

SPECIAL SUPPLEMENT

**ABSTRACTS OF PAPERS TO BE PRESENTED AT THE XXIIIrd CONFERENCE
OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH**

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Presentations in the final Conference Program
may be in a different order, and some will be posters.

Fluoride in the environment and oral health in children

Ecologists, toxicologists and physicians have devoted much attention to the problem of long-term exposure to high and to optimal concentrations of fluoride compounds in fresh water, air and food. Exposure to fluoride, combined with environmental factors, may markedly influence the health status of children during development and growth.

Żuławy is a depression in the Vistula delta near the Baltic Sea coast, which evolved during the Quaternary Period and where fluoride content in fresh water ranges from 1.2 to 5 mg/L. The village of Wiślinka in Żuławy is situated near a phosphorite waste depot, and its source of fresh water contains 1.2 mg F/L.

The purpose of this study was to assess oral health status in 144 children aged 6-14, environmentally exposed to a high level of fluoride. The control group consisted of children from a region with a very low fluoride concentration in drinking water. Both groups were matched for age and gender. The examination protocol was based on the WHO Oral Assessment Form (1987) and consisted of evaluation of dentition, gingiva, periodontium and oral hygiene. Status of dentition was evaluated with the index of dental caries (DMF), gingiva and periodontium with GI and CPITN, and oral hygiene with PI I.

More than 83% of permanent teeth in children from Wiślinka revealed mineralization defects, while in the control group the figure was 38%. Children in the high-fluoride group had 2.31 permanent teeth with a history of dental caries. Plaque Index values were similar in both groups (> 1.0), but children from Wiślinka had healthier gingiva (GI = 0.3) than controls (GI = 0.83).

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**Lymphoid depletion of spleen due to
experimental fluorosis in rats**

Objective: To examine qualitatively and quantitatively the histological changes in spleen of rats given sodium fluoride.

Material and Methods: Seventy-five female Wistar rats, each weighing approx. 200 g, were divided into three equal groups. Animals in groups 1 and 2 received intraperitoneally daily doses of 0.5 and 5 mg NaF, respectively, whereas group 3 (control) received physiological saline. The experiments were run for a

period of 3 months.¹ Afterwards, the spleen was removed for histological and histochemical examination and fixed in 10% formaldehyde solution. Histological sections were stained with haematoxylin and eosin, picrosirius red F3BA,^{2,3} and silver reagent according to Gomori. The ultrastructural changes in collagen fibres were investigated by optical polarisation methods.

Results: The lymphoid tissue mass of the spleen decreased and the relative proportion of reticulin and collagen structures increased. These changes correlated with the dose of fluoride.

Conclusions and Interpretation: The absolute and relative proportion of lymphoid tissue in spleen of rats given NaF decreased in a dose-related manner. Loss of lymphoid tissue may play an important role in the diminished reactivity of the organism and may be regarded as a consequence of fluoride toxicity.

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- 3 Constantine VS, Mowry RW. Selective staining of human dermal collagen. II. The use of Picrosirius red F3BA with polarization microscopy. *J Invest Dermatol* 1968;50:419-23.

Author: M Bély, National Institute of Rheumatology and Physiotherapy, and Dept of Pathology, Budapest, Hungary.

Effects of sodium fluoride on bone mineral mass gain in growing rats

The purpose of this study was to investigate the effect of fluoride in drinking water on bone mineral mass gain in growing rats. Forty 6-week-old female Wistar rats were randomised into four groups. One group served as control and received distilled water, while the other three received water containing sodium fluoride at levels of 8, 30 and 60 mg F⁻/L. After six weeks the animals were weighed and the right femoral bone was removed for measurement of mineral mass by dual-energy X-ray absorptiometry (DXA).

Bone mineral mass was increased and body weight was reduced in groups exposed to fluoride. The femoral mineral mass to body weight ratio revealed a significant correlation with the level of fluoride in water.

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Hardness of cortical bone in fluoride-treated growing rats

The purpose of this study was to investigate the effect of fluoride in drinking water on bone fluoride content and bone hardness in rats. Twenty-eight 6-week-old female rats were divided into four groups. One group served as con-

trol and received distilled water, while the other three received water containing sodium fluoride at levels of 8, 30 and 60 mg F⁻/L. After 6 weeks both femoral bones were removed for cortical fluoride content and Vickers microhardness test in distal diaphysis.

Fluoride concentration in bone was significantly increased in the fluoride groups. Bone microhardness was significantly higher in these groups, with maximal values for the 30 mg F⁻/L group.

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Influence of fluoride on Na⁺-H⁺ exchanger activity in human red blood cells

The purpose of this study was to measure the activity of Na⁺-H⁺ exchanger in red blood cells before and after *in vitro* administration of fluoride.

The Na⁺-H⁺ exchanger is a ubiquitous transport system that is involved in the regulation of intracellular pH, cell growth and proliferation, cell volume, and transepithelial absorption of Na⁺, Cl⁻, and HCO₃⁻. Altered activity of the Na⁺-H⁺ exchanger has been implicated in the pathogenesis of essential hypertension. Several studies have also shown an association between erythrocyte Na⁺-H⁺ exchanger activity and predisposition to nephropathy in patients with insulin-dependent diabetes mellitus. On the other hand, some investigators have observed an inhibitory effect of fluoride on erythrocyte enzymes. Fluoride affects co-transport by activating Na/K/2Cl and inhibiting K-Cl.

Ten healthy patients (5 females, 5 males), aged 27-43 years (mean 30) enrolled in the study. Blood was anticoagulated with heparin. Measurement of Na⁺-H⁺ exchanger activity was according to a modified procedure of Orlov et al. Red blood cells were exposed to 0.25 or 2.5 mmol/L fluoride solution. Statistics were done with the Wilcoxon test.

RESULTS

- Fluoride at 0.25 mmol/L inhibits Na⁺-H⁺ exchanger activity by 21% as compared with control (p<0.05).
- Inhibition of the Na⁺-H⁺ exchanger is not significantly increased by fluoride at 2.5 mmol/L (27% vs. control value).
- Fluoride causes a statistically significant decrease in intracellular pH (p = 0.01 for either concentration of F).
- A significant correlation between fluoride concentration and intracellular pH was observed.
- Further studies on a larger group of patients are needed.

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Rearrangement of 5-(trifluorovinyl) uracil-computational simulation of reaction mechanism

Photochemical properties of 1,3-dimethyl-5-(trifluorovinyl) uracil S1 were studied. Surprisingly, during irradiation with UV light ($\lambda > 300$ nm) in water of S1, we were able to isolate as only stable product with relatively good yield derivative of uracil identified as 1,3-dimethyl-(5,6-dihydrourac-6-yl)-difluoroacetic acid S7. Irradiation of substrate 1,3-dimethyl-5-(trifluorovinyl) uracil S1 with higher energy light ($\lambda = 254$ nm) led to a cleavage of C(5)-C(trifluorovinyl) bond and yields 1,3-dimethyl uracil as a sole product. In this presentation we would like to discuss possible mechanism of this transformation based on computational simulations:

[MOST FIGURES NOT FEATURED ON WEBSITE]

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Fluoride content in dental calculus using EDS x-ray microanalysis

Numerous factors have been shown to stimulate or inhibit the mineralization process in dental plaques. Although its content in dental calculus is insignificant, fluoride appears to play an important role in nucleation and crystal formation. Furthermore, the content in dental calculi varies, depending on intake.

Previous studies revealed that the mineralization of dental deposits in the oral cavity of adults was a concern. We have now evaluated the chemical structure of dental calculus in 12-year-old children (n = 29) living in Poznań, Western Poland. Their drinking water contained 0.1-0.6 mg F/dm³ in 1996 and 0.2-1.0 in 1997.

The material studied was supragingival dental calculus scaled from the lingual surface of lower incisors. Concentrations of elements were measured by EDS X-ray microanalysis using Si (Li) PRISM detector (PGT).

Overall, the following elements were detected: carbon, oxygen, calcium, phosphorus, sodium, magnesium, potassium, sulphur, chloride, silicon and fluoride. Fluoride was present in only one sample. The present results should be regarded as preliminary.

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Longitudinal studies on the exposure to fluorides based on urine samples from infants living in Poznań

Fluoride concentration in urine is regarded as a biomarker of exposure to fluoride compounds. Most studies on fluorides in urine have been performed in men. Few reports have appeared on urinary fluoride content in small children.

The purpose of this study performed in 1996-1999 was to determine the urinary fluoride levels in small children drinking fluoridated water. Forty-four children, 2-3 years of age, living in Poznań, participated. Water fluoride content was at optimal levels in terms of caries prophylaxis. Two years after the first urine sample, when the children were 4-5 years of age, sampling was repeated. Urine was obtained in the morning and a fluoride ion-selective electrode (type OP262, Radelkis) was used.

The mean urinary fluoride concentration was 0.36 ± 0.32 mg F/L, and 0.65 ± 0.44 mg F/L two years later. The fluoride level at two intakes of drinking water in Poznań ranged from 0.20 to 0.35 mg F/L. However, according to the local "Sanepid" station, during the last ten years the content ranged from 0.33 to 0.75 mg F/L. It should be mentioned that "Sanepid" is obligated by law to measure the water fluoride concentration four times per year.

For the first measurement, urinary fluoride concentration correlated with age. After two years, no significant correlation was noted. Fluoride content in urine tended to be lower in breast-fed children. In conclusion, the fluoride level in urine seems to depend on diet, rather than age of child.

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Caries in children living in a region with optimal level of fluoride

The purpose of this study was to evaluate the intensity of caries in children from the Wielkopolska province, where the level of F⁻ ions in drinking water is considered to be optimal from the point of view of caries prevention. According to data from 1991-1995, the level of fluorides in drinking water was: Poznań 0.42-0.82; Środa Wielkopolska 0.7-0.9; in rural areas 0.5-0.9 mg F⁻/L.

456 students of elementary schools, aged 7 and 12 years, living in the large town of Poznań, small town of Środa Wlkp., or village near Środa Wlkp. participated in the study. The study was carried out in 1995, according to WHO recommendations (Oral Health Surveys, Basic Methods, third edition, 1986) and standards of dental surgery, with a probe and mirror. The results were recorded in WHO Oral Health Assessment Forms.

The frequency of caries in mixed teeth was high, with a mean of 85.54% in 7-year-olds and 84.01% in 12-year-olds. The frequency was lowest in 12-year-olds from the small town.

The intensity of caries expressed by mean DMF-t increased with age and was 0.48 for 7-year-olds and 2.94 for 12-year-olds. In the younger group, no case of permanent teeth removal was noted, whereas in the older group the M index was 0.1. In 12-year-olds living in the village, DMF-t values were slightly higher (3.17) than in the large and small town (3.03 and 2.62, respectively). Fillings were found more often in girls. The percentage of 12-year-old children achieving the WHO year 2000 goal of DMF-t <3 was 63.9, whereas the percentage with DMF-t = 0 was 19.1.

