ABSTRACTS OF PAPERS PRESENTED AT THE XXXIVTH CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH, GUIYANG, PEOPLE'S REPUBLIC OF CHINA, OCTOBER 18–20, 2018

NEUROTOXICITY OF FLUORIDE: AUTISM SPECTRUM DISORDERS

Anna Strunecká, Otakar Strunecky
Prague and Ceske Budejovice, Czech Republic

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The rising prevalence of autism spectrum disorder (ASD) during the last decades might reflect the synergistic action of an increased burden of new ecotoxicological factors, such as fluoride, aluminum (Al^{3+}), and the increasing number of vaccines in the period of rapid postnatal brain development. A long-term burden of these ubiquitous environmental toxins is several health effects with a striking resemblance to the symptoms of ASD. While Al^{3+} appears among the key suspicious factors in the aetiology of ASD, fluoride is rarely recognized as a causative culprit. According to our hypothesis, the key mechanism in ASD pathophysiology is the immunoexcitotoxicity. Metabolic and mitochondrial defects caused by fluoride may have toxic effects on brain cells, causing neuronal loss and altered modulation of neurotransmission systems. Mitochondrial dysfunction, oxidative stress, inflammation, and excitotoxicity are key players in the pathogenesis of ASD. In addition, the synergistic action of fluoride with Al^{3+} in molecules of aluminofluoride complexes can affect cell signaling, neurodevelopment, CNS functions, and hormonal regulations at several times lower concentrations than either fluoride or Al^{3+} acting alone. Our suggestions of immunoexcitotoxicity as the key etiopathological feature of ASD opens the door to number of new natural treatment modes.

Authors: Anna Strunecká,[a] Otakar Strunecky.[b]
[a]Charles University, Prague, Czech Republic; [b]The Institute of Technology and Business in Ceske Budejovice, Czech Republic.
Correspondence: Anna Strunecká; E-mail: anna.strunecka@gmail.com
Keywords: Autism spectrum disorders; Aluminum; Aluminofluoride complexes; Fluoride; Glutamatergic neurotransmission; Immunoexcitotoxicity; Microglial activation; Neurodevelopment.

HEALTH RISKS OF FLUORIDE: FROM MOLECULES TO DISEASE

Anna Strunecká
Prague, Czech Republic

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The expanding research provides evidence that fluoride affects life processes from fertilization to ageing, from gene transcription to cognition, and behavior with powerful efficacy. Many epidemiological and clinical studies document the effects of fluoride on humans. Fluoridation of drinking water in addition to the wide use of fluoride in medicine, industry, and agriculture, started the era of supplementation of the living environment with fluoride as never before in the history of human race. Dental fluorosis as the sign of fluoride overload is endemic in at least 25 countries across the globe. Fluoride has long been known to influence the activity of various enzymes *in vitro* and became a useful laboratory tool in our understanding of the mechanisms of enzyme catalysis underlying biological processes. It has no vital biological function in living organisms. Fluoride's ability to damage the brain is one of the most active

areas of fluoride research today. Over 300 studies have found that fluoride is a neurotoxin capable of causing widespread developmental brain disorders such as reduced IQ in children, autism, attention deficit hyperactivity disorder, learning disabilities, and other cognitive impairments.

Children and adolescents with poor nutritional status are exposed to alterations of mental and behavioral functions that can be corrected to a certain extent by dietary measures. Reversal of fluoride-induced cell injury and fluorosis through the elimination of fluoride and consumption of a diet containing essential vitamins, mineral, antioxidants, and various nutraceuticals have been shown by several studies to be beneficial.

Author: Anna Strunecká; Charles University, Prague, Czech Republic.
Correspondence: Anna Strunecká, E-mail: anna.strunecka@gmail.com
Keywords: Cognition; Fluoride; Neurodevelopment; Neurotoxicity; Therapy.

GENETIC, EPIGENETIC AND ENVIRONMENTAL FACTORS: THE TRIANGLE OF HEALTH!

