

**ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE  
XXVIth CONFERENCE OF THE INTERNATIONAL SOCIETY FOR  
FLUORIDE RESEARCH, WIESBADEN, GERMANY,  
SEPTEMBER 26–29, 2005**

The following edited abstracts are of papers that have been accepted for presentation, orally or by poster, at the XXVIth Conference of the International Society for Fluoride Research, Wiesbaden, Germany, September 26–29, 2005. They are numbered and arranged in alphabetical order according to the name of the presenting author which is shown in boldface type.

**EFFECTS OF SODIUM FLUORIDE ON THE INTEGRITY OF THE RAT  
GASTRIC MUCOSA**

Abstract number: **1**  
XXVIth ISFR Conference The stomach is exposed to the highest concentrations of fluoride on a daily basis, and overexposure may lead to structural and functional alterations in this organ. The effects of sodium fluoride (NaF) on gastric ulceration, the adherent gastric mucus, and the electrical potential difference (PD) as an index of the gastric barrier integrity were studied in rats using different concentrations of NaF in the presence of 0.05 M HCl.

Oral administration of acidified aqueous 20 and 40 mM NaF significantly ( $P < 0.05$ ) elevated the ulcer index (measured by a 6-grade severity scale) when compared with control conditions (HCl 0.05 M). No significant difference was observed between the two concentrations of NaF.

The adherent gastric mucus was studied using Alcian blue (a dye that binds to glycoproteins and soluble mucopolysaccharides without penetrating mucosal cells). The recovered amount of Alcian blue (representing the amount of the adherent mucus) from the mucosa was dependent on the NaF concentration. Treatment with 20 mM NaF resulted in a slight decrease (25%) of the recovered amount of Alcian blue, whereas 40 mM NaF significantly ( $P < 0.05$ ) decreased the Alcian blue recovery by about 75%. In addition, a significant negative correlation was observed between the amount of recovered dye and the ulcer index ( $P < 0.05$ ).

Intra-gastric perfusion with 0.05 M HCl had a stable mean PD of 37.7 mV after 30 min. Addition of 1, 5, 10, and 20 mM NaF to the HCl solution produced a concentration dependent decrease in gastric PD. At 1 mM, NaF caused a slight drop in PD that became significant ( $P < 0.05$ ) after 30 min of administration. Perfusion of higher concentrations (5, 10, and 20 mM) induced a rapid and significant fall in the PD from the fifth minute of perfusion with the 10 and 20 mM concentrations and had a maximum reduction of  $28.9 \pm 1.2$  mV in PD.

In conclusion, sodium fluoride at a concentration as low as 1 mM appears to affect the integrity of the gastric mucosa in rats.

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Keywords: Alcian blue mucus binding; Gastric mucus in rats; Gastric potential difference; Sodium fluoride and rats; Stomach ulcer index.

## STRUCTURAL CHANGES OF INTERVERTEBRAL DISCS IN EXPERIMENTAL OSTEO- AND ARTHROFLUOROSIS OF RATS

Abstract number:  
**2**  
 XXVth ISFR Conference

*Introduction:* In previous studies,<sup>1-5</sup> we investigated and discussed experimental osteo- and arthrofluorosis, including qualitative and quantitative changes in the bone, articular cartilage, and joint capsules in rats.

*Present Objective:* The purpose of this study was to examine structural changes of the intervertebral discs in rats administered sodium fluoride.

*Material and Methods:* Seventy-five female Wistar rats, each weighing about 200 g, were equally divided into three groups. Animals in Groups 1 and 2 received daily doses of 0.5 mg and 5 mg of NaF, respectively, by intraperitoneal administration, whereas Group 3 (controls) received physiological saline solution only. The experiments were run for a period of three months.<sup>1</sup> Histological and histochemical studies were carried out on lumbar vertebrae III-V. The tissues were fixed in a 10% formaldehyde solution, decalcified at room temperature in a solution composed of 24 mL of 85% formic acid, 50 mL of 55% HCl, and 126 mL of distilled water. Histologic sections were stained with haematoxylin and eosin, picosirius red F3BA,<sup>6,7</sup> toluidine-blue at pH 6 and pH 3.5,<sup>8</sup> or by the ABT (aldehyde-bisulphite-toluidine blue) reaction at pH 1.<sup>9</sup> Changes of the intervertebral discs were also investigated by polarisation optical methods. The fluoride content of bone tissue was determined by ion selective analysis.<sup>2</sup>

*Results:* Progressive submicroscopic derangement of collagen fibers and aggrecan molecules in the annulus fibrosus of intervertebral discs was found in rats exposed to NaF. The orientation of collagen fibers, polysulfated acidic GAGs, and neutral polysaccharides containing vicinal OH groups decreased within the annulus fibrosus of intervertebral discs compared to controls. The nucleus pulposus was atrophic and collapsed, resulting in narrowed intervertebral spaces in some cases. Structural changes correlated with the dose of NaF and with the fluoride content of bone tissue (1.10 mg F/g in Group 1, 1.44 mg F/g in Group 2, and 0.423 mg F/g in the control Group 3).<sup>2</sup>

*Conclusions and Interpretation:* The submicroscopic disorientation of collagen fibers and aggrecan molecules in the annulus fibrosus of intervertebral discs observed here by fluoride correlate with the reduced metabolic activity and/or necrosis of cellular components. The qualitative and quantitative changes within the intervertebral discs may therefore be regarded as a toxic effect of fluoride. Pathological changes and damage to tissue components in our experimental animals are analogous to human diseases, e.g., spondylarthrosis, and may be considered as complex functional abnormalities caused by fluoride.

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Keywords: Arthrofluorosis in rats; Intervertebral disc changes; Osteofluorosis in rats.

### FLUORIDE REMAINING IN THE ORAL CAVITY OF CHILDREN AFTER BRUSHING WITH FLUORIDE GEL

Abstract number:

**3**

XXVIth ISFR Conference

With increased levels of fluoride in the environment, it has been suggested that the total fluoride exposure from various sources should be precisely monitored. Methods of topical fluoride application, generally considered safe and widely used in caries prevention, involve not only the local action of application but also the absorption of a certain dose of fluoride. Therefore there is a need to determine the amount of fluoride remaining in the oral cavity after the procedure and address an issue of proper standards for fluoride administration.

The objective of this study was to evaluate the amount of fluoride remaining in the oral cavity of children after they brush their teeth with GABA Elmex gel.

The study involved seven groups of seven 11-year-old children and seven groups of seven 7-year-old children. In each study group measured amounts of the gel were applied on the toothbrushes (about 0.6 g of the gel), and the total mass of the product used was recorded. Tooth brushing was carried out according to the manufacturer's recommendations with the younger children especially being asked to rinse their mouth after brushing. All the children were instructed to spit the saliva with the remaining gel and rinsing water into plastic beakers and put their toothbrushes after brushing into a plastic container. After thorough rinsing of the toothbrushes in redistilled water, the content of the container and the contents of the beakers were poured together. Fluoride concentrations in this solution were determined using an ionselective fluoride electrode (MARAT, model 0937) and a silver chloride reference electrode (MARAT, RAE111). On the basis of these results the amount of fluoride remaining in the oral cavity of a child were calculated.

The results of the study indicate that after brushing with Elmex gel, from 0.4 to 1.8 mg of fluoride (mean 1.1 mg) remains in the oral cavity of the 7-year-old participants. Similar results were obtained for the 11-year-old children: 0.5 to 1.8 mg fluoride (mean 1.2 mg).

The study revealed that when the recommended standards of fluoride application are followed, the amount of fluoride remaining in the oral cavity of children is comparable to their estimated daily fluoride intake from diet and dentifrice.

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Keywords: Fluoride gel application; Oral fluoride retention; Tooth brushing.

**ORIGIN AND DEVELOPMENT OF THE ISFR**

Abstract number:  
**4**  
XXVth ISFR Conference

During the 1950s, shortly after water fluoridation began to be widely adopted in the United States, George L Waldbott, MD (1898–1942), an allergy specialist in Eastern Michigan, encountered a variety of reversible illnesses in his patients traceable to fluoride in their drinking water. Although he was well-known for his work on respiratory and skin allergies, he found, to his dismay, that he was obliged to report his well-verified clinical findings of illness from fluoridated water in European medical journals because major US medical journals declined to publish them. In studying the world literature of the biomedical and environmental effects of fluoride, Dr Waldbott came in contact with many leading researchers in these fields and found they were eager for a venue to share and discuss their findings. He therefore arranged a very successful conference on the Toxicology of Fluorine held in Bern, Switzerland in October 1962. In 1966 he founded the American Society for Fluoride Research that held a conference on recent developments in fluoride research in Detroit, after which it became the present International Society for Fluoride Research. The first conference of the ISFR was held in Frankfurt-on-Maine, Germany, in October 1967, followed to the present by 25 stimulating conferences at one- to two-year intervals in various locations in Europe, the United States, India, Japan, China, and New Zealand. In 1968 Dr. Waldbott inaugurated *Fluoride Quarterly Reports* of the ISFR, later changed simply to *Fluoride*. After his death in 1982, his widow Edith M Waldbott (1903–1997) served as “interim editor” until 1991, when she was succeeded by the late Dr John A Colquhoun of Auckland, NZ (1924–1999), followed at the end of 1998 by the present writer with Dr Bruce J Spittle as managing editor. Over the years, *Fluoride* has published a wide range of peer-reviewed research reports and reviews, hundreds of abstracts from the literature, and many significant editorials and communications.

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Keywords: International Society for Fluoride Research (ISFR); ISFR conferences; ISFR journal *Fluoride*.

**EFFECTS OF A PROTEIN DEFICIENT AND PROTEIN RICH DIETS FED ALONG WITH SODIUM FLUORIDE ON SOME SPERM FUNCTIONAL PARAMETERS IN MICE**

Abstract number:  
**5**  
XXVth ISFR Conference

The effects of ingestion of protein deficient and supplemented or rich diets alone and along with sodium fluoride (NaF, 5 mg, 10 mg, 20 mg/kg body wt) on some specific sperm functional parameters in mice were studied and compared with those fed a control protein diet + NaF in the above three doses for 30 days.

Feeding a protein deficient diet to male mice treated for 30 days with NaF (5, 10, 20 mg/kg body wt) caused a significant decrease in sperm motility, count, viability, fertility rate, hyaluronidase activity, sperm mitochondrial activity index (SMAI) and in number of normal spermatozoa. However, acridine orange fluorescence staining for DNA integrity and total acrosin levels were comparatively less affected.

The control protein diet + NaF (3 doses) treatment also caused significant alterations in all the parameters as compared to control Group I. However, the animals fed a protein supplemented diet alone and along with NaF (5,10, 20 mg/kg body wt) for 30 days did not manifest any changes and the values were almost same as in control.

The decrease in sperm motility is related to decline in SMAI and abnormal sperms to their count. The significant decrease in viability along with the above parameters would affect

fertility, whereas, inhibition of hyaluronidase and to some extent of acrosin could affect fertilizing capacity of the sperm.

The results reveal that dietary factors are important in curbing fluoride toxicity.

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Keywords: Fertility rate; Fluoride; Hyaluronidase activity; Protein deficient diet; Protein rich diet; SMAI; Sperm count; Sperm motility; Sperm viability.

## **A SURVEY OF FLUORIDE IN 90 ENDEMIC VILLAGES OF MEHSANA AND BANASKANTHA DISTRICTS OF NORTH GUJARAT, INDIA**

Abstract number:

**6**

XXVIth ISFR Conference

A survey was conducted in 90 endemic fluoride villages of Mehsana and Banaskantha Districts of North Gujarat, India, with reference to fluoride levels in drinking water, serum, and urine as well as several other parameters in serum and urine compared to the control population of low-fluoride Ahmedabad city. In the endemic area 749 cases (590 males and 159 females) were studied with an average of 20–50 cases per parameter in both the endemic and control areas. The analysis of water, blood, and urine showed significantly high fluoride levels in the endemic population as compared to controls. The increased serum catecholamine levels in the endemic population might be due to the stress caused by fluoride and may influence the hypothalamic-gonadal axis as well as disturb carbohydrate metabolism in these individuals. The latter was evident as liver function markers, viz., serum transaminases were significantly increased as compared to control. The decrease in serum protein and calcium but an increase in  $\text{Na}^+$ ,  $\text{K}^+$  suggests electrolyte imbalance and probable loss of water from the body. On the other hand, the increase in protein and potassium in urine suggest their loss in urine due to altered kidney function. The assay of thyroid hormones reveal disturbance in thyroid function leading to change in basal metabolic rate. The haemoglobin content was low with respect to the control suggesting an anaemic state.

The decline in serum testosterone but increase in cholesterol point to altered hypothalamo-hypophysial-gonadal axis, which might affect steroidogenesis, sperm motility, and other reproductive functions and fertility, as evident in several studies. Serum sialic acid, a known marker for detection of fluoride disturbances, was reduced in the endemic populations, probably as a consequence of elevated glycoaminoglycans, which hinder hormone-receptor interaction. Genotoxicity studies have revealed chromosomal aberration, increase in sister chromatid exchange, and changes in replicative index in peripheral blood lymphocytes in fluorotic individuals.

The above data clearly indicate the presence of adverse effects of high water-borne fluoride in endemic population in North Gujarat, India.

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Keywords: Banaskantha; Blood fluoride; Drinking water; Endemic areas; Fluorosis; Mehsana; North Gujarat; Urine fluoride.

## **ANALYTICAL CHEMISTRY OF FLUORIDE: THE RELIABILITY OF ROUTINE DETERMINATIONS AND IMPLICATIONS FOR WATER FLUORIDATION**

Abstract number:  
**7**  
XXVIth ISFR Conference

South Africa is in the process of implementing mandatory fluoridation of water following the final approval by Parliament in September 2001. The target level is 0.7 mg F/L. This emotional issue is still being debated and has sparked numerous studies and investigations of the many aspects of fluoridation. This study directs the attention to the measurement of fluoride in water, a vital, but difficult aspect of water fluoridation which has seemingly been overlooked up to now.

The ability to measure fluoride concentration in water accurately is an obvious prerequisite for the safe and effective implementation of water fluoridation. First, the fluoride content of the raw water must be determined accurately in order to calculate how much supplementation is required, and second it must be checked to ensure that the target dosage is met within allowable tolerances. Any measurement error will be compounded during the feedback loop in the fluoridation process. It can be shown that a constant under measurement of 30% could lead to an overdose of 40% which could significantly increase the health risk of water fluoridation. Here it will be shown that measurement errors can be quite large in the determination of low levels of fluoride in natural water samples typically encountered during fluoridation. Different analytical procedures for fluoride determination will be compared and the pitfalls associated with low level fluoride determinations will be assessed. Results of an interlaboratory proficiency testing scheme for fluoride determination, specially implemented for this study among 66 South African laboratories and administered by the South African Bureau of Standards, show that of the most common methods (ISE, IC, and SPADNS) used for fluoride determination in South Africa, ISE gave the most reliable results and is also the most frequently used method. The risk of Al remobilisation from scaled-up municipal pipes following fluoridation will be assessed and analytical methodology presented which can be used to speciate the resulting fluoro-aluminium complexes.