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Fluoride-induced renal damage and its reversal by some antidotes

Sodium fluoride (NaF) (10 mg/kg body weight) was administered orally to male mice for 30 days to evaluate various biochemical and structural changes in the kidney. The effects of withdrawal of NaF treatment and ingestion of ascorbic acid (AA) and calcium (Ca⁺⁺) were also investigated. NaF treatment caused severe alterations in the structure of kidney as compared to control. Disorganization of glomeruli with increased space between glomeruli and capsule wall

was evident. Renal tubules showed vacuolization and distortion and the number and size of mitochondria in epithelial cells was reduced. Nuclear membrane was indented and showed dense chromatin which was not distributed homogeneously. These changes could be correlated with decrease in the activities of marker enzymes, viz. alkaline and acid phosphatases and levels of creatinine and protein in the kidney, suggesting altered kidney functions.

The withdrawal of NaF gave incomplete recovery. Conversely, supplementation with AA and Ca^{++} in the withdrawal period was found to promote recovery from all NaF-induced effects, thus supporting their beneficial role in the recovery from toxic effects of NaF on renal function. On the whole, Ca^{++} alone and in combination with AA was more effective than AA. Therefore, Ca^{++} and AA could be used for the management of fluoride-induced changes in the kidney.

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Amylase activity in serum and pancreas of rats in acute NaF poisoning

The purpose of this study was to examine amylase activity in serum and pancreas of rats (Wistar FL strain) given a single intraperitoneal dose of 35 mg NaF/kg body mass. Amylase activity was assayed with a commercial test kit (Sigma USA, cat. no. 577-3).

No changes in amylase activity were found in serum, while in the pancreas an increase of 23% was demonstrated, indicating modified metabolic activity of the pancreas under the influence of fluorine. Due to the fact that alpha-amylase in rat serum originates from salivary glands and liver (A. Tomasik *et al* "Origin of serum alpha-amylase isoenzymes from healthy adult rats", *Materia Medica Polona*, vol. 27, Fasc. 2, 1995), we could not observe any correlation between amylase activity in serum (no change) and pancreas (increase).

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Beneficial effects of a protein-supplemented diet on fluoride-induced toxicity in liver of male mice

The effects on the liver of male mice of protein-supplemented and protein-deficient diets with exposure to sodium fluoride at three doses (5, 10 and 20 mg/kg body weight) were investigated.

The results reveal that a protein-deficient diet with NaF for 30 days (any dose) in male mice brought about a significant decrease in glutathione levels and activities of superoxide dismutase (SOD) and catalase along with a significant increase in lipid peroxides (LPO) in the liver. To a lesser extent, these

changes were also observed in groups fed a control protein diet with 5, 10 or 20 mg NaF.

On the other hand, mice on a protein-supplemented diet with NaF showed no change in liver LPO, glutathione level, activities of SOD and catalase, in comparison with animals on a control protein diet.

The present study indicates that protein supplementation has a beneficial effect on the liver and is necessary for recovery from fluoride toxicity. The importance of protein in the diet, especially in developing countries where protein malnutrition may occur, is evident.

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The influence of fluoride and/or aluminium on free radical toxicity in the brain of female mice and beneficial effects of some antidotes

The present study was designed to investigate the effects of sodium fluoride (NaF) and/or aluminium chloride (AlCl_3) at doses of 10 and 200 mg/kg body weight, respectively, for 30 days on the cerebral hemispheres of female mice (*Mus musculus*). The reversibility of induced toxicity by withdrawal of NaF+ AlCl_3 and by administration of ascorbic acid (AA), calcium (Ca) or vitamin E alone or in combination during the withdrawal period were also studied.

Fluoride and aluminium individually and in combination reduced the concentration of free radical scavengers such as glutathione and reduced ascorbic acid, and impaired the activities of protective enzymes: superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) in the brain. This correlated with increased levels of dehydroascorbic acid and lipid peroxides in the organs. Withdrawal of NaF+ AlCl_3 brought about a partial recovery of all parameters studied. However, administration of ascorbic acid, calcium or vitamin E alone or in combination resulted in a complete recovery from the induced toxic effects. Recovery was more pronounced with a combination of these antidotes, possibly due to antioxidant properties of vitamin C and E and their synergistic action. Thus, the present study suggests that NaF+ AlCl_3 -induced cell damage is transient and reversible in the murine brain.

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An outline of possibilities of using fluoride-containing mine waste water for prophylaxis and therapy

The aim of this article is to present possibilities of using mine waste water for prevention and therapy of some diseases. The water, containing significantly higher quantities of fluorine compounds, is drawn from Jurassic layers

at a considerable depth. In the coal mine it combines with salty Carboniferous water and is pre-treated before being discharged to a river. Weakly mineralized Jurassic water with a fluoride content of 6-11 mg F/dm³ can be used for prevention of dental and periodontal disease, particularly in risk groups. Fluoride contained in this water can be beneficial to patients after surgery or undergoing other treatment. Fluoride water from the Lublin Coal Basin can be helpful in bone disease, such as osteoporosis and fractures. According to the literature, reconstruction of normal osseous tissue is possible through balneotherapy. The water, free of harmful chemical compounds and micro-organisms, can be consumed (with controlled uptake of fluoride), used to wash the oral cavity, and for baths. However, the application of fluoride waters in children, due to their unstable metabolism, should be exercised with caution.

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Effect of fluoride on the ultrastructure of rat liver

Fluoride as sodium fluoride (NaF) is used in caries prophylaxis via drinking water, fluoridated salt, or in the form of fluoride tablets. Being one of the most effective forms of caries prevention, it still generates much controversy due to the cytotoxic effects of fluoride.

The purpose of this study was to evaluate the effect of fluoride on the ultrastructure of rat hepatocytes during growth and development. 75 Wistar rats received NaF, either 1.2 or 3.6 mg F/kg b.m., from intrauterine life until maturity. The liver was examined on the 14, 30, 60, and 90 day of life during exposure to fluoride, and 30 days following fluoride withdrawal. Four samples (1 mm³ each) were collected from the left lobe of 41 rats and sections were studied using a transmission electron microscope (OPTON 900 PC).

The results indicate that ultrastructural changes in hepatocytes intensify with increasing dose of fluoride. This applies mainly to nuclei, mitochondria, rough and smooth endoplasmic reticulum, and sinusoids. Glycogen content changes as well. Most of these changes are reversible.

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Pharmacokinetic parameters in rats with acute renal damage caused by intravenous high dose of fluoride

Fluoride(F) complexes are used in some fields of industry and medicine. F excretion depends mainly on kidney function. Urinary F concentration is measured to monitor the health of workers exposed to F. The toxicokinetics of F were studied by analyzing plasma concentration of F after intravenous injection of 2.86, 5.71, and 8.57 mg/kg in male Wistar rats. A dose-response relationship between F and renal tissue injury was obtained. Blood samples were

collected at 0, 10, 20, 30 min, and 1, 2, 3, 4, 5, and 6 h after injection. Plasma concentration-*vs*-time profiles were evaluated by a nonlinear least-square method for fitting data to polyexponential equations, and by calculation of relevant pharmacokinetic parameters. Results indicate that a two-compartment model would best describe the elimination of F from plasma. The β rate constant, total plasma clearance (Cl) and first-order rate constants (K_{21} , K_{el}) decreased and half-time of β phase ($t_{1/2\beta}$) significantly increased with rising dose. The kidney is the main target organ for F toxicity. Acute exposure to high doses of F leads to lesions in renal tissue and renal dysfunction. The Cl of F depends mainly on renal F excretion. Since severe kidney damage markedly affects the toxicokinetics of F and decreases the elimination of F, some other nephrotoxic indicators, as well as measurements of plasma F levels would be necessary for monitoring high-dose F exposure.

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The positron-emitting radioisotope ^{18}F for quantitative imaging of biochemical pathways of fluorine compounds *in vivo*

The fluorine radioisotope ^{18}F has physical half-life of 110 min. Its decay is accompanied by the emission of positrons. It can be produced in high yields at small cyclotrons. Thus it is a very useful radioisotope for positron emission tomography (PET).

As the chemical and biochemical properties of ^{19}F and ^{18}F are identical, the fluorine radioisotope can be used as a radioactive label of fluoride itself or almost any fluorine compound. Furthermore ^{18}F can be introduced into (labeled to) various non-fluorine containing compounds of biochemical interest by either nucleophilic or electrophilic syntheses, resulting in chemically and biochemically similar traces. PET is capable of detecting the distribution of these ^{18}F -compounds *in vivo*, in a dynamic and quantitative way.

The [^{18}F]fluoride anion has not been consistently and continuously applied in the diagnosis and therapy of bone metastases or osteoporosis since its first use in the 1940s. Today the availability of ^{18}F via the (p,n) process on highly enriched H_2^{18}O targets in comparatively common particle accelerators, together with modern, high-resolution PET scanners, offers excellent conditions for a renaissance of this tracer. Detection of even small metastases and their exact localization allows the differentiation between degenerative or metastatic processes. Furthermore, quantification of metabolic bone remodeling by [^{18}F]fluoride compared to metabolic parameters determined via other tracers may result in additional information important for therapy control purposes.

The most prominent example of a labeled tracer is the glucose analogue 2- [^{18}F]fluoro-2-deoxy-D-glucose [FDG]. It has become the most frequently used oncologic PET tracer. The reason for this unequalled success lies in the coinci-

dence of two main chemical concepts a solid biochemical concept of the compounds behavior with respect to intercellular metabolism of carbohydrates, and the reliability of the nuclear chemical synthesis of this glucose tracer.

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Experimental And Clinical Applications Of [¹⁸F]Fluoride Ion Positron Emission Tomography

In recent years, the [¹⁸F]fluoride ion has increasingly been used with positron emission tomography for the *in vivo* measurement of bone metabolism. Two principal methodologies, the static and dynamic approach, are available for research and clinical applications. Static [¹⁸F]fluoride ion PET has clinically been used for the detection of primary and secondary bone tumors and to assess the extent of metabolic bone diseases (renal osteodystrophy, hyperparathyroidism, Paget's disease). Static whole body [¹⁸F]fluoride ion PET delivers superior image quality in the assessment the overall metabolic activity of the skeleton, but the information obtained from these images is similar to conventional bone imaging modalities such as [^{99m}Tc]Tc-diphosphonate compound bone scanning.

On the other hand, dynamic [¹⁸F]fluoride ion PET is able to provide quantitative measures of the initial steps of bone metabolism with the 3-compartment model validated by Hawkins and coworkers (J Nucl Med 33 (1992): 633-642). According to this model, the kinetic parameters K₁ and K₂ describe tracer transport from the vascular to the bone tissue compartment and back. The incorporation of [¹⁸F]fluoride into the bone matrix (bound bone compartment) is described by the parameter k₃ and can be estimated accurately with dynamic PET imaging. The applicability of the this PET modality in the estimation of bone blood flow and bone formation rate was evaluated experimentally. Our data have shown that K₁ is an accurate estimate for bone blood flow under normal and low flow conditions.