Jörg Spitz
Schlangenbad, Germany

Abstract number
3
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Noncommunicable diseases (NCD) have become the biggest issue of health care worldwide. Mainstream medicine is not able to overcome this problem due to its focus on symptoms instead of causes. On the other hand, there is an overwhelming flood of interdisciplinary research leading to a new understanding of the human being with huge implications for our health. We had to learn that our body is not a single being but a temporary aggregation of our environment, consisting of billions of bacteria, viruses, and fungi as well as a similar amount of human cells, forming a microcosm in the macrocosm of our universe and provided with unique abilities. This "human system" is nonlinear, self-regulating, self-regenerating, self-reproducing, and constantly connected to its environment via the exchange of information and material. The next thing we had to learn concerns our genes. The cells are using the genetic information like a textbook, which has been written by evolution. The use of this genetic information is largely dependent on environmental factors which influence the reading of the genes by a mechanism called "epigenetics" which is a kind of intelligent interface to our environment. While the structure of genes is kept stable over long periods, epigenetics are able to react quickly to changes in the environment of the being. We do not live just by chance in a terrestrial environment and these environmental factors are prerequisites of our human life which has been styled by them. As a result, changing the environment by technical progress has not only led to its pollution by a large variety of nonhistorical elements (NHE) but also to the loss of an equally large number of health resources: the long list of these lost natural resources from our environment starts with micronutrients and essential fatty acids in our processed food, goes on with physical factors like gravity and sunlight, and ends up with a variety of social factors in our communities.One example of a negative environmental influence is fluoride, the topic of this conference. While fluoride has been a long known local environmental problem in some areas of China and India, today it has become a worldwide problem as a by-product of the nuclear and fertiliser industries resulting in the poisoning of people all over the world. The same happens with aluminium and other metals. Another typical example of environmental influence on the human body is the interaction of the UVB radiation

with our skin, producing the "solar hormone vitamin D". As all our cells have receptors for the solar hormone, vitamin D is essential for our health and involved in the origin and progress of almost all noncommunicable diseases from diabetes to dementia and cancer. Due to our change from living in a natural environment with extended sun exposure to living in an artificial environment (buildings, cars, et cetera) we have almost completely lost our exposure to the sun and by that the chance to produce the solar hormone and get, as a result, a worldwide disease favouring deficiency. Conclusion: to overcome NCD three actions needed: 1. teaching people the new knowledge to improve their health literacy; 2. providing the lost former natural factors from the environment by intelligent replacement measures and, at the same time, 3. reducing the pollution by nonhistorical elements. These combined procedures will enable our bodies to (re)use their inborn evolutionary skills not only to develop optimum health but also to treat the diseases of diabetes, depression, dementia, and multiple sclerosis. In consequence, noncommunicable diseases are no longer an unsolvable problem. We have the knowledge to overcome them and we should apply it.

Authors: Jörg Spitz; Academy for Human Medicine, Krauskopfallee 27, 65388 Schlangenbad, Germany.
Correspondence: Jörg Spitz; E-mail: info@mip-spitz.de.
Keywords: Environmental factors; Epigenetic factors; Genetic factors; Triangle of health.

EPIDEMIC AND CONTROL OF ENDEMIC FLUOROSIS IN CHINA

Dian-Jun Sun, Yan-Hui Gao, Li-Jun Zhao
Harbin, Heilongjiang Province, People's Republic of China.

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4
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Endemic fluorosis has been regarded as a severe public health issue in China since the 1960s and was listed in the management of major endemic diseases in 1979 by the former Ministry of Health. According to the different high fluoride sources, endemic fluorosis consists of three types, i.e., drinking water type, coal-burning type, and drinking brick-tea type. The latter two types only exist extensively in China. Based on the latest statistical report in 2017, the drinking water type endemic fluorosis is present in 77,290 villages, in 1,039 counties in 28 provinces, and affects 68,120,000 people; the coal-burning type endemic fluorosis affects 8,6000,000 households, in 171 counties in 12 provinces, and affects 30,650,000 people. The drinking brick-tea type endemic fluorosis is prevalent in 7 western ethnic provinces, affecting 13,100,000 people in 13,230 villages, in 229 counties. Actions to reduce the fluoride intake are the fundamental measures for the prevention and control of endemic fluorosis. The basic effective measure for controlling drinking water type endemic fluorosis is changing to a low fluoride water source and/or the defluoridation of drinking water. Endemic fluorosis caused by pollution from burning coal is managed by comprehensive prevention based both on health education and the use of more efficient stoves. The reduction of the fluoride content in brick tea is of fundamental importance for controlling the drinking brick-tea type fluorosis. China's Fluoride Content of Brick Tea, the first national health standard of its kind in the world published in 2005, requires that the fluoride content of brick tea should be no more than 300 mg/kg. From the 1960s, scattered tentative efforts to change water supplies were carried out only in parts of the provinces experiencing serious endemic fluorosis. From 1980s, supplies designed to provide low-fluoride water were carried out throughout China. In 1987–1989, the results of pilot projects to control coal-

