ABSTRACTS FROM THE XXVIIITH CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

FLUORIDE: BONE AND BRAIN EFFECTS

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ENHANCED FLUORIDE IN GROUNDWATER IN EASTERN ANATOLIA: EFFECTS, ORIGIN AND POSSIBILITIES FOR REMEDIATION

In spring 2002 and summer 2003 two research surveys were carried out in eastern Anatolia, Turkey. These were intended to evaluate the situation in the area regarding dental fluorosis and the hydrogeological conditions of ground water resources, thought to be responsible for the enhanced fluoride in the ground water. The hydrogeological/hydrochemical investigations undertaken in spring 2002 revealed a dramatic situation of the water supply in several villages in the Dogubeyazit area, manifesting fluoride concentrations above the WHO limit of 1 ppm in drinking water. This was confirmed by a dental survey with the collection of urine spot-sampling and nail-sampling in three villages where the available water supply contained 6 to 11 ppm fluoride. A mean concentration of 9 ppm F in urine and 19 ppm F in the nails of 119 children aged between 4 and 15 years validated this predicament. The resulting severe dental fluorosis is illustrated by the photographs taken of children (and adults) living in this area. The possible repercussions on the skeleton of the high fluoride concentration in drinking water were not considered in the initial preliminary investigations. However, over dozens of years, the regular intake of fluoride in the concentrations measured will undoubtedly lead to endemic skeletal fluorosis. Since the enhanced fluoride intake from the drinking water is largely responsible for the poor health of the locals, the only solution for an amelioration of their health is to provide low fluoride water to the population of the whole area affected. Consequently, the hydrogeological part of this study will attempt to contribute to the alleviation of this problem by the following: a) to survey the existing situation with regards to the current use of groundwater provided by the existing springs, and boreholes, as well as newly drilled boreholes, all of which are considered potential direct sources of drinking water for both people and livestock; b) to consider and propose a new distribution network of ground water resources with low fluoride concentration in order to lower its intake thus improving health and quality of life of the affected population.

Authors: **Balderer W**,[a] Leuenberger F,[a] Menghini G,[b] Dierauer W.[c]
Correspondence: [a]Institute of Geology/Engineering Geology Unit, Swiss Federal Institute of Technology, ETH Zurich, Switzerland; [b]Division of Applied Prevention, Department of Preventive Dentistry, Periodontology and Cariology, Dental Institute of the University of Zurich, Switzerland; [c]Palu 7, 7023 Haldenstein, Switzerland.
E-mail: Werner.balderer@erdw.ethz.ch
Keywords: Dental fluorosis; Dogubeyazit area; Eastern Anatolia; Fingernail fluoride; Groundwater fluoride.

NEED FOR THE RIGHT CONTROL DIET

Most chronic fluoride laboratory toxicity and other studies on rodents are conducted with standard commercial diets that contain not only substantial amounts of fluoride (often as much as 25 mg F/kg) but also aluminum, silicon, and other elements in uncontrolled or undesirable quantities. Interactions of these elements and combinations of them can greatly complicate and compromise the results of long-term fluoride supplementation with such diets. On the other hand, a nutritionally optimal and superior ultra-low fluoride artificial diet was described and used some years ago by SA Khalawan, JC Elliott, and RW Fearnhead (*Br J Nutr* 1980;44:371-9). In contrast to many other ultra-low F diets, this diet, prepared from green algae (*Chlorella pyrenoidosa*) and yeast (*Saccharaomyces cerevisiae*) plus added minerals, vitamins, and sucrose, contained as little as 0.05 mg F/kg and was well tolerated by both rats and mice, producing normal pregnancies and highly successful weaning. Moreover, unlike many commercial rodent diets, mice raised on this diet showed no signs of obesity and had unusually long life spans and excellent reproduction through four generations. With such a diet, definitive studies can be conducted to determine the effects incremental amounts of fluorides and/or other substances added to it can have on hard and soft tissues, reproduction, learning ability, thyroid and other glandular functions, longevity, and other parameters.

Author: **Burgstahler Albert W.**
Correspondence: Department of Chemistry, The University of Kansas, Lawrence, KS 66045, USA.
E-mail: awburg@ku.edu
Keywords: Chronic fluoride studies; Optimal control diet; Ultra-low fluoride diet.

BONE FLUORIDE CONTENT IN RATS TREATED PRE- AND POSTNATALLY WITH SOY BEAN ISOFLAVONES

Soya isoflavones are substances whose action is similar to weak estrogens. Along with technological progress and changes in lifestyle and habits, danger of exposure to these substances has increased considerably. Additionally, we do not know periods in human life in which exposure to hormonally active compounds is safe. Large amounts of potentially estrogenic substances are consumed by children in a period of their greatest sensitivity. Children from the 4th month of life are capable of digesting and assimilating isoflavones at a level similar to adults. The correct morphology and function of the skeletal system depend on estrogens which influence the mineralization of the osseous tissue. During puberty, an elevated level of estrogens causes an increase in the concentration of somatotropin (growth hormone), which lead to a so called “pubertal growth spurt”. This period of intense growth occurs earlier in girls than in boys, which is associated with a quicker achievement of a suitable level of estrogens necessary to initiate this process. At the same time a high level of estrogens stimulates the emergence of blood-vessels which penetrate epiphysial gristles providing them with oxygen, inorganic substances and contributing to the inflow of cells differentiating into osteoblasts. This in turn causes the mineralization of the chondral matrix, and in effect the formation of the bone. The ossification of epiphysial cartilages in long bones ends when reaching sexual maturity and this is the moment when final body height is established. Although there is no convincing evidence of the influence of early exposure to xenoestrogens on the later development of children, especially boys, the fact that infancy is a period of elevated sensitivity to estrogens increases the probability of changes in the mature stage. Hence the aim of this study is the initial assessment of the influence of soya isoflavones with a proven estrogen potential, administered pre- and postnatally, on the mineral composition of the skeletal system in male rats. The animals for the research were female Wistar rats which, after the ascertainment of the pregnancy until the end of lactation, received soya isoflavones at a dose of 200 mg/kg bw/24 hr, and after the separation of sucklings from mothers, young males received soya isoflavones at the same dose until reaching sexual maturity (for 3 months). After the period of treatment, the animals were sacrificed and blood and bones were taken for analysis. Fluorides were determined with a potentiometric method, calcium and magnesium with atomic absorption spectrometry, and the concentration of estradiol (E2) with “ECLIA” electroluminescence method. In the blood serum of rats treated with phytoestrogens, a statistically significant higher concentration of estradiol ($p=0.026$), lower fluoride concentration ($p=0.032$) and lower calcium concentration ($p=0.008$) were observed in comparison to the control group. Simultaneously, the concentrations of fluoride and magnesium in the bones of the examined animals were significantly higher than in the control group (respectively $p=0.039$ and $p=0.0015$). Calcium concentration in bones increased slightly in the examined rats compared with the control group, however in this case the absolute differences were not statistically significant. Results show that pre- and postnatal supplementation of male rats with soya isoflavones can considerably increase the concentration of estrogens in blood serum, which in turn can affect the composition of elements in bones.

Authors: Gutowska I,[a] Baranowska-Bosiacka I,[b] Noceń I,[c] Czupryńska K,[d] Marchlewicz M,[d] Wiszniewska B,[d] **Chlubek D**.[b]
Correspondence: [a]Laboratory of Biochemistry and Chemistry, [b]Department of Biochemistry, [c]Department of Medical Chemistry, [d]Department of Histology and Embryology, Pomeranian Medical University, al. Powstańców Wlkp. 72, 70-111 Szczecin, Poland.
E-mail: izagut@poczta.onet.pl
Keywords: Bone calcium; Bone fluoride; Bone magnesium; Isoflavones in rats; Serum estrogens; Soya isoflavones; Xenoestrogens.

CAN IMPROVED NUTRITION REDUCE THE RISK OF DENTAL FLUOROSIS? AN EPIDEMIOLOGICAL SURVEY IN JALORE, RAJASTHAN, INDIA

Introduction: India is among 23 nations around the globe where health problems occur due to excess ingestion of fluoride (>1.5 mg/L) in drinking water. In Rajasthan 18 out of 32 districts are classified as fluoride endemic, and 11 million people living there are at risk. Many researchers have assessed dental fluorosis with water fluoride level, but very few have tried to correlate it with nutritional status and other affecting factors. *Present objective:* The study was undertaken in Jalore, Rajasthan: (1) to estimate the fluoride content of drinking water sources; (2) to assess the clinical prevalence of dental fluorosis in relation to drinking water fluoride and the nutritional status of the population; (3) to recommend suitable remedial or nutritional action for prevention of further dental fluorosis. *Materials and methods:* A cross-sectional study based on stratified cluster samples of the Jalore population was done among age cohorts 5, 12, 15, 35–44, and 65–74 years as recommended by the World Health Organization (WHO). A total of 658 permanent residents were examined for dental fluorosis by Dean's fluoride criteria. The concentration of fluoride in drinking water from representative wells was determined by the ion selective electrode method with limit of detection (LOD) of 50 μg/L ± 2%. Nutritional status was recorded by body mass index (BMI) using criteria in agreement with WHO. Data were processed and analyzed by chi-square test and multiple logistic regression analysis using statistical package for the social sciences (SPSS) software. *Results:* The water fluoride level ranged between 3.56 and 4.07 ppm among all regions. The principal diet of the region is bajra and mustard seeds rich in calcium. The prevalence of dental fluorosis was 94.9% and 92.7% among children 5 and 12 years old, while for 15 and 35–44 years it was 97.2% and 95.8%, respectively. Proportional analysis revealed a statistically significant relationship ($p<0.01$) for participants with improved nutritional status and less dental fluorosis. Multiple logistic regression analysis for dental fluorosis showed a higher odds ratio (OR) with water fluoride level (OR=5.08), undernutrition (OR=3.87), and poor education. *Conclusion:* Water defluoridation is required among these high fluoride areas. Improving nutrition and dietary modification is also an important adjunct of fluorosis prevention along with water defluoridation. Avoiding ingestion of fluoride rich foods and tea as well as sufficient intake of calcium containing food can also reduce fluorosis.

Authors: Dagli Rushabh J, Kumar TS, Mathur A, Jain M, Dhanni C, Balasubramanya G, Prabu D, Kulkarni S.
Correspondence: Department of preventive and community dentistry, Darshan Dental College and Hospital, Loyara, Udaipur(313001), Rajasthan, India.
E-mail address: rushabhjdagli@yahoo.co.in
Keywords: Dental fluorosis; Jalore, Rajasthan; Nutrition; Water fluoride.

FLUORIDE AND FLUOROSIS IN TURKEY – A DILEMMA

According to certain documents and also the United Nations Development Programme (UNDP), fluorosis is still a significant health concern in Turkey. With the object of putting the problem of high levels of fluoride ingestion into a wider perspective, some basic facts about fluorine exposure and its distribution in the environment in Turkey have been explored. Previously published studies and data from national and international organizations, agencies, web pages, universities, other relevant research institutes were used to estimate the exposure of people in Turkey to risk factors. After finishing this study I prepared a website related to this problem. This research has assembled data on fluoride in drinking water, beverages, foods, soil (geological effects), industrial sources, blood and urine in humans and animals as well as respective patterns of the excess fluoride in these items to identify and analyze their contribution to the fluorosis problem in Turkey. In some regions of Turkey this problem is very important, but at present this view does not seem to be shared by various branches of the Turkish government or many researchers. Thus the "Official map of fluoride in Turkey–2003" as published by the Ministry of Health of Turkey says there are no high levels of fluoride in water sources in Turkey. Consequently, all the government authorities support setting up a "Fluoride-treatment program throughout Turkey for preventing tooth decay." Clearly, however, there is a need to document the problems in Turkey related to fluorosis. Unfortunately, the issue of fluoride consumption by people and animals in Turkey has never been adequately addressed in all its dimensions, since studies to date, done independently, have lacked

coordination and awareness of the results of one another. Thus the question of whether there is an environmental risk of fluorosis in Turkey has not has not been addressed or answered. The effects of fluoride on people and other living creatures in the environment and the local possible risk level will be examined and discussed. Although the water fluoride recommendations of the World Health Organization should certainly be taken into account, it is a very serious error in my judgment to suggest there is no risk of fluorosis considering only the level of fluoride in the water the people drink. Even more serious is the suggestion of providing the children with extra fluoride orally on the on the grounds that the drinking water is deficient in fluoride. More important than conducting further studies of these health problems is the need to identify and implement practical measures to control and prevent future exposure to excessive levels of fluoride in the natural water supplies and other environmental sources.