Certain limitations in the estimation of bone blood flow under high flow conditions can be resolved using a correction factor derived from combined PET studies with [¹⁵O]H₂O and [¹⁸F]fluoride ion PET (M Piert: *J Bone Mineral Res* 1998;13:1328-36). Transforming these results into the clinical field, dynamic [¹⁸F]fluoride ion PET allows the quantitative *in vivo* assessment of bone blood flow, bone transplant vitality (M Piert. *Eur J Nucl Med* 1999;26:615-24) and bone formation rate (C Messa. *J Clin Endocr Metabol* 1993;77:949-55).

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Fluoride metabolism in plants

There have been various reviews on plants and fluoride in the past 10 years. This review will cover the earliest manifestations of fluoride toxicity in plants including enhanced respiration and ATPase activity.

The most sensitive membrane to fluoride is the tonoplast and this is related to ATPase. Also, ATPase in the plasmalemma is inhibited at very low fluoride concentrations. Subcellular fluoride partitioning occurs in the plant cell resulting in different concentrations in various organelles. Higher concentrations contribute to fluoride damage. This partitioning occurs because of 2 fluoride forms (HF and F⁻). The HF form will pass through the lipid membranes 6x as readily as F⁻. The subcellular distribution of accumulated fluoride and direct influences of fluoride on enzymatic activities prior to the display of any visual damage are discussed.

Fluoride content in different soils is presented and correlated with fluoride content in plants. The uptake of fluoride by plants from these soils is discussed in terms of plant fluoride accumulation, plant structure and plant biomass. A direct correlation was found between water-soluble fluoride in soils and fluoride concentrations in tobacco plants. Plant sensitivity to fluoride is discussed in terms of cellular components that affect fluoride solubility.

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Study of fluoride metabolism by bio-rhythm analysis:

I. Analysis of urinary fluoride excretion with the Cosiner method

The use of radioactive¹⁸F for studying the fate of ingested fluoride (F) is difficult owing to its short half-life. The purpose of this study was to investigate the metabolism of F in humans by following the fate of ingested F through measurement of urinary F levels. For this purpose, human volunteers were examined under feeding and fasting conditions. Urine samples for F analysis were collected every 2 h for 4 days. The results were subjected to rhythm analysis with the Cosiner method. Under fasting conditions, no rhythm was observed in urinary F concentrations, whereas vertex phase times for urinary volume (UrV) and urinary F content showed a rhythmic pattern, being 11.82 and 12.37 h, respectively. Under feeding conditions, a rhythm was observed with respect to vertex phase times for urinary F concentrations, UrV, and urinary F content, being 0.57, 11.78, and 12.14 h, respectively.

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Study of fluoride metabolism by bio-rhythm analysis:

II. Analysis of urinary fluoride, various other minerals, and creatinine by Cosiner method

The aim of this work was to study fluoride (F) metabolism in humans in relation to various minerals and creatinine (Cre). For this purpose, five healthy men were fed space food for 4 days and urinary samples were taken at 2 h in-

tervals for determination of F, Na, K, Cl, Ca, Mg, P, and Cre concentrations and the results were analyzed with the Cosiner method.

During the feeding period, vertex phase times for urinary concentrations of Na, K, Cl, Ca, Mg, P, Cre, and F were 13.66, 9.47, 13.16, 2.4, 0.94, 1.27, 0.91, and 0.57 h, respectively, whereas during the fasting period the values were 12.45, 11.91, 12.33, 10.89, 13.32, 15.54, 10.59, and 13.14 h, respectively.

Under feeding conditions, three rhythm groups were observed: (1) Na, K, and Cl, (2) Ca, Mg, P, and Cre; (3) F. The results suggest that various factors including minerals may influence F metabolism and urinary F excretion.

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Concentration of fluoride ions in blood serum and content of fluorine in bones and teeth of rats chronically exposed to fluorine in drinking water

The purpose of this study was to examine the concentration of fluoride ions (F^-) in blood serum, as well as their content in bones (femur) and teeth (incisors) of rats (Wistar FL strain) after a 4-month exposure to 50 and 100 mg of fluoride ion (as NaF) per litre of distilled drinking water. The concentration of fluoride ions in serum was assayed with Radelkis OP-262 instrument (Hungary), while the fluoride content in bones and teeth was assayed using Orion 96-09 ion-selective electrode (USA). A statistically significant increase in F^- concentration in serum and in the content of fluorine in bones and teeth was found in rats exposed to F^- . An increase of 8.6 times in bones and 6 times in teeth was noted in the low-dose group, while in the high-dose group the content in bones increased 14.3 times and in teeth 11.5 times. We found a stable increase in absorption of fluorine by bones and teeth, relatively higher in the former tissue. These results confirm that fluorine, due to its high chemical and, in consequence, biological activity, and its small size, easily penetrates hard tissues (bones, teeth). The increase in fluorine content of hard tissues and concentration in serum was proportional to the dose of NaF.

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Activity of some anti-oxidation enzymes and concentration of malonic dialdehyde in rats with fluoride-induced hyperglycemia

The purpose of this study was to induce fluoride hyperglycemia in rats and to examine free radical processes in the pancreas by assaying the activity of

anti-oxidation enzymes and the concentration of malonic dialdehyde as an end-product of lipid peroxidation responsible for cell damage.

The study was conducted in 30 rats of the Wistar FL strain which during 4 months received distilled drinking water containing 50 and 100 mg of fluoride ion per litre (as NaF).

A statistically significant increase in serum concentration of fluoride ion (assayed with Radelkis OP-262 instrument (Hungary)) was found. Serum glucose concentration (assayed with Cormay test kit (Poland)) increased by 60% in rats receiving 50 mg F⁻/L and by 78% in animals receiving 100 mg F⁻/L.

The activity of superoxide dismutase (SOD) in pancreas (cytoplasmic – CuZnSOD and mitochondrial – MnSOD isoenzymes) was assayed with Oyanagui's method, selenium-dependent glutathione peroxidase (GSHPox) with Paglio and Valentine's method and malonic dialdehyde concentration (MDA) was assayed with Ohkawa's method. A 50 % (statistically significant) decrease in CuZnSOD activity in the pancreas was found, as well as a decline in MnSOD activity. Histological examination of the pancreas was performed in tissues embedded in paraffin and stained with hematoxylin and eosin. No changes in pancreatic β -cells, cells of the outer secretory part or stroma were revealed. The results seem to rule out pancreas dysfunction as the cause for hyperglycemia. Presumably, considerable loss of CuZnSOD activity was without effect on the oxidation-reduction equilibrium of the cell (large enzyme buffering capacity). In confirmation of this, no change in the concentration of MDA was found. Hyperglycemia should rather be explained by abnormalities in glucose metabolism and its hormonal regulation.

Authors: E Grucka-Mamczar, R Polaniak, E Birkner, W Jacheć, B Stawiarska-Pięta. Dept of Biochemistry, Silesian Medical Academy, Zabrze, Poland

Hepatocyte and neuron apoptosis induced by chronic fluorosis in rats

Fluoride-induced apoptosis has not been reported *in vivo* except for some cultured cells. The objective of the present study was to check whether apoptosis can be induced in rats by chronic exposure to fluoride. The experiment was divided into two parts:

(1) *Hepatocyte apoptosis* Adult Wistar rats were kept for 8 weeks on a balanced diet with adequate calcium and drinking water supplemented with sodium fluoride (200 mg F/L). Apoptosis in hepatocytes was examined by flow cytometry (FCM). The percentage of DNA fragmentation (apoptotic peak value) was 13.73 ± 4.05 % in the group treated with fluoride and 0.67 ± 1.11 % in control ($p < 0.001$). A similar result was obtained in rats maintained for two months on a monotonous diet with low calcium and fluoridized water (100 mg F/L), and no preventive effect was seen when calcium was added to the diet.

(2) *Neuron apoptosis* Rats were treated as described above. Brain samples were dissected from the cerebral cortex, hippocampus, and thalamus and neuron apoptosis was measured by FCM. The percentage of DNA fragmentation in each part of the brain was much higher in the fluoride-treated group than in control. For example, in the cerebral cortex the apoptotic peak value was 49.60 ± 0.70 % in the fluoride-treated group and 25.36 ± 0.80 % in control ($p < 0.01$).

These results demonstrate that apoptosis in hepatocytes and neurons can be induced *in vivo* by chronic fluoride poisoning. Our data also suggest that oxidative stress may play some role in the inducement of hepatocyte apoptosis and that ICE (interleukin- 1β converting enzyme, caspase-1) may participate in the regulation of neuron apoptosis induced by fluoride.

Authors: G Li, X Lu, and L Jing. Norman Bethune Univer of Medical Sciences, Changchun China.

Can selenium at trace levels modify the effects of exposure to sodium fluoride?

Interactions between trace elements like selenium and fluorine compounds have led the authors to check whether sodium selenite exerts any influence on some biochemical parameters in serum and bones of rats chronically exposed to sodium fluoride (NaF) per os. The concentration of fluorine in serum was studied with a fluorine electrode. The activities of aspartate (AST) and alanine (ALT) aminotransferases, alkaline phosphatase (ALP), and γ -glutamyltransferase (GGTP), total bilirubin and total protein concentrations were measured using commercially available tests. The content of calcium, magnesium and inorganic phosphate in bones was also evaluated. Statistics were done with the Kruskal-Wallis test.

Sodium selenite was administered at a dose of 5 and 10 $\mu\text{g}/\text{kg}$ body mass/24 h, NaF was at a dose of 20 mg/kg b.m./24 h. Both compounds were mixed with standard chow and administered either separately or jointly during 4 months. The results show that 10 $\mu\text{g}/\text{kg}$ body mass of sodium selenite markedly reduces the level of fluorine in serum without affecting the mineral content of bone. The remaining parameters remained unchanged in intoxicated rats receiving sodium selenite. It is concluded that large doses of sodium selenite do not exert a beneficial effect in chronic intoxication with sodium fluoride.

Authors: M Grzela,¹ A Put,² J Królewski.¹ ¹Chair and Dept. of Pediatric Orthopedics and ²Dept of Toxicology, Pomeranian Academy of Medicine, Szczecin, Poland.

Measurement of bone calcium, phosphorus and fluoride in rat osteoporosis model by x-ray fluorescence analysis

We reported on the measurements of total bone mineral density (BMD) of the mandible, lumbar spine and femur in osteoporotic rats to clarify the unique

characteristics of the mandible. A significant decrease ($p < 0.01$) has been observed in BMD values for the whole lumbar spine and femur as compared to the mandible. No remarkable changes in BMD of the mandible have been observed, thanks to adequate mechanical stimulation during mastication and the maintenance of occlusion.

