligence Quotient was found in children from areas with medium or severe degrees of dental fluorosis compared to children from areas without dental fluorosis or only slight dental fluorosis. In the other reference by Zhao, Liang, Zhang and Wu[43] children in an area with a high rate of dental fluorosis, 86%, had significantly lower intelligence than those in an area with a low rate of dental fluorosis, 9%. The source of the fluoride in the study by Li *et al*,[42] in the medium and severe fluorosis areas, was the coal used for cooking, heating and drying grain. In the study by Zhao *et al*[43] the area with a high dental fluorosis rate had a water supply with a high fluoride level, 4.12 ppm.

The basic concern of intelligence being affected in some children with dental fluorosis accurately reflects the concerns raised in the studies and it is difficult to see it involving misstatement, or gross misstatement, of the facts of the research. Finding it chilling that fluoride might affect brain development is understandable given that fluoridation was initially promoted as being completely safe. The query about the relevance of the effect of excess fluoride on brain development, because in one of the studies it came from coal smoke rather than water, does not appear to be relevant. The concern is that if fluoride, from whatever source, affects other organs detrimentally as well as causing dental fluorosis, then water fluoridation, which is associated with significant dental fluorosis for many, may be unsafe.

15. *Relevance of animal studies of the effect of fluoride on the brain.* The point is made by Pollick that the study by Mullenix *et al*[44] is irrelevant to water fluoridation because the pregnant rats studied were injected with fluoride, which would not occur with humans, and the weanling and adult rats then drank water with different concentrations of fluoride including, for adult rats, a period of six weeks with water at a concentration at least 100 times that recommended for water fluoridation.

Colquhoun indicates his concern that, because of accumulation within the body, lower doses taken for longer periods of time might have a similar effect to higher doses taken for a short period. He saw a model having been established with other toxins such as lead. In their paper Mullenix *et al*[44] note that it was the fluoride levels in plasma, not fluoride levels of exposure, which best predicted effects on behaviour. Similar plasma fluoride levels of 0.076-0.25 ppm have been found in humans ingesting 5-10 ppm fluoride in drinking water and plasma levels as high as 0.28 to 0.43 ppm have been measured in children drinking water containing 16 ppm fluoride. Fasting serum fluoride levels of 0.2 to 0.3 ppm are used in the treatment of osteoporosis, and plasma fluoride levels as high as

1.44 ppm are found in children 1 hour after receiving topical applications of an acidulated phosphate fluoride (1.23%) gel.

Mullenix *et al*[44] considered that because humans occasionally are exposed to high amounts of fluoride and plasma levels as high as those found in this rat study, neurotoxic risks deserve further evaluation. The closeness of the lower limit of 5 ppm, in the range of 5-10 ppm fluoride in drinking water which produced similar plasma levels to those found in the rats, to the maximum contaminant level for fluoride acceptable for drinking water of 4 ppm[10] is a cause for concern. The evidence thus suggests that the adverse effects on brain function, possibly on that of the hippocampus in particular, are related to the plasma levels found rather than the method of administration.

Thus although the study by Mullenix *et al*[44] may not have been intended, as noted by Pollick, to determine the effects of water fluoridation, its results could be seen as being particularly relevant to assessing whether the fluoridation of water was safe. The findings of Mullenix *et al*[44] are consistent with clinical reports of chronic exposure to fluoride being associated with cerebral impairment, affecting particularly concentration and memory.[45]

This position has been underlined by subsequent events and the publication of two papers[46,47] by Isaacson, Varner, Jensen and Horvath on the effect, in rats, of the chronic administration of aluminium fluoride and sodium fluoride on neuronal and cerebrovascular integrity. In these studies, the chronic administration of aluminium fluoride or sodium fluoride in drinking water in rats resulted in distinct morphological alterations in the brain including effects on neurones and cerebrovasculature. The concentrations of aluminium fluoride, 0.5 ppm (as 0.5 ppm Al), and sodium fluoride, 2.1 ppm (as 2.1 ppm NaF), used gave fluoride ion concentrations of about 1 ppm, the level often used in water fluoridation.