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Keywords: Fluoride determination; Testing proficiency; Water fluoridation.

## **SULFURYL FLUORIDE: A NEW FUMIGANT FOR POST-HARVEST FOOD COMMODITIES IN THE UNITED STATES**

Abstract number:  
**8**  
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This paper will review the US regulatory history of sulfuryl fluoride ( $\text{SO}_2\text{F}_2$ ) as an insecticide fumigant on stored food commodities and in food processing facilities. This use will result in a substantial increase of inorganic fluoride residues in various US foods.

Since 1959, sulfuryl fluoride has been used as an insecticide (Vikane) to fumigate closed structures and their contents such as domestic dwellings, barns, warehouses, ships in port, and railroad cars. On January 23, 2004, the US Environmental Protection Agency (EPA) approved a Final Rule for the first-time use of sulfuryl fluoride as a fumigant (ProFume) on over 40 post-harvest stored food commodities. EPA identified fluoride as the major toxicological endpoint of concern for exposure to sulfuryl fluoride. EPA's assessment for acceptable risks to sulfuryl fluoride was based on the 4 parts-per-million (ppm) US Maximum Contaminant Level for fluoride in drinking water.

This Final Rule approved the highest levels of inorganic fluoride residues in food ever permitted by EPA. For example, 130 ppm wheat germ; 125 ppm wheat flour; 75 ppm rolled

oats; and 75 ppm oat flour. In March 2004, the Fluoride Action Network and Beyond Pesticides submitted formal objections to EPA on its Final Rule. While EPA is obligated to respond to objections, it is not obligated to do so in a timely fashion. This is of some concern as food fumigation with sulfuryl fluoride became effective with the Final Rule and before EPA addressed substantive issues submitted by citizen advocacy groups.

Among the issues raised was EPA's unprecedented decision to allow an acceptable dosage of fluoride for infants (0.571 mg/kg bodyweight/day) which is five times higher than for adults (0.114 mg/kg/day). This decision runs counter to EPA's mandate under the Food Quality Protection Act to set standards that are *more* protective of children, not *less*. Many more stored food commodities are expected to be fumigated with sulfuryl fluoride before objections to the Final Rule are adjudicated. In March 2005, Dow AgroSciences petitioned EPA for new fluoride residue tolerances for over 600 commodities, including 850 ppm in eggs; 98 ppm in rice flour; 70 ppm in processed foods; and 40 ppm in beef meat.

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Keywords: Dow AgroSciences; Fluoride residues; Fumigants; Pesticide tolerances; ProFume; Routes of exposure; Sulfuryl fluoride; US EPA regulations.

## **BLOOD FLUORIDE LEVELS AS A TOOL FOR ASSESSING RISK OF FLUORIDE TOXICITY**

Abstract number:

**9**

XXVth ISFR Conference

The concentration of ionic fluoride in blood (inorganic fluoride in serum or plasma) is considered, in theory, to be an important determinant of fluoride toxicity. However, there appears to be a lack of discussion in the literature concerning the actual blood fluoride levels associated with adverse effects. As a result, blood levels are typically ignored in risk assessments on fluoride toxicity, with reliance placed on water fluoride levels or estimates of total daily intake instead. Since there are several potential advantages of using blood fluoride levels in risk assessment, a literature review was undertaken to determine 1) the inorganic blood fluoride levels associated with harm in animals and humans, and 2) the inorganic blood fluoride levels in humans living in communities with fluoride levels currently considered safe by the US Environmental Protection Agency ( $\leq 4$  ppm). In addition, data on peak fluoride levels in children following ingestion of dental products was also reviewed.

In animals, damage to the kidney and brain has been reported with blood levels as low as 38 and 77 ppb, respectively. In humans, damage to bones (skeletal fluorosis), has been reported with blood levels as low as 74 ppb. This compares to estimated average blood levels of 76 ppb in humans drinking 4 ppm fluoride and 19 ppb in humans drinking 1 ppm fluoride. Reliance on average fluoride levels will be inappropriate, however, due to wide variations that exist within the same community. In particular, individuals with kidney disease, heart disease, osteoporosis, or combinations thereof, may have blood fluoride levels that greatly exceed the average (by a factor of 6 or more). In addition, the peak blood levels (up to 1,450 ppb) in children following ingestion of fluoridated dental products may also exceed the levels associated with acute damage to the kidney (950 ppb). The significance of transient fluoride levels to chronic toxicity, however, has not been determined. In conclusion, blood fluoride levels in humans drinking water with  $< 4$  ppm may equal or exceed the blood levels associated with harm in both animals and humans.

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Keywords: Blood fluoride in animals; Blood fluoride in humans, Fluoride toxicity; Risk assessment.

**THE FLUORIDE-OSTEOSARCOMA CONNECTION REVISITED**

Abstract number:  
**10**  
XXVIth ISFR Conference

The debate over a possible connection between exposure to fluoride and osteosarcoma has a long and contentious history. From a biological perspective it is highly plausible that such a relationship should exist; however, findings have been mixed in both animal and human studies.

This review will revisit this issue in the light of a recent PhD dissertation from Harvard University by Elise Bassin. To date this thesis has neither been cited nor described in the scientific literature. Bassin's work was based on a case-control study, sponsored by the US National Institutes of Health. She found a robust association between an increase in osteosarcoma in young males when exposed during their 6th, 7th, and 8th years of life.

Bassin used a database of osteosarcoma cases and matched controls taken from 11 hospitals around the USA between 1989 and 1992. Controls were matched by age ( $\pm 5$  years), sex, and approximate residence distance from hospital. She analyzed data for subjects aged 20 years and younger at diagnosis. Using conditional logistic regression to maintain matching, Bassin calculated the Odds Ratio (OR) for each age of exposure for the "Medium" (0.30–0.99 ppm F in water, or 30–99% of US optimal fluoridation level, adjusted for climate) and "High" exposure (1.00+ ppm F in water, or 100% and greater of the US optimal level, adjusted for climate) compared to "Low" fluoride exposure ( $0 < 0.30$  ppm F). She found that among males, for almost all ages of exposure, there was an increased risk. In particular, for ages 6, 7, and 8 there was a peak in risk, which was statistically significant at the 95% Confidence Interval (CI) level. For her model with covariates the peak risk for exposure occurred at age 7 and reached an OR of 7.2 (CI 1.7–30.0).

Not only is this study important in itself, but when re-visiting previous studies, with this window of vulnerability in mind, it may help explain the negative findings from some authors. On the basis of this analysis we believe the weight of evidence shifts in favor of a positive relationship between early exposure to fluoride and osteosarcoma in young boys and young men. Adding urgency to this discussion is the fact that osteosarcoma is frequently fatal in young males, and millions of children are currently exposed to fluoride in communities which use water fluoridation.

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Keywords: Fluoride in water; Osteosarcoma case-control study; Osteosarcoma in young males.

**LETHAL INHALATIONAL EXPOSURE TO HYDROFLUORIC ACID DURING A MAINTENANCE WASHING OPERATION OF A HYDROGEN FLUORIDE LIQUEFYING PIPING SYSTEM**

Abstract number:  
**11**  
XXVIth ISFR Conference

Anhydrous hydrogen fluoride (HF, bp 19.5°C) is produced commercially by heating fluorite ( $\text{CaF}_2$ ) with concentrated sulfuric acid according to the following overall reaction:  $\text{CaF}_2 + \text{H}_2\text{SO}_4 \rightarrow 2\text{HF} + \text{CaSO}_4$ . Vaporized HF is liquefied in a refrigerating

tank through pipes to which entrained calcium sulfate adheres and gradually accumulates. Every six months it is regularly washed from the pipes with water jets. In the present instance, the pipes were experimentally washed with water two days before the main washing operation, and the safety of the procedure was confirmed with the release of a mildly acidic washing solution. At the time, no risk management against hydrofluoric acid (HFA) exposure was in place, and a 65-year-old male worker was severely sprayed on his face just after the start of the preliminary washing. Half an hour later he died from acute respiratory failure. At



autopsy, ionized fluoride concentrations ( $\mu\text{g}/\text{mL}$ ) from the body fluids were 61 in the heart, 54 in thoracic cavity and 66 in urine inside the bladder. High serum concentrations of ionized fluoride indicated massive exposure to HFA. Pathological findings revealed severe bilateral pulmonary congestion and edema. We hypothesize that calcium sulfate hardened with the water during the experimental washing caused blockages in the pipes, causing accumulation of diluted HFA in the pipes. As a consequence, clogging of the pipes apparently caused HFA to collect and the washing fluid to run back. At low concentrations ( $<20\%$ ), HFA can become a weak acid that is somewhat irritating to the skin. It is suggested, therefore, that a low concentration of HFA was inhaled massively into the peripheral respiratory passages, resulting in severe dysfunction of blood-gas exchange that was likely caused by direct alveolar exposure. Although hyperkalemia is normally due to severe hemolysis, it is also reported that HFA exposure can often be complicated by hyperkalemia and hypocalcemia. Acute heart failure might be caused by disorders of these electrolytes. The cause of death would be a combination of pulmonary disorders with cardio-dysfunction. In summary, it is especially important to have thorough safety measures in place against inhalation of HFA. It is also essential that there are no blockages in the HF liquefying pipes before washing operations.

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Keywords: Hydrogen fluoride toxicity; Inhalation toxicity; Lethal exposure; Safety measures.

### **FLUORIDE HAZARD OF GROUNDWATER IN THE JAIPUR DISTRICT, RAJASTHAN, INDIA, AND METHODS TO MITIGATE IT**

Abstract number:

**12**

XXVIth ISFR Conference

An extensive study was made of fluoride in groundwater wells of thirty villages in Jaipur district, Rajasthan, India. Analysis of water samples revealed fluoride concentrations above the 1.5-ppm limit recommended by the World Health Organization, with levels ranging between 1.8 and

17 ppm.

The potability of groundwater has deteriorated in these villages creating a severe fluoride problem for 75 percent of the villagers who are suffering from dental fluorosis, skeletal fluorosis, and gastrointestinal fluorosis. This groundwater contamination is caused primarily by leaching of fluoride from fluorite, fluorapatite, and biotite-containing micas, which are abundant in Jaipur. A second source of the high fluoride is from dyeing and printing industries in Jaipur, where five hundred small-scale cotton print industries that have used artificial dyes for the last thirty years are located. These industries discharge their fluoride-containing waste effluents without proper clean-up and recycling into the Amanishah River. Seepage from these effluents increases the fluoride contamination of the groundwater in Jaipur.

Besides fluoride in the water, other factors contribute to the endemic fluoride problem, including nutritional deficiencies, high ambient temperature, high water alkalinity, and low calcium and vitamin C intake. Moreover, there has also been large increase in the use of fluoride-containing sachets of pan masala, gutka (containing tobacco), and mouthwashes and mouthrinses in Jaipur district.

For mitigation of fluoride in the groundwater, the Nalgonda technique, treatment with activated alumina, and the FUC (fluoride uptake capacity) technique are being used. But nano

filtration membrane NF45 is the best for removal of fluoride. NF200, NF270, DS-5 DK, and DS-5 DL polyamide nano filtration membrane are also effective.

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Keywords: Activated alumina; Fluorosis in Jaipur, Fluoride uptake capacity (FUC) method; Groundwater fluoride; Nalgonda technique; NF45 nano filtration membrane; Rajasthan.

## INTERACTIONS BETWEEN $\text{AlF}_x$ AND GDP (GUANOSINE DIPHOSPHATE) IN VARIOUS MEDIA CALCULATED ON THE BASIS OF MOLECULAR MODELLING

Abstract number:  
**13**  
XXVth ISFR Conference

Interactions between fluorine and aluminium have recently become of increasing interest. In particular, the focus of attention is the endotoxic properties of  $\text{AlF}_x$  in connection with reports of its role in the aetiology of Alzheimer's disease (AD). The pathological lesions of this disease are not caused simply by aluminium but by fluoride-aluminium complexes. Fluoride-aluminium complexes ( $\text{AlF}_x$ ) form spontaneously in aqueous solutions containing fluoride and trace quantities of aluminium ions, and they seem to act similarly to inorganic phosphate analogues. The phosphate analogues GDP and GTP (guanosine di- and triphosphate)—known as high-energy compounds—play a fundamental role in bioenergetic processes of living organisms. On the other hand,  $\text{AlF}_x$  complexes react with G-proteins participating in biological neurotransmission systems. Our earlier experimental models of the behaviour of GDP and  $\text{AlF}_3$  molecules in vacuum proved that the main reaction site was a phosphate moiety. The computer images obtained confirmed that an O-P bond in the GDP molecule is attacked by a fluoride ion derived from  $\text{AlF}_3$  dissociation. The resulting  $\text{AlF}_2^+$  moiety then attacks the oxygen atom linking two phosphate moieties in GDP rupturing the O-P bond.

The objective of the current study was to determine the interaction between GDP and  $\text{AlF}_3$  molecules in an aqueous medium. The calculations were performed with the use of the HyperChem program. In an aqueous medium  $\text{AlF}_3$  did not cause the rupture of the O-P bond in the phosphate moiety of the GDP molecule but only associated with it, creating a GDP- $\text{AlF}_3$  complex. Moreover,  $\text{AlF}_3$  may bind to GDP within both phosphate moieties in combining with GDP. On the basis of the distribution of the electrostatic potential it was determined which of the atoms in the molecules studied are the most reactive ones. They were: oxygen atoms of phosphate groups, oxygen and nitrogen atoms in the guanine ring, and fluorine atoms in aluminium-fluoride complexes. Our findings are consistent with the suggestion of other authors on the possibility of formation of a GDP- $\text{AlF}_x$  complex in the aqueous medium, and the processes in question follow other rules than in vacuum. The present studies point to the role of the medium in reactions between  $\text{AlF}_x$  and GDP.

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Keywords: Aluminium; GDP- $\text{AlF}_x$  complexes; Molecular modeling.