The present study measured bone calcium (Ca), phosphorus (P) and fluoride (F) in the rat osteoporosis model. X-ray intensities of Ca, P and F in bone (mandible, lumbar spine, femur) were measured by X-ray fluorescence analysis (XRF, Rigaku RIX 3100). Fifty 14-week-old SPF female Wistar rats were used and divided into two equal groups: low-calcium diet (experimental) and normal diet (control). Rats were maintained on a given diet for 8 weeks. Subsequently, the mandible, lumbar spine, and femur were soaked in 10% formaline for one week, dried and crushed. X-ray intensities of Ca, P and F were measured by XRF. Significantly lower X-ray intensities of Ca, P and F ($P < 0.01$) in the lumbar spine and femur were observed in the experimental group. No significant differences in X-ray intensities of Ca, P and F in the mandible were noted between the experimental and control groups.

Authors: N Hashiguchi,¹ K Kono,² M Shimahara¹, J Sennda,¹ Y Tanaka¹, S Komiyama,¹ T Dote,² K Usuda.² ¹Dept of Oral Surgery and ²Dept of Hygiene and Public Health, Osaka Medical College, Takatuki City, Osaka, Japan.

Fluoride prevention of dental caries – current trends

A short presentation of progress in fluoride prevention of dental caries is made. Over the past 55 years, fluoride prevention has greatly contributed to the reduction in dental caries in many countries. The evolution of fluoride prevention programs has brought changes in indications, methods and fluoride preparations. Methods of fluoride prevention and fluoride preparations should be evaluated not only with regard to the effectiveness, safety and cost, but also to expectations and acceptance. Cost analysis is very important because of great differences between general and individual prevention methods, including office procedures increasingly accepted and widespread.

The type of fluoride prevention program has a firm influence on the results of dental epidemiological investigations performed in different countries and regions. A wide range of frequencies and intensities of dental caries, depending on the region, age, and effectiveness of preventive programs, is revealed.

The present results are helpful for the identification of children and youth at risk of caries. Interesting trends to vary the caries prevention program and respond to the degree of caries risk were observed in some countries. These programs are based on an individual approach using fluoride preparations. Relevant examples are discussed.

Author: Z Janczuk. Dept of Conservative Dentistry, Pomeranian Academy of Medicine, Szczecin, Poland.

The influence of quercetin on the activity of cytochrome p-450 system during chronic exposure to NH₄F

The purpose of this study was to check the influence of quercetin, a natural antioxidant, on the activity of the cytochrome P-450 system during exposure to ammonium fluoride. Male Wistar rats were exposed for 6 months to NH₄F aerosol in a toxicological chamber. The concentration of fluorine in air was 2 mg/m³. Six groups of animals were formed: group I-control; group II with quercetin 5 mg/kg body mass/24 h; group III with quercetin 20 mg/kg b.m./24 h; group IV with NH₄F; group V with NH₄F and quercetin 5 mg/kg/24 h; and group VI with NH₄F and quercetin 20 mg/kg/24 h. After six months the animals were sacrificed, the liver was collected and the microsomal fraction was prepared by ultracentrifugation for the determination of cytochrome P-450 content, cytochrome b₅ content, activity of NADPH-cyt. c reductase, activity of NADH-cyt. b₅ reductase, activity of aminopyrine N-demethylase and activity of aniline hydroxylase.

The following changes were observed:

- Reduced activity of NADPH-cyt. c reductase in the exposed group as compared with control (IV/I)
- Reduced activity of NADH-cyt. b₅ reductase in the 20 mg/kg quercetin group as compared with control (III/I)
- Reduced activity of NADH-cyt. b₅ reductase in the exposed group given quercetin 20 mg/kg in comparison to the exposed group without quercetin (VI/IV).

The results do not support a protective role of quercetin on the cytochrome P-450 system during chronic exposure to NH₄F.

Authors: Z Juzyszyn, B Czerny, Z Myśliwiec, A Machoy-Mokrzyńska, A Put. Dept of Toxicology, Pomeranian Academy of Medicine, Szczecin, Poland.

Brick tea fluorosis in China

China is the only country producing brick tea, with more than 90% sold to Chinese minorities. A new form of fluorosis called brick tea fluorosis was discovered in 1986 in the Sichuang province. Research on brick tea fluorosis conducted in China and presented in 24 papers is summarized. Chinese minorities, including Tibetan, Mongolian Uygur, and Kazakh have a 100-year history of brick tea consumption. The consumption of brick tea, urinary fluoride levels and dental fluorosis in children and teenagers have extensively been studied in the Tibetan and Mongol populations. The results indicate that in spite of low urinary fluoride levels, daily intake of fluoride ranged from 3.41 to 6.05 mg, with 90% originating from brick tea. The incidence of dental fluorosis induced by brick tea was 51.2 - 75.3%. According to the dental fluorosis index, three degrees were identified: noticeable, medium and severe. Experimental brick tea

fluorosis in animals confirmed this new form of the disease. High concentrations of fluoride in brick tea are due to the biological nature of tea trees. The tree absorbs fluorine from the soil and stores it in leaves. The longer absorption takes place, the higher is the fluoride content. Brick tea is usually produced from old and poor quality leaves and thus contains tens of times more fluoride than the common green or black tea. The results also offer some interesting ideas for research and prevention of brick tea fluorosis.

Author: C Jin. Tea and Health Laboratory, Hunan Medical Univer, China.

Inter-Institutional error of measurement using fluoride ion-specific electrodes

The purpose of this study was to examine the error in electric potential readings using fluoride ion-specific electrodes. Identical samples were measured at institutions using the same procedure.

Tap water, 6 brands of commercially available mineral water, TISAB-II and F^- (as NaF) stock solution (1 ppm) were distributed to 5 institutional laboratories. Five investigators prepared F^- standards (0.1, 0.2, 0.3, 0.5, 1.0 ppm) from the stock solution, added 10 g TISAB-II per 100 g and measured approximate potentials. Next, the absolute electric potential with 0.1 mV resolution was recorded every 30 sec from maximum to minimum.

The relative potential (mV) was calculated by subtracting the absolute potential (mV) at 1.0 ppm, meaning that the relative potential at 1.0 ppm is 0.0 mV. A straight line through 0.0 mV and the mean relative potential at 0.1 ppm obtained by the five investigators served as the calibration curve (Nernstian plot). The estimates (5 for each aqueous sample and F^- standard from 5 investigators) were re-read on the line.

The readings deviated between 1.5 mV and 0.7 mV. The deviation of the re-read relative mV ranged from 1.0 mV to 0.3 mV, with the coefficient of variance less than 4%, at each concentration level for 7 water samples and F^- standards (0.2, 0.3, 0.5 ppm).

In conclusion, these findings suggest the inter-institutional agreement of measurements, to the extent of the Nernstian response, using the same procedure and compensated as described above.

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China's battle with crippling waters

Ingested fluoride accumulates in bones and ligaments. Children exposed during tooth enamel formation will develop a characteristic mottling of the teeth. The degree of mottling varies considerably from person to person depending on a number of variables including intake of water, calcium, magne-

sium, protein, black or green tea and/or vitamin C. Reduced dental fluorosis would be expected to result in less skeletal fluorosis as the child grows toward middle age.

Researchers from China Medical University (CMU) in Shenyang, China and China Department of Endemic Disease Control (DEDC), identified fluoride in well water as the probable cause of extensive tooth, bone and joint damage to the residents of over 1026 counties in 28 provinces, autonomous regions and municipalities. This amounts to 36% of the total counties in China. There are 25 million patients with dental fluorosis and 1.25 million with skeletal fluorosis from drinking water alone.

In the early 1980's, the DEDC in conjunction with research by the CMU began a program of lowering water fluoride levels. About one half of the villages received reduced fluoride water. The cost of correcting the problems of fluoride contaminated drinking water has inhibited the complete correction of this problem. This tragic situation has provided an unusual opportunity for research into the physical and economic changes that the low fluoride water produces. The success of the program in improving the quality of life and economic productivity and reducing dental fluorosis has been reported in a number of papers.

When the water fluoride level was reduced to 0.5 ppm, fewer than 10% of the children now have dental fluorosis of the mildest kind. With water fluoride levels of 0.9-1.0 ppm research shows 37.8% of the life-long resident children have dental fluorosis with <1% severe. With water fluoride levels from 4 to 11 ppm, 100% of the examined children had dental fluorosis with >70% moderate and severe. This video documents the dramatic changes in physical well-being and movement of skeletal fluorosis victims and reduction in dental fluorosis with improved water supplies. (A video documentary).

Authors: D Kennedy, B Kennedy, G Dai, G Sun, C Qian, and H Gao.

The influence of fluoride compounds on the growth rate of children residing near an aluminum plant

Several large aluminum processing plants which contribute substantially to industrial pollution of the environment are located in the Ural region. Among the main environmental pollutants in aluminum production waste are hydrogen fluoride and solid fluorides.

An epidemiological study was conducted in two cities: Kamensk-Uralsky and Krasnoturiinsk, where the aluminum plants are located. The purpose was to analyze the intensity and nature of industrial pollution of the environment and its impact on some parameters of physical development in children aged 0-7 years.

A study cohort of 1994 children was formed using the method of multiseried choice. The main parameters of physical development (height and weight) were compared with standard growth curves for children in the Ural region. Three

groups of children were formed: with low, average, and high degree of physical development, and a discriminant multifactor analysis with application of pattern recognition methods was conducted. Discrimination was made based on the contribution of many factors reflecting not only the level of industrial pollution of the environment at the place of residence, but also medicobiological history, family life-style, and conditions during preschool period.

These studies showed that body height and weight tended towards lower values in comparison with standard growth curves. The younger the child, the greater the difference was.

The results of multifactor analysis suggest that industrial pollution plays a significant role in the individual's physical development rates. Depending on the age group, the effect of factors reflecting industrial pollution was from 10 to 40% of the total and was sufficient for reliable discrimination of children with low, average and high rates of physical development. By strength of influence they rank second to factors of medicobiological history (60%) and compete with family factors (30%).

Along with a direct effect of industrial pollution on the child, proof of its indirect influence through parents was found. Migration of parents within 10 years after the child's conception, from ecologically "safe" regions to areas near aluminum-processing plants, increases the probability of slower physical development of their children.

Authors: N Kocheva, L Popova, G Nasybullina, E Polzik. Ural State Medical Academy, Ekaterinburg, Russia.

Background versus reality of the crucial publication pertaining to the "success" of fluoridation in the drinking water of Basel, Switzerland

This is a piece of Science History which I want to show on the basis of the fundamentals (auf der Basis des kleinen Einmaleins, 1x1) and of common sense instead of mathematical statistical methods – reality versus presentation in the crucial publication on the "stupendous success" of the fluoridation of Basel's drinking water.

At the end of the fifties, the Kanton Basel-Stadt Parliament, decided on potable water fluoridation. Even though the project was greeted with enthusiasm (critics were few and outspoken), no statistically useful data were available.

