

## ALUMINOFLUORIDE COMPLEXES

### Fluoride and aluminum: messengers of false information

Intensive laboratory research on the mechanisms of signal transduction has produced experimental data that could change our understanding of the action of fluoride at the cellular level. After reflecting on these laboratory studies, we suggest that some of pathological changes are not produced by fluoride alone but by the synergistic action of fluoride and aluminum. Heterotrimeric G-proteins mediate the transfer of information from heptahelical receptors to effector molecules. The discovery of aluminofluoride complexes ( $\text{AlF}_x$ ) as a new class of phosphate analogues has been followed by demonstrations of their usefulness in laboratory investigations and their pharmacological efficacy.  $\text{AlF}_x$  complexes interact with all known G-protein-activated effector enzymes. G-proteins take part in an enormous variety of biological signaling systems, helping control almost all important life processes.

The family of cell-surface receptors that require coupling to G-protein transducers for functional signaling is vast and diverse.  $\text{AlF}_x$  may clone or potentiate the action of numerous extracellular signals. It appears probable that we will not find any physiological process which is not potentially influenced by  $\text{AlF}_x$ . The aluminofluoride complex acts as the first messenger triggering processes of neurotransmission and potentiating the action of various hormones. It is evident that  $\text{AlF}_x$  are species that convey false information, which is then amplified by processes of signal transmission. Many human diseases have their origin in the malfunctioning of signaling components. Pharmacologists estimate that up to 60% of all medicines used today exert their effects through a G-protein signaling pathway. The synergistic action of fluoride and aluminum in the environment, water, and food can thus evoke multiple pathological symptoms.  $\text{AlF}_x$  might induce alterations in homeostasis, metabolism, growth, and differentiation in living organisms. An awareness of the health risks of this new ecotoxicological phenomenon, an increasing load of aluminum ions and fluoride, would undoubtedly contribute significantly to reducing the risk of a decrease in intelligence of children and adults, and many other disorders in the 21<sup>st</sup> century.

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Keywords: Aluminofluoride complexes, Aluminum, False information, First messenger, G-proteins, Signal transduction.

### Interactions between guanosine diphosphate (GDP) and aluminum fluoride ( $\text{AlF}_3$ )

Fluorine-aluminum compounds are now being studied with increasing interest. The neurotoxic properties of  $\text{AlF}_3$  are most important medically because of reports connecting  $\text{AlF}_3$  with the pathogenesis of Alzheimer's disease. One hypothesis is that the complex of aluminum and fluorine can influence the G-protein receptors and the resulting phosphorylation. Moreover,  $\text{AlF}_3$  activates several guanine nucleotides mimicking the actions of some neurotransmitters and hormones. Some

physico-chemical properties of  $\text{AlF}_3$  and GDP, as well as their antagonist interactions in many biochemical pathways, have been analyzed.

The aim of this study was to investigate of interaction between  $\text{AlF}_3$  and GDP by virtual molecular modeling using the HyperChem computer program. The semi-empiric method (PM3), with Polak-Ribeier's optimization algorithm, was used. The PM3 method is suitable for modeling of the molecules containing elements of the main groups of the periodic table.

The results indicated that the main sites of the reaction in GDP are phosphate groups. The computer analyses obtained from PM3 molecular modeling confirmed that the GDP molecule is attacked initially by one of the  $\text{F}^-$  ions from  $\text{AlF}_3$ . It gets near the phosphate moiety of the phosphate group and pulls it back from GDP (the O-P bond makes itself longer). Next, the remaining part of the  $\text{AlF}_3^{2+}$  attacks the oxygen atom connecting the phosphate groups in GDP and causes breaking of the P-O bond. The two-stage nature of the reaction was confirmed by calculations concerning the length of the bonds, total energy E, and the molecular heat of formation.  $\text{AlF}_3$  can attack GDP in the space of the first and second phosphate rest, although according to our calculations, the outer one has priority.

The mechanism presented clarifies the interactions between the inorganic  $\text{AlF}_3$  complex and the biologically important GDP nucleotide.

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Keywords: Aluminium fluoride, G-proteins, Guanosine diphosphate.

## EFFECTS OF FLUORIDE ON BONE, COLLAGEN, AND CARTILAGE

### Water fluoridation and bone damage: a weight of evidence analysis

A very disturbing omission by those relatively few countries that promote and practice water fluoridation has been the failure to collect comprehensive data on the bone fluoride levels of their citizens as a function of the length of time of their exposure to fluoridated water. This unfortunate situation exists despite the fact that for many years researchers have been aware that approximately 50% of the fluoride ingested each day accumulates in the bones.

Recently, a number of government sponsored reviews have re-examined the risks and benefits of water fluoridation (York Review, 2000; Medical Research Council (UK), 2002; and the Irish Fluoridation Forum, 2002). In each case one of the risks considered was fluoride's potential for damaging bones and making them more subject to fracture. However, each of these reports has been criticized because the authors failed to consider all the available evidence which pertains to this issue.

A "weight of evidence" approach will be used, and the research in the literature which sheds light on the matter is examined. Biochemical studies, animal studies,

clinical studies, case studies of workers exposed to fluoride in industry, and epidemiological studies of people drinking fluoridated water (both natural and artificial) will be included. The author will attempt to answer the following questions:

- 1 At what fluoride concentration in the bone does damage occur?
- 2 Is there any evidence that children's bones have been damaged from fluoride exposure?
- 3 At what cumulative dose of fluoride can we expect an increase in bone fractures in the elderly to occur? And,
- 4 Are people living in artificially fluoridated communities likely to exceed these cumulative doses with lifetime exposure to fluoride (from this and other sources combined)?

By using the "cumulative dose" approach, the author hopes to avoid the pitfall of describing daily doses administered in clinical studies over short periods of time (1 - 5 years) as "high doses", and those administered over extended periods of time (50 - 70 years or more) as "low doses", as many commentators have done. For bone it is the total accumulation of fluoride that appears to be critical in the increase of fractures, especially in the non-vertebrae.

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Keywords: Bone damage, Bone fractures, Cumulative dose, Fluoride in bone, Fluoridation, Weight of evidence analysis.