burning type fluorosis in the Yangtze Three Gorges provided the foundation for the full implementation of control measures all over the country. In this new century, China's government attaches great importance to the prevention and control of endemic fluorosis. Supported by special central funds from the Ministry of Health, the distribution of high fluoride water sources and the prevalence of drinking tea type fluorosis were investigated in detail and comprehensive measures for the control of coal-burning fluorosis were fully implemented. At the same time, plans for water improvement for the control of endemic disease in the western regions, the National Eleventh Five-year Plan, and the Twelfth Five-year Plan for the construction of safe drinking water projects in rural areas were carried out successively by the Ministry of Water Resources, which promote in a major way the progress for the control of drinking water fluorosis in China. At the present time, great achievements have been achieved for the prevention and control of endemic fluorosis in China. A total 595 drinking water fluorosis counties, out of 1039, and 146 coal-burning fluorosis counties, out of 171, have reached the control standard. However, the low-fluoride brick tea was able to be accessed in only 10% of the total drinking brick-tea type of fluorosis villages evaluated. Effort should be taken to promote the popularization of low-fluoride brick tea in endemic areas. Meanwhile, surveillance and evaluation should and will be continued in the areas with all three types of endemic fluorosis.

Authors: Dian-Jun Sun,[a] Yan-Hui Gao,[b] Li-Jun Zhao[c]
[a]Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, Harbin 150081, People's Republic of China; [b]Key Lab of Etiology and Epidemiology, Education Bureau of Heilongjiang Province & Ministry of Health (23618504), Harbin 150081, People's Republic of China; [c]Heilongjiang Provincial Key Laboratory of Trace Elements and Human Health, Harbin Medical University, Harbin 150081, Heilongjiang Province, People's Republic of China.
Correspondence: Dian-Jun Sun; E-mail: hrbmusdj@163.com
Key words: Coal-burning fluorosis; Drinking brick-tea fluorosis; Drinking water fluorosis; Prevention of fluorosis.

THE HARMFUL EFFECT ON THE HUMAN BODY OF HYDROGEN FLUORIDE FOLLOWING THE USE OF SODIUM FLUORIDE IN DENTAL CARIES PREVENTION

Kenji Akiniwa, Kenichi Narita
Tokyo and Niigata City, Japan

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5
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It is well known that sodium fluoride (NaF), which is used prophylactically for the prevention of dental caries, easily changes into molecular hydrogen fluoride (HF) under acidic conditions. HF is the most toxic form of fluoride among the fluorine compounds according to the classifications by Roholm (1937) and Kono (1994). The change from NaF to HF occurs in the oral sites with rich dental plaque and high acidity including carious lesions and tooth necks, the cervical part of dental implants, and the oro-pharyngeal membrane which is close to falling dental plaque. Swallowed NaF also changes to HF in the stomach. The ionized fluoride reacts with gastric juice (hydrochloric acid) to form HF and passes through the stomach wall to diffuse to the whole body with a remarkably high speed which is quite different from the usual absorption of fluoride (Gutknechit et al., 1981). HF is able to permeate the biomembrane of tissues and the concentrations of HF and F reach a dynamic equilibrium depending on the acidity of tissue. The currently accepted theory on fluoride absorption is that inorganic fluoride in the form of the undissociated molecule, hydrofluoric acid (HF), is absorbed by rapid passive diffusion along the entire gastrointestinal tract, without any apparent active transport mechanisms being involved (Singer and Ophaug, 1982; Barbakow, 1983; Whitford and Pashley, 1984).