## FLUORIDE PARTICIPATION IN MINERAL REMODELLING OF PREMOLARS IN THE EUROPEAN ELK

Abstract number:

**14**

XXVth ISFR Conference

The European elk is one of the largest animals in the *Cervidae* family, but at present it is a deer species rarely found in Poland. Its teeth are adapted for tearing, grinding, and chewing up plant food. Deciduous teeth (incisors, canines, and premolars) are replaced in the first years of life by permanent teeth. In young elk, permanent tooth buds may be found in and dissected from the mandible. They constitute a perfect model for studying mineral remodelling of permanent teeth. One of the microelements participating in the mineral remodelling of teeth is fluorine. Its participation in the mineral remodelling of permanent teeth was the objective of this study. The study material consisted of tooth buds from animals below one year of age and of permanent teeth from elk of various ages. After degreasing, drying, and weighing, the tooth buds and teeth were designated for assaying the fluoride content by the potentiometric method with the ion-selective electrode. Twenty-six tooth buds were collected in total. Their weight ranged from 0.0443 g to 0.5284 g. Due to the high affinity of fluorine to calcium, the calcium content was additionally tested in the buds by atomic absorption spectrometry. The mean content of fluoride found in the buds was 49.32 (SD = 11.55) mg/kg, and that of calcium was 155.85 (SD = 26.58) mg/g. For permanent teeth these values for fluoride ranged from 16.1 mg/kg to 240.2 mg/kg, depending on the age of the elk. Fluorine accumulation in tooth buds and permanent teeth differs from the accumulation of calcium. The high total fluoride content in the first periods of formation of the permanent tooth buds should be noted. Within weeks, the fluoride content decreases and becomes equal to the content in deciduous teeth. Potential mechanisms of fluorine behaviour in teeth buds and permanent teeth of European elk in relation to mineral remodelling of teeth during the entire lifespan of an individual animal are discussed in this presentation. Currently, the elk is a strictly protected species in Poland. Therefore, further observations on mineral remodelling of its teeth will be possible only after its population increases.

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Keywords: European elk; Fluoride in permanent teeth; Fluoride in tooth buds; Mineral remodeling; Premolar teeth.

## EFFECTS OF FLUORIDE ON PROLIFERATION, DIFFERENTIATION, AND APOPTOSIS OF CULTURED CAPRINE (GOAT) OSTEOBLASTS

Abstract number:

**15**

XXVth ISFR Conference

In this study we investigated the effect of varying concentrations of fluoride (F) on caprine osteoblasts derived from the long bone periosteum of goat fetuses. The osteoblasts were isolated by incomplete trypsinization and cultured at 37°C in DMEM supplemented with 15% FBS and humidified 5% CO<sub>2</sub>. (The subcultured osteoblasts were studied by Giemsa and HE staining, scanning electron microscopy, and the mineralized nodule by alizarin red and Von Kossa staining.) The effects of different concentrations of F (0,  $1.0 \times 10^{-7}$ ,  $1.0 \times 10^{-6}$ ,  $1.0 \times 10^{-5}$ ,  $1.0 \times 10^{-4}$ ,  $5.0 \times 10^{-4}$ , and  $1.0 \times 10^{-3}$  mol/L) on osteoblastic morphology, proliferation, differentiation, calcification, alkaline phosphatase (ALP) activity, and mineralized nodule count were evaluated. Apoptosis was monitored through DNA fragmentation and the cellular morphology with DNA ladder, Hoechst 33342 and propidium iodide (PI) double staining, and transmission electron microscopy (TEM), and the level was studied with a fluorescence-activated cell sorter (FACS) and PI double staining. Cell-cycle was also examined with FACS and PI staining.

Results showed that the time of adhesion and complete confluence of osteoblasts occurred at 4 hr and 10 days, respectively. Merging osteoblasts and apparent mineralized nodules were found in cultures at day 4 and 14, respectively. Triangular and polyangular cells with large nuclei and 1–3 nucleoli and obvious cell processes were observed. ALP and mineralized nodule staining showed positive reactions. Joined and overlapped cells were shown by SEM. Exposure of F at  $10^{-7}$  and  $10^{-6}$  mol/L for 48 hr promoted osteoblast proliferation, but at  $10^{-3}$  mol/L a significant inhibition occurred. The activity of ALP and mineralization were enhanced by F at  $10^{-7}$ – $10^{-6}$  mol/L, but they were decreased by  $5.0 \times 10^{-4}$  mol F/L. The control osteoblasts showed abundant microvilli and cell protrusions that joined and overlapped with each other. However, incomplete membrane and shrinkage and detachment or break up of cell protrusion were found in cultures exposed to  $10^{-4}$  mol F/L. Whereas the control osteoblasts showed clear nuclear envelope, large nucleoli, karyoplasms, abundant endoplasmic reticulum, and mitochondria, those exposed to F at  $10^{-4}$  mol/L or higher showed cellular membrane disintegration, cytoplasm condensation, chromatin compaction or fragmentation, endoplasmic reticulum expansion, and nucleus shrinkage. F exposure at  $10^{-5}$ – $2.5 \times 10^{-5}$  mol/L induced apoptosis with DNA fragmentation. FACS cell-cycle analysis demonstrated that F potentially caused  $G_0/G_1$  arrest. Few cells could be found in either the S phase or the  $G_2/M$  phase. These findings suggest that F at high concentrations can adversely affect the health of caprine osteoblasts.

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Keywords: Caprine osteoblasts; Fluoride and osteoblasts; Goat fetus; Osteoblast apoptosis; Osteoblast differentiation; Osteoblast proliferation.

## SILICOFLUORIDES AND BLOOD-LEAD: A MECHANISTIC INVESTIGATION

Abstract number:  
**16**  
XXVIth ISFR Conference

Masters and Coplan, Masters et al., and Macek et al. have reported that children living in areas where hydrofluosilicic acid or its sodium salt (silicofluorides) are added to the water supplies are at increased risk of elevated blood-lead levels compared to children not exposed to these chemicals. These reports indicate that the lead appears to be from a source other than the drinking water. Age of housing has been shown to be correlated with the elevated blood lead levels, indicating a possible source to be lead-based paint. This work has been criticized, principally on the basis of a lack of a mechanism to explain how environmental lead can be preferentially absorbed by children exposed to silicofluorides.

The work reported in this presentation describes experiments aimed at elucidating a possible mechanism for such preferential absorption. The hypothetical mechanism involves chelation of ingested lead by partially hydrolyzed silicofluorides. The experimental method uses a modification of a standard environmental test: the octanol-water partitioning of lipid-soluble substances. Silicofluorides and other chelating agents are added to aqueous systems containing lead, and then measurements are made of the amount of lead transported into the octanol, which theoretically mimics the lipid membrane of the gut.

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Keywords: Blood-lead; Lead absorption; Silicofluorides.

## CALCULATION OF TOTAL DAILY FLUORIDE URINARY EXCRETION FROM SPOT SAMPLING

Abstract number:  
**17**  
XXVIth ISFR Conference

Simple and quick methods are required for measuring the amount of fluoride excreted in urine for public health or industrial health surveillance. For this reason this research was conducted using the Cosinor method developed from our previous years of biorhythm research on urinary excretion patterns. This method allows one to take a spot urine sample at a specific time in the day to estimate the total daily urinary fluoride excretion.

*Materials and Methods:* Five healthy male adults, 20 years age, volunteered for the study with signed consent forms after the purpose of this study was fully explained to them. They assigned to consume given amounts of standard space foods for six days at 0830, 1230, and 1830. During these six days urine sample were collected every two hours from 0700 to 2300. The determination of the fluoride in urine was made by the Orion 96-09 type combination fluoride selective electrode and ion meter. Rhythm analysis was based on the Cosinor method using this result. The Cosinor method analyzes the frequency (F) of cycle changes and the position of a phase for a circadian phenomenon in relation to a given variable. In the case of urinary fluoride it uses the Halbert-dependent rhythm analysis plotted graphically according to the equation:

$$F = C_o + C_{\cos}(\omega t + \phi)$$

where,  $C_o$  = average daily urinary fluoride output;  $C_{\cos}$  = amplitude, both in the same units as F;  $\omega$  = angular frequency;  $t$  = time (hr), and  $\phi$  = computative acrophase.

*Results:* Our previous studies have clarified fluoride biorhythm excretion into urine. Then, from the amount of fluoride excreted every two hours, the peak phase is found to occur at 0080 and 1208. Since these rhythms have a 24-hr cycle, the peak of the peak phase computed by the Cosinor method appears every 12th  $\pm$  hour. If the peak phase of the  $\pm$  is seen on the Y axis, the middle point of the width will be the average value. Since the average value is a one-hour value, the amount of fluoride excretion per day is 24 times this value:

$$AD = M \times 24$$

where AD = Amount of a daily fluoride excretion; M = Hourly amount of fluoride excreted.

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Keywords: Cosinor method; Daily fluoride excretion; Spot urine sampling.

## DIRECT DETERMINATION OF FLUORIDE BY GAF MOLECULAR ABSORPTION SPECTRA AND HIGH-RESOLUTION CONTINUUM SOURCE AIR-ACETYLENE FLAME ATOMIC ABSORPTION SPECTROMETERS

Abstract number:  
**18**  
XXVIth ISFR Conference

Fluoride cannot be determined directly by conventional atomic absorption spectrometry. An attractive alternative way is the utilization of molecular absorption of a fluorine-containing diatomic molecule. In this work, a high-resolution continuum source atomic absorption spectrometer equipped with an ordinary air-acetylene flame offers a simple and rapid method for the direct determination of fluoride based on measurement of the molecular absorption of gallium mono-fluoride (GaF). To this end, the absorption spectrum of GaF between 211 and

213 nm was utilized, and the most sensitive band head at 211.248 nm was evaluated for direct analytical determination of fluoride.

Both the robustness and reliability of the new method were verified studying the possible matrix and spectral interferences caused by concentrations of up to 5% nitric, hydrochloric, phosphoric, and sulphuric acids, and aqueous solutions containing up to 2000 mg/L of Al, Ca, Co, Cr, Cu, Fe, In, K, Mg, Mn, Na, Ni, Pb, and Sn. No serious interferences were found for the metallic matrices; only high concentrations of hydrochloric acid cause matrix interference by forming relatively stable GaCl. However, as the presence of 3% (v/v) hydrochloric acid results in a depression of 8% in the molecular absorption of GaF, the interference is of minor significance. A stronger effect arises from phosphoric acid of more than 3% (v/v), generating spectral interference by molecular absorption of PO. In this case, adding calcium can effectively reduce the interference.

The detection limit is about 1.0 mg F/L using the GaF band head at 211.248 nm in combination with a measurement time of 5 sec; the linear dynamic range was found to be more than 3 orders of magnitude. By analysis of standard reference materials the performance of the method was verified, yielding good precision and accuracy. The detection limit can be further improved to a level below 1 µg F/L for sample amounts of 10 – 20 µL, using a graphite furnace atomizer.

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Keywords: Air-acetylene flame; Continuum source; Fluoride determination; Gallium mono-fluoride molecular absorption; High-resolution atomic absorption spectrometer.

## **EPIDEMIOLOGICAL TRACKING SURVEYS AND FLUOROSIS CONTROL STRATEGY IN SHENYANG, CHINA**

Abstract number:

**19**

XXVIth ISFR Conference

Shenyang, located in Liaoning Province in NE China, is one of the endemic fluorosis areas with elevated fluoride in the drinking water. The main strategy to correct the problem has been to change the source of water. We have carried out three epidemiological surveys in the moderate and severe fluorosis area: (1) in 1980 before the water source was changed, (2) in 1993 after the water supply was changed and (3) between 2003 and 2004. The results show that before changing the water, the prevalence of dental fluorosis among children between 8 and 15 years of age was 89.2%, and 45.6% among adults, while the overall prevalence of skeletal fluorosis was 6.65%, and the average level of fluoride ion in the urine was 5.9 mg/L.

For children aged 8–15 born after the water source was changed, the prevalence of dental fluorosis decreased to only 3.9%, and their urinary fluoride fell to 0.88 mg/L. Moreover, no new patients were afflicted with skeletal fluorosis. In the 2003–2004 survey, however, the prevalence of dental fluorosis among children between 8 and 15 years of age who had stopped using the new low-fluoride well had risen to 38.0% and their urinary fluoride to 1.82 mg/L. Among adults who had stopped using the low-fluoride water the overall prevalence of dental fluorosis was 28.5% and skeletal fluorosis was 9.0% whereas among adults who did not revert to the old water supply the prevalence of dental fluorosis was 30.3% and skeletal fluorosis 7.5%.

These results suggest that drilling a new, low-fluoride well is an effective strategy to overcome fluorosis caused by fluoride in the drinking water. When people stop using the new well, the prevalence of skeletal fluorosis of the adults will increase as before. In 2004, the

government of Shenyang invested 118 million RMB in drilling a new well to change the source of water in the moderate and severe grade fluorosis area to prevent any further high-level exposure to fluoride.

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Keywords: Epidemiological tracking survey; Fluorosis control; Fluoride in water; Shenyang, China.

## HIGH LEVELS OF FLUORIDE, ARSENIC, AND SELENIUM IN GROUNDWATER IN LOS ALTOS DE JALISCO, MEXICO

Abstract number:

**20**

XXVIth ISFR Conference

Groundwater in some localities of Los Altos de Jalisco, a 20-county region in the west-central Mexican state of Jalisco, is contaminated with deep geothermal waters containing high levels of fluoride, arsenic, and selenium. Water for urban areas in Los Altos de Jalisco comes mainly from untreated groundwater, thus exposing a significant number of people in these areas to potentially toxic levels of these contaminants.

In order to determine the levels of fluoride, arsenic, and selenium in the groundwater, the water in 133 public water wells (81% of the total number of wells in the study area) were sampled and analyzed. Fluoride was determined electrochemically by the fluoride ion selective electrode method; the other elements were determined by inductively coupled plasma optical emission spectroscopy.

The results showed that more than 46% of the sampled wells have a fluoride concentration greater than 1.5 mg/L, which is the maximum Mexican drinking water standard for fluoride. At least 34% of the wells exceeded the national guideline value of 25 µg/L for arsenic, and more than 95% of the sampled wells have selenium concentrations higher than 10 µg/L, which is the World Health Organization guideline value.

Daily exposure doses to fluoride, arsenic, and selenium were calculated from well water analyses and the most important risk factors obtained from data in the literature. In the cases of fluoride and arsenic, the doses are greater than the upper tolerable limits recommended by international health organizations. There is no public information on epidemiological studies performed in Los Altos de Jalisco relative to the chronic ingestion of fluoride and arsenic. Nevertheless, calculation of exposure doses of these contaminants suggests that a significant portion of the total population, mainly babies and children, are exposed to toxic levels of fluoride and arsenic in their drinking water. In the case of selenium, exposure doses are not high enough to prognosticate severe health effects.

Adverse health effects from fluoride and arsenic in the drinking water in Los Altos de Jalisco could be reduced by improving the enforcement of the law. Additionally, a risk communication program addressed to mitigate toxic effects of these contaminants water should be implemented through the promotion of healthy nutritional practices. Epidemiological studies are mandatory to determine the prevalence of health problems caused by high levels of toxic elements present in the drinking water.

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Keywords: Arsenic in groundwater; Fluoride in groundwater; Los Altos de Jalisco, Mexico; Selenium in groundwater.

**FLUORIDE DISTRIBUTION AND FLUOROSIS IN CENTRAL RAJASTHAN, INDIA**

Abstract number:

**21**

XXVIth ISFR Conference

India is among 23 nations around the globe where health problems occur due to excess ingestion of fluoride (> 1.5 mg/L) in drinking water. In Rajasthan, 18 out of 32 districts are classified as fluoride endemic, and 11 million people living there are at risk. An exploratory qualitative survey was conducted of the community regarding fluoride and related health problems in central Rajasthan.