### **Parathyroid hormone and osteosclerosis in endemic skeletal fluorosis**

Endemic skeletal fluorosis is well known as a chronic metabolic disease caused by ingesting large amounts of fluoride (F) in water, or less often in foods. Varying degrees of osteosclerosis, osteomalacia, and osteoporosis characterize the bone lesions. Although many reports have described osteosclerotic cases resulting from high cumulative F intake, crippling skeletal fluorosis has also been reported in tropical countries in association with low concentrations of F in drinking water suggesting that other dietary factors were contributing. Excessive bone formation in osteosclerosis was considered to require specific conditions. In addition to F intake, other factors likely to contribute to osteosclerotic change need to be investigated. In this study, laboratory abnormalities were analyzed in human endemic skeletal fluorosis. Several hypotheses were formulated about the causation of osteosclerosis.

Seventy-three article-length publications between 1966 and 2001 concerning human endemic skeletal fluorosis were retrieved from Medline. Articles were selected for containing information about eight parameters including serum concentrations of parathyroid hormone (PTH) and ionized calcium ( $\text{Ca}^{2+}$ ). PTH was high in most patients with fluorosis compared to normal subjects. Low dietary calcium (Ca) intake has been suggested to exacerbate the severity of the bone lesions in children, who live in areas endemic for fluorosis. Skeletal fluorosis has been linked to the combination of excess F, low Ca, and high PTH. The increase in PTH correlated well with excess F ingestion.

Four mechanisms for the development of osteosclerosis in fluorosis are hypothesized. Firstly, the intermittent secretion of PTH accelerates bone formation. Secondly, nutritional factors and various metabolic states may affect PTH levels and responsiveness. Chronically low  $\text{Ca}^{2+}$  raises the serum  $\text{Ca}^{2+}$  concentration required for a 50% reduction in the PTH secretion set point. A decrease in activated vitamin D leads to decreases in the receptors in the kidneys and intestine for PTH and also makes bone more resistant to PTH. Thirdly, PTH secretion continues to be high after normalization of  $\text{Ca}^{2+}$ . Fourthly, PTH secretion is prolonged despite sufficient  $\text{Ca}^{2+}$  supply, resulting from a high PTH secretion set point. Thus, intermittently high serum concentrations of PTH accelerate bone formation.

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Keywords: Bone formation, Calcium, Endemic fluorosis, Osteosclerosis, Parathyroid hormone, Skeletal fluorosis.

### **A review and a recent study of the effects of fluoride on bone strength in the rat model**

The effects of fluoride on the mechanical properties of bone in various animal models have been investigated, with most authors reporting a negative influence on bone strength. In the present study the effect of different fluoride concentrations in drinking water on bone mechanical strength in young growing rats was examined.

Ninety-six 6-week-old female Wistar rats were randomized into four groups. One group was given distilled water and the other three groups were exposed to fluoridated water at different concentrations (8, 30 and 60 mg  $\text{F}^-/\text{L}$ ). After six weeks all rats were sacrificed and tests were performed. The strength of the femoral body and neck was measured, microhardness was determined according to Vickers, and a penetration test was used to evaluate mechanical properties of the trabecular bone.

The results of this study show that high fluoride intake decreases bone quality, both cortical and cancellous. On the other hand, sodium fluoride administered in lower concentration increases the strength of both the femoral neck and cancellous bone in the appendicular skeleton in growing rats.

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Keywords: Bone strength, Compressive strength, Femur, Fluoride exposure, Microhardness, Rat model, Penetration test.

### **Effects of sodium fluoride on bone metabolism and structural changes of the mandible in ovariectomized rats**

The aim of this study was to investigate the effect of fluoride on selected biochemical markers of bone remodeling, bone mass, and structural changes of the mandible in ovariectomized rats.

Forty-six 6-week-old female Wistar rats were randomized into four groups. Two groups were given distilled water, and the other two groups were exposed to fluori-

dated water at concentrations of 8 and 60 mg F<sup>-</sup>/L. After six weeks three groups were ovariectomized (one group exposed to distilled and both groups receiving fluoridated water). After another six weeks all rats were sacrificed. Serum was then collected for measurement of fluoride concentration, serum total alkaline phosphatase activity (ALP), and the concentration of telopeptide of rat type I collagen (ICTP). The mandibles were extracted, the mineral mass was determined, and radiological and histomorphometric examinations were performed.

The rats with higher plasma fluoride concentrations after ovariectomy demonstrated lower ICTP concentrations and higher ALP activity than animals with lower fluoride concentrations. The exposure to fluoride led after the ovariectomy to higher mineral mass and lower porosity in mandibles compared to animals receiving only distilled water. After ovariectomy the alveolar height decreased in rats exposed to 8mg F<sup>-</sup>/L as compared with rats drinking distilled water or 60 mg F<sup>-</sup>/L.

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Keywords: Alkaline phosphatase, Alveolar height, Bone metabolism, Fluoride exposure.

### Osteofluorosis in rats

Experimental osteofluorosis was induced in 75 female Wistar rats by the intraperitoneal administration of sodium fluoride (NaF) in a dose of 0.5 or 5 mg daily for three months. A control group received saline injections.

The results showed an increased volume of the tibia, femur, and vertebrae in the treated animals. The bone mass, osteoid surface, and osteoid volume all increased. Mineralisation of osteoid was delayed with the proportion of newly formed woven bone increasing with the higher dose of administered fluoride. Sporadic scattered necrosis of osteocytes and chondrocytes occurred in association with fluoride administration together with a disarrangement of collagen fibres. Focal, scattered calcification was seen in the articular capsules and tendons together with some fibrocartilaginous transformation. In the intervertebral discs a reduced orientation of collagen fibres in the annulus fibrosus occurred together with atrophy and collapse of the nucleus pulposus resulting in narrowing of some intervertebral spaces.

Bone fluoride levels were 1.10 mg F/g bone tissue and 1.44 mg F/g bone tissue in the groups receiving NaF in doses of 0.5 and 5 mg daily compared to a bone fluoride level of 0.42 mg F/g in the control group.

Increased bone and osteoid volume were apparently caused by decreased bone resorption. The qualitative and quantitative changes in the subchondral bone tissue, articular cartilage, capsules, tendons, and intervertebral discs may be regarded as toxic effects of fluoride. The pathological changes and damage to the tissue components lead to progressive joint destruction.