HF concentrates via stomach-artery route as proposed by Takahashi (1996). Salivary A high level of fluoride in the serum results in fluoride being secreted in the saliva. After the saliva is swallowed, HF is formed in the acidic environment of the stomach. The HF is then absorbed resulting in the occurrence of a vicious circle. Consequently, the serum fluoride concentration would increase to be several times higher than the normal level. We calculated the absorption speed of the HF and found it to be approximately 3 million-fold higher compared to that of ionized F (Narita. 2017). Recent evidence suggests that absorbed F may affect the developing brain of the fetus.

Authors: Kenji Akiniwa,[a] Kenichi Narita.[b]
[a]Japanese Society for Fluoride Research, Machida City, Tokyo, Japan; [b]Akiha Ward, Niigata City, Niigata Prefecture, Japan.
Correspondence: Kenji Akiniwa; E-mail: sky.aknw@gmail.com.
Key words: Absorption speed; Harmful effects of fluoride; Molecular hydrogen fluoride (HF); Sodium Fluoride (NaF).

FLUORIDE-INDUCED REPRODUCTIVE TOXICITY

Jun-Dong Wang
Taigu, Shanxi Province, People's Republic of China

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Fluorine is the most reactive element in the periodic table and exists widely in nature and life, either as inorganic fluorides or organic fluoride compounds. Because the margin of safety is extremely narrow, an elevated consumption of fluoride in daily life will increase the bodily burden and the risk of fluorosis, leading to dental fluorosis, skeletal fluorosis, and nonskeletal fluorosis such as alterations in the liver, kidney, immune system, central nervous system, and reproductive system, etc. Here, we summarize the adverse effects and underlying mechanisms of fluoride on the reproductive system and highlight the effects on testis and sperm. The testis is an organ for producing sperm and the process is called spermatogenesis. The cell types of the testis consist of the spermatogenic cell (SMC), the Sertoli cell (SC), the Leydig cell (LC), and the immune cell (IC). It has been proven that fluoride influences apoptosis, the Y chromosome, the heat shock proteins in the SMC; apoptosis, the antioxidant system, the blood-testis barrier and immune privilege in the SC; apoptosis, oxidative stress, steroidogenesis and autophagy in the LC; as well as the inflammatory response in IC; which can partly explain the structural and functional damages of testis. Sperm generated in the process of spermatogenesis are highly specialized and condensed cells, which function in transporting and delivering the male genetic information to the descendant during the fertilization of the oocyte. Fluoride affects sperm motility through decreasing ATP generation, sperm hyperactivation and chemotaxis by disturbing the Ca^{2+} signaling pathway, which results in failed fertilization, and finally in infertility. In addition, omics technologies are increasingly applied to investigate molecular mechanisms by which fluoride produces reproductive dysfunction. Our labs have finished the deep sequencing analysis, gene expression profiling, and proteomics analysis in the testis and sperm of mice who have been administered fluoride. Some important mRNAs, miRNAs, and proteins were found in these studies, which may potentially provide information for clarifying fluoride-induced reproductive toxicity.

Author: Jun-Dong Wang; Shanxi Key Laboratory of Ecological Animal Science and Environmental Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi, People's Republic of China.
Correspondence: Jun-Dong Wang; E-mail: wangjd53@outlook.com.
Key words: Fluoride-induced reproductive toxicity; Sperm; Testis. neurotransmission; Immunoexcitotoxicity; Microglial activation; Neurodevelopment.

INTEGRATED FLUOROSIS MITIGATION PROGRAMME FOR ABATEMENT OF FLUOROSIS IN SELECTED FLUOROSIS ENDEMIC VILLAGES OF NAWADA DISTRICT, BIHAR