Author: **Demirel Umit.**
Correspondence: Istanbul, Turkey.
E-mail: udedmirel@istanbul.edu.tr
Keywords: Fluoride intake patterns; Fluoride and health; Fluorosis in Turkey.

FLUORIDE EFFECTS ON TOOTH ENAMEL FORMING CELLS AND MATRIX

Fluorosed enamel is hypomineralized, and with increased severity, becomes pitted with secondary staining. The mechanisms by which fluoride, an electronegative highly charged ion, can alter tooth enamel formation remain unclear. Animal and cell culture model systems have been used to understand the biological effects of fluoride on tooth enamel formation. These systems have shown that when fluoride is incorporated into the forming apatite, it can alter amelogenin/mineral interactions. At high levels, fluoride has been shown to affect developing ameloblasts, resulting in localized cyst formation in the developing enamel organ, and alter ameloblast modulation at the maturation stage. However, the question remains as to what is the unifying mechanism that could account for these effects of high levels of fluoride exposure on enamel development. Furthermore, why are there individual variable responses to the effects of fluoride on tooth enamel formation? The goal of this presentation is to review the known effects of fluoride on tooth enamel development, and to propose a model for fluoride interactions in enamel development.

Author: **Den Besten Pamela K.**
Correspondence: Department of Preventive and Restorative Dental Sciences, University of California, 513 Parnasuss Avenue, San Francisco, CA 94143, USA.
E-mail: Pamela.denbesten@ucsf.edu
Keywords: Amelogenin/mineral interactions; Dental fluorosis; Enamel maturation; Hypomineralization.

ANALYSIS OF METABOLISM CHARARACTERISTICS IN RATS POISONED WITH COMBINED FLUORIDE AND ALUMINUM

In order to analysis of metabolism characteristic in the rats poisoned with combined fluoride and aluminum. 80 male Wistar rats were randomly divided into 4 groups: control group （A） was given tap water, fluoride group（B）was given distilled water with 100 mg/L F in the form of sodium, aluminum group （C） was given distilled water with 200 mg/L Al in the form of chloride，F+Al group (D) was given distilled water with 100 mg/L F in the form of sodium and 200 mg/L Al in the form of chloride. The animals were fed standard rodent chow and water ad libitum for 3 months. At the end of the experiment, all four groups of rats were sacrificed and the serum, ashed bone and urine were collected, the contents of fluoride were determined by the F-ion selective electrode (ISE) method, the contents of aluminum were detected by atomic absorption spectrophotometry. In the sodium fluoride group, the serum fluoride, bone fluoride and urine fluoride were significantly increased compared with the control group ($p<0.01$, 0.71±0.13 mg/L vs 0.06±0.01 mg/L in serum fluoride, $p<0.01$, 5135.92±525.04 mg/kg vs 391.73±42.58 mg/kg in bone fluoride, $p<0.01$,10.19±17.30 mg/L vs 6.46±1.35 mg/L in urine fluoride, respectively). In the F+Al group, serum fluoride, bone fluoride and urine fluoride were 0.44±0.10 mg/L, 2907.70±34 mg/kg and 81.21±14.78 mg/L, which is significantly higher than that in the control group ($p<0.01$), but significantly lower than that in the fluoride group ($p<0.01$). In the aluminum group, the serum aluminum, bone aluminum and urine aluminum were significantly increased compared with the control group ($p<0.01$, 11.60±1.34 μg/L vs 6.60±1.09 μg/L in serum aluminum, $p<0.01$, 11.00±1.64 mg/kg vs 7.98±0.58 mg/kg in bone

aluminum, $p<0.01$, 356.98±42.94 μg/L vs 123.80±40.88 μg/L in urine aluminum, respectively). In the F+Al group, serum aluminum, bone aluminum and urine aluminum were 13.16±1.71 μg/L, 14.58±1.81 mg/kg and 416.54±64.25 μg/L, which is significantly higher than that in the control group ($p<0.01$), also significantly higher than that in the fluoride group ($p<0.05$). With simultaneous exposure to fluoride and aluminum, aluminum decreased the absorption, accumulation, and excretion of fluoride; but fluoride increased the absorption, accumulation, and excretion of aluminum.

Authors: **Gao Yanhui**, Sun Dianjun, Lin Lin.
Correspondence: The Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention. Harbin Medical University, Harbin 150081, PR of China.
E-mail:gaoyh411@163.com
Keywords: Aluminum and fluoride; Bone aluminum; Bone fluoride; Rat Al and F metabolism; Serum aluminum; Serum fluoride; Urinary aluminum; Urinary fluoride.

EARTH SCIENCE FOUNDATIONS IN FLUORIDE RESEARCH: A 100 YEAR-LONG MEDICAL GEOLOGY INVESTIGATION. (DANIEL BOYLE MEMORIAL LECTURE IN MEDICAL GEOLOGY)

Because fluorine (F) is the most electronegative element of the Periodic Table, its occurrence, distribution, "behaviour", and fate in the natural environment (i.e. geosphere, hydrosphere, atmosphere and biosphere) is of great interest to the field of earth science. Owing to its unique properties, F is considered the "Rosetta Stone" of biogeochemistry, and the spatial distribution of variations in concentration in earth materials is routinely used as an indicator of i) differences in depositional environment of sedimentary rocks, ii) economically-significant mineralization deep in the subsurface, iii) presence of important structural features (faults, fractures, units of high hydraulic conductivity), or iv) anthropogenic contamination. Temporal variation in F is sometimes also a precursor of volcanic and earthquake activity or a signal of an increase in man-made pollution. Given the multitude of potential sources of F in modern times, great care must be taken in distinguishing between naturally-occurring F in the environment and anthropogenic contamination when undertaking biological investigations. This is handily accomplished through the application of the principles of medical geology, the study of health problems related to "place" or location. The International Medical Geology Association has been active in the field of biogeochemical F research since its inception at the turn of the millennium and brings together the expertise of both biological and earth scientists to bear on the problem of F toxicity wherever it may occur. In this inaugural Daniel Boyle Memorial Lecture in Medical Geology, the biogeochemistry of F will be summarized, the most significant contributions of medical geology to the past century of F research will be reviewed, and foundational resource materials for researchers will be shared.

Author: **Gingerich Heather**.
Correspondence: International Medical Geology Association of North America, 172 Albert Street, Ingersoll, ON Canada N5C 2Y4.
E-mail: heather_gingerich@yahoo.com
Keywords: Anthropogenic fluoride; Biogeochemistry; Environmental fluoride; Medical geology.

FLUORIDE EFFECTS ON BONE QUALITY ARE INFLUENCED BY GENETICS AND ENVIRONMENTAL FACTORS

Studies on humans given fluoride (F) as a therapeutic treatment for osteoporosis have shown that approximately one-third of patients do not respond to the drug. In addition, studies on skeletal fluorosis in geographic area with naturally high F content in the water have shown that not all children residing in these areas are affected by the disease. In order to study the influence of genetics on bone quality, F at 0, 25, 50, and 100 ppm in the drinking water was administered to three inbred strains of mice with different susceptibility to dental fluorosis: A/J, a susceptible strain; SWR/J an intermediate strain, and 129P3/J, a resistant strain. The bone F content of the three strains was similar, but the mechanical properties of the susceptible strain deteriorated significantly while they remained unchanged in the resistant strain. F treatment had no effect on bone microarchitecture, but it caused an increase in osteoid formation and a decrease in mineralization heterogeneity in all three strains. Interestingly, with increased dosages of F, we found increases in bone mineral crystals in cross-sections that correlate with most of the changes in mechanical properties. We hypothesize that an increase in bone fluoride affects the mineral-organic interfacial bonding and/or bone matrix proteins, interfering with

bone crystal growth and causing inhibition on the crystallite faces as well as bonding between the mineral and the collagen.

Author: **Grynpas Marc.**
Correspondence: Laboratory Medicine and Pathobiology, Samuel Lunenfeld Research Institute, Toronto, ON Canada.
E-mail: GRYNPAS@mshri.on.ca
Keywords: Bone fluoride; Bone quality; Environmental factors; Genetic effects; Skeletal fluorosis.

THE POLITICAL ECONOMY OF FLUORIDE TOXICOLOGY

This paper reviews the political nature of fluoride-related hearings and research and demonstrates the value-laden aspects of the controversy. Fluoride-emitting industries and their allies have relied upon corporate-funded science, slick public relations tactics, and friends in high places to help them to minimize environmental and occupational health problems associated with mining and producing fluoride-containing products, and with consuming substances that contain fluoride. This pattern of industry suppressing information about the toxicity of its products is not new—a long trail of deception and deceit on the part of industry and its government allies occurred with lead, vinyl chloride, asbestos, cigarettes, and a number of other contaminants. Members of fluoride-contaminated communities, workers with occupational health concerns, and consumers with fluoride concerns have been chastised and discredited for questioning fluoride's toxicity. Meanwhile, industrial fluoride emissions and exposures have been allowed to continue. Medical doctors, dentists, and research scientists who have tried to help people with fluoride-related health concerns have also been attacked and have paid a price in their careers. Science is not neutral. Scientists, hearing officers, policy makers, and medical personnel bring core values to their research and practice, whether or not they are aware of it. This paper illustrates the influence of corporate funding and politics on investigations into fluoride toxicity.

Author: **Haley Ella.**
Correspondence: Department of Sociology, Health, and Environment, Wilfrid Laurier University, 75 University Avenue West, Waterloo, ON, Canada N2L 3C5.
E-mail: ella@athabascau.ca
Keywords: Fluoride hearings; Fluoride toxicology; Industrial influence; Politics of fluoride.

EFFECT OF FLUORIDE ON THE EXPRESSION OF COLLAGEN TYPE I AND DENTIN PHOSPHOPROTEIN IN TOOTH QUALITY

To study the effect of fluoride on the expression of collagen (COL) type I and dentin phosphoprotein (DPP) in tooth both in natural and experimental fluorosis animals, we chose four female fluorosed Inner Mongolia small fat-tail cold sheep aged three years for fluorosed group, and four healthy for control; twenty male Hartley albino guinea pigs were divided randomly into two groups of ten, the control group were fed with normal diet and the fluorosed group added 150 mg/L NaF in water for 90 days. The incisors and molars from sheep and guinea pigs were collected to observe the pathological damage by HE, Masson and determine the expression of COL1A1 and DSPP genes by QRT-PCR, COL type I and DPP proteins by IHC. The results indicated that we successfully made the mottled enamel and wave-formed mouth model in guinea pigs, compared with the control group. Serious structural changes were observed in collagen, dentinoblasts, enamel and endodontics of fluorosed dentine. High fluoride can affect the expression of COL type I and DPP in incisor and molar of sheep and guinea pigs, inducing lower expression of COL1A1 gene and COL type I, DSPP gene and DPP in incisor and molar. We believed that industrial fluoride pollution showed more inhibition on the expression of COL1A1 gene and COL type I in the molar of sheep than those in incisor. In conclusion, it appears that fluoride adversely affected the expression of COL type 1 and DPP, then the calcification, and finally decreased the quality of the teeth.

Authors: **Han Tianlong**,[a] Wang Min,[a] Yan Xiaoyan,[a] Hong Jian-hua,[b] Wang Jung-dong.[a]
Correspondence: [a] Prof Jun-dong Wang, Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, PR of China. [b]Campus Hospital of Shanxi Agricultural University, Taigu, Shanxi 030801, P.R. of China.
E-mail: wangjd@sxau.edu.cn
Keywords: COL1A1 gene; Collagen; Dentin phosphoprotein; DSPP gene; Fluoride and guinea pigs; Guinea pig incisors.

FLUORIDE AND THE BRAIN: DANGERS AND DIVERSITIES

This presentation emphasizes the complexity of the brain and the many ways in which fluorides can disrupt its structure and function. A modern way of looking at the nervous system is presented based on three semi-independent systems—the traditional nervous system, the visceral nervous system, and the endocrine nervous system. All these systems collaborate to produce our actions, thoughts, affections, and moods. Attention is paid to how fluorides can disrupt one, two, or all three systems. Individual differences in system susceptibility, as well as genetic inheritance, probably are main contributors to the differences in reactions of people to fluoride exposure. Attention is also given to the interaction of fluorine with other elements, especially certain metals. Of special interest are the anatomical changes induced by fluorides in the brain that resemble alterations found in the brain of Alzheimer's patients. The hypothesis is offered that the main cause of all dementias is a reduction in the metabolic activity of the entire brain caused by alterations in blood flow and reductions in chemicals essential to aerobic metabolism.

Author: **Isaacson Robert L.**
Correspondence: Department of Psychology, Binghamton University, Binghamton, NY 13902.
E-mail: Isaacson@binghamton.edu
Keywords: Dementias; Fluoride and the brain; Nervous system.