The osteoarthritis and spondylarthrosis produced in the experimental animals are analogous to those seen in human disease and are undoubtedly complex functional abnormalities caused by fluoride.

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Keywords: Fluoride in bone, Osteofluorosis, Osteoarthritis, Rat study.

## FLUORIDE AND TEETH

### Fluoride and dental caries: systemic and topical effects

A major paradigm shift has occurred regarding the mechanism of action of fluoride in preventing dental caries. The shift has been a slow one, taking nearly three decades. Nevertheless, it is now well recognized in the literature that the predominant, if not the entire, anti-caries effect of fluoride is a topical one, after the teeth emerge into the oral cavity. It appears that even a very small increase in intra-oral fluoride concentration, which can result, for example, from repeated exposure to fluoridated water or by the intra-oral release of fluoride from fluoride-rich dental fillings, has the effect of promoting remineralization of demineralized dental hard tissues. The end result is a remineralized white spot incipient lesion which is rich in fluorapatite but low in carbonate and magnesium. The latter two are apatite mineral destabilizers, while fluoride stabilizes the mineral phase. There is some evidence that fluoride in low daily doses may affect the metabolism of bacteria believed to be responsible for dental caries, but this effect is a minor one.

Exposure of the teeth to topical fluorides with high concentrations of various fluoride agents (toothpaste, mouth rinses, professional fluorides) appears to work by providing a deposit of calcium fluoride that releases fluoride in small increments each time plaque bacteria metabolize sugars to acids.

The benefits of fluoridated water, however, if they even exist today, no longer seem to be of clinical significance, especially when placed in the context of the damage that fluoride ingestion from drinking water is doing to teeth. Ingested fluoride, from any source, during the developmental years in children, results in dental fluorosis in a dose-dependent manner. Not only is the incidence of fluorosis increasing at alarming rates, but so is the severity. The effect of long-term fluoride ingestion may be having similar effects on other hard tissues in the body. In this review a discussion of the systemic effects of fluoride ingestion will be limited to dental fluorosis, an area we are only now starting to understand more fully.

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Keywords: Dental caries, Dental fluorosis, Fluoridation, Systemic effects, Topical effects, Caries prevention.

### Serum and urine fluoride levels after topical dental fluoride application

The study was designed to examine the fluoride level in serum and urine of adults after topical application of an aminofluoride solution to the teeth. The tests were made in adults randomly divided into three equal groups. In group A patients

brushed their teeth with a dentifrice without fluoride; in group B patients brushed with an aminofluoride solution, recommended for topical application; in group C the patients drank 200 mL of milk after brushing with the aminofluoride solution. Samples of blood and urine were collected before applying the fluoride solution and hourly for the following six hours. Fluoride content was determined using an ion-selective fluoride electrode (Orion Research Inc.).

The results indicated that after topical fluoride application the level of fluoride in serum and urine increased, with the highest content occurring one hour after the application. Fluoride was absorbed systemically from the oral cavity despite being applied only topically to the crowns of the teeth. The influence of the milk is still being investigated.

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Keywords: Aminofluoride, Fluoride in urine, Fluoride in serum, Topical fluoride.

#### AMELIORATION OF FLUORIDE TOXICITY

##### **Beneficial effects of certain antidotes in mitigating fluoride and/or arsenic induced hepatotoxicity in mice**

The effects of the coadministration of sodium fluoride (NaF) (5 mg/kg body weight) and/or arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) (0.5 mg/kg body weight) for 30 days on the liver of female mice (*Mus musculus*) were investigated. The degree of recovery one month after of treatment withdrawal and the beneficial effects of some antidotes - calcium, ascorbic acid, and vitamin E, alone and in combination - were also studied.

Histopathological examination of the treated groups revealed hepatocellular necrosis, extensive vacuolization, and fatty deposition in the hepatocytes. The central veins and sinusoids of the liver were dilated and engorged with blood. These effects were not observed in the control group. The treatments also caused marked alterations in liver function with an increase in serum transaminases, accumulation of glycogen, and inhibition of phosphorylase activity, thus indicating that carbohydrate metabolism was affected.

Withdrawal of the NaF and As<sub>2</sub>O<sub>3</sub> treatments for 30 days resulted in some recovery in the histological and biochemical changes. Amongst the various antidotes, ascorbic acid was the most beneficial in bringing about pronounced recovery, followed vitamin E and calcium. However, all three antidotes administered together resulted in significant recovery in the histoarchitecture and carbohydrate metabolism of liver and serum transaminases. The values with the antidotes were comparable to those of the controls. Thus fluoride and/or arsenic induced effects were transient and reversible.

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Keywords: Antidotes, Arsenic, Fluoride toxicity, Hepatotoxicity, Mouse study.

### **Micronuclei and aneuploidy frequencies in human peripheral blood lymphocytes treated with sodium fluoride and arsenic trioxide**

The present study was carried out with a view to score aneuploidy and chromosomal aberrations along with a micronuclei assay in peripheral blood lymphocyte cultures to observe the effects of sodium fluoride (NaF) and arsenic trioxide ( $\text{As}_2\text{O}_3$ ). The protective effect of ascorbic acid, if any, was also studied.

The *in vitro* addition of NaF alone and in combination with  $\text{As}_2\text{O}_3$  at doses of 10  $\mu\text{g}$  and 0.001  $\mu\text{g}$  respectively to 7 mL of culture medium increased micronuclei frequency in binucleate cells. A significant increase ( $p < 0.02$ ;  $p < 0.001$ ) in the microfrequency was observed in the fluoride and arsenic treated cultures (Groups III-IV). However a highly significant increase ( $p < 0.001$ ) in the micronucleus frequency occurred after the combination treatment of NaF and  $\text{As}_2\text{O}_3$  (Group V) in both male and female individuals suggesting their genotoxic effects. Micronucleated cells could be the result of disturbances in the cell division process or due to chromosomal aberrations involving the formation of acentric fragments. Micronucleus formation is closely correlated with chromosomal breakage. This may be one of the reasons for the increased frequency ( $p < 0.001$ ) of chromosomal aberrations and aneuploidy after fluoride and arsenic treatments. However the addition of ascorbic acid (AA) at a dosage of 12  $\mu\text{g}/7\text{mL}$  culture medium in Group VI (NaF +  $\text{As}_2\text{O}_3$  + AA) showed a significant decline ( $p < 0.001$ ) in the mean frequency of micronuclei indicating some protective effects of ascorbic acid.