Thus, with the benefit of hindsight, it is clear that Colquhoun was correct to be alarmed about the studies implicating fluoride as adversely affecting brain development, even though the source of the fluoride was not always in the drinking water but included injections[44] (for pregnant rats) and coal smoke (in the study by Li *et al*[42]).

16. *Omission of details of dose and concentration in discussion on toxicology.* Pollick argues that Colquhoun consistently alleges harm from fluoride without stating the dose or concentration of fluoride. Colquhoun indicates that low intakes for long periods might be as deleterious as high intakes for short periods. In his paper[1], when referring to harm from fluoride, he gives references which provide details of the fluoride exposure involved. For example, in the study by Zhao *et al*[43], on intelligence in children, the water fluoride concentrations in the two groups is given as 4.12 ppm and 0.91 ppm.

Colquhoun appears to be explaining what influenced him to change his mind from being an ardent advocate of water fluoridation, who fiercely poured scorn on a lay person who had heard and accepted the case against fluoridation[1], to one who was opposed to it. Given that there are space restrictions, which limit how much detail can be included from any paper referred to, it is appropriate to omit details about dose and concentration of fluoride where such information is readily available in the publications that are cited. In some instances, reference is made[1] to "fluoridated Auckland" and "fluoridated areas of America" and the

meaning is conveyed that the level of fluoride in the water would be in the region of 1 ppm.

The reader, after considering the two sides of the debate, will be in a position to decide what their own view is. Colquhoun required some courage to change his mind. Somerset Maugham[48] would not have had him in mind when he wrote, in *Of Human Bondage*, "Like all weak men, he laid an exaggerated stress on not changing one's mind".

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Letter to the Editor

NATURAL AND ARTIFICIAL

I would very much like to see some explanation of the difference between natural calcium fluoride and the artificial fluorides used in water fluoridation.

Eileen Adelman

Managing Editor replies: There is a belief in some natural health circles that naturally occurring calcium fluoride is less harmful than other fluorides used for artificial water fluoridation. My understanding is that, in general, the fluoride ion is equally toxic, whatever its origin. In India and China naturally occurring fluorides cause much ill health, including crippling fluorosis. However, there are some differences:

1 The industrial waste products used for artificial water fluoridation are much more soluble than calcium fluoride, so are much more dangerous if there is an accidental overdosing of the water supply. Such accidents have occurred, causing sickness and sometimes deaths. However, at the relatively low concentrations occurring in artificially fluoridated water, calcium fluoride and the other fluorides are both soluble.
2 Sometimes naturally fluoridated water also contains minerals like calcium and magnesium, which are known to mitigate the toxic effects of the fluoride. Conversely, the industrial waste products used for water fluoridation almost always contain trace amounts of other toxins such as cadmium, lead and arsenic.
3 Recent research (see Editorial in this issue) indicates that silicofluorides, commonly used for fluoridation, are much more dangerous than previously realized, in that they increase the lead content of the water.

John Colquhoun

SCHATZ: PARADOXICAL EFFECTS

I do not understand Neil Jenkins' two letters to the editor in *Fluoride 30* (4) 1997. With respect, to paradoxical effects, Neil has conveniently forgotten his own 1963 publication.[1] I discussed it in an article in 1964.[2]

Neil not only reported anomalous results in his own research with fluoride but also referred to anomalous results which others got with fluoride. He called them "some unexplained anomalies."

Neil himself therefore conveniently disregarded his own data and erroneously concluded that "The solubility of enamel has been found to be related to its fluoride content."

I sent Neil a copy of everything I have ever published on fluoride and on paradoxical effects, including the 1964 article.

Albert Schatz PhD
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1 Jenkins GN. *Journal of Dental Research* 42 (supplement to No. 1) 444 1963.
2 Schatz A. The importance of paradoxical effects of fluoride with respect to fluoridation and the toxicology of fluoride. *Pakistan Dental Review 15* (4) October 1964.