AMELIORATION OF FLUORIDE-INDUCED NEURONAL OXIDATIVE STRESS BY ADMINISTRATION OF SELECTED ANTIOXIDANTS

Fluoride-induced oxidative stress contributes to the pathogenesis of fluorosis by disrupting the delicate pro-oxidant/anti-oxidant balance that exists within mammalian cells. Increased production of reactive oxygen species (ROS) after fluoride treatment in *in-vivo* studies suggest that fluoride exposure causes generation of ROS and alteration of antioxidant defense systems in animals and endemic victims. The mechanisms of oxidative stress include the effect of fluoride on membrane, DNA, and antioxidant defense systems of cells. From low to high doses of fluoride exposure, there are different responses of fluoride-induced oxidative stress in various target sites including teeth, bones, testes, thyroid, liver, and brain in epidemiological as well as animal studies. Therefore, reducing the possibility of fluoride interacting with critical biomolecules and inducing oxidative damage, or bolstering the antioxidant defenses cells might be associated with the beneficial role of antioxidant nutrients through exogenous supplementation of antioxidant molecules. Although many researchers have investigated the benefit of antioxidants in preventing fluoride toxicity, the mechanisms of antioxidant nutrients being effective via rebalancing the impaired prooxidant/antioxidant ratio are not completely clear. Although various studies have been reported on antioxidant nutrients such as vitamin E, vitamin C, and selenium, their mode of action is still incompletely defined. The present study was undertaken to analyze the effect of fluoride on the developing brain and determine the ability of clinoptilolite along with vitamin E, selenium, and zinc to reverse fluoride-induced neurotoxicity. Treatment of fluoride-exposed animals with clinoptilolite along with vitamin E appeared to reverse fluoride-induced alterations in different compartments of brain monitored by activity levels of catalase, malondialdehyde (MDA), reduced glutathione, and oxidized glutathione. Combined treatment with clinoptilolite with vitamin E was more effective than with zinc and selenium in enhancing restoration of all these parameters indicative of fluoride-induced oxidative stress. Tissue specific changes, following fluoride exposure and responses to the treatment with different antioxidants were recorded in the parameters of oxidative damage. As the reversal of these parameters by clinoptilolite and vitamin E was independent and may decrease the toxic actions of accumulated fluoride-mobilizing capability in neuronal cells, this ought to be mainly due to their strong antioxidant property. Thus the ideal treatment of fluorosis may an appropriate combination of antioxidant supplementation.

Authors: **Jayaprakash**, Basha PM,[a] Madhusudhan N, Puja Rai, Shabana Begum.
Correspondence: [a]Department of Zoology, Bangalore University, Bangalore-560 056, India.
E-mail: pmbashabub@rediffmail.com
Keywords: Clinoptilolite; Fluorosis; Free radicals; Oxidative stress; Vitamins C/E; Zinc.

FLUORIDE CONCENTRATIONS IN BONE AND SERUM OF ANIMALS MANAGED WITHIN A RESTRICTED GEOGRAPHIC AREA IN SOUTH AFRICA

Fluoride occurs in various amounts in soils, water, the atmosphere, vegetation, and body tissues. Inorganic fluoride compounds are the most important sources as far as fluoride toxicosis in animals is concerned. Animals normally ingest small amounts of fluoride without adverse effect. It may indeed be beneficial in small amounts, but it is harmful when ingested in excess, and the hazards of excessive fluoride intake in animals have been known for a long time. In mammals, 99% of the body burden of fluoride is found in bones and teeth. However, the levels of fluoride in plasma, serum, and urine have been considered useful biomarkers for fluoride exposure. Routine bone and soft tissue (blood) samples were collected from different antelope species (*Connochaetes taurinus*, *Damaliscus dorcas phillipsi* and *Aepyceros melampus*) managed within a restricted geographic area of approximately 700 ha. The area has fluoride producing facilities on site. Control bone (*Damaliscus dorcas phillipsi* and *Aepyceros melampus*) and blood (*Connochaetes taurinus*) samples were acquired off site. The normal plasma fluoride concentration in cattle is < 0.2 ppm (or mg/L) and normal bone concentrations range from 400 to 1200 ppm. Control bone samples had a mean fluoride level of 1053 mg/kg (260 – 2020 mg/kg), and the bone samples collected within the study area had a mean fluoride level of 5673 mg/kg (4240 – 7520 mg/kg). Control serum fluoride levels had a mean of 0.4 mg/L, whereas the serum samples collected within the study area had a mean fluoride level of 2.16 mg/L(1.6 – 2.8 mg/L). The bone and serum fluoride values in the study area samples significantly exceeded the controls collected at the off-site locations. Although the numbers of evaporation pans on site in operation have been reduced, and the average stack emissions are within discharge limits and are trending lower, the resident antelope are still showing excessive accumulated fluoride levels. By determining these effects and having academic results, this research may assist the implementation of various management strategies in areas where naturally high levels of fluoride are present, for example Pialnesberg National Park and a number of privately owned game ranches. Follow up research is still needed to ascertain the effect of decreasing the emissions in the area, and what effect this will have on the fluoride levels found in the resident antelope, as well as other introduced animal species and the vegetation on site.

Authors: **Labuschagne K**,[a] Bain C,[b] Bartels P.[a]
Correspondence: [a]Wildlife Biological Resource Centre, National Zoological Gardens of South Africa, Pretoria, South Africa; [b]Safety, Health and Environmental Quality, P.O Box 582, Pretoria, South Africa.
E-mail: info@wbrc.org.za
Keywords: Antelope; Bone fluoride; Serum fluoride; South African wildlife.

IS THE PINEAL GLAND A TARGET FOR FLUORIDE TOXICITY?

Background: Fluoride (F) has an avid affinity for hydroxyapatite, the mineral component of bone, teeth and pineal gland. Pineal calcification, which is a normal physiological occurrence, starts during early childhood. The current increase in the incidence of dental fluorosis signifies that a significant proportion of the population have received too much F during tooth development in early childhood. If plasma F levels are high enough to prevent normal formation of enamel, do they have a concomitant effect on the pineal gland? Specifically, does F interfere in the metabolic pathway of tryptophan, an essential amino acid in the human diet, to melatonin, an important neurohormone? Significantly, plasma melatonin-levels are normally highest in young children; and interact with pubertal development. *Method:* A controlled longitudinal experimental study compared the 24-hour levels of sulphatoxymelatonin, (aMT6s), the major urinary melatonin metabolite, excreted by fluoridated and control Mongolian gerbils, (N=12 females, 12 males/group) at four ages from prepubescence (7 weeks), to puberty (9 and 11¾ weeks), to adulthood (16 weeks). Fluoridated gerbils received 2.3 µg F/g body weight/day orally from birth until 24 days and thereafter food containing 37 mg F/kg. Concentrations of aMT6s were measured by radioimmunoassay. Onset of pubertal development was assessed using broad physiological markers: body weights, ages at vaginal opening, development of scent glands, testes weights. *Results:* Compared to the controls, both male and female fluoridated gerbils excreted highly significantly less aMT6s at 7 and 9 weeks; males also at 11¾ weeks. In agreement with previous studies, the controls excreted constant absolute levels of

aMT6s/24h and relative to body weight, significantly less aMT6s from 7 to 16 weeks. The fluoridated female gerbils had a significantly earlier onset of pubertal development. Testes weights were significantly lower than the controls at 16 weeks. *Conclusions:* Fluoride inhibits pineal melatonin synthesis in prepubescent gerbils causing an earlier onset of pubertal development in females and decreasing testes weights in males.

Author: **Luke Jennifer.**
Correspondence: Wretham Practice, Forest Row, East Sussex, RH18 5HR, UK
E-mail address: jenniluke@onetel.com
Keywords: Fluoride; Melatonin; Pineal gland; Puberty.

EXPLORING THE FLUORIFICATION PROCESS OF METABOLIC DISEASES

In humans, fluoride even as a trace element can be found in varying accumulative quantities over time throughout the body. The specific roles that it plays are diverse. Since the Thrombogenic Theory (Rokitansky, 1842) first acknowledged deposits of varying components within the arteries, the build-up of excess substances includes mechanisms of involving plaque formation and osteo-related metabolic diseases as well as fluorification of soft tissues. Mechanisms of these processes are illustrated in retrospect in this paper. Fluorification is linked to dietary and medication interactions as well as modern-day industrial practices, extending from environmental (water) fluoridation to additive intake in consumer products such as toothpaste, dental hygiene products, and even consumer beverages. As such, these produce trends verified experimentally that continue to contentiously deepen our integration of fluoride in human life in the 21st century.

Author: **Mohamedally Sheik M.**
Correspondence: 27 Woodside Gardens, Tottenham, London N17 6UY, United Kingdom.
E-Mail: drsm@mohamedally.com
Keywords: Fluoride accumulation; Fluoride exposure; Fluorification mechanisms; Thrombogenic theory.

CURRENT EPIDEMIOLOGICAL RESEARCH ON A LINK BETWEEN FLUORIDE AND OSTEOSARCOMA

In 2006 EB Bassin published a case-control study showing a strong association between fluoridation and osteosarcoma. It found a 5-fold increased risk in boys exposed during a window of vulnerability between ages 5–10. A related study, led by CW Douglass and RN Hoover, is still unpublished. Their case-control study focuses on bone fluoride as the measure of exposure. Douglass has stated that preliminary results found no association with osteosarcoma. We examine five possible explanations for these conflicting results. 1.) Bassin's study might have suffered from selection bias leading to a spurious finding, but only if two conditions existed: i) most of her hospital control subjects had bone fractures, and ii) fluoride at higher exposure levels substantially prevented bone fractures. Sensitivity analyses show that to explain away Bassin's results would require the unlikely scenario where 75% of controls were bone fracture patients and the low fluoride subjects had 5-fold greater risk of bone fractures. 2.) Douglass' study approach may be unable to detect an association between fluoride and osteosarcoma, even if one exists. His main exposure measure, bone fluoride concentration, reflects cumulative lifetime exposure, but cannot identify exposure during a specific age window, and could thus dilute effects. 3.) Douglass' work may suffer selection bias because all control subjects for which he obtained bone fluoride levels had bone tumors other than osteosarcoma. If fluoride is a risk factor for any of these other types of bone tumor, this will bias results toward the null. It is not only biologically plausible that fluoride may cause other types of bone tumors but also some research supports the link, especially for Ewing's sarcoma, the second most common bone cancer in the US after osteosarcoma. 4.) Another weakness in Douglass' methodology is that he may have included subjects over age 20. Older patients may not be susceptible to fluoride-induced osteosarcoma so their inclusion would dilute any effect. 5.) Finally, even if Douglass uses residential history data to try to measure age-specific exposures, similar to Bassin's approach, his results may be biased toward the null. Selection bias could arise in a restricted analysis of his second set of controls from non-cancer orthopedic department patients. Not all these controls were matched to cases on distance from enrollment hospital. His osteosarcoma cases likely traveled longer distances for treatment at major urban hospitals than his orthopedic

controls whose less serious problems would not justify such long travel. Whereas 85% of controls were enrolled at hospitals located in fluoridated cities, only about 55% of people in the US have fluoridated water. If Douglass fails to adjust for geographic distance or has insufficient orthopedic controls from beyond the metropolitan areas, his results will again be biased toward the null.

Authors: **Neurath Chris**, Connett Paul.
Correspondence: American Environmental Health Studies Project, 82 Judson St, Canton NY 13617 USA.
E-mail: cneurath@AmericanHealthStudies.org
Keywords: Bone cancer; Epidemiology; Fluoride and cancer; Investigational bias; Osteosarcoma.

EFFECTS OF FLUORIDE AND LEAD ON THE EXPRESSION OF N-METHYL-D-ASPARTATE RECEPTOR 1 IN THE HIPPOCAMPUS AND ON LEARNING-MEMORY OF RATS

A growing number of studies indicate that excessive ingestion of fluoride can result in dysfunction of the central nervous system (CNS), and alterations in mental work capacity. The neurotoxicity of fluoride may involve a central mechanism called excitotoxicity, which is considered to be a common mechanism in various metal-related neurotoxic reactions as from lead. Interestingly, fluoride has been reported to increase the accumulation of neurotoxicant lead in the body. Therefore, in order to explore mechanisms by which fluoride and/or lead impact on the learning and memory ability, two studies were performed in adult rats and offspring rat pups. Healthy adult Wistar rats were randomly divided into four groups. With one group as control, the other three groups were given sodium fluoride (150 mg/L) and lead acetate (300 mg/L), and both together, respectively. Spontaneous behavior, learning ability, and memory retention were tested by open-field and Y-maze tests. The results showed that fluoride exposure altered behaviors patterns and impacted recognition and memory ability. To determinate the brain disorders induced by fluoride and lead during early development, the gene and protein expression of N-methyl-D-aspartate receptor 1 (NMDAR1) in the hippocampus of offspring rat pups at postnatal day 14 and 28 exposed to sodium fluoride and/or lead acetate were determined by quantitative real-time polymerase chain reaction (QRT-PCR) and immunochemistry. The results indicated that at postnatal day 28 both NMDAR1 gene and protein expression level increased significantly compared with the control. These results can be seen as evidence of a relationship between the excitotoxicity and neurotoxicity of fluoride and/or lead.