A study on distribution and health hazards by fluoride in groundwater was performed in 1030 villages of Bhilwara district of Central Rajasthan. A total of 1286 water samples were collected and analyzed for fluoride concentration. The fluoride concentration ranged from 0.2 to 13.0 mg/L. Among the villages, 756 (73.4%) have fluoride concentrations above 1.0 mg/L, 60 (5.83%) have fluoride concentrations above 5.0 mg/L, with a maximum number of 24 (19.5%) in Shahpura of Benera Tehsil. A detailed fluorosis study was conducted in 43 out of 60 villages having water fluoride levels above 5.0 mg/L.

A total of 4252 individuals above 5 years age were examined for evidence of dental fluorosis, and 1998 individuals above 21 years were examined for evidence of skeletal fluorosis. The overall prevalence of dental fluorosis and skeletal fluorosis was 3270/4252 (76.9%) and 949/1998 (47.5%), respectively. A maximum of 1016 individuals (23.9%) were diagnosed with mild dental fluorosis by Dean's classification and 378 (8.8%) with severe dental fluorosis. A Dean Community Fluorosis Index of 1.62 was calculated for the total study area and 3.0 for Surajpura of Banera Tehsil. A total of 566 individuals (23.3%) were diagnosed with Grade I type skeletal fluorosis as graded according to Teotia et al., but only 12 individuals (0.6%) had Grade III (crippling) skeletal fluorosis.

In conclusion, the prevalence and severity of fluorosis increased with increasing fluoride concentration. It is interesting to note that in some villages, the prevalence and severity of fluorosis were highest in subjects belonging to the economically poor community. Similarly, male labourers showed the highest prevalence of fluorosis. The prevalence and severity of fluorosis were higher in subjects using tobacco, betel nuts, and alcoholic drinks. In contrast, subjects using citrus fruits and a good nutritional status showed low prevalence.

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Keywords: Central Rajasthan, Community Fluorosis Index; Dental fluorosis; Fluoride water; India; Skeletal fluorosis.

**EFFECTS OF FLUORIDE ON MURINE MACROPHAGE CELLS (I):  
CYTOTOXICITY AND ALTERATION IN THE PRODUCTION OF  
PRO-INFLAMMATORY CYTOKINES**

Abstract number:

**22**

XXVIth ISFR Conference

Endemic fluorosis occurs in many parts of China and India. The immune system may play a role for induction of skeletal fluorosis via cytokine inhibition related to bone metabolism. In this study we evaluated the effects of sodium fluoride (NaF) on cell viability, cytokine production, and mRNA expression in the murine macrophage lineage cell line J774.1.

J774.1 cells were cultured in RPMI1640 with 5% fetal bovine serum. The cells were aliquoted in a 24-well culture plate with  $1\text{Å}\sim 10^6$  cells/well. NaF was added to the wells at



concentrations of 0, 10, 100, and 1000  $\mu\text{M}$ . The plate was incubated in a humidified incubator at 37°C with 5%  $\text{CO}_2$  for 18 hr, after which lipopolysaccharide was added to each well. The cells were then incubated for an additional 6 and 24 hr. Cell viability was determined by the trypan blue exclusion method. Total RNA was extracted from the J774.1 cells after an additional 6 hr of incubation. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to analyze the mRNA expression for tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and  $\beta$ -actin (internal control). For the cells incubated for an additional 24 hr, the concentration of TNF $\alpha$  or IL-1 $\beta$  in the supernatant was determined by enzyme-linked immunosorbent assay (ELISA). Data were analyzed by ANOVA.

The mean value of cell viability for the 1000- $\mu\text{M}$  group at 24 hr (18 hr plus additional 6 hr) or 42 hr (18 hr plus additional 24 hr) following NaF exposure was significantly lower than that of the respective controls (The mean values of viability for 24 hr were 93.3% for the control and 84.4% for the 1000- $\mu\text{M}$  group, and those for 42 hr were 92.8% for the control and 86.3% for the 1000- $\mu\text{M}$  group). The mean value of TNF $\alpha$  in the supernatant of the 1000- $\mu\text{M}$  group was significantly lower than that of the control. For IL-1 $\beta$ , the mean value of the 1000- $\mu\text{M}$  group tended to be lower than that of the control, while there were no significant differences for relative mRNA expression of TNF $\alpha$  and IL-1 $\beta$  among the groups. Translation from mRNA may be inhibited by fluoride. Since TNF $\alpha$  and IL-1 $\beta$  activate osteoclasts, the inhibition of the production of these cytokine may induce bone sclerosis.

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Keywords: Fluoride cytotoxicity; Interleukin-1 $\beta$  (IL-1 $\beta$ ); Murine macrophages; Reverse transcriptase polymerase chain reaction (RT-PCR); Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ).

## K-BENTONITES AS A POSSIBLE SOURCE OF FLUORIDE-RICH GROUNDWATER IN ESTONIA: FIRST LEACHING EXPERIMENTS

Abstract number:

**23**

XXVth ISFR Conference

Groundwater provides two thirds of the drinking and domestic water supply in Estonia. The Silurian-Ordovician (S-O) aquifer system is often the only source of drinking water in central and western Estonia.

It consists of diverse formations of limestone and dolomite with clayey interlayers. Water from wells is distributed directly to consumers without removal of fluoride.

The most serious water quality problems in the S-O aquifer system are associated with the presence of fluoride, the content of which extends up 7.2 mg/L. Approximately 50% of the  $\text{F}^-$  values are above 1.5 mg/L. The concentration of fluoride in the aquifer system is related with the depth of the wells and the main factors controlling dissolved fluoride in groundwater are pH and chemical type of water.

There are 2–40 cm thick K-bentonite beds in Ordovician and Silurian carbonate rocks, the composition is very similar to Scandinavian bentonite layers. Estonian K-bentonites are composed of altered volcanic material mixed with terrigenous material. Volcanic gases and ash are rich in fluorine, and thus clay-rich K-bentonite beds provide adsorption and ion exchange sites for F-ions.

In order to study the fluoride content of aquifer material limestone, dolomite, and K-bentonite samples were analysed using a sequential leaching method (previously boiled 18-megohm water and 0.5 M Tiron). The Tiron is employed to dissolve F associated with oxidic Fe and Al sites of the bentonites. The total fluorine content of the rock samples was also determined. The amount of F leached into the water varies between 4 and 10 mg/kg from limestone and dolomite and between 25 and 51 mg/kg from K-bentonite. During the second extraction with 0.5 M Tiron, 55–126 mg/kg of F was solubilized from K-bentonites. The total F content of carbonate rocks that were examined is mostly 100–400 mg/kg, the highest occurring in clayey dolomite (1100 mg/kg). K-bentonites are also rich in total F: 2800–4500 mg/kg. Thus, the dissolution of fluorides from host rocks is the probable source of fluoride-rich groundwater.

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Keywords: Estonia; Fluoride; Groundwater; K-bentonite.

## WATER DEFLUORIDATION BY KAOLIN AS AN ADSORBENT

Abstract number:

**24**

XXVIth ISFR Conference

The commercially available clay mineral, Kaolin (hydrated aluminium silicate,  $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ ) was examined for its defluoridation efficiency. Batch experiments were conducted to determine the effect of contact time, temperature, and pH on the adsorption of fluoride from water at 30, 40, and 50°C by Kaolin having a particle size of 0.1–0.4  $\mu\text{m}$ . The kinetics of adsorption and the adsorption isotherms at different temperatures were also determined. Kaolin required a minimum contact time of thirty minutes for optimum fluoride removal from water. The high rate of fluoride adsorption is due to the availability of more specific surface area of the material, since adsorption efficiency is dependent on the surface morphology. The effect of pH on the defluoridation capacity of Kaolin was examined over a wide range. This mineral possesses appreciable defluoridation efficiency under both acidic and neutral conditions.

The influence of pH on the pronounced adsorption at acidic ranges is attributed to the domination of chemisorption over other forces of adsorption. However, under neutral conditions chemisorption and physisorption are responsible for adsorption of fluoride. This is confirmed by the zero point of charge of Kaolin at pH 7. The presence of common anions like chloride, sulphate, bicarbonate, and nitrate did not significantly influence the fluoride adsorption of the material. Temperature studies on fluoride adsorption indicated a reduction in the adsorption rate at high temperature. Thermodynamic studies revealed that the adsorption process is exothermic in nature and spontaneous at lower concentrations, as evidenced by the negative values of G. The adsorption of fluoride occurs on the surface of the material and also through intraparticle diffusion of the adsorbent material. The surface morphology of the treated and untreated samples was studied by scanning electron microscopy. X-ray diffraction also confirmed a deposition of fluoride on the surface of clay material.

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Keywords: Chemisorption; Defluoridation; Kaolin fluoride adsorbent.

## **BIOMONITORING FLUORIDE AIR POLLUTION BY GLADIOLUS LEAF TIP NECROSIS: A NEW STANDARDIZED METHOD BASED ON DECADES OF RESEARCH EXPERIENCE**

Abstract number:

**25**

XXVIth ISFR Conference

Injurious effects of fluoride air pollution on plants have been well known for more than a century. Owing to their strong phytotoxicity, inorganic fluorides can cause visible plant damage at concentrations as low as  $1 \mu\text{g}/\text{m}^3$ . Sensitive plant species, particularly *Liliaceae* and *Iridaceae*, have been used for decades to detect fluoride air pollution effects based on visible leaf symptoms. The gladiolus is well suited as a sensitive bioindicator or biomonitor of plants, combining high susceptibility to atmospheric fluorides with a characteristic leaf shape that facilitates quantitative assessment of fluoride-induced leaf tip necrosis.

Quick and simple measurements of the extent of leaf tip injury to gladiolus can be carried out repeatedly during the exposure period by placing them in self-watering exposure pots for several weeks, making possible measurement of a "Leaf Injury Rate" (= progress of leaf injury in mm/day). Leaf Injury Rates are directly proportional to the impact of gaseous fluorides as shown under controlled exposure to low concentrations of hydrogen fluoride (HF). This method has been applied repeatedly in the surroundings of fluoride emission sources (e.g., brickyards and aluminium smelters) and has proved useful (1) to delineate and differentiate zones of varying fluoride impact, and (2) to demonstrate the consequences of quantitative changes in fluoride emissions at these sources, e.g. due to measures to reduce emissions.

Without need for chemical analysis, this very specific and sensitive bioindicator also provides sound information to assess risks toward vegetation due to fluoride air pollution. The method described here has been standardized and is about to be published as VDI guideline 3957 No. 14 (VDI = The Association of German Engineers) in Fall 2005, complementing the VDI Guideline Series 3957 on the use of plant biomonitors for ambient air pollution, both on the national and subsequently on the European level. The "Standardized Exposure of Gladiolus" is an inexpensive yet scientifically sound visual tool to assess risk of plant injury caused by inorganic fluorides.

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Keywords: Atmospheric fluorides; Biomonitoring; Fluoride air pollution; Fluoride bioindicator; Gladiolus leaf injury; Leaf injury rate.

## **EFFECT OF FLUORIDE POLLUTION FROM INDOOR COAL BURNING ON TOOTH, BONE, AND BLOOD PARAMETERS OF FARMERS IN CHINA**

Abstract number:

**26**

XXVIth ISFR Conference

Four groups of Chinese farmers with different fluoride exposure from indoor fluoride pollution by burning coal were selected from four rural villages (two control and two fluoride polluted) in China. Except for fluorosis, the subjects were healthy male and female adults between the ages of 35 and 60, with 119 to 122 local residents in each group.

Fluoride exposure of each study population was determined by analysis of fluoride in food, drinking water, and indoor air using a combination fluoride-specific electrode. Dental fluorosis was determined using the Dean Index as well as the Total Surface Index of Fluorosis. For skeletal fluorosis, the Singh and Jolly diagnostic criteria based on clinical evidence and radiological examination were used. A blood sample from each subject was analyzed for sodium, potassium, and chloride using an EPX Automatic Analyzer.

Additionally, urea nitrogen, creatinine, calcium, phosphorus, uric acid, total protein, albumin, total bilirubin, alkaline phosphatase, glutamic-oxaloacetic transaminase, cholesterol, and creatinine kinase were analyzed using the National 7150 Automatic Analyzer. A two-way analysis of variance (ANOVA) model was used to examine the effects of coal fluoride levels (normal or high). Statistically significant values were recorded.

*Results:* (1) Daily fluoride intake: The mean total fluoride intake of the farmers in control groups A and B was estimated to be 2.42 and 2.48 mg/day, respectively. In the polluted groups C and D it was significantly higher ( $P < 0.001$ ) at 6.57 and 8.54 mg/day, respectively. These higher values greatly exceed the Chinese hygienic standard of 3 mg F/day for persons over age 15.

(2) Prevalence of fluorosis: Control groups A and B had dental fluorosis rates of 14.81 and 14.13%, respectively, but no cases of skeletal fluorosis. In polluted groups C and D dental fluorosis was more severe, and the rates were significantly higher ( $P < 0.001$ ) at 92.86 and 99.02%, respectively. Moreover, the incidence of skeletal fluorosis was 44.44% in Group C and 95.0% in Group D.

(3) Biochemical effects: Judged by Chinese reference values, all blood sample parameters appeared to be in the normal clinical range regardless of fluoride exposure. Statistical analysis of the blood chemistry data indicated no significant differences between the four groups ( $P > 0.05$ ). It was noted, however, that the potassium and alkaline phosphatase levels were generally higher in the polluted groups C and D than in the control groups A and B.

*Conclusion:* Since the values of the blood chemistry were within the normal ranges for these otherwise healthy subjects, the main effect of fluoride exposure from indoor air pollution appears to be the high risk of dental and skeletal fluorosis. Installation of stoves with chimneys, improved home ventilation, and use of nonpolluting methods for drying food are a public health priority for areas with indoor fluoride pollution.

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Keywords: Coal-burning fluorosis; Dental fluorosis; Farmers in China; Skeletal fluorosis.

## **SIGNIFICANCE OF SIALIC ACID AND GLYCOSAMINOGLYCAN LEVELS IN EARLY DIAGNOSIS OF CHRONIC FLUOROSIS IN FARM CHICKS**

Abstract number:

**27**

XXVth ISFR Conference

To study the significance of the serum sialic acid (SA) and glycosaminoglycan (GAG) levels in chronic fluorosed farm chicks in early diagnosis and to select the sensitive index for diagnosing chronic fluorosis, 250 one-day-old Roman chicks were randomly divided into five groups of 50 per group. One group served as the control, and the other four groups were fed 500, 1000, 1500, and 2000 mg NaF/kg diet, respectively, for 150 days. Serum specimens were sampled every 30 days for measuring serum fluoride levels by the F-ion selective electrode method (ORION Research, Model, EA 940 digital ion analyzer provided with an F-selective electrode). Serum SA and GAG contents were determined by standard methods, and the clinic signs of the tested chicks were observed.