JENKINS REPLIES

Al Schatz suggests that in my review[1] of the relationship between the fluoride concentration of enamel and its solubility I have overlooked the existence of paradoxical effects. The results I quoted from Isaacs *et al*[2] mostly consisted in showing that the higher the fluoride in enamel the lower is its solubility. The results on only two samples out of a total of 24, each pooled from 40 teeth, were unexpected, Which I still think can be regarded as "unexplained anomalies" which may occur in the collection of any experimental data.

The only results of Isaac *et al* that might be regarded as paradoxical refer to the four results comparing enamel from persons over 50 and under 20 years of age. The fluoride of the enamel is lower in the younger group (as expected) but in three out od the four comparisons the solubility is also lower. However, as Isaac *et al* point out, unknown changes may take place during aging which affect the solubility of enamel. Also, it is possible that dietary changes (for example, in trace elements) between the 1900s and the 1940s (when these teeth were forming) may influence the composition and solubility of enamel and over-ride the effect of fluoride.

I conclude that these results do not demonstrate a paradoxical effect of fluoride but show that many substances other than fluoride may affect enamel solubility. Provided that like is compared with like, the solubility of enamel has been found to be related to its fluoride concentration although this is only one means by which fluoride protects against caries.

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1 Jenkins GN. *Journal of Dental Research 42* 444 1963
2 Isaac J, Brudevolt F, Smith FA, Gardner DE. *Journal of Dental Research 37* 254 1958.

I refer to the letter of Gillian Durham,[1] New Zealand Director-General of Health, which criticized the article "New evidence on fluoridation" [2]. To justify her allegation that the article lacks "veracity" and is selective, Durham refers to the 1994 Public Health Commission report *Water fluoridation in New Zealand* [3]. That report itself states that it "was essentially written by one person with experience in particular areas only" and "places some reliance on the quality of previous reviews that have been conducted. It is important to acknowledge that there are limitations with some of the reviews. Some may have tended to place unwarranted weight on the findings of previous expert reviews and lack wide representation of all the areas requiring expertise." This weakness is revealed by the report's one-side reliance on small-scale pro-fluoridation studies to claim that fluoridation is cost-effective, rather than the various comprehensive studies which indicate little if any benefit. Yet Dr Durham implies that the PHC report is a watershed document and that only later papers have relevance.

However, the PHC report conceded that:

- "It is possible that there is a small increased risk of hip fracture associated with fluoridation"
- "a small increased risk of osteosarcoma in young men cannot be ruled out."
- "Fluoride intake from food is likely to be relevant to skeletal fluorosis in some areas (especially when vegetables are grown in fluorotic soil)"
- "individuals with longstanding and severe renal disease are theoretically at risk in temperate countries at usual water fluoridation levels"
- "Skeletal fluorosis might be occurring in individuals with either a long term intake or a metabolic susceptibility."

It was also admitted that skeletal fluorosis can occur at fluoride levels as low as 0.7-2.5 ppm, though it was qualified by the fact that the countries involved are nearly all tropical and therefore tend to have high water intakes. Many people in Australia and New Zealand also have high liquid intakes.

One has to question Durham's judgement that the benefits of possibly preventing some dental caries outweigh the above admitted risks.

There has been recent publicity in New Zealand that dental caries is increasing with increasing poverty, in both fluoridated and nonfluoridated places. This fact disproves the assertion that fluoride is particularly advantageous to the lower socio-economic groups.

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118 Forrest Hill Road
North Shore City
New Zealand

1 Durham G. Review of evidence on fluoridation (letter). *Australian and New Zealand Journal of Public Health 21* 548 1997. Reprinted in *Fluoride 31* (3) 163-165 August 1998.

2 Diesendorf M, Colquhoun J, Spittle BJ, Everingham DN, Clutterbuck FW. New evidence on fluoridation. *Australian and New Zealand Journal of Public Health 21* (2) 187-190 1997. Reprinted in *Fluoride 30* (3) 179-183 August 1997.

3 *Water Fluoridation in New Zealand. An Analysis and Monitoring Report.* Public Health Commission, Wellington 1994.

Supplement numbers (S) are in the No.3 August *Fluoride* after page 174

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