Authors: **Niu Ruiyan**,[a] Sun Z,[a] Cheng Z,[a] Hong J,[b] Wang JD.[a]
[a]Correspondence: Prof Jundong Wang, Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi, P.R. of China.
[b]Campus Hospital of Shanxi Agricultural University, Taigu, Shanxi, P.R. of China.
E-mail: wangjd@sxau.edu.cn
Keywords: D-Aspartate receptor 1; Excitotoxicity; Fluoride neurotoxicity; Hippocampus; Memory-learning in rats.

CAN THE EFFECT OF SODIUM FLUORIDE ON AGGRESSION, SEXUAL ACTIVITY, AND FERTILITY IN RATS BE REVERSED BY VITAMIN E?

Vitamin E is a fat-soluble vitamin. Alpha-tocopherol (α-tocopherol) is the name of the most active form of vitamin E in humans. It is a powerful biological antioxidant. Antioxidants act to protect cells against the effects of free radicals, which are potentially damaging by-products of energy metabolism. Free radicals can damage cells and may contribute to the development of cardiovascular disease, CNS inhibition, and cancer.

Fluoride is known to decrease the levels of antioxidant enzymes in the brain. Previous study carried out in our lab, have demonstrated that fluoride intake in Rats (300 ppm) significantly decreased aggression, sexual behavior, and fertility in adult male Sprague-Dawley rats. Our current study is underway to determine whether vitamin E, through its ability to limit production of free radicals, might lower or prevent the effects produced by fluoride intake in Rats. The effect of ingestion of sodium fluoride (NaF) in drinking water at a concentration of 300 ppm for 12 weeks is being investigated on aggression, sexual behavior, and fertility in adult male Sprague-Dawley rats, in the presence and absence of vitamin E (12.5 mg/kg). Our initial results showed that simultaneous intake of vitamin E with fluoride did not reverse those effects produced by NaF ingestion in Rat. However, others have reported a protective role of vitamin E in opposition to the effect of NaF on fertility in mice. Those observed differences might be

due to genetic variations between rat and mice. Currently, work is still undergoing with a new set of rats in addition to a set of mice to test latter hypothesis.

Authors: **Nusier Mohamad**, Alami Jamil.
Correspondence: Prof Mohamed Nusier, Department of Biochemistry and Molecular Biology, Jordan University of Science and Technology, School of Medicine, Irbid 22110, Jordan.
Email: mick@just.edu.jo.
Keywords: Fluoride and fertility. Fluoride and male rats; Sexual aggression; Vitamin E.

A GRAPHICAL REPRESENTATION OF SOME FLUORIDATION RISK FACTORS

For over half century water fluoridation has been ethically and factually controversial for both benefits and risks. The percentageof thewhole population in each state of the USA who receive fluoridated water varies from 2%to 88%.Graphically, the 50states can be ranked in order of the percentage of the whole population fluoridated andlistingthe reported prevalence ofsuspected risk factors, such as mental retardation, diabetes, obesity,and cancer, along with lack of improved dental health can be easily examined.The effectiveness of a public health intervention must be measurable in the public at large, and either the risks notdetected orelse be significantly less than the measured benefit. Otherwise the intervention must be stopped. Graphical representations of fluoridation in relation to significant health risks and lack ofimprovement in dental health orreduction in dental expensesare importantpublic health educational considerationswhen making a judgment call to discontinue water fluoridation.

Author: **Osmunson Bill.**
Correspondence: Aesthetic Dentistry of Bellevue and Lake Oswego, Bellevue, WA 98004, USA.
E-mail: bill@smilesofbellvue.com
Keywords: Fluoridation risk factors; Graphical representation; National assessment; Water fluoridation.

PERINATAL MORTALITY AND FLUORIDATION IN THE WEST MIDLANDS, U.K.

Perinatal mortality statistics are published annually by all countries in conformity with international law. Any clearcut effects due to fluoridation should be impossible to hide. This evidence from~70%-fluoridated West Midlands shows clearly that the excess perinatal losses reported there are of specific kinds, only one of which is significantly amplified by an effect that is confined to the *fluoridated* areas. The remaining *unfluoridated* areas, apart from North Staffordshire, follow more closely the *net* rates for England and Wales. West Midlands Perinatal Audit [WMPA] was required to investigate possible causes and remedies for a persistent and highly significant (average 45%) annual excess of neonatal deaths, established by OPCS regional records. WMPA's Report "Stillbirth and Neonatal Death 1991-1994" (1996) concluded that the observed excesses other than congenital anomalies [A] were due to an unexplained excess of < 1 kg premature deliveries. No attempt was made to compare rates in the context of fluoridation. This paper re-analyses the data against percent fluoridation and averaged Jarman Scores for the 15 DHAs using 1991-4 data corrected via OPCS data, and Wigglesworth losses [B] to [E]. Correlation charts and Jarman Score plots show that statistically significant ($p < 0.01$) excess neonatal deaths in Wigglesworth category [D] occur *inevitably* and *only* under fluoridation, while there are only relatively small and non-significant net differences in the remaining [BCE] losses. [CE] losses (representing intranatal/postnatal losses other than [D]) have rates roughly equidistant from 1993/4 [CE] rates for England and Wales (fluoridated above, unfluoridated below) but net stillbirth rates [B] are substantially higher in both unfluoridated and fluoridated West Midlands, with a small excess in the former. This excess [B] is more than cancelled out by the extra [CE] deaths in the fluoridated areas. Persistently poorer air quality around central Birmingham may be involved. Other than [D] none of these differences achieve statistical significance. North Staffordshire (unfluoridated, $p < 0.05$) and Worcester (fluoridated) DHAs show further excess [D] losses reasonably attributable to fluoride air pollution from local ceramics industries. The excess deaths in West Midlands RHA do reflect exceptionally high loss-rates for fetuses weighing less than 1 kg. Their proportions are non-significantly higher in the fluoridated areas in all loss categories but only [D] losses show the highly specific and significant reported excesses attributed here to fluoridation status. These significant ($p < 0.01$) excesses persist in [D] loss for neonates weighing up to 1.5 kg. N.B. All [stillbirth + early neonatal death] records can be used to confirm a strongly augmenting effect

of fluoridation on the latter rates, but it seems clear that deaths *attributed* to the consequences of extreme prematurity—Wigglesworth [D]—represent a unique and reliable marker for one of the more extreme adverse effects of mass fluoridation on human gestation. Biological causation seems likely to involve the inhibiting effect of internalised fluoride ions on the enzyme systems which control the transformations of thyroxine T4 either to T3 or to reverse-T3. As parturition approaches, changes in the production of these enzymes normally ensure that, instead of reverse-T3 (the fetal form) a cascade of T3 becomes available to the fetus just before delivery. This activates all but the most highly premature and physically weak neonates for independent life. Respiration failure causes most [D] loss. These extra losses in fluoridated areas are strongly Jarman Score-dependent, and must reflect maternal social and e.g. nutritional deprivation. Poorer second trimester fetal growth and higher premature delivery rates (under persistent gestational stress) must follow. Yet it is not higher net premature delivery rates *per se* that promote [D] loss in fluoridated areas but rather the greater probability ($p < 0.05$) that these neonates will die, despite all available resuscitation techniques and neonatal intensive care facilities.

Author: **Packington Ian E**.
Correspondence: 42 Huntington Road, York, YO31 8RE, U.K.
E-mail: ianpackington@hotmail.com
Keywords: Fluoridation; Perinatal mortality; West Midlands, U.K.; Wigglesworth [D] losses.

INFLUENCE OF FLUORIDE ON EPITHELIAL STRATIFICATION AND WOUND HEALING IN CULTURE

Objectives: Oral epithelia and dental enamel are exposed daily to fluoride in oral health products. Compared with teeth and bones, the biological effects of fluoride on oral epithelia are poorly investigated. In the present study, we performed the effects of sodium fluoride (NaF) at low and high doses on the differentiation of human epithelial cell line, HaCaT that expresses the keratins (Ks) of oral mucosa. *Methods:* The profile of keratin expression at the protein level was assessed by 2D gel electrophoresis, immunocytochemistry, and Western blotting, and at the mRNA level by RT-PCR. Stratification was investigated by electron microscopy and DNA synthesis by BrdU incorporation and an ELISA test assay. The influence of fluoride was also analyzed in a wound healing scratch assay. *Results:* High dose of fluoride treatment inhibited stratification, and decreased expression of K15, a keratin associated with stratification. Both RNA and protein expression were affected. In parallel, expression of specific keratins for terminal differentiation, K1 and K10, was also dwindled. This reduction was related, in time and dose, to a cutback in expression of the K1/K10 regulating enhancer binding protein c/EBP alpha. Finally, fluoride enhanced the closure of the in vitro wound at high dose of NaF due to an increased proliferation rate. *Conclusion:* We showed for the first time that NaF influences keratinocyte differentiation in vitro, retarding the process of stratification and blocking the expression of some keratins of terminal differentiation. The presence of fluoride may enhance proliferation of keratinocyte precursors and improve healing capacities of mucosa.

Authors: **Prado Euridice**, Shabana H, Forest N, Berdal A.
Correspondence: INSERM, Paris, France.
E-mail: euridice_prado@yahoo.fr
Keywords: Epithelial cell stratification; Fluoride and oral mucosa; Keratinocyte differentiation; Oral epithelia; Wound healing.

AMELIORATION OF FLUORIDE TOXICITY IN RABBITS BY *MORINGA OLEIFERA* AND *TAMARINDUS INDICA* EXTRACT

An experimental study was undertaken to assess the ameliorative efficacy of aqueous extracts of *Tamarindus indica* fruit pulp and *Moringa oleifera* seeds in fluoride intoxication in rabbits. New Zealand white male rabbits (n = 24), 4 to 6 weeks old, weighing between 600 and 800 g were randomly divided into four equal groups comprising 6 animals each. The rabbits were maintained in a laboratory animal house with a 12-hr day and night photoperiod and were provided with rabbit feed *ad libitum*. Group I animals received drinking water (fluoride concentration less than 0.28 ppm) with no added sodium fluoride and served as negative control. Animals of group II received drinking water with 200 ppm added sodium fluoride to serve as positive control. Groups III and IV animals received drinking water containing 200 ppm added sodium fluoride and aqueous extracts of *Tamarindus indica* fruit pulp @ 100 mg/kg and

Moringa oleifera seed @ 50 mg/kg body weight once daily, respectively. Blood samples were collected on day 0, 45, and 90, and the animals were sacrificed after taking radiographs on day 90. Plasma fluoride concentration in groups III and IV was significantly ($p<0.05$) lower compared to that of group II on day 45 and 90. Cortical indices and metaphysical width of the group III and IV rabbits also revealed beneficial effects of these two extracts in the fluoride intoxicated rabbits. Reduced hepatic and renal damages in the group III and IV animals in comparison to positive controls were also evident from the plasma enzymes activity. From these results, it can be concluded that administration of either of these two plant extracts reduces the toxic effects of concurrent fluoride administration.

Authors: **Ranjan Rakesh**,[a,b] Swarup D,[a] Patra RC,[a] Dey S.[a]
Correspondence: [a]Division of Veterinary Medicine, IVRI, Izatnagar, UP, India; [b]Department of Clinical Veterinary Medicine, College of Veterinary Sciences GADVASU, Ludhiana, Punjab, India.
E-mail: rakesh_ranjan3@rediffmail.com
Keywords: Ameliorative effects; Fluoride intoxication; Moringa oleifera; New Zealand rabbits; *Tamarindus indica*.