Results were as follows: An animal model of chronic fluorosed chicks was successfully established; serum F levels exhibited an obvious direct dose-effect pattern. Compared with the control group, the serum SA content in all fluorosed groups decreased, whereas the GAG level increased. Serum F levels significantly correlated with serum SA and GAG contents (p

<0.01), and the correlative coefficients were  $r = -0.773$  and  $r = 0.798$ , respectively. The SA/GAG ratios in all fluorosed groups decreased by 30% or more than in the control.

In conclusion, this work has shown that SA and GAG metabolism in NaF-treated chicks is significantly altered and that a 30% or higher ratio of serum SA/GAG is a sensitive and reliable index for diagnosing fluorosis in chicks and chickens.

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Keywords: Farm chicks; Fluorosis diagnosis; Serum glycosaminoglycan; Serum sialic acid.

### STEM CELL MOBILIZATION WITH G-CSF IN MICE EXPOSED TO SODIUM FLUORIDE

Abstract number:

**28**

XXVIth ISFR Conference

Granulocyte colony stimulating factor (G-CSF) is employed in the clinic as a mobilizing agent to stimulate egress of hematopoietic stem progenitor cells (HSPC) from bone marrow into peripheral blood. However, effects of fluoride on the process of stem cell mobilization are unknown. Hence the aim of the present study was to evaluate the influence of short-term exposure to sodium fluoride (NaF) on the kinetics of HSPC egress from bone marrow to peripheral blood in G-CSF-mobilized mice. We evaluated the percentage of circulating Sca-1<sup>+</sup> cells coexpressing c-kit receptor by fluorescence activated cell sorter (FACS) and the number of circulating hematopoietic progenitors in clonogenic tests after 2, 4, and 6 days of mobilization with G-CSF of mice initially exposed to NaF. Moreover, we described the morphology of the bone marrow, spleen, and liver in the exposed mice, and the localization of early hematopoietic (Thy-1- or c-Kit-R-positive) as well as apoptotic cells in spleen and liver. We found that NaF noticeably influenced the process of HSPCs mobilization resulting in enhancement of the HSPC population in peripheral blood and detectable morphological changes in the spleen of the treated mice.

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Keywords: Bone marrow in mice; Granulocyte colony stimulating factor (G-CSF); Hematopoietic stem/progenitor cells (HSPC); Sodium fluoride in mice; Stem cell mobilization.

### PROTECTIVE ACTION OF MILK PROTEINS AGAINST FLUORIDE INTOXICATION IN RATS

Abstract number:

**29**

XXVIth ISFR Conference

In this report we present a study of the effect of a standard diet (LSM) supplemented with milk proteins (100 mg protein/100 g body weight of animal) on the fluoride content in tissues of rats exposed to a small inhalation concentration of ammonium fluoride. A total of 80 adult Wistar male rats were divided into eight groups of ten, out of which four groups were exposed to an ammonium fluoride aerosol (2 mg F<sup>-</sup>/m<sup>3</sup>) in toxicological chamber six hours a day, five days a week, for 25 weeks with supplementation of their diet with albumin, casein, and whey types of milk proteins. The remaining four groups were the controls given the same diet as for each group of F-exposed animals. Exposure to ammonium fluoride by inhalation was used to

exclude the possibility of milk protein-fluoride interactions in the alimentary canal. When the experiment was completed, the animals were sacrificed and dissected to collect samples of blood, femur bone tissue, and urine that afterwards were subjected to laboratory tests. The effect of LSM feed supplemented with albumin, casein, and whey proteins on the fluoride content in blood serum, bone, and urine was evaluated. It appeared that the small  $2 \text{ mg F}^-/\text{m}^3$  concentration of ammonium fluoride did not noticeably disturb general systemic processes evaluated on the basis of selected physical and biochemical parameters. However, all milk proteins added to the LSM standard feed lowered the content of endogenous and exogenous blood serum fluoride, increased bone fluoride accumulation, and had various effects, though not large, on urinary fluoride excretion. Statistical analysis by a neural network computer program established the relative importance of the individual proteins in relation to their effect on these fluoride parameters.

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Keywords: Albumin; Ammonium fluoride aerosol; Casein; Fluoride in rats; Milk whey.

## **FLUORIDE IN GROUNDWATER AND PREVALENCE OF DENTAL FLUOROSIS AMONG CHILDREN IN VILLAGES OF HARYANA, INDIA**

Abstract number:

**30**

XXVth ISFR Conference

The problem of high fluoride in groundwater is one of the most important health related geo-environmental issues in India. About 17 states have high fluoride levels in their groundwater, including Haryana where such water is the major source of drinking water. The present study was conducted in Jind district of Haryana to determine the fluoride content of groundwater wells and to survey the prevalence of dental fluorosis in three villages of the district. Other water quality parameters, including pH, electrical conductivity, total dissolved salts, total hardness, and total alkalinity as well as sodium, potassium, calcium, magnesium, carbonate, bicarbonate, chloride, and sulfate concentrations, were also measured. A systematic calculation of correlation coefficients among the different physico-chemical parameters was also performed. The analytical results revealed considerable variations in the chemical composition of the water samples. The majority of them do not comply with Indian as well as WHO standards for most of the water quality parameters measured. The fluoride concentration in the underground water of these villages varied from 0.84 mg/L to 5.9 mg/L, causing dental fluorosis among people, especially children, in these villages. A total of 1523 resident children were surveyed for dental fluorosis, out of which 1281 ( 84.11%) were found affected with various stages of dental fluorosis.

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Keywords: Dental fluorosis; Drinking water; Haryana, India; Underground water.

## FLUORIDE THERAPY FOR OSTEOPOROSIS REVISITED

Abstract number: Present in only trace amounts in soft tissues of the body, fluoride

**31**

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normally accumulates to a significant extent with increasing age in metabolically active skeletal and dental tissues. With this fact in mind, elevated doses of fluoride (e.g., 50–60 mg NaF/day) have been advocated for treatment of post-menopausal and old-age onset osteoporosis, even though this condition is known to result not from lack of fluoride but mainly from long-term dietary deficiencies, inadequate physical activity, and hormonal imbalances. Although relief from pain associated with osteoporosis by fluoride therapy has been reported, and bone density is increased by such treatment, the resulting bone lacks tensile strength and is often more susceptible to fractures. Under certain circumstances fluoride can also promote arterial calcification leading to thromboses as first described by Rokitansky in 1842. Consequently, for these and other reasons, the use of fluorides for treatment of osteoporosis remains controversial scientifically.

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Keywords: Arterial thrombosis; Fluoride therapy; Osteoporosis treatment.

## CORRELATION BETWEEN THE INCIDENCE OF DENTAL FLUOROSIS AND OSTEOSARCOMA IN PROVINCES OF KENYA

Abstract number: Recent research showing an association between fluoride exposure and

**32**

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osteosarcoma prompted a re-examination of older studies to see if any such link had been overlooked. Although Kenya has no artificial fluoridation, previous investigations in Kenya are of particular interest given the high natural fluoride levels found in many regions of the country.

Data were examined from two unrelated studies published in the 1980s. One determined the incidence rates of osteosarcoma and the other the prevalence rates of dental fluorosis in Kenya's eight provinces. Linear regression analysis revealed an extremely strong positive association between the two sets of data, yielding a Pearson correlation coefficient of  $R^2 = 0.91$  at  $p < 0.001$ .

The dental fluorosis study included a large representative sample of people ( $n = 34,287$ ) from all provinces of Kenya. Subjects were restricted to those born and raised in the same district so that geographical misclassification due to migration was eliminated. The prevalence of dental fluorosis (all degrees) among the population ranged widely from 11.7 to 56.5% between provinces and corresponded closely to drinking water fluoride levels.

The osteosarcoma study reported incidence rates by province and by ethnic group using data from the Kenya Cancer Registry (251 cases). Within single ethnic groups with presumably similar genetics, significantly different osteosarcoma rates were found in different provinces (range: 6 to 23/million people/year). The authors surmised that some "geomedical variable" was the likely cause of the variation in osteosarcoma.

Recent research suggests a confounding factor could be radium in drinking water. Published and unpublished levels of radionuclides in Kenyan drinking water were therefore examined. Since no measurements of radium were found, its decay product, radon ( $^{222}\text{Ra}$ ), was used as a surrogate. A moderately strong positive correlation between average province radon levels and osteosarcoma rates was found ( $R^2 = 0.52$ ).

Kenya has the potential to be an excellent study area for the relationship between fluoride, radium, and osteosarcoma, as it contains regions with widely ranging levels of all three of

these variables. The currently available data from Kenya, however, support a link between fluoride and osteosarcoma.

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Keywords: Cancer registry in Kenya; Dental fluorosis; Osteosarcoma; Radon in Kenyan waters; Water fluoride in Kenya.

## EFFECT OF FLUORIDE ON THE PROLIFERATION OF OSTEOBLASTS

Abstract number:

**33**

XXVth ISFR Conference

Although the anti-caries effects of low concentrations of fluoride have been studied extensively, fluoride at higher concentrations sometimes significantly alters the properties of hard tissues such as bones and teeth.

Osteoblasts play a key role in bone formation, which is often promoted or retarded by the presence of trace elements. Since the behavior of osteoblasts in the presence of fluoride is not yet fully understood, it is important to investigate the effect of fluorides on the proliferation of osteoblasts. Culture media containing 0~0.05 mol/L of fluoride were adjusted by mixing with HF solution. At the same time, media containing 0~0.05 mol/L of chloride as an indicator of pH were also adjusted by mixing with HCl solution. Each 100  $\mu$ L of F-containing or Cl-containing medium was added to 3000 osteoblast cells in each well and cultured for three days. Optimal density was observed, and the cell numbers were calculated. Osteoblasts grew well at a fluoride level below 0.001 mol/L. However, above 0.005 mol/L of fluoride, the osteoblast numbers decreased dramatically to almost negligible. The pH at 0.005 mol/L was 7.71 and scarcely decreased compared to the control pH of 7.81. However, osteoblasts showed favorable proliferation with 0.05 mol/L of chloride (pH 6.81). Therefore, fluoride affected osteoblast proliferation more strongly than the pH in the solution. Light microscopic observation showed quite different features between control cells and those at higher concentrations of fluoride. These findings indicate that fluoride dramatically inhibits osteoblast growth at concentrations above 0.005 mol/L ( 95 ppm F) in the medium solution.

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Keywords: Osteoblasts; Osteoblast proliferation.

## ASSESSMENT OF FLUORIDE EXPOSURE IN PREGNANT WOMEN IN POZNAN BY FLUORIDE LEVELS IN THEIR URINE

Abstract number:

**34**

XXVth ISFR Conference

In discussions of the safety and effectiveness of fluoride for caries prevention in pregnant women, there is a need to assess the amount of fluoride exposure in future mothers and, consequently, the fetus in their living environment. Accordingly, the aim of this study was to evaluate the fluoride exposure in pregnant women living in Poznan, where the level of fluoride in drinking water ranges from 0.4 to 0.8 mg/L.

The assessment was made on the basis of fluoride levels determined in fasting morning urine samples. The subjects of the study were 31 pregnant women aged 22–34 in the regular course of pregnancy and 30 healthy non-pregnant women aged 21–34 as the control group. On the basis of a questionnaire the sources of fluoride exposure of all subjects were determined. Urine samples were collected twice: in 28<sup>th</sup> and 33<sup>rd</sup> week of pregnancy in the



study group, and once in the control group. Measurements of fluoride concentrations were performed with an ion-selective electrode Orion 96-09. The data were statistically analyzed using Statistica for Windows program ( $p < 0.05$ ). The mean fluoride levels in urine in the 28<sup>th</sup> week of pregnancy were significantly lower than the mean levels in urine in the 33<sup>rd</sup> week (34.33 mol/L and 44.08 mol/L, respectively). In the control group the values were higher than in the study group (68.40 mol/L). Statistically significant differences between mean concentrations of fluorides in urine of patients consuming different amounts of tea, poultry and fish were observed.

Fluoride levels of women residing in Poznan, assessed on the basis of fluoride concentrations in urine samples, reached levels observed in areas with fluoridated water. The differences between fluoride concentrations in the study and control group samples as well as those from the 28<sup>th</sup> and 33<sup>rd</sup> week of pregnancy may be explained by fetal metabolism of fluorides. This research confirmed that diet is an important source of fluoride exposure, and the results show there is no need for implementing systemic fluoride, still recommended by some authors, for caries prevention in pregnant women living in Poznan.

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Keywords: Fluoride exposure; Fluoride in urine; Pregnant women; Women in Poznan.

## **SODIUM FLUORIDE-INDUCED OXIDATIVE STRESS IN RAT HEPATOCYTES AND ITS AMELIORATION BY VITAMIN C**

Abstract number:

**35**

XXVIth ISFR Conference

Fluoride inhibits the activity of many enzymes. Its adverse effects on the structure and functioning of many organs suggest that fluoride may generate free radicals and, in consequence, interfere with the antioxidant defense mechanism in a living cell. The aim of our research was to examine whether in the presence of fluorides, ascorbic acid also changes the activity of oxidative enzymes in hepatocytes.

For this study, hepatocytes were sampled from Wistar rats by means of an enzymatic method. The isolated hepatocytes were incubated for 120 and 240 min in L-15 medium containing fluoride ions at a concentration  $6.0 \times 10^{-3}$  mol/L and/or ascorbic acid (AA) at a concentration  $3.0 \times 10^{-3}$  mol/L and in pure medium (control trials). After incubation the cells were centrifuged and mechanically homogenized. In the homogenate the activity of superoxide dismutase (SOD) was measured by the Beauchamp and Fridowich method (1971), glutathione peroxidase (GPx) by the Paglia and Valentine method (1967), and glutathione reductase (GR) by the Goldberg and Spooner method (1983). Protein was determined by Lowry's method (1975), and enzyme activity was expressed in international units (U) converted to mg of cell protein.

A decrease was observed in SOD and GPx activity in hepatocytes incubated with fluoride for 120 min. Extending the incubation time to 240 min did not increase the changes in the activities of the enzymes. AA also lowered SOD's activity, but it did not alter GPx activity. In hepatocytes incubated with fluoride and AA, no increased inhibition of SOD's activity was observed, thereby indicating there was no synergistic effect of fluoride with AA. The changes in SOD and GPx activities were not accompanied by any statistically significant changes in GR activity.

The results indicate that the mechanism of lowering SOD activity by fluoride ions is different from than by ascorbic acid. When SOD activity is lowered by fluoride, the

concentration of superoxide anion radical increases, and AA is beneficial because of its ability to react with reactive forms of oxygen.

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Keywords: Antioxidative enzymes; Ascorbic acid; Fluoride and enzymes; Oxidative stress; Rat hepatocytes.

## RELATIONSHIPS AMONG THE CHLOROPHYLL, FLUORIDE, AND SULFUR CONTENT OF NEEDLES IN THREE SPECIES OF CONIFERS IN POLAND

Abstract number:

**36**

XXVIth ISFR Conference

This report concerns relationships among the chlorophyll, fluoride and sulfur content of needles in Scots pine (*Pinus sylvestris* L.), European spruce (*Picea abies* L. Karst.), and Colorado spruce (*Picea pungens* Engelm.) growing in Poland. The one- and two-year needles were collected in November and February between 2001 and 2003 in the southern towns of Strzemieszyce, Ustroń Polana, and Zebrzydowice located in areas of iron, steel, and heat-power generating plants and also in Brzezna near Nowy Sącz situated in a non-industrial area.