Bihari Singh, Kamal Kishor Singh
Patna, India

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7
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Fluorine in the form of fluoride is a potent trace element which, when it accumulates in the human body above the permissible limit, has serious health implications. In the fluorosis endemic villages of our study, drinking water is the main source of fluoride intake. Intake of fluoride contaminated water for a prolong period causes fluorosis. Our previous studies on fluorosis problems in Bihar have revealed fluorosis endemicity in several villages of Rajauli, Nawada, and Bihar. Among the villages, Kachhariadih, Muslimtola, Hanuman Nagar and some parts of old Hardia village have been found to be severely affected by fluorosis. All the three types of fluorosis—skeletal, non-skeletal and dental—have been observed in many inhabitants of these villages. Almost the entire population of these villages have complaints of pain in bones and joints, stiffness of body parts, weakness, and loss of appetite, etc. Physico-chemical analysis of water samples of the drinking water sources have shown fluoride concentrations in them from 1.9 ppm to as high as 6.4 ppm which are much above the maximum permissible limit of 1.5 ppm. Recently a study cum mitigation programme entitled "Integrated Fluorosis Mitigation (IFM) Programme in Fluorosis Endemic Selected Villages of Nawada District Bihar," has been launched. It is a collaborative socio-scientific research programme of the Centre for Fluorosis Research, Anugrah Narayan College (A.N. College), Patna, CSIR-NEERI, Nagpur, NIRTH, Jabalpur, PHED, Government of Bihar and UNICEF, Patna. The IFM programme combines three widely recognized mitigation measures: (i) Provision for uninterrupted supply of fluoride-safe water to the entire population of the study villages; (ii) Nutritional intervention in the form of some plants, calcium, vitamin C, vitamin D, and iron which have been found to be highly effective in abating the health problems of fluorosis patients; and (iii) Extensive awareness cum-interaction programme with the villagers to educate them about the ill effects of ingestion of fluoride-contaminated drinking water for a longer period, and the benefits of consumption of fluoride-safe water coupled with the regular use of nutritional supplements. For the regular availability of fluoride-safe drinking water, the villagers have been provided with hand pump attachable adsorbent based defluoridation units made available by CSIR-NEERI, Nagpur. The IFM Programme is running satisfactorily as is evident from the positive response of the villagers and also through questionnaire sheets to record the improvement in the health status of the people there.

Authors: Bihari Singh[a]; Kamal Kishor Singh[b].
[a]Centre-Incharge, Centre for Fluorosis Research, A. N. College, Patna-800013, India; [b]Member, Executive Committee, Centre for Fluorosis Research, A. N. College, Patna-800013, India.
Correspondence: Bihari Singh; E-mail: bihari_singh2001@yahoo.com.
Keywords: Fluorosis; Nawada villages; Nutritional interventions; Safe water.

FLUORIDE DETERIORATES TUBULOINTERSTITIAL NEPHROPATHY OF RATS CAUSED BY UNILATERAL OBSTRUCTION AS AN EXPERIMENTAL MODEL

M Tsunoda, T Kido, C Sugaya, H Yanagisawa
Tokorozawa, Saitama, and Shinbashi, Tokyo, Japan

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The contamination of ground water by fluoride (F) has been reported worldwide. Since F is filtered by kidneys, human or experimental animals with renal damage may be more affected by F than those with normal kidney function. Tubulointerstitial fibrosis of kidneys can be induced in rats by unilateral ureteral obstruction (UUO) as an experimental animal model for renal damage and urinary stone disease. We examined the effects of F on tubulointerstitial fibrosis in the obstructed kidney of UUO rats. The left ureters of male rats, aged 6-weeks-old, were ligated using silk sutures. Fluoride was administered to the rats for 2 weeks at the concentrations of 0, 75, and 150 ppm in the drinking water. After 2 weeks of administration, the kidneys of the rats were sampled and histological and immunohistochemical staining were used to identify positive areas within the renal cortex and staining-positive cells by image analysis. We found a significant increase in the obstructed kidneys of the UUO rats exposed to 150 ppm of F for areas or number of cells stained with antibody against collagen type I compared with the controls. It is suggested that F exacerbates tubulointerstitial nephropathy resulting from UUO. The UUO rats are useful animal model for the elucidation of the toxic effects of F on humans with renal damage.

M Tsunoda,[a] T Kido,[b] C Sugaya,[a] H Yanagisawa[b]
[a]Department of Preventive Medicine and Public Health, National Defense Medical College, Tokorozawa, Saitama, Japan; [b]Department of Public Health and Environmental Medicine, Jikei University School of Medicine, Shinbashi, Tokyo, Japan.
Correspondence: Masashi Tsunoda; E-mail: mtsundoda@ndmc,ac.jp
Key words: Animal model; Fluoride; Tubulointerstitial fibrosis; Unilateral ureteral obstruction.