EFFECT OF MELATONIN AGAINST FLUORIDE-INDUCED OXIDATIVE STRESS IN THE MOUSE OVARY

In this study, we evaluated the protective effect of melatonin against ovarian oxidative stress induced by fluoride in mice. The animals were divided into four groups with a 30-day treatment period for each group. The first group served as control. The second group was intraperitoneally injected with melatonin (10 mg/kg bw/day). The third group was orally administered sodium fluoride (NaF; 10 mg/kg bw/day). The fourth group was given both melatonin and NaF with melatonin preceding NaF by 30 minutes. The results showed a significant reduction in body and organ weights in the NaF-treated group, which also showed an enhanced level of ovarian lipid peroxides accompanied by a significant decline in the levels of total proteins, total ascorbic acid, reduced glutathione, and the activities of superoxide dismutase and catalase. Compared to NaF alone, the combined treatment significantly lowered the level of lipid peroxides and enhanced the antioxidant status. Similarly, the histopathological changes of ovary and fluoride levels were also revived by the combined treatment. The second group of mice given melatonin without exposure to NaF exhibited no significant changes in the above indices as compared to the control group. Thus the results obtained from this study suggest that melatonin exerted a protective action against fluoride-induced oxidative stress in the mouse ovary.

Authors: **Rao Mandava V**, Chawla SL.
Correspondence: Zoology Department, University School of Sciences, Gujarat University, Ahmedabad, India.
E-mail: zooldeptgu@satyam.net.in
Keywords: Fluoride effects; Melatonin as antioxidant; Mouse ovary; Ovarian oxidative stress.

STUDY OF INDOMETHACIN AND CALCIUM GROUP MEDICATIONS IN ADULTS CHRONICALLY EXPOSED TO FLUORIDE USING FINGER AND TOE NAILS AS BIOMARKERS

In Akodiya and Laxmipura villages of Tonk District, Rajasthan, India, having water fluoride levels ranging from 2.01 to 6.80 ppm, fluoride level changes in finger and/or toe nails and serum were studied after administration of the following medications: Group I – sustained release Indomethacin (75mg/day); Group II – calcium (500 mg), vitamin D (800 IU) and vitamin C (500 mg) per day. Nails were clipped and collected during periodic visits of 35 randomly selected chronic fluorosis cases while supplying the drugs. Blood & urine samples were also collected as the subjects permitted. Procured nails were individually pyrohydrolysed by putting them in furnace at 400^{o}Cfor 12 hr; afterward, 5 mg of each aliquot was dissolved in 25% HCl and then buffered with 10 mL of TISAB for measurement of fluoride by an ion selective electrode (9609 BNWP Thermo Scientific Orion). Following administration of the medications, finger and/or toe nails showed significant reduction in fluoride level commensurate with serum fluoride level, suggesting beneficial effects of these treatments in chronically fluoride exposed villagers.

Authors: **Sankhala Sohan Singh**, Rakesh B, Harshwal RK.
Correspondence: Department of Orthopaedics, SMS Medical College & Hospital, Jaipur, Rajasthan, India.
E-mail: drsankhalass@gmail.com
Keywords: Fluorosis patients; Fingernail fluoride; Toenail fluoride; Tonk District, Rajasthan, India.

GASTRO-INTESTINAL DISCOMFORTS IN HUMAN POPULATION EXPOSED TO FLUORIDE IN DRINKING WATER

Rajasthan is one of the fluoride endemic states of India where fluoride concentration in groundwater ranges from 0.4 to 12.5 ppm. The maximum permissible range for fluoride in drinking water has been set at 1.0–1.5 ppm by WHO. A human health survey was carried out in villages of Sanganer Tehsil, Rajasthan, to examine the gastro-intestinal discomfort caused by high fluoride in drinking water. The study was carried out by interviewing persons living in the study area wherein the fluoride concentration was low (<1.0 ppm), intermediate (1.0–1.5 ppm) and high (>1.5 ppm). A total 10 villages were selected from each group the Sanganer Tehsil to study gastro-intestinal discomfort, viz. loss of appetite, bloated feeling, stomachache, diarrhea, and constipation. The results revealed that, in children in the low-fluoride villages, no cases of gastro-intestinal discomfort were found and very few cases in the intermediate fluoride villages. However, among the children in the high fluoride villages, loss of appetite was present in 14.40%, bloated feeling in 8.47%, stomachache in 5.08%, diarrhea in 1.69%, and constipation in 1.69%. Few cases of gastric discomfort in adults were observed in the low and intermediate fluoride villages, whereas in the high fluoride villages among adults, loss of appetite was present in 40.21%, bloated feeling in 34.02%, stomachache in 17.52%, diarrhea in 5.67%, and constipation in 16.49%. The prevalence of gastric discomfort in children and adults, especially in the low and optimum fluoride area may be due to poor nutrition and low economic status. However, the maximum cases of gastric discomfort were observed in children and adults in the high fluoride villages, which may be correlated with poor nutrition as well as high fluoride concentration in drinking water. When fluoride along with water reaches the stomach, it forms hydrofluoric acid, which is highly corrosive, and hence the stomach and intestinal lining (mucosa) is destroyed with loss of microvilli. Thus more gastric discomforts were observed in both children and adults in the high fluoride villages.

Authors: **Sharma Jashwanti D**, Jain P, Sohu D.
Correspondence: Reproductive Physiology and Environmental Toxicology Laboratory, Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, India
E-mail: jaishree_ajay@yahoo.co.in
Keywords: Bloated feeling; Constipation; Diarrhea; Fluoride in water; Gastrointestinal discomfort; Sanganer Tehsil, Rajasthan; Stomachache; Water fluoride.

PREVALENCE OF NEUROLOGICAL MANIFESTATIONS IN LOCAL HUMAN POPULATIONS EXPOSED TO FLUORIDE IN DRINKING WATER

Excess fluoride intake through food, water, and other sources causes dental, skeletal, and non-skeletal fluorosis in human as well as in animals. A health survey was conducted in a human population exposed to low (<1.0 ppm), intermediate (1.0–1.2 ppm), and high (1.2–6.4 ppm) fluoride concentration in the drinking water of villages in Sanganer Tehsil, Rajasthan, India. A total of 2691 subjects were personally interrogated and the data were recorded for the villages having the three fluoride concentrations. A total 1145 children aged 12–18 years and 1546 adults aged >18 years were interviewed for various neurological ailments, viz., headache, insomnia, lethargy, polyuria, and polydipsia. Among children in the low and intermediate fluoride villages, there were no neurological manifestations, but in the high fluoride villages 9.48% of the children were found to have headache, 1.21% insomnia, and 3.23% lethargy. No cases of polyuria and polydipsia in children were reported. Among adults, in the low fluoride villages1.56% reported suffering from headache, 2.51% in the intermediate fluoride villages, and 26.96% in the high fluoride villages. For insomnia the figures were 1.17%, 1.12%, and 24.74%, respectively. For lethargy they were 2.73%, 3.63%, and 23.70%. Polyuria and polydipsia were absent in the low and intermediate fluoride villages, but in the high fluoride villages their occurrence was 0.74% and 1.19%, respectively. The severity of the ailments increased with the increasing fluoride concentration in drinking water, making survival the toughest task for the inhabitants. As the data indicate, among children in the endemic villages, the most severe malady was headache, followed by lethargy and insomnia, without any cases of polyuria and polydipsia. Among adults, the percentages with headache, insomnia, and lethargy were very small in both the low and intermediate fluoride villages, whereas about 25% of adults in the endemic villages reported these neurological manifestations, clearly pointing to fluoride

maximum cases were reported in fluoride endemic area, clearly indicative of role of fluoride in them. In contrast to children, polyuria and polydipsia were also observed in adults in the high fluoride villages.

Authors: **Sharma Jashwanti D**, Jain P, Sohu D.
Correspondence: Reproductive Physiology and Environmental Toxicology Laboratory,
Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur. India.
E-mail: jaishree_ajay@yahoo.co.in
Keywords: Fluoride in water; Headache; Insomnia; Lethargy; Neorological manisfestations; Polydipsia; Polyuria; Sanganer Tehsil, Rajasthan, India.

MITIGATION OF FLUORIDE TOXICITY BY VITAMIN C, VITAMINS C+D, AND CALCIUM IN ALBINO RATS (*RATTUS NORVEGICUS*)

Healthy male albino rats (*Rattus norvegicus*) weighing between 200 and 250 g were divided into five groups and studied for 60 days. Group I rats received only tap water (0.96 ppm F); Group II rats were given 5.8 ppm F water collected from Watika Village of Sanganer Tehsil in Rajasthan; Group III rats were treated with vitamin C (6 mg) daily for 60 days along with the 5.8 ppm F water; Group IV rats were administered vitamins C (6 mg daily) + vitamin D (6 mg once a week) and calcium (6 mg/day) along with the 5.8 ppm F water; Group V rats were given the 5.8 ppm F water for 60 days and then the 0.96 ppm F tap water for the next 30 days. After the respective treatments, the animals were sacrificed, autopsied, and blood was extracted. The liver, kidney, lung, heart, and adrenal glands were excised, weighed, and used for tissue biochemistry. The results revealed that exposure of rats to 5.8 ppm F water for 60 days decreased body and organ weights (liver, kidney), total RBC, hemoglobin, hematocrit, concentration of protein, ascorbic acid, cholesterol, and glycogen as compared to control rats. The enzyme activity of acid and alkaline phosphatase decreased in the liver, but increased in the kidney. The serum concentrations of protein, cholesterol, triglycerides, and glucose were reduced, whereas electrolytes (Na^+, K^+, and Cl^-) increased significantly after fluoride water treatment. Serum alkaline phosphatase activity remained depressed, whereas SGOT and SGPT were elevated above the control level. On the other hand, exogenous feeding of vitamin C and vitamins C+D and calcium along with the 5.8 ppm F water for 60 days resulted in altered biochemical parameters almost to control level, indicating beneficial role of vitamins and calcium in fluorosed animals. However, when the withdrawal period was extended it was not enough to overcome the fluoride induced stress in rats in all the hematology, tissue, and serum parameters. There was better recovery in animals treated with vitamins C+D and calcium in comparison to only vitamin C treatment along with the F water. Therefore, the data suggests that in fluoride toxicity, vitamins C+D plus calcium can be a better option to maintain normal body physiology.

Authors: **Sharma Jashwanti D**, Jain P, Sohu D.
Correspondence: Reproductive Physiology and Environmental Toxicology Laboratory,
Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur. India.
E-mail: jaishree_ajay@yahoo.co.in
Keywords: Biochemical parameters; Calcium antidote; Fluoride toxicity in rats; Hematology; Serum enzymes (SGOT, SGPT); Toxicity mitigation; Vitamins C and D.

TOOTH DEVELOPMENT, DENTAL FLUOROSIS, AND AMELOGENESIS IMPERFECTA

Tooth formation involves a series of developmental events that genetic defects can disrupt at various stages. Early tooth development is characterized by epithelial-mesenchymal interactions and genetic anomalies in signaling systems, and transcription factors lead to tooth agenesis or supernumerary teeth. Later, during extracellular biomineralization, genetic anomalies specifically interfere with dentin, enamel, and cementum deposition, as each of these tissues makes use of its own set of extracellular matrix molecules. Among the three mineralized tissues in teeth, dental enamel relies upon the largest number of specialized genes for its formation. Based upon the dental phenotype and pattern of inheritance, 14 clinical forms of non-syndromic amelogenesis imperfecta (AI) are recognized, but there may be more. In practice it is difficult to pigeonhole an AI pattern into this classification. Of particular interest is AI that looks like dental fluorosis and can be mistaken for it. However, it is inherited, not acquired, and the teeth readily develop caries. We hypothesize that fluoride binds to a protein material that is specialized for dental enamel formation and interferes with its function. Defects in the gene

encoding this protein cause an AI phenotype that mimics dental fluorosis.

Author: **Simmer James P.**
Correspondence: Department of Biologic and Materials Sciences, University of Michigan School of Dentistry, Ann Arbor, MI, USA.
E-mail: jsmmer@umich.edu
Keywords: Amelogenesis imperfecta; Biomineralization; Dental fluorosis; Dental genetics; Genetic anomalies; Tooth development.

PREVALENCE OF FLUOROSIS IN KACHHARIADIH AND MUSLIMTOLA VILLAGES OF NAWADAH DISTRICT BIHAR: A CASE STUDY TO MITIGATE SUFFERINGS

Kachhariadih and Muslimtola villages of Nawadah district, Bihar, India have been reported to have visible cases of skeletal and dental fluorosis. These villages are inhabited by poor schedule caste people, and ground water is the only source of drinking water. The Postgraduate Department of Environmental Sciences, A. N. College, Patna formed a team of teachers and students to survey the health status of the people there and to undertake a detailed physico-chemical analysis of the drinking water from all the bore wells in order to correlate the occurrence of fluorosis with the fluoride level in the drinking water. It was also planned to approach some philanthropic organization to adopt these villages for supply of fluoride free drinking water to the villagers. A health checkup team of doctors led by Dr V S Singh, former Director-in-Chief, Health Services, Bihar, found that about 60–70% of the people of all age group and of both genders suffer from skeletal and dental fluorosis. Almost the entire population complained of pain in bone and joints, and of general fatigue. Physico-chemical analysis of water samples from all the hand pumps showed presence of fluoride between 2.2 ppm and 5.9 ppm, well above the maximum permissible limit of 1.5 ppm. However, the water samples were free from arsenic, iron, and nitrate contamination. With few exceptions, the pH, conductivity, alkalinity, hardness, turbidity, total dissolved solids, etc. were within the permissible limits. When these concerns were brought to kind attention of authorities of the Patna branch of Mahindra & Mahindra Ltd., a Corporate Sector of high repute in India, they procured and installed three hand pumps with fluoride removal attachments. This humanitarian gesture has ensured a regular supply of low-fluoride drinking and cooking water for the entire population of these villages. The Department of Environmental Sciences, A. N. College, Patna has assumed responsibility for monitoring, cleaning, and recharging these defluoridation units at regular intervals. The Department also has future plans to extend the socio-scientific study to the entire Rajauli subdivision, which has suspected cases of fluorosis in other villages as well.