Fluoride ion (F) concentration was determined potentiometrically with a fluoride selective electrode (Orion, USA) and sulfur (S) concentration was determined nephelometrically as sulfate, (SO<sub>4</sub><sup>2-</sup>), after dry ashing of needles in nickel crucibles. Fresh needles were extracted with acetone for spectrophotometric determination of chlorophyll (chl).

F concentration in all the needle samples ranged from 4.44 µg/g to 41.87 µg/g dry weight (dw) (mean 13.91), SO<sub>4</sub><sup>2-</sup> concentration, calculated as S, ranged from 842 µg/g dw to 2662 µg/g dw (mean 1837), and chl from 0.97 mg/g wet weight to 2.81 mg/g ww (mean 1.75). The lowest concentrations of all the elements analyzed were found in the Scots pine needles. However, in the Colorado spruce needles the F and chl concentrations were the highest, and in the European spruce needles the S concentrations were the highest.

The results were analyzed for their interdependency between the chl and F and S content. The interdependency between F and chl with F concentrations up to 10 µg/g dw was not significant ( $r = 0.1122$ ,  $n = 152$ ). Above this concentration, the correlation was positive and statistically significant ( $r = 0.7339$ ,  $p = 0.05$ ,  $n = 49$ ). The interdependence between S and chl concentration was also positive and statistically significant ( $r = 0.3270$ ,  $p = 0.05$ ,  $n = 201$ ). The interdependency between F and S concentrations with F concentrations up to 10 µg/g dw also proved negative and was statistically significant ( $r = -0.3756$ ,  $p = 0.05$ ,  $n = 152$ ). However, above this concentration, it was not significant ( $r = 0.0800$ ,  $n = 49$ ).

From the results, it appears that, apart from the known adverse effects of fluoride and sulfur pollution on plants (Mikhailova 2000), once their accumulation levels are determined, increases in chlorophyll concentration in conifer needles appear to be stimulated by fluorine and sulfur compounds, as reported by others (Oleksyn 1989, Karolewski 1992).

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Keywords: Airborne fluoride; Chlorophyll in conifer needles; Fluoride pollution; Industrial pollution; Sulfur in conifer needles.

## **CHRONIC EFFECTS OF FLUORIDE ON GROWTH, BLOOD CHEMISTRY, AND THYROID HORMONES IN ADOLESCENTS RESIDING IN THREE COMMUNITIES IN NORTHERN MEXICO**

Abstract number: **37**  
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This study was designed to evaluate adverse health effects in adolescents from chronic exposure to various water fluoride concentrations in three communities located in northern Mexico: Ciudad Juarez, Samalayuca, and Villa Ahumada. In these communities the fluoride concentration in water averages 0.3, 1.0, and 5.3 mg/L, respectively. The residents of Villa Ahumada have been exposed to excessive levels of fluoride in drinking water since their birth.

Using urinary fluoride as biomarker, we evaluated the effect of fluoride on dental fluorosis, growth, thyroid hormones, hepatic function, lipids, uric acid, and electrolytes. A total of 201 adolescents (106 female, 95 male), aged 15–20, were included in the study. Each participant donated blood and urine samples, was weighed, measured for height, and answered a questionnaire which requested sociodemographic information and water intake practices. The fluoride concentration in water and in urine samples was analyzed using the US EPA Ion-Selective Method. Dental fluorosis was assessed using the Dean's Index. Biochemical parameters and thyroid hormones levels were measured by standard procedures. ANOVA, Chi Square, and Student's t test were used in the statistical analysis of results.

The mean urinary fluoride concentration in adolescents living in these communities was  $0.792 \pm 0.39$  mg F/g creatinine ( $n = 66$ ),  $1.32 \pm 0.57$  mg F/g creatinine ( $n = 65$ ), and  $2.22 \pm 1.16$  mg F/g creatinine ( $n = 70$ ), in Cd. Juarez, Samalayuca, and Villa Ahumada, respectively. In Villa Ahumada 97 percent of all adolescents exhibited dental fluorosis, and 18 percent of them have serious damage to their teeth. In Samalayuca 53 percent of all adolescents exhibited mild dental fluorosis, 15 percent of them have moderate dental fluorosis, and 2 percent showed serious damage in their teeth. In Villa Ahumada a significant inverse relationship was found between urine fluoride levels and stature; this association suggests that fluoride exposure may affect the teeth but also the growth of adolescents. Serum samples of these individuals showed elevated levels of alkaline phosphatase (ALP), potassium, magnesium, calcium, and phosphate, and decreased levels of thyroid hormone  $T_3$  and uric acid.

These findings show that high fluoride ingestion has a definite relationship with the prevalence and severity of dental fluorosis, decrease of stature, and decrease of thyroid hormone secretion. Uric acid is one of the important antioxidants of plasma, and its level was lower in fluorotic adolescents, indicating that fluoride toxicity may involve a reduction on certain intrinsic scavengers resulting in an increased vulnerability to oxygen free radical toxicity.

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Keywords: Adolescents; Blood chemistry; Dental fluorosis; Fluoride in drinking water; Growth retardation; Northern Mexico; Thyroid hormone  $T_3$ ; Urinary fluoride.

## **FLUORIDE ION ENHANCEMENT OF MONOCYTE DIFFERENTIATION BY TRANSCRIPTION FACTOR $\kappa B$**

Abstract number: **38**  
XXVIth ISFR Conference

Reactive oxygen species (ROS) may stimulate intracellular signalling during cell growth, differentiation, cellular apoptosis, and gene expression. The objective of this study was to determine the role of

fluoride in the process of differentiation of monocytes into macrophages and generation of ROS in these cells.

Human THP-1 cells (a human promyelomonocytic cell line of mature cells in the monocyte/macrophage lineage, originally isolated from a child with acute leukaemia) were treated with 100 nM PMA (phorbol myristic acetate) to facilitate differentiation into macrophages. After PMA treatment, the adhering macrophages were incubated with NaF. Mononuclear cells from blood were separated by density gradient. NaF at 2.5  $\mu$ M was added, and cells were cultured for 48, 72, or 144 hr.

Formation of ROS was measured by flow cytometry with 2',7'-dichlorofluorescein diacetate. Apoptosis was assayed with an Annexin V-fluorescein isothiocyanate binding kit. The percentage of CD 68 cells was determined by flow cytometry. Activation of transcription factor NF $\kappa$ B (the eukaryotic nuclear factor  $\kappa$ B which plays an important role in inflammation, autoimmune response, cell proliferation, and apoptosis by regulating the expression of genes involved in these processes) in cells was measured using a TransAM NF $\kappa$ B Kit. Significant differences were initially determined with Friedmann's ANOVA, and significant results were then subjected to the Wilcoxon matched-pairs test.

Fluoride in relatively low concentrations is a stimulator of the process of differentiation of monocytes into macrophages. This is indicated by increased expression of the CD 68 antigen and increased generation of ROS in monocytes cultivated in the presence of fluoride. Generation of ROS is associated with the effect on expression of enzymatic systems characteristic of mature macrophages. It appears that fluoride may act as potential activator of NF $\kappa$ B because it contributes to more rapid differentiation of monocytes (reflected in CD 68 expression) and generation of ROS caused by Cox-2 expression. Cox-2 expression in the cell is connected with activation of the NF $\kappa$ B pathway and is coincident with down-regulation of peroxisome proliferator-activated receptor  $\gamma$  (PPAR  $\gamma$ ). Cox-2 induction by the NF $\kappa$ B pathway activation takes place during monocyte differentiation, which contributes to additional stimulation of Cox-2 transcription.

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Keywords: CD 68; Cell differentiation; Cox-1; Cox-2; Fluoride stimulation; Monocyte/macrophage conversion; Reactive oxygen species; Transcription factors.

## **A PRELIMINARY STUDY OF DENTAL FLUOROSIS AND CARIES AMONG SECONDARY SCHOOLCHILDREN IN KOTA KINABALU, SABAH, MALAYSIA AFTER CESSATION OF WATER FLUORIDATION**

Abstract number: Fluoridation of drinking water ceased in the state of Sabah, Malaysia in

**39**  
XXVIth ISFR Conference

the mid-1980s. This preliminary study, conducted in 2004, was aimed at assessing the prevalence and severity of dental fluorosis and the incidence of dental caries among 13-year-old children in a secondary school in Kota Kinabalu, the capital of Sabah. A total of 117 respondents took part in this study after fulfilling inclusive and exclusive criteria. A calibrated dentist performed visual identification of both dental fluorosis and caries in the respondents, using the Horowitz et. al (1984) Tooth Surface Index of Fluorosis (TSIF) and Decayed, Missing, and Filled Teeth (DMFT) scores, respectively. The concentration of fluoride in the drinking water was 0.078  $\pm$  0.006 mg/L.

Among the 117 respondents, 72 (61.5%) were males and 45 (38.5%) were females. The mean TSIF score was 0.24 and the DMFT score was 3.60. The range in severity of dental fluorosis was 0 to 3, while for dental caries it was 0 to 12. Only 19 (16.2%) of the respondents had dental fluorosis compared to 102 (87.2%) who had dental caries. More males had dental fluorosis (14/72 = 19.4%) than females (5/45 = 11.1%). However, the incidence of dental caries among males (63/72 = 87.5%) was about the same as among females (39/45 = 86.7%). The severity of dental fluorosis was low, since the highest score was 3, while for dental caries the highest DMFT score was 12.

Although the findings of this survey suggest that cessation of fluoridation resulted in an increase in dental caries, contrary to other reports (e.g., from communities in Canada, Cuba, East Germany, Finland, and Holland), this conclusion cannot be drawn without adequate baseline data and surveys of appropriate controls for comparison. The study also provides no direct evidence concerning changes in the prevalence of dental fluorosis.

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Keywords: Cessation of fluoridation; Dental caries; Dental fluorosis, Kota Kinabalu, Malaysia.

### AMELIORATION OF FLUORIDE TOXICITY BY VITAMINS C AND D PLUS CALCIUM IN FEMALE RAT REPRODUCTION

Abstract number:

**40**

XXVIth ISFR Conference

An investigation was conducted of normal reproductive cyclic of female rats (*Rattus norvegicus*) weighing between 150–200 g. The experimental rats were given fluoride (5.8 ppm) contaminated drinking water (FW), sodium fluoride (6 ppm, prepared in distilled water), vitamin C (6 mg), and combinations of vitamins C (6 mg) and D plus calcium (vitamin D, 6 mg, plus calcium, 6 mg, once a week) for 15 and 30 days. Sodium fluoride and fluoride water treatment caused reduction in the weights of ovary, uterus, vagina, and adrenal gland, circulating level of estrogen, and the number of litters, fertility rate, and altered tissue and serum biochemistry as compared to control values. However, body weights were not appreciably affected and only non-significant change in the weights of liver and heart occurred. Kidney weights were diminished significantly following FW (fluoride contaminated drinking water) treatment for 15 and 30 days. Major haematological parameters (RBC, WBC, Hb, and Ht) remained within normal range.

Cholesterol is a precursor of steroidal hormones, and in the present study the concentration of cholesterol in ovary and adrenal was found to be increased significantly following administration of NaF and FW, indicative of non-utilization by the system and thereby decreasing the estrogen level and fertility in rats. The above altered parameters were restored partially or completely after exogenous feeding with vitamin C, or vitamins C and D plus calcium. The data suggest that administration of NaF and FW to female rats caused toxic effects in reproductive and vital organs, but vitamin C alone and in combination with vitamin D and calcium helped significantly to overcome this toxicity and thereby maintain normal physiology of reproductive and vital organs. Vitamins C and D plus calcium can therefore have an important role in prophylactic treatment of fluorosis.

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Keywords: Amelioration of fluoride toxicity; Calcium prophylaxis; Female rats; Reproductive effects; Vitamins C and D.

## GEOLOGICAL STUDY OF FLUORIDE IN GROUNDWATER OF SANGANER TEHSIL OF JAIPUR DISTRICT, RAJASTHAN, INDIA

Abstract number: **41**  
XXVth ISFR Conference

The occurrence of high fluoride concentration in groundwater has become one of the most important health related geo-environmental issues in many countries of the world including India. The high fluoride concentration in groundwater resources and the resultant disease “fluorosis” is widely distributed in nearly 20 states of the country. In Rajasthan, groundwater of the western and some southern parts of the state is contaminated with high levels of fluoride. The extent of severity of dental fluorosis has been surveyed in Nagaur, Pali, Barmer, Jalore, Jaipur, Dausa, Bikaner, Sirohi, and Sikar districts of Rajasthan.

In the present investigation, determinations of fluoride in drinking water were conducted in villages of Sanganer Tehsil, Jaipur District, Rajasthan. A total of 300 potable water samples were collected from 100 villages and analyzed by standard methods for pH, fluoride (F), electrical conductivity (EC), and total dissolved solids (TDS).

The water samples were alkaline with pH ranging from 7.7 to 9.1, all within the official limit of 6.9 to 9.2. The fluoride concentration of groundwater varied from 0.2 to 5.4 ppm, and 18% of the villages of Sanganer Tehsil were endemic for fluoride, with F concentrations that were above the permissible limit of 1–1.5 ppm. EC ranged from 483 to 9570  $\mu\text{mhos/cm}$  indicative of high concentration of dissolved solids and salts in the water samples. The EC of all the samples was beyond the optimum limit of 300  $\mu\text{mhos/cm}$ . TDS varied from 242 to 4650 mg/L, and 16% of the villages had TDS that exceeded the permissible limit of 500–1500 mg/L. There was positive correlation between pH and F, and between EC and TDS, but F and pH exhibited an inverse correlation with EC and TDS. Overall, the data indicate that the groundwater of 100 villages in Sanganer Tehsil is unfit for human consumption because it has elevated F, EC, and TDS. The water can be used for drinking and cooking only after treatment with lime and alum, which can reduce the fluoride concentration.

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Keywords: Electrical conductivity; Groundwater fluoride; Sanganer Tehsil, Rajasthan; Total dissolved solids.