Hence it is concluded that in the present *in vitro* study, fluoride and arsenic modified chromosome structure and segregation leading to the induction of micronuclei in binucleate cells. The addition of ascorbic acid to the culture medium had a mitigating effect.

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Keywords: Aneuploidy frequency, Arsenic trioxide toxicity, Ascorbic acid, Lymphocytes, Micronuclei frequency, Sodium fluoride toxicity.

### ***Moringa oleifera* (radish tree) seed extract as an antidote for fluoride toxicity**

The aim of this study was to assess the efficacy of *M. oleifera* (radish tree) extract to prevent or alleviate fluoride toxicity in Wistar rats. Groups of animals were treated by oral intubation with fluoride (NaF) (25 mg/kg bw), seed extract (1g/kg bw) or a combination of fluoride and seed extract (25mg/kg bw, and 1g/kg bw, respectively) for 14 days. Other groups received the fluoride and seed extract at the same dose levels of 25 and 1 g/kg bw respectively, for 14 days each sequentially, with both orders of administration being studied (reversal phase group). A control group received distilled water.

No change occurred in the treated groups in the body weight, feed consumption, or haematological parameters. Alkaline phosphatase was significantly increased in

the fluoride treated group, particularly in females, compared to the seed extract group. There were no significant changes in albumin, glucose, or BUN, but GPT and GOT were increased in both sexes. The biochemical alterations were consistent with the histopathological changes in liver, testis, and femoral bone induced by fluoride ingestion.

Administration of the seed extract reduced the fluoride-induced changes. No correlation was found between the plasma mineral and fluoride levels. There were no significant changes in iron, manganese, zinc, or copper levels. Changes in sodium and potassium were not of clinical significance. Calcium was increased in the group given the seed extract alone. Focal necrosis in liver, hypospermatogenesis in testis, and osteopenia in femoral bone were found in the fluoride treated group but were either not seen or were accompanied by evidence of regeneration in the groups given seed extract in combination with or after the fluoride (reversal phase group).

In conclusion the study found that the seed extract of *M. oleifera* could protect against or ameliorate fluoride toxicity in Wistar rats.

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Keywords: Ameliorating factors, Fluoride toxicity, *Moringa oleifera*, Rat study, Seed extracts.

## ENVIRONMENTAL FLUORIDE

### Fluoride and environmental protection

In the United States, fluoride risks to the environment and public health are controlled by regulations issued under several environmental statutes. These regulations are administered by the U.S. Environmental Protection Agency (USEPA), which also undertakes other measures related to risk control, such its Integrated Risk Information System (IRIS). But the framework of regulatory and non-regulatory risk control as applied to fluoride is peculiarly skewed and internally inconsistent. For instance, while USEPA's Assistant Administrator for Water, Robert Perciasepe, wrote to a U.S. Congressman saying that he had asked the American Dental Association to remove the USEPA from the Association's list of endorsers of fluoridation, the USEPA's framework of regulatory and non-regulatory risk control policies clearly expresses a bias toward fluoridation.

This presentation will explore the USEPA's regulatory and non-regulatory approaches to controlling risks from fluoride with an eye toward elucidating inconsistencies in the policies, and will, to the extent possible, compare policies in the United States with those of other countries.

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Keywords: Environmental Protection Agency, USA. Fluoride risk control, USEPA.

### Urinary fluoride of school children in Gdańsk

Exposure to excessive amounts of fluoride may be caused not only by elevated fluoride levels in drinking water and foodstuffs but also by pollution from fluoride compounds of industrial origin. In the Gdańsk region both kinds of exposure are found. On one hand, a large phosphate fertilizer plant is located within the city. Emission of fluoride from the plant depends on both the magnitude of production and technical innovations in the production process. In recent years several improvements were introduced which substantially decreased fluoride air pollution. On the other hand, the eastern districts of the city are situated in an area with relatively high water fluoride levels (1-3 mg F<sup>-</sup>/L) of natural origin. Moreover, in Wiślinka, a suburb near the city border, there is a phosphate fertilizer waste disposal site. The wastes contain about 0.2% of soluble fluorine compounds. As a result, area inhabitants may be exposed to excessive amounts of fluoride, and local monitoring of exposure to this pollutant is advisable.

The aim of this study was to determine the urinary fluoride concentration of 1240 children (635 boys, 605 girls), aged 7-14, living in Gdańsk, Poland, and to examine whether a correlation exists between age and gender, school location, and fluoride level in the urine. Urinary F<sup>-</sup> was determined with a fluoride ion specific electrode.

The mean urinary F<sup>-</sup> concentration in children attending two schools near a fluoride-bearing phosphate fertilizer waste disposal site with 1.0 – 1.5 mg F<sup>-</sup>/L in the drinking water was 2.14 ± 1.14 mg F<sup>-</sup>/L. At three other schools near a phosphate fertilizer plant but with only 0.2 – 0.5 mg F<sup>-</sup>/L in the drinking water, the mean urinary F concentration was 1.05 ± 0.49 mg F<sup>-</sup>/L. In the first two schools the urinary F concentration in boys was significantly higher than in girls. No age-dependent differences were found in children in any of the schools. These findings of elevated urinary fluoride levels in children require further monitoring of exposure to fluorine compounds.

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Keywords: Children in Gdańsk, Environmental fluoride pollution, Fluoride in urine.

### Fluoride adsorption chemistry and the defluoridation of contaminated natural waters

Excessive fluoride in natural waters, in particular borehole waters, used for drinking water and the associated risk of fluorosis, is a well-known problem afflicting many people in rural areas of South Africa, the African Rift Valley and other parts of the world such as India and Sri Lanka. Excellent defluoridation technologies based on fluoride adsorption onto activated alumina or reverse osmosis are available to solve this problem, but due to cost and the degree of technology involved they are not always suitable for application in rural communities in Africa and elsewhere. In many countries the quest was therefore to develop simple, inexpensive methods for fluoride removal, preferably using local materials, such as suitable clays, as adsorbents for removal of excess fluoride. These endeavours have been only moderately successful, probably because not enough attention was given

to the fundamental chemical and physical aspects of fluoride adsorption. This study focussed on the fundamental aspects of fluoride adsorption chemistry and the efficiencies of clays for fluoride removal from natural waters. The following aspects were addressed:

- The elucidation of adsorption mechanisms and the derivation of a proper modelling algorithm for fluoride adsorption onto mineral surfaces. A thermodynamic model for fluoride adsorption based on the surface chemistry of the adsorbent, the solution chemistry of fluoride, and a surface complexation model for adsorption was developed and verified.
- The effect of mineralogical and structural characteristics of clays on fluoride adsorption capacity. A link between the availability of exchangeable hydroxide on clay surfaces and fluoride adsorption capacity was confirmed.
- The effect of physical and chemical pretreatment procedures on enhancing adsorption efficiencies of clays and the mechanistic explanations for these procedures.