Authors: **Singh Bihari**,[a] Singh KK,[b] Kumar A,[a] Kumar N.[a]
Correspondence: [a]Department of Environmental Sciences, A. N. College, Patna, India; [b]Department of Chemistry.
E-mail: bihari_singh2001@yahoo.com
Keywords: Bihar, India; Bone pain; Dental fluorosis; Fluoride illness; Joint pain; Kachhariadih village; Muslimtola village; Nawadah district, Bihar, India; Skeletal fluorosis.

CHRONIC FLUORIDE TOXICITY FROM FLUORIDATED DRINKING WATER

The case is presented of a 67-year-old woman who repeatedly had multiple symptoms when using her municipal fluoridated drinking water (FDW) source, with 0.85 ppm F, but became largely symptom free when using spring water (SW) with 0.1 ppm fluoride. Several cycles occurred of developing symptoms after 1–4 weeks of exposure to FDW and remission of most symptoms within 1–2 weeks following the use of SW. She had used predominantly FDW for 40 years and had experienced some symptoms for several years. She became aware of the relationship between being symptomatic and using FDW after being relatively symptom free when living away from Dunedin in areas that did not have FDW and then experiencing a return of her symptoms after recommencing on FDW. Her symptoms included: anorexia; nausea; constipation; headaches; a feeling of nervousness; palpitations; impaired balance; dizziness; nocturnal toe paraesthesiae; oral and pharyngeal dryness; increased water consumption; visual scotomata; tiredness; right upper abdominal pain; jaw pain; pain in her lower-mid thoracic spine; a bluish spot on the medial aspect of her left arm that came and went, was about 10 mm in diameter, was not a bruise due to an injury, and did not turn yellow or brown; weakness in her arms and poorer performance at sport involving her arms; and having brittle nails that broke easily. On 30 July 2007, she commenced a trial of avoiding FDW by using SW, not using

fluoridated toothpaste, and not drinking tea. On 13 August 2007 she reported that her balance was ìso much betterî and that she was less tired than before. She reported decreases in the nausea, constipation, pain in her mid-lower thoracic spine, the right upper abdominal pain, and the tingling in her legs at night, which she had had for a long time and which had been getting worse. She had complete relief from the headaches, nervousness, palpitations, jaw pain, mouth and throat dryness, visual difficulties, and lack of energy. Her ability to play sport improved, and she was better able to anticipate her opponents' moves. She said that the improvement had been "remarkable." The symptoms and the pattern of improvement are consistent with those described for chronic fluoride toxicity.

Author: **Spittle Bruce.**
Correspondence: 727 Brighton Road, Ocean View, Dunedin 9035, New Zealand.
E-mail: spittle@es.co.nz
Keywords: Chronic fluoride toxicity; Fluoridated drinking water; Reversible fluoride illness.

FLUORIDE – A GROWING PROBLEM OF MODERN LIFESTYLE?

There is a growing body of evidence that chronic disorders like diabetes, stroke, and cerebrovascular diseases but also neurological diseases like attention deficit syndrome and dementia are promoted or even due to human lifestyle. The lack of exercise, micronutrients from plants, essential fatty acids from plants and animals, and vitamin D – the solar hormone and too much social stress. On the other hand, environmental changes and exposure to toxic molecules like fluoride and aluminum are also involved. This paper tries to give a bridging link between the two aspects. It is well documented, that as long as metabolic stress (including exposure to toxins) is opposed by an intact supply of (above all nutritional) resources for the cell metabolism, the metabolic balance will remain stable. Unbalance will make the single cell fail, the function of the organs weak and the body ill. Unfortunately, modern lifestyle and urbanization support many negative factors. For example the production and distribution of fluoride as well as aluminum products in every day life stresses cell metabolism extraordinarily. In addition, processed food cannot deliver the necessary micronutrients. An impaired cellular reaction is programmed. The most important function which decompensates early on is our immune system. The growing knowledge about fetal programming and nutrition as one of the most relevant parameters in that context clearly states that it is not the genes but the epigenetics that have the chance to become the key to our health problems. It is hard to believe, but obviously effective is a nutritional proposal that has been made by our late colleague NJ Chinoy from India: just eating watermelon in endemic fluorosis areas helps to compensate. *Conclusion:* Scientific effort should not focus on toxic effect of environmental factors like fluoride and aluminum only but also on measures how to compensate for by simple means in every day life! On the other hand, unnecessary stress factors to the cell metabolism like fluoride in drinking water have to be eliminated.

Author: **Spitz Jörg.**
Correspondence: Society for Medical Information and Prevention, Kornweg 13, 65388 Schlangenbad, Germany.
E-mail: info@mip-spitz.de
Keywords: Chronic diseases; Compromised lifestyle; Environmental fluoride; Micronutrients; Nutritional deficiencies.

ALTERATIONS AND MECHANISM IN THE ARTICULAR CARTILAGE TISSUE OF RATS CAUSED BY COMBINED EFFECTS OF FLUORIDE AND ALUMINUM

The epidemiological characteristics of brick-tea type fluorosis in China are different from the other types of endemic fluorosis. In this research, two main components of brick tea were selected for study, namely fluorine and aluminum. The purpose was to examine lesions of the articular cartilage tissue caused by fluorine and aluminum and to explore the mechanism involved. Eighty Wistar rats were divided into 4 groups, consisted of control group (tap water), fluoride group (100 mg F^{-}/L), aluminum group (200 mg Al^{3+}/L) and F+Al group (100 mg F^{-}/L+200 mg Al^{3+}/L). After 3 months, all the rats were sacrificed. An optical microscope and a transmission electron microscope (TEM) were used to observe the articular cartilage cells and the collagen fibers. Type II collagen and the proteoglycan (PG) in cartilage were detected by histological special staining. The mRNA and protein expressions of Matrix metalloproteases-13

(MMP-13) and aggrecanase-4 (ADAMTS-4) were detected by reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry technology. The urinary hydroxyproline (HPY) was detected by biochemical methods. In the F+Al group,compared with the fluoride group, the damage to cartilage cells and collagen fibers were increased, the urine excretion of HPY increased, less collagen and PG contents existed in the articular cartilage, both the mRNA and protein expression of MMP-13 did not increase significantly. Both the mRNA and protein expression of ADAMTS-4 increased. In summary, fluorosis lesions in the articular cartilage tissue caused by the combination of fluoride and aluminum occurred more easily than with fluoride alone.

Author: **Sun DianJun.**
Address: The Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention. Harbin Medical University, Harbin, China.
E-mail: hrbmusdj@163.com
Keywords: Aluminum and bone; Articular cartilage; Brick-tea fluorosis; Collagen; Fluoride and bone; Rat bone study.

FLUORIDE ACTION ON HARD AND SOFT TISSUE MATRIX MOLECULES: IMPLICATIONS FOR THE UNDERSTANDING OF FLUOROSIS (CONFERENCE KEYNOTE ADDRESS – DAY ONE)

When fluoride ingestion exceeds certain limits, soft tissue ligaments as well as bones and teeth are adversely affected. The ìlimitsî refer to ìtoleranceî and/or ìsusceptibilityî that vary from individual to individual depending upon physiological and environmental factors. The 200 odd bones in the body are either of the cancellous (spongy) or cortical (compact) type. Both types of structures co-exist in certain parts of the same bone. These bones are different in their inorganic and organic constituents. Due to copious blood supply to the spongy bone, more fluoride accumulates compared to the compact bone. The pathological lesions induced by fluoride in these two types of bones are also distinctly different.

Bone matrix whether cancellous or cortical is interwoven with collagen and non-collagen protein, i.e. glycoprotein and proteoglycans. Both the constituents are adversely affected. The collagen, the bulk of the matrix of both bone and tooth are of poor quality. In bone, there is reduction in collagen content, reduction of hydroxylation of amino acids proline and lysine, reduction in collagen cross-link precursors and reduction in collagen bound collagenase activity. The content of glycosaminoglycan (GAG, the carbohydrate side chain in the collagen fiber) is altered significantly in cancellous bone. The process of sulphation is increased. Enhanced sulphation is reflected through sulphated GAG and one of its isomers. The most significant abnormality detected, is the high content of Dermatan sulphate in cancellous bone but not in cortical.

Dermatan sulphate is present in embryonic bone but is non-detectable in normal mature bone. Dermatan sulphate upon accumulation in cancellous bone leads to cartilageous loci formation in the matrix. These are referred to as osteoids. Osteoid formation resemble chondrocytes. The intercellular matrix of osteoids are loaded with sulphated GAG, i.e. dermatan sulphate. Due to dermatan sulphate formation, mineralization of the matrix in unlikely to take place. The cartilageous lesions persist rendering the bone week, resulting in multiple fracture in patients with fluorosis.

Fluorosed human teeth also have reduction in total glycosaminoglycan (GAG) disaccharide molecules and an increase in dermatan sulphate content. Increased dermatan sulphate may be an important factor in clinical manifestations of dental fluorosis. High concentrations of dermatan sulphate induce demineralization as observed in the cartilageous lesions of the cancellous bony regions. In the tooth matrix, calcium content is lost resulting in hypomineralized loci, leading to pitting, fissures, cavity formation, and chipping. The reduced calcium content in human fluorosed teeth substantiates the above observation of enhanced dermatan sulphate formation in human tooth matrix.

Fluorosis, though conventionally known to be a disease of bones and teeth, does not spare soft tissues. The focus is on ligaments, a fine connective tissue, closely associated with bone and articular surfaces. Fluoride accumulates in ligaments, reduces organic constituents; enhances

inorganic constituents such as calcium, phosphorus, magnesium and Ca/P ratio but pyrophosphate content is reduced. Reduced pyrophosphate content, provides a favorable milieu for mineralization as its presence is an inhibitor for the process of mineralization. Ligaments are severely affected upon fluoride ingestion. Calcified ligaments are detected radiologically in fluorosis prior to any other soft tissue calcification. Calcification of ligaments in the articular surfaces of joints and of the vertebral column leads to restricted, painful movements and is used as a parameter for diagnosis of fluorosis.

In conclusion, the derangements in molecular configuration of sulphated GAG and increase in dermatin sulphate in bone and tooth matrices and reduction of the same in soft tissue ligaments, which are diametrically opposite molecular events, appear to be one of the major issues. The opposing molecular biochemical events with focus on sulphated GAGs and how to reverse to normalcy shall remain a challenge for a long time to come. Therefore amelioration of the disease is based on other soft tissue derangements which are reverted to normal much before the irreversible damage takes place in bone and ligament.

Author: **Susheela AK.**
Correspondence: Fluorosis Research and Rural Development Foundation, B-1 Saransh, 34 Indrapasthra Extension, Delhi – 110 092, India.)
E-mail: susheela@bol.net.in
Keywords: Dental fluorosis; Dermatin sulphate; Glycosamino glycan; Ligament calcification; Skeletal fluorosis; Sulphated glycosamino glycans Tissue matrix molecules.

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CLINICAL MANIFESTATIONS AND DIAGNOSIS OF FLUOROSIS: MANAGEMENT OF PATIENTS (CONFERENCE KEYNOTE ADDRESS – DAY TWO)

Fluorosis, one of the most complicated diseases that has baffled the medical fraternity because it does not lie in the domain of any one medical discipline or speciality. Therefore it may be appropriate to commence this presentation with a new definition of Fluorosis: “a neglected, untreatable disease in the public health domain; affects multiple tissues, organs and systems; afflicts young and old without gender discrimination, no treatment but easily preventable through practice of interventions; upon diagnosis at early stages; recovery within a fortnight.”

Fluorosis occurs in three forms: a) Skeletal, b) Dental, and c) Non-skeletal. Considerable importance is attached to the Non-skeletal form of the disease because manifestations arise at very early stages. Suspecting fluorosis at an early stage is a pre-requisite for prevention and control of the disease.