My contribution represents an elementary analysis of the manuscript by Dr. med.dent. Max Gutherz (then Director of the School's Dental Clinic):

“Sozialmedizinische Aspekte der Trinkwasserfluoridierung – Ihre Auswirkungen nach 5 jährigem Bestehen auf das Gebiss des Kleinkindes und der Kinder der 1. Primarstufe im Kanton Basel-Stadt.” (Schweizerische Monatsschrift für Zahnheilkunde 1967;77:492-514).

In the eyes of European dentists, the publication of Gutherz has been, and still is, an “overwhelming proof” of the benefits of fluoridation of drinking water in the Kanton Basel-Stadt and two Communities in the Kanton Basel-Land (with around 250 000 inhabitants).

At the start of his investigation, Gutherz had an extraordinary collection of data on the state of dental health and tooth decay from thousands of school children in the region over several decades. What did he do with it when fluoridation was introduced?

The purpose of this presentation is to make the arithmetical operations visible in the original (documentary) paper of Gutherz, which led him to the commonly accepted famous story of success. No better results have ever since been presented in Switzerland.

*Aspects of social medicine in drinking water fluoridation - its consequences (results) after 5 years on the teeth of infants (kindergarten) and children of the first class in primary schools of Kanton Basel-Stadt.

Author: K Kreuzer. Forum für verantwortbare Anwendung der Wissenschaft, Basel/Flüh/Schweiz Forum for the Responsible Application of Science.

Environmental and occupational exposure to fluoride in the Gdańsk region

The purpose of this study was to evaluate the degree of environmental and occupational exposure of inhabitants to fluoride in the Gdańsk region.

The fluoride intake of the general population is chiefly from drinking water and diet. The mean water fluoride level in most of the communities in the Gdańsk region is 0.40 mg/L, except for Wiślinka, a community located near a phosphate plant waste disposal site, having 1.53 mg/L, and the Żuławy area with a high fluoride level in the soil, having 2.07 mg/L.

The water fluoride level correlated well with fluoride levels in urine and hair of the inhabitants, irrespective of natural or industrial origin. A significant difference in the fluoride levels in urine was ascertained between children residing in the central and eastern districts of Gdańsk. The post-mortem level of fluoride in the rib bone and hair of residents in the Gdańsk region was 625 µg/g and 1.35 µg/g, respectively. The results of these studies are in agreement with moderate exposure to fluoride in this area.

The main sources of occupational exposure to fluoride in the Gdańsk region are: (1) production of phosphate fertilizers, (2) transport of phosphorites, and (3) welding, especially in shipyards.

The highest exposure in a fertilizer plant was at workplaces where superphosphate, fluorosilicate, and phosphoric acid were produced. In 1989 the fluoride concentration in urine, hair, and nails of workers was significantly elevated and was exceeded in all workers producing fluorosilicate. Six years later the exposure was markedly lower.

Workers employed in transport and loading of phosphorites were exposed to high levels of dust containing fluoride. The mean urinary fluoride level in this group was significantly higher than in controls.

Fluorine compounds are used as fluxes in automatic submerged arc welding and in the coating of alkaline electrodes in manual welding. Fluoride levels in blood and urine of welders were significantly elevated but levels in urine were exceeded in only 46% of operators of automatic welding machines and 6 % of manual welders.

A decline in the occupational exposure to fluorine compounds in the Gdańsk region has occurred in the last decade.

Author: J Krechniak. Dept of Toxicology, Medical Univer of Gdańsk, Poland.

The impact of fluoride on bone mineral density in rats

The purpose of this study was to assess whether a correlation exists between bone fluoride and bone mineral density (BMD). Also the impact of fluoride on different bone markers: activity of alkaline phosphatase in blood, level of hydroxyproline in urine, and concentration of calcium and magnesium in blood were investigated. The study was performed on male Wistar rats. The animals were divided into three groups: 1) controls, 2) rats that inhaled hydrogen fluoride, and 3) rats given 20 ppm F⁻ in drinking water.

The animals were exposed for 6 months. Rats from group 2 were placed in whole body exposure chambers and exposed daily for 2 hrs to hydrogen fluoride. The mean concentration of fluoride in air was 8.7 µg/L. Fluoride concentrations were measured by a fluoride specific electrode. Bone mineral density was measured with a Tomoscan LX densitometer.

After the exposure period a significant increase in bone fluoride in both groups of rats exposed to fluorine compounds was ascertained. However, no differences in BMD were found between exposed and control animals. The activity of alkaline phosphatase and concentration of calcium in blood as well as the level of hydroxyproline in urine of exposed animals decreased during the exposure period, whereas the concentration of magnesium in blood increased during the course of the experiment.

Authors: B Urbańska,¹ W Czarnowski,¹ A Muraszko-Klaudel.² ¹Dept of Toxicology and ²Dept of Radiology, Medical Univer of Gdańsk, Poland.

Hair as an index of exposure to fluorine compounds

The purpose of this study was to investigate the impact of exposure to fluorine compounds on fluoride accumulation in hair. The study was performed on male Wistar rats. The animals were divided into three groups: (1) controls, (2) rats that inhaled hydrogen fluoride, and (3) rats that were given 20 ppm F⁻ in drinking water.

The animals were exposed for 6 months. Rats from Group 2 were placed in whole-body exposure chambers and exposed to hydrogen fluoride daily for 2 hrs. The mean concentration of fluoride in air was 8.7 µg/L. Every month, hair was collected from the dorsal part of the animals. One part of each sample of hair was rinsed with acetone, detergent, 2 N sulfuric acid, and redistilled water. Another part of hair was analyzed without washing. Fluoride concentrations were measured by a fluoride specific electrode.

The fluoride content in the hair of animals which were exposed to hydrogen fluoride was several times higher than in rats that received sodium fluoride orally. About 95 % of the fluoride was washed out from the hair of animals that inhaled hydrogen fluoride, and about 40 % from the hair of animals given sodium fluoride in drinking water.

The fluoride content in bone and urine of the exposed animals was also determined. The highest urinary fluoride was in rats exposed to hydrogen fluoride. The concentration of fluoride in bone was significantly higher in both groups of exposed animals than in controls. A significant positive correlation was found between the fluoride concentration in bone and hair of exposed animals.

Authors: K Stolarska, W Czarnowski. Department of Toxicology, Medical University of Gdańsk, Poland.

The role of ¹⁸FDG-PET in the management of oral squamous cell carcinoma

World-wide, oral and pharyngeal squamous cell carcinoma accounts for an estimated annual incidence of some 575 000 tumors, representing the majority of head and neck neoplasms. We studied the value of ¹⁸FDG for initial staging and follow-up in this form of oral cancer.

Fifty one patients were pretherapeutically staged for cervical lymph node involvement and underwent a total of 73 PET examinations for screening and staging with respect to recurrence. All PET results were correlated to define disease status as revealed by histological specimens or clinical follow-up for at least 12 months.

Of the 102 neck sides, 89 were correctly staged as N₊ or N₀, seven were false positive and seven were false negative, resulting in a sensitivity of 76% and specificity of 90%. Histological findings and follow-up showed 54 sites of local recurrence, metachronous secondary tumors, secondary lymph node involvement or distant metastases. 46 of them were identified by FDG-PET imaging, resulting in an overall sensitivity of 85%. Metabolic detection of recurrence or metastasis preceded morphological detection by up to 6 months. However, a substantial proportion, particularly in the cervicofacial region, were false positive sites.

Although PET revealed some metastases as small as 5 mm, we could not safely rely on detection of "micro"-metastases and, therefore, the major clinical

problem of occult cervical metastases remains unresolved. The high specificity of PET, however, may justify an aggressive therapeutic approach in spite of operative risk, as long as therapeutical objectives are met. ^{18}F FDG-PET can be claimed as a very interesting diagnostic tool for the follow-up of oral cancer patients. Early detection of recurrence and identification of additional incurable tumor sites contributes substantially to rational therapeutic management. On these grounds, we recommend ^{18}F FDG-PET for screening and re-staging of recurrent oral cancer.

Authors: M Kunkel,¹ U Wahlmann,¹ P Benz,² GJ Förster,³ J Spitz,² W Wagner.¹ ¹Dept of Maxillofacial Surgery, ²PET Unit, and ³ Dept of Nuclear Med, Univ of Mainz, Germany.

Fluorine accumulation in some fruit trees during their vegetation period and its influence on chlorophyll content

Fluorine (as F^-) and chlorophyll a and b concentrations were determined in leaves of the following fruit trees: apple (*Malus domestica*), plum (*Prunus domestica*), and cherry (*Prunus cerasus*). Samples were collected after 3, 4 and 5 vegetation months (in July, August, and September) from thirty-year-old fruit trees of domestic cultivation in Strzemieszyce, located 6 km from the "Katowice" Steel Works, and from trees in the city of Nowy Sącz (with relatively low fluoride pollution), 200 km away. The concentration of F^- ions was measured with a potentiometer and a fluoride-selective electrode (Orion, USA). Chlorophyll a and b concentrations were assayed in acetone extracts from fresh leaves, using a spectrophotometer (Secomam s. 750, France) at three wavelengths: 645, 652 and 663 nm. The fluorine concentration in leaves depended on the vegetation time, the highest found in plum leaves from Strzemieszyce. The F^- content in leaves from Strzemieszyce and Nowy Sącz ranged from 20 mg to 51 mg F^-/kg dry weight.

Besides higher fluorine levels during the vegetation period, changes were observed in chlorophyll a and b concentration. Comparing plum leaves from Strzemieszyce and Nowy Sącz, higher fluorine levels were accompanied by lower total chlorophyll concentrations. The results demonstrate that environmental contamination with fluorine compounds may lead to suppression of chlorophyll synthesis in plants during their vegetation period.

Authors: Z Kusa, J Sochacka, K Pawłowska-Góral, K Bober. Dept of General and Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Med, Sosnowiec, Poland.

Fluoride level in enamel and saliva in teenagers representing different caries risk groups

The aim of the study was to measure the fluoride level in enamel and saliva in 12-year-old children representing low, medium and high caries risk groups, and repeat the measurements in the same children two years later.

Caries risk in 42 randomly chosen children was diagnosed based on DMF index and salivary microbiological tests. Microsamples of enamel were taken from upper right central incisors. Biopsy with 0.5 M HClO₄ was performed by the same examiner on every occasion. Samples of saliva were collected 1 h after breakfast. Measurements were done using an ion-selective fluoride electrode (Orion Research Inc.).

The fluoride level in enamel of 12-year-old children was similar in all groups (99.6 to 110.2 mmol F/kg). A decrease was noted two years later but the difference was statistically significant only in the high-risk group. The fluoride level in saliva was similar in all groups (2.63 to 3.07 µmol/L). It increased during two years and the difference was statistically significant in the medium and high-risk groups.

Authors: K Lisiecka, Z Janczuk, A Suszczewicz, K Opalko. Dept of Pediatric Dentistry, Pomeranian Academy of Medicine, Szczecin, Poland.