BASIC INVESTIGATION AND CLINIC TREATMENT FOR THE COAL-BURNING TYPE OF ENDEMIC FLUOROSIS IN GUIZHOU, CHINA

Zhi-Zhong Guan1,2*, Yong Wang3, Qing-Hong Duan4, Rui-Guang Liu5, Fu-Cheng Li6, Shi-Xing Xu7, Gui-Ling Yang8, Jie Deng2, Yi Li2, Chang-Xue Wu2, Yan-Jie Liu1, Na We1, Yang-Ting Dong2, Xiao-Lan Qi2, Wen-Feng Yu2
Guiyang, Liupanshui City, and Qizingguan County, People's Republic of China

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Endemic fluorosis occurs widely in the world and is characterized by skeletal and dental fluorosis and a vast array of bodily pathological changes. The coal-burning type of endemic fluorosis is the severest form of fluorosis and was confirmed to be present in China in the 1970's. This type of endemic fluorosis is primarily induced by fluoride-contamination of food and air indoor by smoke emitted during the burning, by the residents in the areas, of coal, containing a high amount of fluoride in open non-flued stoves for drying food during the autumn harvest and for heating in the winter. In Guizhou Province of China, about 15 million people live in such areas of the coal-burning type of endemic fluorosis. Among the population, 10 million suffer from dental fluorosis and 800,000 from skeletal fluorosis. Since 1980, an efficient strategy with integrated control has been carried out for eliminating the disease in these areas of China. Firstly, adapted stoves have been set up which can burn coal without pollution resulting in a significant decline of the fluoride contamination of food and air indoor. Secondly, education has been carried out with the residents on the harmful

effects of fluoride pollution on the body from coal burning. After following this strategy for many years, the number of the patients with dental or skeletal fluorosis has been significantly decreased, indicating that the coal-burning type of endemic fluorosis in China has been efficiently controlled. In addition, basic research has been carried out to understand the pathological changes and the molecular pathogenesis of the condition. Besides the occurrence of skeletal and dental fluorosis, excessive fluoride has been found to result in damage to multiple organs in the patients, In addition, we suggested earlier that the increased level of free radicals resulting from fluoride might the main mechanism involved in the damage in fluorosis to multiple organs or systems. This has subsequently been confirmed by a large number of investigations. In our results, as compared to control cases, the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), and the expressions of SOD and GST mRNAs in the blood of the population living in the areas with severe coal-burning endemic fluorosis were significantly increased. In addition, the raised malondialdehyde (MDA) level in the blood of the population living in the areas with severe coal-burning endemic fluorosis was significantly decreased. Changes in hepatic and renal function were detected in the residents living in the areas of endemic fluorosis. Interestingly, the health conditions of the residents in the endemic fluorosis area has been greatly improved after several years of the integrated control. Furthermore, clinical treatments for the patients with dental and skeletal fluorosis have been performed by dentists and surgeons. Diagnostic imaging has been improved by the imaging doctors in our hospital. This has led to better results being achieved. The pharmaceutical drugs used for the treatment of the patients with chronic fluorosis have also been investigated and preclinical results have been obtained in our group. Importantly, we should notice that we should note that a long period of integrated control is required to efficiently eliminate the hazard of the coal-burning type of endemic fluorosis.