First and foremost it is very important to obtain a good clinical history. Based on this history, leads are often obtained to suspect fluorosis. The suspicion then requires confirmation through diagnostic tests. Once the disease is confirmed, it is necessary to retrieve more detailed information, viz., the source of fluoride entry. It could be water, food, dental products, drugs, and/or industrial emission.

Two major interventions to practise are: a) withdrawal of fluoride source(s) by which the progression of the disease would be arrested; b) promotion of a nutritive diet with adequate intake of calcium, iron, folic acid, vitamins C and E, and other antioxidants through dairy products, vegetables, and fruits. By this intervention, the damage caused to various tissues is minimized and rectified. Pharmaceutical products, i.e., synthetic drugs, should be avoided as recovery is faster through consumption of nutrients through dietary sources. The management of the patient should commence by monitoring and assessing the impact of interventions. Before dealing with monitoring, one ought to know the diagnostic tests.

Diagnostic tests: For the correct diagnosis of Fluorosis certain tests need to be carried out. Most importantly, fluoride needs to be tested in a) blood, b) urine, and c) drinking water, besides a fore-arm x-ray to detect any signs of ligamental calcification. It is also necessary to test for d) hemoglobin, as it is used as an index for assessing health improvement, during monitoring and impact assessment. If the disease is suspected in children, certain additional tests are required, viz., the T_3, T_4 and TSH besides iodide in urine.

*Differential diagnosis:*Fluorosis may have overlapping clinical manifestations with other disorders, viz.: arthritis, spondylitis, osteoporosis and osteomalacia, irritable bowel syndrome (non-ulcer dyspepsia, NUD), diabetes mellitus, iron deficiency anemia, iodine deficiency disorders, and rickets in children.

The dilemma facing a physician of clinical manifestations overlapping with other disorders mentioned above can be resolved by conducting the appropriate diagnostic tests. When enhanced fluoride levels in body fluids are detected the disease is likely to be Fluorosis. The confirmatory test comes when the source of fluoride is withdrawn and the health complaints disappear in a matter of a few days. This aspect will be discussed in greater detail during the presentation.

Monitoring and Impact Assessment: This should be done within 15–20 days after introducing the interventions by determining the fluoride levels in serum and urine. The levels should decrease and he hemoglobin level increase. After 8 or 12 weeks these tests should be repeated. This is a confidence-building exercise. Once the patient understands the necessity to keep away from fluoride, he/she would know how to look after the diet, and recurrence is very rare, unless fluoride is ingested unknowingly.

Author: **Susheela AK.**
Correspondence: Fluorosis Foundation of India, Delhi, India. (Use fuller address? Fluorosis Research and Rural Development Foundation, B-1 Saransh, 34 Indrapasthra Extension, Delhi – 110 092, India.)
E-mail: susheela@bol.net.in
Keywords: Clinical manifestations of fluorosis; Differential diagnosis; Non-skeletal fluorosis; Reversible fluoride intoxication; Tests for non-skeletal fluorosis.

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ENDOCRINE EFFECTS FROM FLUORIDE EXPOSURE: IMPLICATIONS FOR BONE AND BRAIN

The US National Research Council (2006) concluded that fluoride is an endocrine disruptor: normal endocrine function or response in humans is altered by exposure to fluoride. The major effects examined to date include alterations of thyroid function, calcium metabolism, pineal function, and glucose metabolism. Both direct and indirect mechanisms of action appear likely, including direct stimulation or inhibition of hormone secretion, indirect stimulation or inhibition of hormone secretion, and inhibition of peripheral enzymes necessary for activation of a normal hormone. People with reduced thyroid function are at higher risk of clinical hypothyroidism and cardiac disease, among other things. In particular, reduced thyroid function in a pregnant woman, even if asymptomatic, can result in lowered IQ of her offspring. Fluoride exposure results in an increased requirement for calcium and a higher risk for calcium deficiency and consequent problems. Altered pineal function could impact a number of bodily processes or systems, including calcium metabolism, bone growth, and central nervous system function. Fluoride exposure appears to result in increased blood glucose levels or impaired glucose tolerance in some individuals. Also, diabetic individuals often have higher than normal intake of drinking water and consequent higher fluoride exposures. Many endocrine effects are associated with fluoride intakes in the range typically observed in the U.S. In general, adverse effects are more likely in the presence of dietary deficiencies (e.g., iodine or calcium). The increasing "epidemics" of thyroid disease, diabetes, and other health problems in the U.S. warrant examination of the possible role of nearly universal exposure of the population to fluoride. *Reference:* National Research Council (2006). Fluoride in Drinking water: A Scientific Review of EPA's Standards. Washington, DC: The National Academies Press.

Author: **Thiessen Kathleen.**
Correspondence: SENES Oak Ridge, Inc., Center for Risk Analysis, 102 Donner Drive, Oak Ridge, TN 37830, USA
E-mail: kmt@senes.com
Keywords: Calcium metabolism; Endocrine disruptor; Fluoride toxicology; Glucose metabolism; Thyroid function.

***IN VITRO* EFFECT OF MELATONIN AND CURCUMIN ON FLUORIDE TOXICITY IN HUMAN LYMPHOCYTES**

Fluoride is a widely-known water contaminant causing a serious health threat in India and the world over. The present study was aimed to study the role of melatonin and curcumin in mitigating the genotoxic effects of fluoride *in vitro* in human lymphocyte cell cultures. The study consisted of a control group with no treatment, fluoride group with 3.4 μM NaF and antioxidant groups where melatonin (0.2 mM) and curcumin (7.7 μM) alone were added to the cultures with and without fluoride. Ethyl methane sulphonate (EMS) was used as the positive control. All treatments were given for 24 hr. The cultures were then harvested for analysis of sister chromatid exchanges (SCEs), cell cycle proliferative index (CCPI), and comet tail length.

The data for all the groups was compared with control and that of fluoride group was compared with antioxidant groups. The significance was evaluated using student's t test. The importance of the data will be discussed.

Authors: **Tiwari H**, Rao MV.
Correspondence: Zoology Department, University School of Sciences, Gujarat University, Ahmedabad, India.
E-mail: zooldeptgu@satyam.net.in
Keywords: Curcumin; Genotoxic effects; Lymphocyte cell cultures; Melatonin.

DEFLUORIDATION BY BACTERIOGENIC IRON OXIDES: SORPTION STUDIES

At concentrations above 1 mg/L, fluoride in drinking water can lead to various forms of dental and skeletal fluorosis, a disease that causes mottling of the teeth, calcification of ligaments, crippling bone deformities and many other physiological disorders that can, ultimately, lead to death. Conservative estimates are that fluorosis afflicts tens of millions of people worldwide. As there is no effective treatment for fluorosis, especially in the more advanced s tages, prevention is the only means of controlling the disease. While numerous defluoridation techniques have been explored, no one method has been found to be both effective and inexpensive enough to implement widely. Our research began in India, with a large-scale geochemical study of the groundwater in a fluoride-contaminated region of Orissa. Having developed a clear understanding of the geochemical relationships that exist between fluoride and other parameters present in an affected area, as well as the complex relationships that obtain between those other parameters that can, in turn, impact the presence of fluoride, we began investigating certain remediation scenarios involving iron oxides. A common approach to remediation involves the partitioning of fluoride from groundwater by sorption onto a variety of materials, one of the most effective of which is iron oxide whose surface area acts as a scavenger for fluoride. In the presence of iron oxidizing bacteria, the oxidation rate of iron has been shown to be ~6 times greater than in their absence; fluoride should, therefore, be removed from an aqueous environment by bacteriogenic iron oxides (BIOS) much more quickly than by abiotic iron oxides. Most recently, sorption studies have been conducted using both BIOS and synthetic hydrous ferric oxides in order to compare the behavior between biotic and abiotic iron oxides. From these studies we are deriving sorption isotherms that will allow us to compare how much fluoride will be removed by sorption from BIOS versus synthetic iron oxides. Sorption affinity constants have also been determined, which will allow for the prediction of fluoride removal in a wide variety of groundwater systems.

Authors: **Tokaryk Kerry E**, Ferris F Grant.
Correspondence: Geology Dept., Earth Sciences Centre, University of Toronto, Toronto, ON, Canada M5S 3B1.
E-mail: kerry.tokaryk@utoronto.ca
Keywords: Bacteriogenic iron oxides; Fluoride and iron oxides; Water defluoridation.

EFFECTS OF ORAL ADMINISTRATION FLUORIDE ON VIABILITY AND SPLENIC CYTOKINE PRODUCTION IN MALE HEREDITARY NEPHROTIC MICE

The ICR-derived glomerulonephritis (ICGN) mouse is a strain of mice with a hereditary nephrotic syndrome. In the previous ISFR conference, we reported the effects of fluoride administration for 1 month on the viability of female ICGN mice. All female ICGN mice exposed to 150 ppm of fluoride were dead before the end of administration. In this study, we examined the effects of fluoride on splenic cytokinine production and the viability of male ICGN mice with the nephrotic syndrome. Male ICGN mice (n=8 per group) with the nephrotic syndrome were administered fluoride at 0, 25, 50, 100, and 150 ppm in their drinking water for 1 month. The development of the nephrotic syndrome in each mouse was confirmed by BUN. Male ICR mice, the type of progenitors of ICGN mice, were also exposed to fluoride at 0 and 150 ppm in their drinking water for 1 month. The body weights of mice were checked every day. The concentrations of TNF alpha and IFN-gamma in the supernatant of the activated splenic macrophages and T cells and B cells from mice were determined by ELISA. All ICGN mice exposed to 150 ppm of fluoride and one mouse exposed to 100 ppm were dead before the end of one month of F administration. The mean body weight of the ICGN mice exposed to 150 ppm at their death was 16.91g, and the body weight of the dead ICCN mouse exposed to 100

ppm was 12.61 g. There were no significant differences in body weight among the ICGN groups at the end of administration. The mean body weights were 29.01 g for the 0 ppm, 28.73 g for the 25 ppm, 26.68 g for the 50 ppm, and 25.59 g for the 100 ppm group. There were also no significant differences in body weights between the groups of ICR mice. There were no significant differences in any cytokines among the ICGN groups or between the ICR groups. It was confirmed that the administration of 150 ppm fluoride in the drinking water for 1 month is fatal for mice with a nephrotic syndrome.

Authors: **Tsunoda M**,[a] Tsunoda H,[b] Itai K[c] Zhang Y,[a,d] Kodama Y,[e] Sugita-Konishi Y,[e] Katagiri H,[f] Hosokawa M,[a] Sugaya C,[a] Inoue Y,[a] Miki T,[a] Kudo Y,[a] Satoh T,[a] Aizawa Y.[a]
Correspondence: Prof Masashi Tsunoda, [a]Dept. of Preventive Medicine and Public Health, Kitasato University School of Medicine, Kitasato 1-15-1, Sagamihara, Kanagawa 228-8555, Japan; [b]Iwate Medical University; [c]Dept. of Preventive Medicine and Public Health, Iwate Medical University; [d]Fukushima Rehabilitation Academy; [e]National Institute of Health Science;
[f]Dept. of Public Health, Kitasato University School of Allied Health Sciences
E-mail: mtsunoda@med.kitasato-u.ac.jp
Keywords: Hereditary nephrotic mice; ICR mice; ICGN (glomerulonephritic) mice; Splenic cytokinine production.

EFFECTS OF NUTRITION AND FLUORIDE ON SKELETAL DEVELOPMENT IN RABBITS

Epidemiological investigation has demonstrated that skeletal fluorosis in different countries varies owing to regional disparity. It is mainly prevalent in underdeveloped malnutrition areas concomitant with thyroid dysfunction, especially in high fluoride regions with protein (Pr) and calcium (Ca) deficiency. The thyroid gland, one of the most sensitive organs in its functional responses to excessive amounts of fluoride, exhibits important effects on skeletal growth, development, and turnover as well as the metabolism of virtually every body tissue in both children and adults. Many studies also indicate that Pr and/or Ca supplementation can help alleviate F-induced osteoporosis and thyroid dysfunction. That being the case, how does high fluoride affect skeletal development by impaired thyroid function, and how does protein or calcium counteract skeletal fluorosis? The aim of this research is to investigate the effects of Pr and/or Ca supplementation on bone development and thyroid function in Pr and Ca-deficient (malnutrition) rabbits exposed to fluoride. Eighty 30-day-old New Zealand rabbits were divided randomly into four groups of twenty, maintained on distilled water and fed the following diets for 120 days: (1) a malnutrition control (MC) diet (8.58% Pr, 0.49% Ca); (2) the MC diet plus HiF (200 mg F ion/kg from NaF); (3) a Ca deficient MC diet plus HiPr+HiF (0.46% Ca, 18.41% Pr, plus HiF); and (4) a Pr deficient MC diet plus HiCa+HiF (2.23% Ca, 8.35% Pr, plus HiF). After the 30, 60, 90, and 120 days, the serum bone gla-protein (BGP) and thyroid hormone were measured using radioimmunoassay kits. The femoral morphology was observed by X-ray and scanning electron microscopy. The results indicated that the HiF diet markedly increased changes in TSH and thyroid hormone levels. Supplemental Pr and/or Ca were able to counteract the toxic effects of high F intake on the unsound bone development and decreased TSH change and alleviated the thyroid dysfunction. In conclusion, fluoride exhibits obvious toxic effects on thyroid function and skeletal development, resulting in disordered secretion of thyroid hormone and accelerated bone turnover. Pr and Ca supplementation was able to counter the fluoride toxicity, reducing changes of thyroid hormone and relieving and stabilizing bone development. Macroscopically, Pr appeared to be superior to Ca.