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Keywords: Adsorption chemistry, Defluoridation, Environmental fluoride pollution, Fluoride in water, Natural waters, South Africa.

### **Endemic fluorosis in Kaveripattinam and Dharmapuri, Dharmapuri district, South India**

Dharmapuri district, in the endemic fluorotic Tamil Nadu region of South India, is a less developed drought-prone area with extremely high levels of fluoride in the groundwaters in its villages. These villages have rocky strata rich in fluoride bearing minerals from which fluoride leaches resulting in high fluoride levels in the groundwater. Drinking water sources from 254 villages in 62 panchayats of the Dharmapuri block and 231 villages in 31 panchayats of the Kaveripattinam block were analyzed for fluoride content and other important constituents and parameters. In the Dharmapuri block 71% of groundwater sources were contaminated with excess fluoride while 90% of water sources of the Kaveripattinam block contained fluoride above the tolerance limits. For 18 villages in the Kaveripattinam block, with severe endemicity and an alarming level of dental fluorosis, the water sources had extremely high levels of fluoride of 7-8 mg/L. The regions containing high levels of fluoride in the two revenue blocks were identified by established methods of mapping. An explanation for the dependence of fluoride on total alkalinity and total hardness is offered.

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Keywords: Dharmapuri block, Endemic fluorosis, India, Kaveripattinam block, Tamil Nadu region.

### Endemic fluorosis from brick-tea fluoride

Endemic fluorosis from fluoride in brick-tea among Tibetans living in Kamba, Sichuan, was first reported in the early 1980s. We studied both the fluoride content of brick-tea and the health of heavy consumers of it including Tibetans, Mongolians, Yugus, Hasaks, and Uygurs.

The diet for the herdsmen, who lived in a cold area at high altitude, comprised highland barley flour, milk, butter, and brick-tea. Vegetables and fruit were usually unavailable. The old coarse tea leaves, from which brick-tea is made, were found to have a higher fluoride content (>550 mg/kg) than the leaves used for ordinary green and black teas (15-50 mg/kg).

The fluoride intake for children and adults was 5-12 mg/day. Urine fluoride was up to 2-11 mg/L. The prevalence of dental fluorosis in children was 50-88%. Skeletal fluorosis in adults had a prevalence of 83% with the condition commonly being disabling at a late stage. There was a high prevalence of subclinical skeletal fluorosis among children.

Fluorosis from brick-tea is a public health problem. Reducing the fluoride content of brick-tea has been done by adding an adsorptive ingredient (approved Chinese Patent No. ZL 99 1 15669.2). A four-year program to expand the use of this low-fluoride brick-tea has been sponsored by the China Medical Board of New York Inc., USA, but the level of support is insufficient to match vast size of the endemic area. Further research is needed on how fluoride accumulates in tea plants and how tea-borne fluoride affects bones and teeth.

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Keywords: Brick-tea, Dental fluorosis, Endemic fluorosis, Fluoride intake, Tibetans, Urinary fluoride.

### Organofluorine compounds in nature

Many people are alarmed by evidence for the toxicity of fluoride and the potency of new fluorine-containing products, but few are aware that nature itself produces dangerous organofluorine compounds. The halogens chlorine, bromine, and iodine appear in many organic natural compounds, while fluorine, the 13<sup>th</sup> most abundant element in the earth's lithosphere, has been identified as a component of several natural compounds. All these compounds are toxic for mammals, including humans, and represent a small but toxicologically very interesting group of poisons.

Most of these substances occur in plants and, to a lesser degree, in bacteria. The best-known fluorine-containing natural compound is fluoroacetic acid or as a fluoroacetate. It is found in a wide variety of plants in low concentrations; however, in certain plants it accumulates in very high concentrations and these are poisonous for livestock. A well-known plant of this species is the South African shrub known as gifblaar (*Dichapetalum cymosum*).

Fluoroacetate is toxic because it is converted *in vivo* in mitochondria into fluorocitrate, which occurs through condensation of fluoroacetyl-CoA with oxaloacetate

by the enzyme citrate synthetase, which normally supplies acetyl-CoA into the citric acid cycle. Fluorocitrate is a strong inhibitor of aconitase, a further important enzyme of the Krebs cycle, and the result is inhibition of a vital biochemical pathway for energy production in the organism..

All  $\omega$ -fluorinated fatty acids with an even number of carbon atoms in their molecule possess similar toxicity, and some of them, for example,  $\omega$ -fluoropalmitic,  $\omega$ -fluorostearic, and  $\omega$ -fluorooleic acids, are the principal toxic substances of another West Africa shrub, *Dichapetalum toxicarium*, also known as ratsbane. The toxicity of  $\omega$ -fluorinated fatty acids results from their metabolization to fluoroacetate; nevertheless, some of them are more toxic than fluoroacetate. The most toxic  $\omega$ -fluoro-fatty acid is probably  $\omega$ -fluorooctanoic acid, but even  $\omega$ -fluorobutyric acid is approximately four times more toxic than fluoroacetate.

Another fluorine-containing natural compound is an adenosine derivative product with antibiotic activity. This was isolated from the organism *Streptomyces calvus*. This organofluorine substance, nucleocidin, is a very effective broad-spectrum antibiotic, but too toxic for clinical use.

Fluorinated bioactive compounds thus play an important role in human and veterinary medicine and in agro chemistry.

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Keywords: 1080, *Dichapetalum cymosum*, *Dichapetalum toxicarium*, Fluorinated fatty acids, Fluoroacetate, Fluororganic compounds, Fluoride in plants, Nucleocidin.