The effects of fluoride on collagen in rat bone

Collagen represents the bulk of bone tissue, accounting for 90% of organic matrix. In order to reveal the effects of fluoride on different levels of collagen metabolism, collagen content, collagen distribution, collagen fiber crosslinking, and collagen mRNA level were studied in rats. NaF was given in drinking water at a concentration of 100 ppm. Rats were sacrificed after 0.5, 1, and 2 months. The ratio of organic to inorganic compounds and the content of osteocalcin in bone revealed no significant change after 0.5 and 1 month. After 2 months, the ratio increased ($p < 0.05$) and osteocalcin content decreased ($p < 0.05$) in the fluoride group as compared with control.

Collagen fiber distributions was studied using Picrosirius polarization technique. Collagen was analyzed by SDS-PAGE electrophoresis to assess the degree of crosslinking. Type I and Type II collagen cDNA probes were prepared and collagen mRNA levels in bone and cartilage were measured using *in situ* hybridization. Changes in collagen protein and mRNA indicate that fluoride affects collagen metabolism and consequently interferes with bone formation.

Authors: BC Liu, Q Miao, M Xu, XD Wu, BH Yuan, BR You. Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing, China.

***In vitro* and *in vivo* studies on the potential toxicity of sodium fluoride to human hematopoiesis**

The influence of fluoride on human hematopoiesis has not been addressed sufficiently in the literature. However, environmental pollution with fluorine compounds has already been associated with increasing morbidity due to hematological diseases. The relationship between fluoride and human hematopoiesis is of interest for several reasons, including insight into the potential

toxicity of fluoride to normal hematopoietic cell growth, viability, differentiation and apoptosis. With these issues in mind, we analyzed the influence of sodium fluoride on human hematopoiesis using a variety of techniques. We also evaluated the influence of NaF on the morphology of several organs in a murine model.

Human marrow hematopoietic cells were exposed *in vivo* to different doses of NaF at 37°C and 4°C for 30 and 120 min. and subsequently examined for the effect on clonogenicity of human CFU-GM (Colony Forming Unit of Granulocytes-Macrophages) and BFU-E (Burst Forming Unit of Erythrocytes). Relatively high concentrations of sodium fluoride (10 and 50 mg/L) were toxic to marrow myeloid (CFU-GM) and erythroid (BFU-E) progenitors. Inhibition of colony growth was more evident after incubation at 37°C than 4°C. A similar effect was observed when viability of the cells was checked with the trypan blue exclusion test. Phases of apoptosis were studied in bone marrow (BM) and cord blood (CB) hematopoietic cells (early phase with Annexin V test and FACS, executive phase with ELISA). Both phases of apoptosis were detectable in CB and BM hematopoietic cells exposed to sodium fluoride. Finally, we injected normal mice with increasing doses of NaF and searched for any morphological changes in the liver, spleen, kidneys, lungs and bones. Sodium fluoride-induced morphological changes in the spleen and lungs. In contrast, the liver, kidneys and bones appear to be relatively resistant to this substance.

The present results suggest that sodium fluoride is potentially harmful to human cells involved in hematopoiesis.

Authors: B Machaliński, V Dziedziejko, M Marchlewicz, W Marlicz, I Stecewicz, L Wenda-Różewicka, ZT Machoy. Dept of General Pathology, Pomeranian Academy of Medicine, Szczecin, Poland.

Effects of sodium fluoride ingestion on some serum parameters and bone tissue components in growing rats

The influence of sodium fluoride in drinking water on fluoride, calcium, magnesium, bilirubin, urea and creatinine concentrations, aspartate aminotransferase (AspAT), alanine aminotransferase (AlAT) and cholinesterase (ChE) activities in serum of growing rats was studied. Additionally, fluoride, calcium and magnesium concentrations in bone (femur) were assayed.

Forty 6-week-old female Wistar rats were randomised into four equal groups. One group served as control receiving distilled water *ad libitum* and the other three received water containing NaF at levels of 8, 30 and 60 mg F⁻/L.

After six weeks of the experiment the group receiving 60 mg F⁻/L showed the highest bilirubin, urea and creatinine concentrations, and lowest ChE activity in serum.

A significant and dose-dependent increase in serum and bone fluoride was found, more pronounced in trabecular than cortical bone. Fluoride intake at 30

and 60 mg F⁻/L in drinking water was associated with significant increases in calcium and magnesium concentrations in cortical bone, compared with the control group.

Authors: A Machoy-Mokrzyńska,¹ A Bohatyrewicz,² P Białecki,² M Kędziński.²

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Effect of sodium fluoride on bone marrow transplant engraftment - in vivo studies

Our previous studies have suggested that NaF might be toxic to early human cord blood and bone marrow hematopoietic cells. Extending our preliminary report, we performed additional experiments to evaluate whether hematopoietic cells treated with sodium fluoride in *ex vivo* conditions are able to engraft into marrow cavities of lethally irradiated mice and successfully reconstitute hematopoiesis. Bone marrow was aspirated from freshly decapitated Balb C mice, the adherent depleted mononuclear cell (A⁻MNC) fraction was isolated and subsequently incubated with different doses of sodium fluoride for 12 h at 37°C. Other groups of mice were exposed to 800 cGy gamma irradiation source 24 h prior to transplantation. Cells exposed to NaF *in vivo* were evaluated for their ability to engraft in the irradiated mice. Untreated cells and cells exposed to a low (1 mg/L) concentration of NaF established a significant number of new hematopoietic islets on the surface of spleen. In contrast, much fewer hematopoietic islets were found when cells were exposed to higher levels of sodium fluoride (10, 50 mg/L). Hematology values in peripheral blood were in line with this finding. We conclude that relatively high doses of fluoride may significantly affect hematopoiesis.

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Effects of diet and fluoride on the development of dental cells and tissues in rats

Particular phases of tooth-bud growth and maturation and apposition of dentin and enamel, are stimulated by exogenous factors like diet and fluoride.

The purpose of the study was to assess stereologically the volume fractions of dental cells and tissues in the first molar in the mandible of 14-day old rats. In the first control group, pregnant rats (mothers) and their offspring were fed with a standard diet and given water with 0.16 mg F/L. In the second, third, and fourth group, pregnant rats from the 13th day of pregnancy and then their offspring were fed with Keyes Experimental Diet 2000 which was deficient in

proteins, lipids, carbohydrates. The second group drank water without fluoride while the third and fourth groups were given water with 10 mg NaF/L and 110 mg NaF/L respectively. Of these offspring, four groups of 14 day old Wistar male rats were used (20 animals, 5 per group)

Under ether anaesthesia, the 14-day old male rats were decapitated. Mandibles were decalcified, fixed, dehydrated and embedded in paraffin. Serial 6 µm thick sections were cut in the frontal and sagittal plane and stained with haematoxylin and Bosin as well as with azan after Heidenhain. Sections were inspected under a microscope Pictoval Carl Zeiss Jena.

The volume fraction of the dental components were estimated stereologically using point-counting method based on randomly selected sections. The microscopic pictures were projected on the sheet of paper and profiles of dental components were drawn around. Along with the estimation of the volume fraction, additionally the coefficient of error of the estimate was calculated. The volume fraction of the enamel was the largest in the group fed a deficient diet and water without fluoride, smaller in the rest of the groups and the smallest in the control group. The difference was statistically significant.

The biggest volume of the dentin was in the group fed with the standard diet and watered with 0.16 F/L, the smallest in the group fed with a deficient diet and given water without fluoride. The difference was statistically significant. The difference of the volume fraction of ameloblasts was statistically significant between the group fed with a deficient diet and given water with 110 mg NaF/L and all remaining groups.

Sodium fluoride dissolved in drinking water during pre- and postnatal life modified odontogenesis in the rats. The deficient diet and high doses of sodium fluoride in drinking water applied simultaneously during prenatal and postnatal life significantly restricted the apposition of the dentin. Stereological estimation gives objective results of dentin and enamel apposition.

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Fluoride and/or aluminium toxicity in liver and gastrocnemius muscle of male mice and its amelioration by some antidotes

In the present study adult male albino mice (*Mus musculus*) were treated with sodium fluoride (NaF) (10 mg/kg body weight) or aluminium chloride (AlCl₃) (200 mg/kg body weight) alone or in combination for 30 days to investigate their effects on nucleic acid metabolism in liver and gastrocnemius muscle. Treatment caused a significant decline in the levels of protein, DNA and RNA, reflecting alterations in nucleic acid and protein metabolism in these organs. The DNA/RNA and RNA/protein ratios in liver and gastrocnemius muscle were altered as well, indicating disturbances in the process of transcription and translation caused by NaF, AlCl₃ and NaF+AlCl₃. Withdrawal of the com-

bined treatments resulted in partial recovery. However, administration of ascorbic acid (AA), calcium (Ca^{2+}), or vitamin E (vit. E) alone or in combination during the withdrawal period, helped in maintaining the *status quo* of all parameters in liver and muscle at almost control levels. The results reveal that the induced effects were reversed by combined, synergistic/additive action of the antidotes.

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The influence of Chrysin on some biochemical parameters in serum of rats subchronically exposed to sodium fluoride

Fluorine is known for its strong electronegativity which facilitates interactions of this element with biologically active substances. Chrysin is a flavonoid compound from maracua (*Passiflora incarnata*) capable of forming complexes with metals and exhibiting antioxidant action. Flavonoids appear to exert a beneficial effect in chronic exposure to industrial toxins, including fluorine compounds.

The purpose of this work was to study the influence of chrysin on some biochemical parameters in serum of rats intoxicated orally with 100 mg/dm³ solution of NaF in water. 60 male Wistar rats were divided into 6 equal groups: group I-control on standard chow and water *ad libitum*; group II with chrysin in globules 10 mg/kg body mass/24 h; group III with chrysin 20 mg/kg b.m./24 h; group IV with NaF; group V with NaF and chrysin 10 mg/kg/24 h; and group VI with NaF and chrysin 20 mg/kg/24 h. After 3 months blood was obtained and the activities of aspartate aminotransferase (AspAT), alanine aminotransferase (AlAT), cholinesterase (ChE), alkaline phosphatase (AP), and bilirubin concentration in serum were measured. Intoxicated animals had higher levels of AspAT, AlAT and ChE. This increase was suppressed by chrysin. AP activity was reduced in intoxicated animals. The administration of chrysin in intoxicated animals increased the activity of AP. The present results support the supplementation of the diet with chrysin as a preventive measure in exposure to fluorine compounds.

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Effects of continuous intravenous administration of sodium fluoride on rat kidney

Many reports have appeared on experimental administration of fluoride (F) using oral, intraperitoneal or single intravenous injection routes. As F is rapidly excreted through the kidneys, it is difficult in this way to study the effects of long-term exposure to high plasma levels of F. We have used continuous intra-

venous infusion to study the influence of F on renal function and nephrotoxicity in 3 groups of male Wistar rats. Sodium fluoride solution (3 ml/h for 6 h, 18 or 36 mg F/kg body mass) was administered through jugular veins with an infusion pump. Urine samples were collected from the bladder every 2 hours.