## THERAPEUTIC DIFFERENCES BETWEEN THE TOXICITY OF MONOCHLORO- AND MONOFLUOROACETIC ACID IN RATS

Abstract number: **42**  
XXVth ISFR Conference

Monochloroacetic acid (MCAA,  $\text{ClCH}_2\text{COOH}$ ) is used as an intermediate in the synthesis of drugs, dyes, and other substances. The sodium salt of monofluoroacetic acid (MFAA,  $\text{FCH}_2\text{COOH}$ ) is used as a very potent rodenticide. Many accidental fatal exposures to MCAA and MFAA or its salts have been recorded (Routt J, et al., 1975). Previously we reported that sodium monochloroacetate (SMFA) causes hypoglycemia and lactic acidosis, and that these symptoms can be prevented by infusion of a glucose solution (Shimizu H et al., 2002). Because of its structural similarity to MFAA, it has been proposed that MCAA might inhibit aconitase (European Commission Health & Consumer Protection Directorate-General, 2003). However, we found that MCAA did not inhibit aconitase but did inhibit the formation of glyceraldehyde-3-phosphate (GAPDH), and that this appears to be the main cause of the inhibition of liver gluconeogenesis (Sakai A, 2005). It has also been proposed that MFAA may inhibit GAPDH and cause hypoglycemia. This study focused on the effects of glucose infusion for MFAA exposure and how they differ from exposure to MCAA.

**Materials and methods:** (1) Male Sprague-Dawley rats (10 weeks old) and chemically pure sodium monofluoroacetate (SMFA) were used. Thirty-six rats were divided into six equal groups and were injected simultaneously with SMFA in amounts ranging from 1 to 6 mg/kg bw. A probit dose-mortality curve was drawn on the basis of mortality at 14 days to determine the LD50 of SMFA. (2) Fifteen rats underwent ten hours of 10% glucose infusion therapy at the rate of 2 mL/hr after administration of an LD50 dose of SMFA. Blood glucose and lactate were monitored every hour during the experiment, and the survival rate was determined at 14 days. (3) Thirteen rats, three hours after administration of an LD50 dose of SMFA to six of them (plus seven controls), had blood samples taken and analyzed for blood sugar.

**Results:** The LD50 of SMFA after 14 days was 2.6 mg/kg bw. The survival rate of the glucose-treated group at 14 days was 53%. Blood glucose in the glucose-treated group during glucose infusion remained higher than fasted blood glucose, and the blood lactate level increased during infusion. Thus, blood glucose and lactate did not decrease 3 hours after administration of SMFA.

**Conclusion:** The survival rate of the glucose-treated group was not higher than the estimated survival rate at an LD50 dose of SMFA. Blood glucose was not decreased 3 hours after SMFA administration. Thus the main cause of death was probably not due to hypoglycemia caused by inhibition of GAPDH. Because blood lactate level increased, lactic acidosis can be the cause of fatal toxicity, resulting from inhibition of aconitase. Toxicity could also be due to the difference of affinity to the enzymes that SMFA inhibits. The effectiveness of therapies combining glucose infusion with other reported antidotes is an important area for future study.

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Keywords: Blood glucose; Monochloroacetic acid; Monofluoroacetic acid; LD50 in rats; Sodium monofluoroacetate.

## FLUORIDATION, PARADIGM CHANGE, AND TARDIVE PHOTOPSIA

Abstract number:

**43**

XXVth ISFR Conference

Although water fluoridation as a public health measure has been widely promoted for over 50 years as a “safe and effective” way to reduce dental caries, it has been and remains highly controversial. From the start there have been concerns about risks of adverse health effects. These were not allayed, for example, by finding a reduced age for the menarche in an early trial. Moreover, there is unrefuted clinical evidence of individuals who have experienced reversible ill health from drinking fluoridated water, verified by appropriate challenge, dechallenge, and rechallenge tests. As a result, polarization continues between those who insist fluoridation is safe, claiming it to be one of the top ten public health interventions ever, and those who see it as harmful and relatively ineffective and offer 50 unanswered reasons why it should be discontinued. Such unresolved differences have prevented any consensus between the two parties. In examining this conflict, the work by Thomas Kuhn on *The structure of scientific revolutions* may be helpful. He argued that where major conflicts occur, rather than indicating some scientists are frail humans who cannot admit their errors, even when confronted with strict contrary findings, neither proof nor error is at issue. The transfer of allegiance from a widely-held paradigm to one that contradicts it is a conversion experience that cannot be forced. According to Kuhn, lifelong resistance to contrary evidence, particularly among those

with productive scientific careers, is not necessarily a violation of scientific standards but really a reflection of the nature of scientific research itself. The source of the resistance is the implicit assurance that the older paradigm will ultimately solve all its problems, and although this assurance at times appears stubborn and pigheaded, it is what makes normal puzzle-solving in science possible. Because scientists are human, however, conversions are often slow and occur only a few at a time, until a new generation of researchers, sooner or later, emerges as a single group. Thus a conversion experience, like that of Saul of Tarsus on the road to Damascus, rather than a logical analysis of information, may be involved in accepting a new paradigm on fluoridation. Bearing in mind the maxim that there are none so blind as those that will not see, a suitable term to describe this slowness to see the light is *tardive photopsia*.

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Keywords: Fluoridation mindset; Kuhn, Thomas; Paradigm change; Scientific revolutions; Tardive photopsia.

### WHY DOES ALUMINUM AMPLIFY BIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF FLUORIDE?

Abstract number: 44  
XXVIth ISFR Conference

During recent decades serious dangers from the synergistic action of aluminum and fluoride to human health has emerged as a new conclusion from research on cell signaling. Epidemiological studies and the impact of local environmental disasters around the whole world demonstrate health hazards associated with chronic exposure to fluoride and aluminum from environmental sources.

Fluoride has long been known to influence the activity of various enzymes *in vitro*. Later it was demonstrated that many effects primarily attributed to fluoride are caused by synergistic action of fluoride plus aluminum. In water, aluminum ions with an excess of fluoride forms aluminofluoride complexes (AlF<sub>x</sub>), whose average stoichiometry depends on the fluoride concentration and the pH of the solution. Numerous papers show that AlF<sub>x</sub> complexes influence various functions and biochemical reactions of all cells and tissues of the animal or human organisms with powerful pharmacological efficacy. AlF<sub>x</sub> may clone or potentiate the action of numerous extracellular signals. Moreover, the signal of every AlF<sub>x</sub> molecule is greatly amplified during the processes of signal transduction inside the cell. Fluoride in the presence of trace amounts of aluminum can thus act as the messenger of false information, activating G proteins and evoking numerous pathological alterations.

Assessment of the health risks linked to the chronic but cumulative intake of small doses of fluoride and aluminum from food should be focused on sensitive groups such as infants, children, and women in various risky periods of their life, the elderly, those who are ill with the aim of determining how to prevent and minimize the risks of the fluoride burden.

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Keywords: Aluminofluoride complexes; Aluminum; Cell signaling; Fluoride health risks; G proteins.



## BIOLOGICAL PARALLELS AND OPPOSITES OF FLUORIDE AND LITHIUM

Abstract number: **45**  
XXVIth ISFR Conference  
Fluoride and lithium have been used in medicine for over 50 years. Yet, despite their abundance in the earth's crust, these elements have not been involved in the evolution of life on Earth as components of organic compounds in living organisms. The question of their biogenic role has not been fully explored. Since World War II there has been an immense rise in the number of new fluorine-containing compounds and a wide use of both fluorine and lithium in many industrial applications characterize post war development.<sup>1</sup>

Despite intensive laboratory and clinical research investigating the mechanism of the therapeutic action of both fluoride and lithium, it is still a mystery how these simple ions exert such profound biological and pharmacological effects. It is interesting that the observed effects of fluoride and lithium on enzymes, G-proteins, and second messenger molecules are opposite. Both fluoride and lithium can affect the etiopathology of Alzheimer's disease.<sup>2,3</sup> Whereas fluoride has neurotoxic effects on the brain,<sup>4,5</sup> lithium has been found to be neuroprotective. On the other hand, both elements have serious adverse effects, which must be taken into account in their application to humans.

The increased burden of fluoride and lithium from many sources might evoke long-term health problems linked to this exposure. The expected benefits might well become a hidden biological bomb for human civilization.

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Keywords: Alzheimer's disease; Biological effects of F and Li; Cell signalling; Lithium; Neuroprotection; Neurotoxicity.

## DOSE-RESPONSE RELATIONSHIP BETWEEN SKELETAL FLUOROSIS AND FLUORIDE IN BRICK-TEA

Abstract number: **46**  
XXVIth ISFR Conference  
The dose-response relationship between fluoride in brick-tea and the prevalence of skeletal fluorosis (SF) in adults was studied to determine a safe upper limit for fluoride intake from brick-tea. In brick-tea drinking endemic fluorosis areas of the Tibetan pastoral areas of Sichuan province, cluster sampling was conducted of residents above age thirty in Amu, Jiangrong, Anqu, Longrang, and Maiwashi villages of Hongyuan County in Aba state. X-ray technology was used to diagnose SF, and the daily fluoride intake of each person from brick-tea infusions was determined by a retrospective cohort study. Results: Among the 207 residents examined, the X-ray standardized prevalence rate of SF was 49.76%, which increased with age and poor health, especially among persons over age forty. Both the amount of drinking brick-tea infusions and the amount of fluoride intake from them increased with

age and were higher in the SF group than that in the unaffected group. The average daily fluoride intake was  $4.49 \pm 1.51$  mg/person/day in the SF group and  $1.86 \pm 1.46$  mg/person/day in the unaffected group. The average daily fluoride intake of subjects with different stages or grades of SF was  $3.36 \pm 1.35$  mg (stage I),  $4.96 \pm 1.44$  mg (stage II), and  $6.42 \pm 1.33$  mg (stage III), respectively. There was a significant ( $p < 0.05$ ) positive linear correlation between the logarithm of daily fluoride intake from brick-tea and the prevalence of SF in each age group. The 95% normal upper-limit for daily fluoride intake from brick-tea was 3.37 mg/person/day, but the 90% unilateral upper-limit was 2.94 mg/person/day, which reflected the SF status more truly and avoided missing and misjudging diagnosis. Conclusion: (1) Along with increasing age, the amount of drinking brick-tea infusions and the amount of fluoride intake also increased; the prevalence of SF was greater, and the degree of fluorosis was more severe—all showing a high regularity and correlation among them. (2) The normal upper-limit of fluoride intake from brick-tea among person without SF was lower than the total fluoride intake allowed in the health criteria for drinking water in endemic fluorosis areas.

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Keywords: Brick-tea fluoride; Dose-response to fluoride; Skeletal fluorosis; Upper intake limit.

## DOSE-RESPONSE RELATIONSHIP BETWEEN DENTAL FLUOROSIS AND FLUORIDE IN BRICK-TEA

Abstract number:

**47**  
XXVIth ISFR Conference

The dose-response relationship between fluoride in brick-tea and the prevalence of dental fluorosis was studied to determine a safe upper limit for children for fluoride intake from brick-tea. In brick-tea drinking endemic fluorosis areas of the Tibetan pastoral areas of Sichuan province, cluster sampling was conducted of children aged from 8–12 years in primary schools at MaiEr-ma, GeDeng Temple in Aba county, AMu, JiangRong, LongRang, and XiWang in HongYuan county. The daily fluoride intake of each child was determined by a retrospective cohort study. Dental fluorosis was rated with Dean's criteria and a fluoride ion selective electrode was used to measure fluoride in urine and brick-tea water and urine. Results: Dental fluorosis was usually mild, not significantly related to age, and had a prevalence of 52.15%. Fluoride intake and brick-tea consumption increased with age but remained at relatively low levels. The average daily fluoride intake from drinking brick-tea in the children without dental fluorosis was  $0.873 \pm 0.249$  mg compared to  $1.386 \pm 0.699$  mg in the children with dental fluorosis. A positive correlation was present between the dental fluorosis index and the logarithmic mean of the daily fluoride intake from brick-tea for each age group,  $r = 0.919$ ,  $p < 0.05$ . The threshold for the presence of dental fluorosis was a total fluoride intake was 2.243 mg/day. Conclusions: 1. Although dental fluorosis had a high prevalence of over 50% it was usually only mild in severity. 2. The dental fluorosis present in the children was characteristic of that associated with drinking brick tea. 3. Dental fluorosis developed in children with a daily intake of fluoride from brick tea which was lower than the total fluoride intake allowed in the health criteria for drinking water in endemic fluorosis areas.

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Keywords: Brick-tea fluoride; Children, Dental fluorosis; Dose-response to fluoride; Dental fluorosis threshold; Upper intake limit.

## EXPERIMENTAL STUDY OF FLUORIDE TOXICITY ON OSTEOBLASTS DURING BONE FORMATION

Abstract number: **48**  
XXVIth ISFR Conference  
In many areas of the world, there is a high incidence of endemic fluorosis, including various stages of skeletal fluorosis affecting millions of people exposed to excessive fluoride intake. Although the mechanisms of fluoride toxicity on bone remain unclear, it is well known that bone-forming cells (osteoblasts) play an important role in various stages of bone formation.

The present study was designed to investigate the toxicity of fluoride in rat osteoblasts during the initial bone-forming stage. We established methods for isolating, culturing, and identifying osteoblasts from the newborn rat skull-cap bone (calvaria). The proliferation and differentiation of these osteoblasts were examined to determine the effects of fluoride on the initiation of bone-formation at the cellular level. Moreover, the influence of fluoride on cell growth cell cycle patterns, and apoptosis was investigated through both *in vitro* and *in vivo* experiments. In addition, fluoride-induced genotoxicity was also explored. The results of these studies provide basic data for further investigations of the mechanisms of skeletal damage induced by fluoride.

Through our experiments we established a suitable technique for the isolation, culturing, and identification of calvarial osteoblasts *in vitro*, thereby providing experimental material for analyzing the mechanism of fluoride toxicity in osteoblasts. At this stage our conclusions are:

1. Fluoride has a two-phase, non-monotonic direct effect on osteoblasts. At low doses it promotes the proliferation and differentiation of osteoblasts (hormesis effects), whereas at high doses it has significant adverse effects.

2. Excessive fluoride can disturb cell activity and cell cycle, arrest the cell cycle in S phase, and induce apoptosis of osteoblasts.

3. Excessive fluoride induces DNA damage in osteoblasts.

4. Excessive fluoride can penetrate the placental barrier and thereby affect the cell structure and cell cycle of rat offspring by rendering the cell cycle stagnant in the S phase and inducing apoptosis.

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Keywords: Bone formation; Fluoride toxicity on osteoblasts; Osteoblasts.

## EFFECTS OF FLUORIDE ON MURINE MACROPHAGE CELLS (II): ANALYSIS OF CYTOKINES BY REAL-TIME PCR AND ELISA

Abstract number: **49**  
XXVIth ISFR Conference  
Macrophage function varies widely according to the cytokines produced. Among macrophage-producing cytokines, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) are pro-inflammatory and osteoclastic cytokines. IL-10 inhibits the production of cytokines by T cells. IL-12 drives the development of Th0 cells toward Th1 cells. We evaluated the effects of sodium fluoride (NaF) on production of these cytokines and mRNA expression in a murine macrophage lineage cell line, J774.1. The mRNA expression of cytokines was analyzed by quantitative real-time polymerase chain reaction (PCR).