## BIOLOGICAL EFFECTS OF FLUORIDE

### The influence of fluoride on human leukemic cell lines

Our previous findings indicated that fluoride might be potentially toxic toward normal early human hematopoietic cells. On the other hand, data on the potential influence of fluoride on leukemic blasts are not available in the literature. To address this issue, we exposed different human leukemic cell lines to increasing doses of sodium fluoride (NaF) as well as sodium hexafluorosilicate (Na<sub>2</sub>SiF<sub>6</sub>) for 2 hr at 37°C.

We employed HL-60 (AML-M3 according to FAB classification), TF-1 (AML-M6), HEL (AML-M6) and K562 (CML in blastic phase) cell lines. After the exposure, the cells were washed out and plated in the methylcellulose cultures to grow blastic colonies. TF-1 cells required an addition of growth factors to *ex vivo* culture. In contrast, HEL as well as the rest of the cell lines used did not.

We found that higher concentration of both NaF and Na<sub>2</sub>SiF<sub>6</sub> may potentially affect the clonogenicity of human leukemic cells. The most visible effect was noted in the case of HL-60 cells.

Finally, in order to obtain more information on the influence of fluoride on neoplastic blasts, we estimated the induction of apoptosis in the human leukemic cell lines exposed to NaF or Na<sub>2</sub>SiF<sub>6</sub>. We employed two different assays to evaluate early phase of apoptosis. The cells were exposed to increasing doses of the fluorine

compounds and stored at 37°C for 24 h. The next day the percent of apoptotic cells was assessed. Apoptotic and necrotic cells were detected after Annexin-V and propidium iodide staining, respectively, and evaluated by FACS (Flow Activated Cell Sorter). Moreover, apoptosis was evaluated using RT-PCR method for semi-quantitative detection of human proapoptotic (Bax) and anti-apoptotic (Bcl-X<sub>L</sub>) gene expression.

Apoptosis was detectable both after exposure to NaF and Na<sub>2</sub>SiF<sub>6</sub>. Higher doses of fluorine compounds induced a larger number of the cells to enter the early phase of apoptosis, measured by FACS as well as RT-PCR.

In conclusion, our findings revealed that human leukemic cells could be influenced and damaged by fluorine compounds.

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Keywords: Apoptosis, Clonogenicity, Fluoride toxicity, Human leukemic cells.

### **Patterns of urinary fluoride excretion after consumption of space food and Japanese menu food**

When the urinary excretion of fluoride (F) is monitored in relation to dietary F intake it can be difficult to measure accurately the fluoride content of the food consumed. In this study the pattern of urinary F excretion was studied after the ingestion of either typical Japanese food or space food. Space food involving freeze-dry technology has been developed by Oregon Freeze Dry Inc. in conjunction with the National Aeronautics and Space Administration (NASA) to meet the requirements of NASA's space program. It is reconstituted with water.

The subjects were four healthy young adult males, aged 20. They were fed either Japanese or space food three times daily, at 0830, 1230, and 1830 hr, respectively, for four consecutive days. The subjects and the investigator stayed in the same house, carrying out their routine activities. During the 4 days urine was collected at 4-hr intervals except at night. After measuring the volume a portion was placed in a polyethylene bottle and kept freeze-dried until analysis.

The menu for the first and second days was general food of mainly Japanese-style, and for days three and four space food. The amount of F in the space food was measured using a flow-injection method developed by Itai, and the amount of urinary F was measured using an ion-electrode direct measurement method. The day-to-day variation in urinary fluoride excretion showed a similar pattern with both types of food.

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Keywords: Dietary fluoride, Fluoride in urine, Japanese food, Space food.

### **Hemodialyzability of the bound and ionizable fractions of serum fluoride**

Patients undergoing long-term hemodialysis (HD) are known to develop high serum fluoride (F) levels in spite of the increased popularity of using reverse osmosis-treated water (RO water).

In this study, serum and dialysate ionic F were determined by a F-ion selective electrode method (ORION Research, Model, EA 940 digital ion analyzer provided with an F-selective electrode) in 29 patients undergoing hemodialysis treatment. To 1 mL of each of sample, 100  $\mu$ L of total ionic strength adjustment buffer (TISAB) were added so that the pH and ionic strength were sufficiently high to liberate any F that might be bound or in complex form. The movement of F across the HD membrane was investigated. The percentage of the theoretically filterable and unfilterable F throughout the HD session was evaluated by the equation of Graf *et al* (1981). The percentage of the actually filtered or unfilterable F was evaluated by regression analysis.

An abnormally high serum fluoride was found at both the beginning ( $65.9 \pm 28.3$   $\mu$ g/L) and at the completion of the hemodialysis sessions ( $46.5 \pm 26.7$   $\mu$ g/L). The results showed that  $60.0 \pm 23.9\%$  of the serum fluoride at the beginning of the session was in theoretically filterable fraction, and that  $80.8 \pm 42.4\%$  of this fraction was actually filtered during the hemodialysis session.

In conclusion, the dialysate and the dialysis procedure used in the study were considered to be safe and adequate for serum fluoride removal. The high serum fluoride at the completion of the hemodialysis session was thought to have originated from the fraction of unfilterable bound fluoride. To further improve the removal of fluoride from the serum during HD, a strong focus on the bound fraction of serum fluoride will be necessary.

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Keywords: Bound fluoride, Fluoride in serum, Hemodialysis, Ionizable fluoride.

### **Fluoride levels in liver, kidney, and brain of mice after subacute oral treatment with fluoride**

Contamination of groundwater by fluoride has been reported in China and India. It is of interest to determine the levels of fluoride in organs after exposure to fluoride via drinking water. It is also interesting to know whether neurological effects of fluoride can be induced or not by the oral exposure. The purpose of this study was to determine fluoride levels in organs of mice exposed to fluoride via drinking water. The neurological effects of fluoride was also studied by determining neurotransmitter levels.

Adult male BALB/c mice (six per group) were exposed to 0, 1, 5, 25, 125 ppm of sodium fluoride in drinking water for a month. Following the treatment period,

mice were euthanized, and the liver, kidneys, and brain removed. Brain samples were dissected into six regions. Half the cerebrum was used for the determination of fluoride ion concentration. Fluoride in each organ was isolated by pyrohydrolysis, recovered into water solution, and determined using a flow-injection apparatus with a fluoride ion selective electrode as the detector. The concentrations of neurotransmitters and their metabolites in brain regions were determined by HPLC.