Authors: **Wang Jinming**,[a] Cheng Xiaofang,[b] Niu Ruiyan,[a] Wang Jundong.[a]
Correspondence: Prof Jundong Wang, [a]Shanxi Key Laboratory of Ecological Anima| Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi, PR of China; [b]Information College of Shanxi Agricultural University, Jinzhong, Shanxi, PR of China.
E-mail: wangjd@sxau.edu.cn
Keywords: Bone metabolism; Calcium supplementation; High fluoride intake; Rabbit bone development; Nutritional deficiency; Protein supplementation; Thyroid hormone disturbances.

FLUORIDE, ARSENIC, AND SELENIUM LEVELS IN THE ENVIRONMENT AND THE POPULATION OF THE ANKANG AREA, SHAANXI PROVINCE, CHINA

In the Ankang area of Shaanxi Province, China, the inhabitants burn raw coal for daily cooking and indoor heating. For this study, in order to measure the levels of fluoride, arsenic, and selenium in the environment and in the local inhabitants, 198 persons were randomly selected from two villages in the Ankang area, while, for the control group, 88 persons were also

randomly selected in areas where raw coal is not used as fuel. For the analysis of arsenic and selenium, a graphite furnace atomic absorption spectrophotometer was used; for the determination of fluoride, a fluoride ion-selective electrode was used. Samples representing the environmental situation were taken from coal, drinking water, soil, corn, and pepper, and samples of serum, hair and urine as indications of the population exposure were taken from residents. In the Ankang area, the fluoride levels in coal, soil, corn, pepper, urine, hair, and serum were all significantly higher than those from the control site or control group, with 1757.5±634.6 mg/kg in coal (10 times higher than that in control site), 1.5±1.3 mg/kg in dissolved soil, 4.8±1.6 mg/kg in corn, 31.8±21.8 mg/kg in pepper, 2.7±1.9 mg/L in urine, 24.5±21.8 mg/kg in hair, and 0.3±0.2 mg/L in serum, respectively. The arsenic levels in all samples except in drinking water samples from the Ankang area were significantly higher than those from the control site. The selenium levels in all samples representing the environmental situation in the Ankang area were remarkably greater than those from the control site. However, it was found that no significant difference in selenium levels in the serum, hair, and urine samples taken from the study and the control subjects. The data obtained from this study indicate that higher arsenic and fluoride in the Ankang area of Shaanxi province could be the natural occurrence of geological phenomena. The selenium concentration in the environment was found to be moderate. The higher levels of arsenic and fluoride found in the study subjects from the study site could be due to their burning of raw coal containing greater arsenic and fluoride.

Authors: **Wang Zhi-fang**,[a] Guo Xiong,[a] Lei Yan-xia,[a] Bai Guang-lu,[b] Wang Yu-dong,[a] Fan Zhong-xue,[b] Zhang Qiang,[a] Ding Yue-qing.[c]
Correspondence: [a]Department of Public Health, Medical college, Key Laboratory of Environment and Genes Related to Disease (Xi'an Jiaotong University), Ministry of Education, Xi'an City 710061, China; [b]Institute of Endemic Disease of Shaanxi province, China; [c]Center for Disease Control and Prevention of Ankang City, Shaanxi province, China.
E-mail: chinachichi@gmail.com
Keywords: Ankang, Shaanxi Province, China; Arsenic; Coal burning; Arsenic; Environmental fluoride; Selenium.

NON-TOXIC DOSE EFFECT OF FLUORIDE ON ODONTOBLAST GENE EXPRESSION *IN VITRO*

Elevated fluoride intake may lead to local tissue disturbances known as fluorosis. Towards an understanding of this effect, fluoride-responses were analyzed in MO6-G3 cultured odontoblast cells. Up to 1 mM NaF, cell proliferation as measured by DNA accumulation was not inhibited, whereas at 3 mM, cells detached from their support and did not proliferate. Intracellular structures, characterized by electron microscopy, were normal up to 1 mM, but at 3 mM, necrotic features appeared. No sign of apoptotic transformation occurred at any NaF concentration. Fluoride-sensitive genes were identified by microarray analysis: expression of about 1000 out of 46 000 target-defined RNA species was enhanced or reduced. The sensitivity of several RNA species likely to be implicated in tissue formation was confirmed by conventional RT-PCR, and their expression levels were determined by real-time RT-PCR. At 1 mM fluoride, RNAs encoding the extracellular matrix proteins asporin and fibromodulin, and the cell membrane associated proteins periostin and IMT2A were 10-fold reduced. RNA coding for signaling factor TNF-receptor 9 was diminished to one-third, whereas that for the chemokine Scya-5 was enhanced 2.5-fold. These RNAs are present *in vivo* in tooth forming cells, as demonstrated by *in situ* hybridization and RT-PCR on RNA from dissected tissue samples. The presence and functioning of fibromodulin in dentin matrix has earlier been studied (Goldberg et al., 2006). Expression of stress factor coding RNAs was not altered. Taken together, these findings indicate that fluoride influences the transcription pattern of MO6-G3 cells without inducing cell stress or apoptosis. *In vivo*, such effects may have consequences on tissues: aberrant expression of fluoride-sensitive genes in odontoblasts may impair the formation of the mineralized dentin matrix and alter cell communication. Given hitherto-proposed mechanisms of tissue formation, patterns of normal and deviant fluorotic dentin may thus be obtained. The selectivity of the fluoride response is explained by the concept that cells gain robustness by fluctuating between discrete physiological states, biological attractors, and that the equilibrium between the states is influenced by fluoride.

Authors: **Wurtz Tilmann**,[a] Sophia Houari,[a] Mauro Nicole,[a] MacDougall Mary,[b] Peters Heiko,[c] Berdal Ariane.[a]
[a]Correspondence: Centre de Recherche des Cordeliers, UMRS 872 - Equipe 5, Paris, France; [b]Institute of Oral and Health Research, University of Alabama at Birmingham School of Dentistry, USA; [c]Institute of Human Genetics, International Centre for Life, Newcastle upon Tyne, NE1 3BZ, UK.
E-mail: tilmann.wurtz@crc.jussieu.fr
Keywords: Cultured MO6-G3 osteoblasts; Dental microarrays; Dentin; Fluoride and odontoblasts; Odontoblast cell culture; Tissue patterning.

SALIVARY GLAND RESPONSE TO FLUORIDE-INDUCED OXIDATIVE STRESS AND LIPID PEROXIDATION

The remarkable decline in dental caries in developed countries is widely believed to be due largely to the widespread use of fluoride (F) supplementation. However, excessive intake of F, apart from causing fluorosis, can inhibit the activity of many antioxidant enzymes in F-intoxicated animals, suggesting an adverse effect on major metabolic pathways. Generation of free radicals, lipid peroxidation (LP), and altered antioxidant defense systems are also regarded as toxic effects of F. *Objectives:* The purpose of this investigation was to evaluate the effects of a single low-dose of sodium fluoride (NaF) acute administration on the antioxidant status and lipid peroxidation of parotid and submandibular salivary glands of rats. *Methods:* Two-month-old male Wistar rats were injected intraperitoneally with NaF solution (F group; 15 mg F^-/kg bw). The control (C group) was administered same volume of sodium chloride solution (3.05%). Animals were euthanized 1, 3, 6, 12, and 24 hours after injection. PA (n=6) and SM (n=10) were extracted and analyzed for superoxide dismutase (SOD) and catalase (CAT) activities, malondialdehyde, and protein levels, and the results were statistically analyzed (Student's t-test, $p<0.05$). *Results:* Increased LP was observed in both PA (F_3, F_6, F_{12}) and SM (F_1, F_6, F_{12}, F_{24}) gland groups ($p<0.05$). Exposure to NaF produced a significant reduction of SOD (31.59% F_6) and CAT (10.00% F_1) activities in PA gland groups ($p<0.05$). For SM gland groups, a significant decrease in SOD was observed only in F_1, F_{12} and F_{24} ($p<0.05$), and no statistically significant difference was noted in CAT activity at all. No significant differences were found in proteins levels for either type of gland. *Conclusions:* The acute exposure to a single low dose administration of NaF impaired the antioxidant enzyme defense system and enhanced lipid peroxidation in both parotid and submandibular salivary glands.

Authors: **Yamaguti PM,** Ganzerla E, Nogueira FN, Nesadal D, Nicolau J.
Correspondence: Oral Biology Research Centre, School of Dentistry, University of São Paulo, Avenida Professor Lineu Pretes, 2227 CEP 05508-000 São Paulo, Brazil.
E-mail: mochidome@usp.br
Keywords: Antioxidant enzymes; Fluoride-induced oxidative stress; Lipid peroxidation; Submandibular salivary glands.

EFFECT OF FLUORIDE ON THE APOPTOSIS AND EXPRESSION OF COLLAGEN 1A1 GENES IN RAT OSTEOBLASTS

Introduction: In previous studies, we investigated and discussed effects of fluoride (F) on collagen I gene expression in rib of goats and rabbits, and cell apoptosis in rat brain and mice testis. *Objectives:* In this study, we investigated the changes in cell proliferation, differentiation, apoptosis, and expression of collagen I genes in neonatal rat osteoblasts (OB) induced by varying dosages of F. *Material and Methods:* OB from the calvarias of neonatal wistar rats were isolated by incomplete trypsinization and cultured at 37×C in DMEM supplemented with 10% FBS and humidified 5% CO_2. After subculture, the OB were exposed to gradient treatment with NaF incubated in 5% FBS-containing medium for 72 hr. Proliferation of the OB was measured by methyl thiazolyl tetrazolium (MTT) assay. The cell cycle and levels of apoptosis were analyzed with a fluorescence-activated cell sorter (FACS) by the Annexin-V-FITC and the propidium iodine (PI) double staining method. The expression of collagen I genes was determined by quantitative real-time polymerase chain reaction (QRT-PCR). *Results:* F inhibited cell proliferation at higher concentrations and enhanced the levels of apoptosis with increasing dosages of F, compared with the control group. FACS cell-cycle analysis demonstrated that high F potentially decreased cell number of G_2/M phase and inhibited the transformation from S phase into G_2/M phase. After treatment with fluoride for 72 hr, the level of COL1A1 mRNA was markedly decreased, whereas no significant change occurred in

COL1A2. *Conclusion:* This *in vitro* study revealed that F induced apoptosis in rat OB and caused the alteration of gene expression of type I collagen.

Authors: **Yan Xiao-yan**,[a] Chen Qing-lin,[a] Hong Jian-hua,[b] Wang Jundong.[a]
Correspondence: Prof Jundong Wang, [a]Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi, PR China; [b]Campus Hospital of Shanxi Agricultural University, Taigu, Shanxi, PR of China.
E-mail: wangjd@sxau.edu.cn
Keywords: Collagen 1A1 gene; Fluoride and osteoblasts; Neonatal rat osteoblasts; Osteoblast apoptosis; Rat osteoblasts.

Index of presenting authors:

INTEGRITY IN SCIENCE AWARD

In November 2007, in Washington, DC, USA, Dr Phyllis Mullenix was presented with a Wise Traditions 2007 Integrity in Science Award by the Weston A. Price Foundation[1] (Figure). Dr Mullenix submitted her research findings on the neurotoxic effects of fluoride for publication even though they had the potential to—and actually did—very adversely affect her future employment as a research scientist.[2]

Figure. Phyllis Mullenix, PhD, (on left), receiving the "coveted Weston A. Price Foundation Integrity in Science Award for her studies on fluoride, and Dr. Natasha Campbell-McBride for her work on treating autism."

Photo from the Winter 2007 issue of Wise Traditions, Vol. 8, No. 4, page 8, by permission of Sally Fallon, MA, co-editor with Katherine Czapp.

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