J774.1 cells were cultured in RPMI1640 with 5% fetal bovine serum. The cells were aliquoted in a 24-well culture plate at  $1 \times 10^6$  cells/well. NaF was added to the wells at concentrations of 0, 100, 300, and 1000  $\mu$ M. The cells were incubated in a humidified incubator at 37°C with 5% CO<sub>2</sub> for 18 hr and activated by lipopolysaccharide (LPS). The cells were then incubated for an additional 6 and 24 hr. Cell viability was determined by the trypan blue exclusion method. Total RNA was extracted from the J774.1 cells after an

additional 6 hr of incubation. Real-time PCR was performed to analyze mRNA expressions of TNF $\alpha$ , IL-1 $\beta$ , IL-10, IL-12, and GAPDH (reference gene). The calibrator-normalized relative ratios for cytokines were computed. For the cells incubated for an additional 24 hr, the concentrations of TNF $\alpha$ , IL-1 $\beta$ , IL-10, and IL-12p40 in the supernatant were determined by enzyme linked immunosorbent assay (ELISA).

The mean values of cell viability for the 1000- $\mu$ M groups were lower than those for the respective controls. For mRNA expression, the mean value of the calibrator-normalized relative ratio of TNF $\alpha$ , IL-1 $\beta$ , and IL-10 for the 1000- $\mu$ M group was significantly higher than that of the respective controls. The mean value of TNF $\alpha$  in the supernatant of the 1000- $\mu$ M group, and that of IL-1 $\beta$  in all NaF-exposed groups was significantly lower than that of the respective control. For IL-12p40, the mean value of the 300- or 1000- $\mu$ M group was higher than that of the control. For TNF $\alpha$  and IL-1 $\beta$ , discrepancies between the mRNA expression and protein production were observed. Fluoride may affect allergies by altering mRNA expressions and protein productions of IL-10 and IL-12.

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Keywords: Fluoride cytotoxicity; Interleukin-1 $\beta$  (IL-1 $\beta$ ); Interleukin-12 (IL-12); Murine macrophages; Real-time PCR; Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ).

### **EFFICACY OF CALCIUM GLUCONATE SOLUTION NEBULIZER TREATMENT AGAINST CHEMICAL PNEUMONIA INDUCED BY HYDROFLUORIC ACID INHALATION EXPOSURE**

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**50**

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Hydrofluoric acid (HF) is an important chemical in electronic and chemical industries. For example, it is used in the semiconductor industry to etch quartz and to clean metal. However, its recent great increase in demand is also a potential risk for occupational exposure among workers using it. In Japan the industrial safety and health law Ordinance on Prevention of Hazards Due to Specified Chemical Substances (Ministry of Labour Ordinance No. 39 of September 30, 1972) states that employers shall promote betterment of the working environment and carefully safeguard the health of workers to prevent health impairments from the use of hydrogen fluoride (inclusive of chemicals or substances which contain HF of more than 5% by weight).

The authors present a case report of a patient with severe lung injury after HF inhalation exposure that was successfully treated by administering a 5% calcium gluconate solution with a nebulizer.

A 52-year old worker used HF for 30 minutes in metal surface cleaning in the manufacture of transformer condenser cylinders. He showed a rapid onset of severe dyspnoea and was hospitalized. On arrival at the emergency medical center, he had shortness of breath, widespread wheezing, and crackles (abnormal breath sound produced by air passing over airway secretions) in his lungs. Both his lung X-ray and CT image showed a fine diffuse veiling over pulmonary fields due to HF chemical pneumonia. Laboratory tests revealed severe hypocalcemia of 8.1 mg/dL, high serum F of 74.6  $\mu$ g/L, and high urine F of 80 mg/L.

First aid and medical treatment for HF exposure is very specific and critical. As first aid for the HF inhalation poisoning, the worker was immediately given 5% calcium gluconate solution by intermittent positive-pressure breathing (IPPB), utilizing a nebulizer. This

treatment was continued for four days. On the 5th hospital day, his respiratory function had improved and the treatment was discontinued. On the 21st hospital day, chest film and CT scan were normal, and he was discharged very much improved on the 22nd hospital day.

Fluoride ions from HF can penetrate tissues, and once absorbed into the body, they rapidly bind to calcium and magnesium and form insoluble fluoride salts that can cause failure of various internal organs. As calcium becomes bound to fluoride, calcium depletion can result in hypocalcemia. With the onset of clinical hypocalcemia, heart function is impaired and the heartbeat becomes abnormal, resulting in cardiac dysrhythmia. Calcium gluconate is very effective and the only HF poisoning detoxication drug. The theory behind this is that as the calcium can complex with the fluoride anion and precipitate out as a salt thus preventing the fluoride from scavenging the body's own calcium stores.

HF is one of the most corrosive acids known. It is necessary to use personal protective equipment, which are effective to protect against HF exposure. The present case indicates that an adequate method of emergency treatment for accidental HF poisoning is necessary.

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Keywords: Calcium gluconate; Hydrogen fluoride exposure; Lung injury from HF.

## DOSE-EFFECT RELATIONSHIP OF FLUORIDE IN DRINKING WATER TO LIVER AND RENAL FUNCTION DAMAGE IN CHILDREN

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**51**

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In this research we investigated the relationship of drinking water fluoride (DWF) to liver and kidney function damage in children with and without dental fluorosis (DF). A total of 210 children 10–12 years old from four areas in Henan Province, China, were selected to form seven groups of 30 in each group. The control group consisted of healthy children without DF from the area with the DWF below 1.0 mg/L. Group L1 consisted of healthy children with DF from the area with the DWF ranging from 1.0 mg/L to 2.0 mg/L. Group L2 consisted of children with DF from the same area as group L1. Group M1 comprised healthy children from the area in which the DWF ranges from 2.0 to 3.0 mg/L. Group M2 comprised children with DF from the same area as group M1. Group H1 comprised healthy children from the area with DWF above 3.0 mg/L. Group H2 comprised children with DF from the same area as group H1. Fluoride levels in the urine and serum were estimated by a fluoride ion selective electrode. Serum contents of total protein and albumin (ALB) and the activities of aspartate transaminase (AST), alanine transaminase (ALT), and lactic dehydrogenase (LDH) were determined by standard methods with the ACTO1 auto-analyzer (ACTA, Italy). The activities of N-acetyl- $\beta$ -glucosaminidase (NAG),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT), and creatinine (Cre) in urine samples were analyzed according to the manufacturer's instructions (Nanjing Jianchen Bioengineering Institute, China).

The results of this investigation were as follows: (1) The fluoride contents in the urine and serum of the high-fluoride children and those with DF were higher than those of the control group. Moreover, the fluoride in urine and serum increased gradually with the increase in DWF. (2) The levels of TP, ALB, ALT, and AST in serum were essentially the same among the different groups. The activities of LDH were increased significantly in children with DF from the area with 2.58 mg/L DWF and in two groups from the area with 4.51 mg/L DWF. Moreover, there was an obvious dose-effect relationship between the DWF and the activity of LDH. In contrast to the high-fluoride children from the areas with 2.58 mg/L and 4.51 mg/L DWF, serum LDH activities of children with DF from those two areas were higher. (3) The

activities of NAG and  $\gamma$ -GT in urine were significantly increased in children with DF from the area with 2.58 mg/L DWF and in the two groups from the area with 4.51 mg/L DWF. In addition, there was an obvious dose-effect relationship between the concentration of DWF and the activity of  $\gamma$ -GT in urine. However, the activities of NAG and  $\gamma$ -GT in urine of two groups from the same area showed no significant difference.

The following conclusions can be drawn from these results. DWF above 2.0 mg/L can cause hepatic function damage in children with a significant dose-effect relationship. The degree of hepatic damage correlated with not only the DWF but also with DF or with its absence. Over 2.0 mg/L DWF can also cause renal function damage in children, again with an obvious dose-effect relationship. However, renal damage degree is not related to DF but the concentration of DWF.

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Keywords: Children in Henan, China; Dental fluorosis; Dose-effect relationship; Drinking water fluoride; Liver function damage; Renal function damage.

## SCREENING OF ENVIRONMENTAL RESPONSE GENES RELATED TO FLUORIDE AND DENTAL FLUOROSIS

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**52**

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In an investigation to determine the susceptibility or resistance of human genes to fluoride, 30 children 10–12 years old were selected from two areas in Anyang County, Henan Province, China. Then, according to the level of fluoride in their drinking water and whether they were afflicted with dental fluorosis (DF), they were divided into three groups: a low-fluoride control group, a high-loaded fluoride group, and a DF group. A 3-mL sample of blood was collected from each child via the elbow vein into a sterile centrifuge tube containing sodium heparin. The leukocytes were isolated from each blood sample, and Total RNA was extracted from each leucocyte sample. The leukocyte gene expression profiles of each group were determined by the gene chip HG-U133A assay kit of the Affymetrix Company, and the results were analyzed by bioinformatical methods.

The gene chip results indicated that a total of 1057 genes were differentially expressed in the high-fluoride group as compared with the low-fluoride control group. Of these genes, 148 were robustly up-regulated and 61 were robustly down-regulated. In the DF group compared to the control group, a total of 964 genes were differentially expressed, including 71 robustly up-regulated and 60 robustly down-regulated genes. Compared with high-loaded fluoride group, 633 genes in the DF group were identified as differentially expressed, of which 15 were robustly up-regulated and 67 were robustly down-regulated genes. The differentially expressed genes mainly included transcription factors, genes related to signal transduction, genes related to immunity, cancer genes, genes related to apoptosis, structure proteins, and transport proteins. From these gene chip results it can be concluded that multiple gene expressions are related to dental fluorosis.

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Keywords: Dental fluorosis; Environmental fluoride; Gene chip expression; Gene response to fluoride.

### **A FLUOROSIS SURVEY AND DEFLUORIDATION OF DRINKING WATER BY CALCIUM OXIDE**

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**53**

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Fluorosis is a disease caused by excessive intake of fluoride through various avenues, water generally being the principal source. The presence of fluoride in drinking water above (and sometimes even below) the permissible limit of 1.5 ppm set by the World Health Organization leads to fluorosis. In the modern world both Man and Nature are responsible for this disease. In Rajasthan, India, the worst affected districts are Nagaur, Jaipur, Ajmer, Sirohi, Jhunjhunu, Churu, Dungarpur, Udaipur, Sikar, and Alwar (Govindgarh). In this study, samples of drinking water collected in plastic bottles in summer and winter season from Ramgarh (Sikar) District, in Rajasthan, were analyzed. These samples were taken from hand pumps and wells. The concentration of fluoride was determined by the fluoride ion selective electrode method before and after adding calcium oxide. The fluoride concentration in the samples was decreased by the addition of calcium oxide. In this paper we also report case studies carried out by the authors on fluorosis in nearby areas. These surveys showed that out of 548 persons (above age five), 319 (58.2%) were afflicted with dental fluorosis and 78 (15.1%) by skeletal fluorosis.

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Keywords: Calcium oxide; Defluoridation; Fluorosis survey.

### **MEAN URINARY FLUORIDE OF VARIOUS POPULATIONS IN CHINA**

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**54**

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Urinary fluoride levels reflect fluoride ingestion and absorption as well as fluoride released from calcified tissues. Although individual urinary fluoride concentrations vary greatly, urinary fluoride of populations as a whole has a certain degree of stability. Epidemiological data indicate that urinary fluoride increases with fluorosis in regions of endemic fluorosis and is therefore frequently used to evaluate the extent and severity of fluorosis in such areas.

In this study, for reference purposes, reports of mean urinary fluoride of Chinese populations in areas without fluorosis were reviewed. A total of 114 mean urinary fluoride levels of children aged 8–15 years and 105 of adults over 18 years of age in different geographical regions ranging from northern to the southern China were collected and analyzed.

In our investigation, urine spot samples of children and adults of the same ages were collected randomly in 100-mL plastic bottles in three endemic fluorosis regions including: (1) ten drinking water type, (2) nine coal-burning type, (3) five brick-tea drinking type. For comparisons, urine sample were also collected in twelve nonfluorosis regions. Urinary fluoride was measured with a fluoride ion specific electrode.

By analysis of the literature data, we found that the frequency distribution of mean urinary fluoride levels of children and adults in nonfluorosis areas did not have a normal distribution. The medium, 95-percentile, and 99-percentile of urinary fluoride means of the children in

these areas were, respectively, 0.70 mg/L, 1.43 mg/L, and 1.79 mg/L; of the adults they were 0.83 mg/L, 1.59 mg/L, and 1.89 mg/L.

Our field investigation revealed that the mean urinary fluoride of adults in all the three types fluorosis areas was higher than 1.68 mg/L, and the mean urinary fluoride of children exceeded 1.49 mg/L in drinking water type and coal-burning type fluorosis areas, but the urinary fluoride means of children in brick-tea drinking type fluorosis areas was as low as 0.79 mg/L. The mean urinary fluoride levels of children in the twelve nonfluorosis areas were all lower than 1.20 mg/L, and those of adults were all lower than 1.49 mg/L.

In conclusion, our study showed that mean urinary fluoride of populations in China differed markedly between fluorosis and nonfluorosis areas. The mean urinary fluoride can serve as an indicator for identifying endemic fluorosis areas: 1.6 mg/L for adults, and 1.4 mg/L for children, except in brick-tea drinking areas.

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Keywords: China; Endemic fluorosis; Population; Threshold; Urinary fluoride.

### **IN VITRO INFLUENCE OF SODIUM FLUORIDE ON ADENOSINE TRIPHOSPHATE (ATP) IN RAM SEMEN**

Abstract number: Various concentrations of NaF were added to ram sperm collected by an artificial vagina to determine the influence of fluoride on the sperm motility and ATP content.

**55**

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In semen there appears to be a connection between the sperm motility and cyclic AMP which probably involves the following sequence of events: (i) generation of cyclic AMP from ATP by adenylate cyclase; (ii) phosphorylation of specific endogenous sperm protein by ATP in plasmalemma and possibly also in microtubules; (iii) transphosphorylation between free ATP and tubulin-bound GDP; and—at a later stage—(iv) energy-yielding dephosphorylation of ATP to ADP, catalyzed by dynein ATPase (Mann et al., 1981).

The ATP content was determined by using the firefly luciferin-luciferase system in a Lumat LB 9807 Berthold luminometer by Method 1 of Gottlieb et al (1987). A high correlation was found between the ATP content and motility of the sperm ( $r = 0.577$ ). The semen (after dilution with 0.9% NaCl) contained 12.43  $\mu\text{mol ATP}/10^9$  spermatozoa (average from 18 rams). The dependence of ATP content on the NaF concentration was as follows: at 20  $\mu\text{mol/L}$ , the ATP content decreased by 74.65%, at 100  $\mu\text{mol/L}$  by 75.46%, at 200  $\mu\text{mol/L}$  by 90.82%, and at 10<sup>5</sup>  $\mu\text{mol/L}$  by 99.90%.

Since the content of ATP in sperm results from both its synthesis and utilization, it is difficult to determine whether the fluoride affects its synthesis or its use. Consequently, it appears necessary to assess the dynamic balance between ATP synthesis and hydrolysis.

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Keywords: Adenosine triphosphate; Fluoride and ATP; Ram semen; Sperm motility.