The mean value of fluoride concentration in liver was 0.087  $\mu\text{g/g}$  for control, 0.077  $\mu\text{g/g}$  for 1 ppm, 0.085  $\mu\text{g/g}$  for 5 ppm, 0.085  $\mu\text{g/g}$  for 25 ppm and 0.228  $\mu\text{g/g}$  for 125 ppm group. The 125 ppm group had a significantly higher mean value compared to other groups. The mean value of fluoride concentration in kidney was 0.143  $\mu\text{g/g}$  for control, 0.135  $\mu\text{g/g}$  for 1 ppm, 0.122  $\mu\text{g/g}$  for 5 ppm, 0.150  $\mu\text{g/g}$  for 25 ppm and 0.350  $\mu\text{g/g}$  for 125 ppm group. The 125 ppm group also had a significantly higher mean value compared to other groups. There were no significant differences of fluoride concentration in cerebrum among the different groups. There were no significant differences in the levels of neurotransmitters or their metabolites in any brain regions among the groups.

The higher concentration of fluoride in kidney among the 125 ppm group may be related to renal damage. The absence of an elevation in fluoride in brain was consistent with there being no alteration in the levels of neurotransmitters and their metabolites in brain regions among the groups.

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Keywords: Fluoride in brain, Fluoride in drinking water, Fluoride in kidney, Fluoride in liver, Mouse study.

### **Early and prolonged toxic effects of silicofluoridated water on chinchillas, caimans, alligators, and rats in captivity**

For about six months during the early 1970s, co-author RFF operated a successful 50-animal fur-trade chinchilla farm in Auburn, Kansas. Within days after the low-fluoride Auburn well water was changed to the nearby  $\text{H}_2\text{SiF}_6$  fluoridated Topeka system, the animals began drinking more than twice as much water as before. Their fur became scruffy and unmarketable, and stillbirths began to occur. Half the colony was then changed to distilled water, resulting in decreased and then normal water consumption. No further stillbirths occurred in this group, and their fur quality was restored. In the Topeka water group, however, high water intake and increased stillborn litters continued. Even the adults in this group began to die prematurely. With a move to another community the chinchilla colony was disbanded because of a decline in the pelt market.

In Kansas City, Missouri, after more than 20 years of optimal health care by co-author PNJ, many of the two dozen caimans and 3 alligators at Parrot Hill Croc Farm began to exhibit toxic effects within three days following the start of water fluoridation with  $\text{H}_2\text{SiF}_6$  on April 9, 1981. Eye membranes became swollen and

ulcerated. The animals tried to avoid the water, moving from tank to tank, evidently in search of less irritating water. By 1983 some had bloated bellies and spinal deformities. Premature deaths began to occur, many before age 10 (normal life span 25 years or more). Autopsies revealed severe disintegration of the GI tract, silicosis, and other abnormalities. Caiman eggs laid since 1981 have all been infertile, in contrast to successful fertilization and hatching before 1981. Hatchlings acquired after 1981 raised in distilled water were healthy until, because of their size, they were switched to the fluoridated tap water.

After fluoridation began, the health of the rats at the croc farm also declined dramatically. Within six months over 200 tumors were counted, with as many as 6 per rat. In October 1981 the rats were given only distilled water to drink. Their condition rapidly improved, and no new tumors appeared. Their life spans increased significantly, with some of the rats reaching more than 7 years of age.

With no other changes having occurred, these findings demonstrate an unequivocal toxicity of silicofluoridated tap water.

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Keywords: Caimans, Chinchillas, Fluoride toxicity, Fluoridation, Rats, Silicofluorides, Water intake.

## FLUORIDE ANALYSIS

### Fluoride measurement in small samples by semi-micro automatic distillation

The study of the biological effects of fluoride often involves measuring fluoride levels in food or the organs of experimental animals. In Japan measurement of the fluoride level in solid samples using the Japan Industrial Standard (JIS) is by the Willard-Winter method using a distillation method. Although this method has the advantage of being able to use samples of a small size, it is difficult by manual operation to keep the system stable with respect to parameters such as the temperature of perchloric acid and the infusion volume of steam. We have developed a new apparatus using an automatic distillation method to obtain a more rapid and accurate determination of fluoride in samples of a small size. The practical application of the method was confirmed by measuring the fluoride content of some samples.

In the present study, 1-3 g of biological solid material were ashed with calcium oxide at 600°C for 3 hours. The final ash product of 0.05-0.15 g was introduced into a steam distillation duct kept at 140°C in a silicone oil bath with electronic temperature control. The final ash product was dissolved with 10 mL of perchloric acid and its temperature kept at 135°C with a C-A thermoelectronic couple monitor. A water pump (W12, ISCO) sent water into a steam gas generator tube at constant flow rate (0.7-1.75 mL/min). The tube was made of quartz glass and directly heated by an iron-chrome band heater. The final steam gas temperature at the output port was 200°C. A condensed steam sample of hydrofluorosilicic acid was collected in a Teflon container.

A difficulty in operating this analysis system is an adverse flow of heated perchloric acid solution produced by fluctuations in the generated steam pressure. This phenomenon easily occurs with multiple factors such as a prolongation of steam heating on-off time, a decrease of steam gas, and cooling down of the system. To prevent this adverse effect, we put a buffer tube and a back flow stop bulb in the steam circuit. With this system four samples can be analysed simultaneously.

Fluoride levels in fillet of salmon (1-3g) were examined by distillation for 20 min and measuring the fluoride ion concentrations with an Orion fluoride meter and electrodes. The result was  $0.469 \pm 0.106$  mg/kg (N=7, Mean  $\pm$  SD).

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Keywords: Fluoride analysis, Distillation sample size, Semi-micro automatic distillation.