Urinary parameters studied included volume, excretion of F, creatinine (Cr), N-acetyl-beta-glucosaminidase (NAG), and alpha-glutathione-S-transferase (GST). Reduced urinary volume and Cr excretion were observed in the 36 mg/kg group. When the high and low F groups were compared, F excretion was not found to be dose-related. GST showed remarkably elevated values in the high-dose group. The NAG/Cr ratio was significantly higher in this group.

The early stage of continuous exposure to high F levels was marked by severe nephrotoxicity seen in both glomeruli and tubules. During this period of our study, GST was a more sensitive indicator of acute proximal tubular damage than NAG. Because F elimination depends mainly on kidney function, acute renal failure would further contribute to accumulation of F. A sensitive marker for the early diagnosis of F poisoning is needed in addition to the monitoring of urinary F levels.

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Oxidation homeostasis in hepatocytes exposed to fluoride ions in the presence of Fe³⁺ ions

Reports have indicated that fluoride ions may indirectly disturb cellular oxidation homeostasis. We decided to investigate whether this will be reflected in changes of the activities of some basic anti-oxidation enzymes of the cell, like superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR), when hepatocytes are exposed to iron(III) and/or fluoride ions in the incubation medium.

Experiments were conducted in hepatocytes isolated from livers of 3-month-old Wistar rats using enzyme digestion. After 30 and 120 minutes of incubation with Fe³⁺ ions at 25 or 75 µmol/dm³, fluoride ions were added at 6 mmol/dm³ and incubation continued for 30 and 120 minutes. In parallel, hepatocytes were incubated for 30, 60, 120 and 240 minutes exclusively with Fe³⁺ ions, or in a medium without any additives (control). Next, the cells were centrifuged, mechanically homogenised and used to determine the activity of oxidation enzymes and protein content.

A significant loss of SOD activity in hepatocytes in the presence of Fe³⁺ ions, proportional to incubation time, was observed. Higher Fe³⁺ concentration was accompanied by greater inhibition of SOD activity. This effect may be explained by interference of Fe³⁺ ions with the Haber-Weiss reaction. Exposure of hepatocytes to fluoride ions did not affect SOD activity after 30 min. incubation, but significantly reduced the activity after 240 min. Incubation of

hepatocytes first with Fe^{3+} , next with fluoride ions, resulted in a slight increase in SOD activity, as compared with hepatocytes exposed to iron ions only. This effect is probably due to complexing of Fe^{3+} with fluoride ions.

In the light of these findings it is difficult to explain the observed changes in glutathione peroxidase (GPx) and glutathione reductase activities caused by Fe^{3+} ions, considering the concerted action of both enzymes. Glutathione disulphide produced with the participation of GPx is a substrate for GR, and in turn GR generates reduced glutathione, which is one of the substrates for GPx. Some authors believe that despite this co-operation, both enzyme activities are not absolutely correlated.

The results of our study support this possibility and point to separate mechanisms by which iron and fluoride ions interfere with the activity of these enzymes. Presumably, the effects are not direct as in the case of SOD. Changes in the activity of cellular anti-oxidation enzymes observed in our study indicate that Fe^{3+} and fluoride ions interfere with oxidation reactions in the hepatocyte and in consequence disturb the oxidation homeostasis of the whole organ.

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Genetic and non-genetic risk factors of occupational fluorosis in workers of aluminium and cryolite plants

The purpose of this study was to evaluate a large set of risk factors influencing the development of occupational fluorosis and to elucidate the prominent role of the human phenotype and its peculiarities. The results of two investigations, carried out in workers of cryolite and aluminum plants, are presented. Comparison of the most informative sub-complexes of dermatoglyphic features (finger and palm prints) from each investigation confirmed that they were identical. A significant coincidence was also found when the type of relationship of each dermatoglyphic feature with fluorosis was studied. Reproducibility of the results of both investigations allows one to consider dermatoglyphics as a reliable marker of genetic predisposition to occupational fluorosis.

Comparison of the results of two multifactor studies has proved that individual predisposition to occupational fluorosis depends on a large number of conditions and is predictable with a high degree of certainty on the basis of a complex of factors. The role of genetic predisposition is quite prominent in respect to other factors, but without a combination of unfavorable environmental factors, it is in fact insufficient to determine the fate of the individual.

As appears from the Table, an analysis of various risk factors proves their significant coincidence in the development of fluorosis in "aluminum" and "cryolite" workers. If we take the influence of all 15 factors in these industries on the development of fluorosis as 100%, the impact of genetic predisposition

will be 26-30%, fluoride exposure 35-37%, social factors 26%, medicobiological factors 9-10%.

Table. Influence of various risk factors on predisposition to occupational fluorosis

| Factor | Aluminum production | | Cryolite production | |
|--------------------------------------|---------------------|------|---------------------|------|
| | Informative- | Rank | Informative- | Rank |
| Genetic predisposition | 1.00 | 1 | 1.00 | 1 |
| Personal life-style | 0.65 | 2 | 0.25 | 5 |
| Residence in fluoride polluted areas | 0.58 | 3 | 0.26 | 4 |
| Occupational exposure to fluoride | 0.48 | 4 | 0.64 | 2 |
| Occupation | 0.32 | 5 | 0.12 | 6 |
| Smoking | 0.16 | 6 | 0.02 | 13 |
| Nationality | 0.14 | 7 | 0.07 | 10 |
| History of locomotor disease | 0.12 | 8 | 0.08 | 9 |
| Housing conditions | 0.10 | 9 | 0.59 | 3 |
| Alcohol abuse | 0.09 | 10 | 0.02 | 12 |
| History of liver disease | 0.08 | 11 | 0.001 | 15 |
| History of kidney disease | 0.06 | 12 | 0.004 | 14 |
| Continuity of exposure to fluoride | 0.02 | 13 | 0.12 | 7 |
| History of endocrine disease | 0.006 | 14 | 0.05 | 11 |
| Age at onset of exposure | 0.002 | 15 | 0.1 | 8 |

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Effect of fluoride on superoxide dismutase (SOD) activity and GSH levels in the earthworm *Eisenia fetida*

The earthworm is widely known for its ecological importance due to its role in the soil and decomposer community. However, little information is available concerning the impact of fluoride (F) on the organism. The purpose of this study was to investigate the influence of NaF on the activity of superoxide dismutase and the concentrations of GSH in the earthworm (*Eisenia fetida*).

E. Fetida were exposed to sublethal concentrations of NaF at 0.1, 1.0 and 5.0 mM respectively, for 24, 48, and 72h under laboratory conditions. A filter paper contact test method was used to achieve dermal exposure and at the end of each experimental period, crude extracts were prepared from the whole tissue for determination of both SOD activity and GSH concentrations. The SOD of *E. fetida* was found to be of the Cu-ZN form. Exposure to NaF inhibited SOD activity and increased GSH concentrations in NaF concentration-dependent manner. The inhibition of SOD by NaF is hypothesized to involve a competitive inhibition of the enzyme through binding to the active site, whereas the increase in GSH concentrations is attributed in part to decreases in H₂O₂ levels caused by SOD inhibition.

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Fluoride diffusion in alluvial soil: dependence on some crucial factors

Diffusion of ions in soil is an important phenomenon governing the uptake of F by plants. In the present study the effects of soil water content (θ) varying from 0.10 to 0.25 $\text{cm}^3\text{cm}^{-3}$, soil pH (\approx 4-8), temperature (288-318K) and incubation time (96-3600 h) on diffusive mobility of F in alluvial soil were studied. Experiments were performed by joining two open ends of F admixed soil core (source section: length 5 cm, diameter 2.15 cm) with native soil cores (soil section: 6 cm, diameter 2.15 cm). Proper sealing was ascertained to avoid any moisture loss. After incubation, water-soluble F was measured in each 1 cm segment of this system. Employing the simple mass balance principle, the values for porous diffusion coefficient (D_p) were computed. The results indicate that diffusive mobility of F increases with increase in soil water content and decrease in soil pH. F mobility was minimal at 303K and increased with rising and falling temperature. The rate of F diffusion across the source-soil junction was initially high and decreased exponentially. Plausible mechanisms have been suggested to explain the observed variation in F mobility.

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Influence of fluoride on growth and pigment content of three plant species tested in liquid culture

The purpose of this study was to assess the influence of two doses of NaF on growth, fluoride accumulation and photosynthetic pigment content of pea cv. Kaliski, bean cv. Wiejska, and maize cv. Tandem. Plants were cultivated in 250 mL plastic containers using Hoagland's medium intermittently aerated by means of an aquarium pump.

Initially, the seeds germinated on filter paper moistened with distilled water only. From the 2nd to 4th week the seedlings were exposed to NaF at 2 and 33 mg F^- per litre in Hoagland's medium. A control experiment (without NaF) was run in parallel. Subsequently, the length and fresh weight of shoots and roots was measured.

For fluoride determinations, dried samples were digested with CaO at 400-800°C and the ash was distilled with 2N HClO_4 at 165°C. Fluoride concentration in acid distillates (pH 5.5) was measured with an ion-selective electrode. Leaves were homogenized in 80% acetone, centrifuged at 7800 x g for 3 min., and the content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids was measured spectrophotometrically at 440, 645 and 663 nm using 80% acetone as reference. Quantities of particular pigments were calculated according to appropriate formulas.

The results show that fluoride did not exert any statistically significant influence on bean shoot growth. However, root length was significantly reduced in

the presence of 33 ppm F⁻ in the medium. Significant reduction of pea shoot and root length was observed at 33 ppm F⁻. Maize shoot length was greatest at 2 ppm F⁻ in the medium, while no significant differences in root length were observed. Fresh weight of bean shoots was significantly greater in the presence of fluoride (either dose) than in controls. The reaction of bean roots was different. Exposure to 33 ppm F⁻ caused a significant decrease in fresh weight of roots, while 2 ppm F⁻ was without effect. Shoots and roots of pea weighed most at 2 ppm F⁻ and least at 33 ppm F⁻. Maize shoots and roots had the greatest fresh weight in the control experiment. The reduction in fresh weight was very considerable at 33 ppm F⁻ in the medium.

The present findings indicate that the biological response to fluoride is genetically determined. All tested species showed a similar tendency to accumulate fluoride when grown at 2 ppm F⁻ *i.e.* 285 - 235 % of control values. At 33 ppm F⁻, the greatest tendency to accumulate fluoride was in the pea (757 %), followed by maize (532 %), and bean (312%). A statistically significant influence of fluoride on chlorophyll a, chlorophyll b, and total chlorophyll content was observed only in maize, with the greatest content at 2 ppm F⁻ in the medium. NaF concentrations used had no influence on carotenoid content. Our results may help to plan crop structure in fluoride polluted regions.