### Comparison of hydroxyapatite and fluoroapatite using time-of-flight secondary ion mass spectrometry (TOF-SIMS)

Chemical analysis of hydroxyapatite and fluorapatite was carried out using time-of-flight secondary ion mass spectrometry (TOF-SIMS) which is a useful technique because of its high chemical sensitivity and selectivity. Hydroxyapatite and fluorapatite were synthesized at  $80 \pm 1^\circ\text{C}$  by introducing 0.5 L of 100 mmol/L  $\text{Ca}(\text{CH}_3\text{COO})_2\text{H}_2\text{O}$  solution and 0.5 L of 60 mmol/L  $\text{NH}_4\text{H}_2\text{PO}_4$  solution containing 0 or 20 mmol/L HF at a rate of 250 mL/hr with a peristaltic pump into 1.0 L of mechanically stirred 1.3 mol/L  $\text{CH}_3\text{COONH}_4$  solution. pH was maintained at  $7.4 \pm 0.2$  with the occasional addition of concentrated  $\text{NH}_4\text{OH}$  solution. The suspensions were stirred for 3 hr, then kept at  $25^\circ\text{C}$  for 24 hr. X-ray diffraction analysis of both hydroxyapatite and fluoroapatite showed typical well-crystallized apatitic patterns. Fluoroapatite was much more crystallized. The fluoride content was similar to that of theoretical fluoroapatite. Scanning electron microscopic observation showed the needle-like crystals of both the apatites. High-resolution transmission electron spectroscopy clearly revealed the slender hexagonal shape at cross-section perpendicular to the c-axis of hydroxyapatite and the typical hexagonal shape for fluoroapatite. FT-IR spectra of fluorapatite indicated the disappearance of the absorption peak observed for hydroxyapatite at about  $3570\text{ cm}^{-1}$ . TOF-SIMS mass-spectrum of calcium was observed just at 40 amu. On the other hand, mass-spectrum of fluorine could be observed clearly at 19 amu, although the intensities of  $\text{PO}$ ,  $\text{PO}_2$ , and  $\text{PO}_3$  were very low. It was confirmed that TOF-SIMS showed much more clearly the difference between the positive and negative mass-spectra of hydroxyapatite and fluorapatite, especially for  $\text{F}^-$ , compared with other methods of chemical analysis.

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Keywords: Chemical analysis, Fluoroapatite, Hydroxyapatite, Mass spectrometry, TOF-SIMS.

### Fluoride in drinking water of Bangladesh

Contamination of water and food by fluoride poses a serious health problem in many areas of South Asia and other regions of the world. Proton Induced Gamma-ray Emission (PIGE), which is a sensitive and reliable method for fluoride analysis in water samples, was used for measuring the fluoride concentration in 304 water samples collected from rural and urban areas in Bangladesh.

The fluoride concentration in the samples was found to lie in the range of 0.02 to 2.32 mg/L with a mean of  $0.43 \pm 0.40$  mg/L. Surface water contained the lowest amount of fluoride with a mean of  $0.14 \pm 0.10$  mg/L for 23 samples. The mean fluoride content in rain water was  $0.26 \pm 0.13$  mg/L, a level higher than that for the surface water. The concentration of fluoride in 102 samples from the city water supplies ranged from 0.03 to 1.10 mg/L with a mean of  $0.33 \pm 0.21$  mg/L. The fluoride concentration in 163 tube well water samples was the highest with a mean of  $0.56 \pm 0.48$  mg/L, and a range of 0.02 to 2.32 mg/L.

In 61% of the samples the fluoride concentration was below 0.5 mg/L and in 94% of the samples it was below the WHO maximum permissible limit (MPL) of 1.5 mg/L for drinking water. Only 16% of the samples exceeded the MPL of 1.0 mg/L set for drinking water in Bangladesh.

The present study indicates that the fluoride concentration in groundwater in Bangladesh is, in general, lower than the MPL of 1 mg/L. For a better understanding of the significance of the findings of the present study, measurements of the fluoride concentrations in food, vegetables, chewing sticks, dental products, etc have to be made, in the localities where the fluoride concentrations in drinking water exceed the MPL. Research in these areas is in progress.

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Keywords: Bangladesh, Fluoride in drinking water, Fluoride analysis, Proton induced gamma emission, Tubewell water.

### Atherosclerotic fluorification

Fluoride levels were measured in post-mortem human tissues with a fluoride selective electrode. Low levels were found in soft tissues and high levels in bone, teeth, and atherosclerotic tissue. Fluoride was first detected in human hard and soft tissues by Gay-Lussac in 1805. A thrombogenic theory for the formation of atherosclerotic plaque was proposed by Rokitansky in 1842 and is consistent with findings presented here.

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Keywords: Fluoride content in atheromatous plaque.

**ETHICS OF FLUORIDATION****The ethics of water fluoridation: a scientific-logical analysis**

Some of the fundamental questions about the fluoridation of public water supplies are ethical in nature: *e.g.* Is medication with an uncontrolled dose wrong?, Is mass medication, which is either compulsory or expensive to avoid, wrong?, Is fluoridation right if its risks are less than its benefits?

Some leading proponents of fluoridation attempt to evade such ethical issues by quasi-scientific argument. For instance, they claim that fluoridation is not medication, but merely an 'adjustment' of the natural fluoride concentrations in drinking water to the 'optimal' level for reducing tooth decay. Or they allege that fluoride is an essential nutrient, rather than a medication.

However, ethical questions cannot be so easily transformed into scientific and technical ones to be answered glibly by dentists and medical practitioners. This paper assists the elucidation of several ethical questions about fluoridation by first clarifying several related questions of science, technology, and logic. This clarification leads to the conclusions that fluoride, at the levels recommended by pro-fluoridationists for reducing tooth decay, is not an essential nutrient; is not a natural substance for babies or for most adults; is not a compulsory medication, but is an expensive-to-avoid medication with an uncontrolled dose; and is harmful to some people. There is scientific evidence that the benefits of fluoridation have been greatly overestimated, but the actual magnitude of benefits is still unclear. It is now clear that any benefit comes from the action of fluoride on the surface of teeth, and there is negligible benefit from swallowing fluoride. It is not possible to weigh risks against benefits in a value-free manner.

These scientific, technical, and logical conclusions prepare the way for ethicists and others to examine the fluoridation issue, unencumbered by the usual 'scientific' myths. The original ethical concerns about fluoridation are found to be well-posed questions, an ethical question used by proponents to justify fluoridation is found to be improperly posed, and a new ethical question arises from the analysis: Is it right for medical, dental, and public health professionals to mislead people that fluoride is an essential nutrient, when fluoridated drinking water has negligible systemic benefit, but may at best have a small topical benefit?

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