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Quantity of bone measured with Microfocus X-ray television system in the rat osteoporosis model

With the recent aging of society, elderly patients with osteoporosis place an increasing demand on orthopedic surgery. Although weak external force is considered to cause bone fracture in these patients, an increased incidence of jaw fracture has not been reported in oral surgery. Furthermore, we have rarely observed osteoporosis in the mandible after motoric restriction by intermaxillary fixation in the rat osteoporosis model.

In the present study, we induced osteoporosis in 14-week-old female Wistar rats with a low calcium diet (3.26 mg F/g) given for 12 weeks. Quantity of bone and density distribution on X-ray photographs of the mandible, lumbar spine and femur of normal (control group) and osteoporotic rats (experimental group) were measured using HITEX MFX-130H made by HITEX and Sigma-III made by Japan Avionix.

A difference in the density distribution in lumbar spine and femur was observed between the experimental and control groups ($p < 0.01$). In the case of the mandible, no difference could be observed after 12 weeks of the experiment.

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Aging and health of the oral cavity

Life styles that characterize our society do play a role in the health and/or aging of the oral cavity, but to what extent? This problem, specifically in terms of prevention of dental caries, remains unresolved by opponents and proponents of fluorine and fluorine-based compounds. The stabilizing action of fluoride on the enamel mineral architecture has long been known, but the stability of dentin structure is closely linked to additional factors, such as the state of periodontium. In addition, the role of fluorine in stabilization of the crystal lattice of the mineral part of dentin is still unclear.

Decay at the level of tooth necks and roots is a serious problem, especially in elderly individuals, in whom periodontal retraction uncovers the boundary between dentin and enamel, exposing dentin on the tooth's surface. Hence, the use by the elderly of compounds that can reinforce the mineral structure of the teeth to limit or prevent the onset of caries seems important.

A scanning image-analysis method was used in this study to assess morphological changes brought about by fluoride removal with perchloric acid (0.5 or 1M for a few seconds) and the reversibility of this process. Our SEM data confirm the accuracy of this procedure.

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Long-term (1977-1998) changes in fluorine deposition on sylvan and agricultural areas caused by emissions from the "Police" chemical plant

The main aim of the study was to determine the deposition of fluorine by bulk precipitation between 1977 and 1998 on sylvan and agricultural areas, caused by emissions from "Police" Chemical Plant and to evaluate time changes in F deposition. The terrestrial ecosystem in the Szczecin region most susceptible to industrial pollution is the coniferous forest on highly acidic sandy soils with a low nutrient content. Arable sandy soils with a low organic content are also vulnerable to acid accumulation (Borowiec, Zabłocki; 1991).

Bulk precipitation was collected by 31 precipitation gauges located within the Forestry Inspectorates of Trzebież and Goleniów, and on agricultural land in the vicinity of "Police" Chemical Plant. Based on monthly measurements of chemical concentrations, annual amounts of fluorine compounds deposited on the soil surface were calculated. Average deposition during 1977-1980, 1981-1985, 1986-1990, 1991-1995 and 1996-1998 was obtained for each of 31 sites and analyzed with the SURFER program. Changes in F deposition depending on time and location are presented graphically.

The highest fluorine emission (average of 67 t y⁻¹) was found in 1981-1985. Afterwards, an approx. 2.0-fold decrease in F emission (28 t y⁻¹) was observed

in 1991-1995 and 1996-1998. The highest fluorine deposition (approx. $462 \text{ kg km}^{-2} \text{ y}^{-1}$) was observed in 1977-1980. Fluorine deposition diminished systematically in subsequent periods of the study: 3.1, 3.4, 6.1 and 5.2-fold in 1981-1985, 1986-1990, 1991-1995, and 1996-1998, respectively. During these periods the average load of fluorine deposited on sylvan areas located more than 10 km from the source of emission ranged from 16 to $95 \text{ kg km}^{-2} \text{ y}^{-1}$. These figures are 2.4-5.8-fold lower than for fluorine deposition on sylvan areas located 2.5-4.0 km from the emitter. The highest deposition of fluorine compounds (average of $265\text{-}944 \text{ kg km}^{-2} \text{ y}^{-1}$) was observed on agricultural land and small forests in the vicinity (0.8-2.5 km) of "Police" Chemical Plant.

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Study of fluoride motion law with generalized difference methods in three-dimensional numeric model of groundwater flow field in Changchun city and its suburbs

To improve drinking water properties, we have conducted tests using high-fluoride water in Changchun and its suburbs in China. This paper examines water usage with the systems analysis method proposed by systems engineering theory, with respect to the three-dimensional structure, the environment and boundary conditions of groundwater flow. On this basis, a hydrogeological conceptual model for elemental fluorine and fluoride is proposed, together with a three-dimensional model of fluorine motion and optimized control of its content. A simulation model of the groundwater system is created at the same time. The output value of the well and benefits to the water user are taken as constant conditions in the economic management model. The relationship between groundwater mining yield and economical benefit has provided new possibilities for water management in the region. Basing on the hydraulic management model, linear and non-linear formulae, trends and groundwater quality management model, the mining yield of 28 well groups was varied to obtain an optimal mining plan. The above model provides for optimal control of content in each well and leads to economical and social benefits. The above results, combined with engineering physics, forecasting and hydrogeological research of regional groundwater have enabled us to draw conclusions regarding the use of groundwater resources.

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Influence of environmental conditions on the content of fluoride in hair of two animal species in the Pomerania region

The purpose of this study was to measure the fluoride content in wild boars (57 animals forming 5 groups) living near Gryfice, Gryfino, Stargard Szczeciń-

ski, Myślibórz, and Trzebiatów; and to compare the results with 20 pigs (control group) raised near Pырzyce, centrally in respect to the former 5 towns. Hair samples were mineralized in perchloric acid and fluorides were quantitated with an ion-selective electrode (Orion, USA).

The significance of differences was calculated using Statistica for Biologists software, single-factor analysis of variance and Duncan test.

The highest fluoride content was found in the hair of wild boar from Starogard Szczeciński district (13.38 ± 6.66 mg/kg), significantly more than in pig hair (7.94 ± 2.01 mg/kg). The lowest content was found in the hair of wild boar from Myślibórz district (5.40 ± 1.22 mg/kg). These figures suggest that the environment exerts a major influence on the content of fluoride in animal hair.

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Fluorine content in dietary intake of small children

Food and water are natural sources of fluorine. Additionally, daily fluorine intake is modified by application of fluoride preparations for oral hygiene. According to National Academy of Sciences, USA, and Institute of Food and Nutrition, Poland, the Recommended Daily Allowances (RDA) of fluorine are the following: for children under 1 year old - 0.7 mg, and for children aged 1-3 years - 1.5 mg. Insufficient fluorine intake may increase the incidence of dental caries in the population.

The purpose of the study was to estimate fluorine content in total daily diet of small children, as well as to investigate the relationship between fluorine level in the diet and its level in drinking water and composition of diet. Material for the study were meals served to children aged 1-3 years, collected during subsequent 10 days of the study in Orphanages for small children in 17 large cities in Poland, as well as water used for cooking.

Measurement of fluorine content was performed using Ion Selective Fluoride Electrode and Orion Research Inc. meter. Samples were prepared according to Polish Standard PN-90-A-867785 „Raw Materials and Products of Fish and Other Water Animals. Fluorine Determination.” Samples were extracted in temperature of 95°C with EDTA and TISAB (Total Ionic Strength Adjuster Buffer - NaCl, acetic acid, trisodium citrate).

The limit of detection of the method was 0.04 mg/L. The recovery was estimated as 98.6%. For example, in samples collected in Warsaw, mean fluorine content in diet varied between 0.35-0.55 mg/kg, total weight of daily meals was 1900-2100 g and fluorine content in drinking water was 0.14 mg/L. In samples collected in Gdynia, mean fluorine content varied between 0.86-1.61 mg/kg, total weight of daily meals was 1800-2100 g and fluorine content in drinking

water - 0.2 mg/L. The high content of fluorine in samples from Gdynia was probably due to the fact that tea was served to the children everyday.

In conclusion, the potential daily dietary fluorine intake by children under three years old may range between 0.67 - 1.16 mg in Warsaw, and 1.55 - 3.38 mg in Gdynia.

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Evaluating the effect of defluoridation measures for endemic fluorosis in China

In order to understand the management conditions which are required in the application of defluoridation projects in China, as well as to prevent endemic fluorosis in 10 provinces and cities which are severe endemic fluorosis areas, 1960 different engineering projects were investigated. A sampling rate of 10% of the total water improvement projects was used. Standard uniform methods were carried out by retrospective epidemiology investigation. All data were input into a computer database and analyzed statistically to evaluate the effect.

The result indicates that defluoridation projects are effective for the prevention of endemic fluorosis. The concentrations of water fluoride are controlled within 1 mg/L, and the prevalence rates of dental fluorosis are within 30-40% with the dental fluorosis appearing in mild or very mild form.

Changing water sources especially drilling wells is the key component of all defluoridation projects. If water sources, financial and material resources are available, drawing low fluoride water and spring water are reliable measures. Physical defluoridation can only be applied in a few regions with high-fluoride water because it is hard to apply in rural villages due to its high cost, the complex management skills needed, and the small water flow.

In time, the relaxation of management and monitoring in some defluoridation projects, may lead to an increase of fluoride levels in water; thus resulting in a rise in the prevalence of dental fluorosis. Therefore, using low fluoride surface waters such as river water or reservoir water is a preferred and important measure for preventing endemic fluorosis. At the same time, it should enhance the water management skills required by personnel because the contents of drinking water fluoride will be continuously monitored together with the children's dental fluorosis rates. In addition, computer monitoring systems will be constructed so as to ensure the measures of defluoridation are executed more efficiently.

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Effect of fluoride solutions on the dentin: a physical, chemical and crystallographical study

The aim of this work was to study in detail differences in the crystalline and energetic structures of enamel and, in particular, dentin after treatment with fluoride solutions (NaF or SnF₂). X-ray diffraction (XRD), infra-red (FTIR), thermal (TG, DTG, DTA) and emitted gas (EGA) analyses were done. The results confirm that enamel and dentin are systems that differ from the energetic point of view, and hence undergo different reactions. XRD data indicate that treatment with SnF₂ resulted in fluorite formation in dentin (approx. 10% by weight) and re-precipitation of SnF₂ with a low degree of crystallinity. In the enamel, instead, an amorphous calcium phosphate compound was observed. EGA and thermal analyses highlighted a different response of enamel and dentin following fluoridation. The enamel was energetically stabilized after treatment with NaF thanks to partial replacement of OH⁻ with F⁻ producing a more stable fluorapatite, at least on the surface. The behaviour of dentin revealed some destabilization at least at temperatures causing loss of CO₂.

In our opinion, treatment of dentin with fluoride requires further study. Our preliminary data suggest the triggering of lattice destabilization phenomena, with concurrent erosion and dissolution. The next step should be a systematic *in vitro* investigation of the effect of fluoride solutions on dentin from different topographical areas (cervical and crown dentin) and on dentin affected by various pathologies (decayed dentin).

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