Authors: Zhi-Zhong Guan,[a,b] Yong Wang,[c] Qing-Hong Duan,[d] Rui-Guang Liu,[e] Fu-Cheng Li,[f] Shi-Xing Xu,[g] Gui-Ling Yang,[h] Jie Deng,[b] Yi Li,[b] Chang-Xue Wu,[b] Yan-Jie Liu,[a] Na We,[a] Yang-Ting Dong,[b] Xiao-Lan Qi,[b] Wen-Feng Yu[b]
[a]Dept. of Pathology at Affiliate Hospital; [b]Key Lab of Endemic and Ethnic Diseases of the Ministry of Education; [c]Affiliated Stomatological Hospital, [d]Dept. of Imaging at Affiliate Hospital and [e]Dept. of Orthopedics at Affiliate Hospital in Guizhou Medical University, People's Republic of China; [f]Center for Disease Control and Prevention of Liupanshui City, People's Republic of China; [g]Center for Disease Control and Prevention of Qixingguan County, People's Republic of China; [h]Guiyang Rui-Feng Pharmaceutical co. LTD, People's Republic of China.
Correspondence: Zhi-Zhong Guan; E-mail: 1457658298@qq.com
Keywords: Clinical treatments; Coal-burning type of endemic fluorosis; Damage to multiple organs; Integrated control; Molecular pathogenesis.

ROLE OF PTH ON FLUORIDE-AFFECTED OSTEOCLASTIC DIFFERENTIATION INDUCED BY CO-CULTURE WITH OSTEOCYTES

Ning-Ning Jiang, Hui Xu, Feng-Yang Guo, Xiu-Yun Zhang
Changchun, People's Republic of China

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This aim of this study was to investigate the mechanism underlying osteocyte-modulated osteoclastogenesis under fluoride treatment *in vitro* and to understand the action of PTH in this process. The IDG-SW3 cell line, a kind gift from Dr Lynda F Bonewald, proved to be useful for studying the osteoblast-to-osteocyte transition and osteocyte function through mineralization induction. The RAW264.7 cell line was cultured with 25ng/mL soluble RANKL to induce osteoclast differentiation in a single culture environment. The cell viability of IDG-SW3 and RAW264.7 cells exposed to fluoride, with and without PTH, was measured by the MTT method. Under the same

experimental conditions, the apoptosis rate of the two types of cells was tested by Flow Cytometry with the FITC Annexin V Apoptosis Detection Kit. The protein expression of RANKL and Sclerostin (Sost) in the IDG-SW3 cells were detected by Western Blotting and analyzed by Gel-Pro analyzer software. The Transwell assay used 8 um pores on a 24 mm polycarbonate membrane insert. The IDG-SW3 cells were placed in the bottom well and cultured with the mineralization induction medium for 21 days. Following this, 105 of the RAW264.7 cells were loaded into the top chamber in DMEM/F12. Four mg/L of fluoride, with and without PTH, was used to treat the co-culture of the IDG-SW3 cells and the RAW264.7 cells for 7 days. In the end, the two types of cells were separately lysed with TRIzol reagent and collected for realtime PCR analysis. The IDG-SW3 cells exhibited the characteristics of osteocytes with detection of SOST protein. Cotreatment with PTH and a fluoride dose ranging from 0.5~16 mg/L significantly decreased the IDG-SW3 cell viability by comparison with the same dose of single fluoride treatment. Correspondingly, the apoptosis rate was markedly higher in the IDG-SW3 cells treated with PTH and fluoride compared to same dose of the single fluoride treatment. A fluoride range of 0.1~4 mg/L had mild effect on cell viability the RAW264.7 cells while exposure of the cells to 8, 16, and 32 mg/L of fluoride greatly inhibited cell viability. The apoptosis rate also significantly increased in the RAW264.7 cells exposed to 16 mg/L of fluoride compared to the control. Next, it was found that different doses of fluoride treatment have a mild effect on RANKL protein expression in IDG-SW3 cells, and cotreatment with PTH and 4 and 16 mg/L of fluoride moderately increased its expression. The protein expression of OPG was mildly decreased with the increase in the fluoride exposure dose and cotreatment of PTH significantly aggravated this reduction. These data implies that it was an anabolic action of PTH on the IDG-SW3 cells that modulated osteoclastogenesis. In the transwell assay, the PCR results on the IDG-SW3 cells showed that fluoride, with and without PTH, increased RANKL expression, but decreased the OPG expression. PTH administration significantly improved the RANKL: OPG ratio compared to the single fluoride treatment. The PCR analysis of RAW264.7 from the top chamber provided valuable data. Tartrate-resistant acid phosphatase (TRAP) as an osteoclast marker markedly increased in cells exposed to fluoride with and without PTH. The key transcription factor of osteoclasogenesis, RANK expression markedly increased in the fluoride groups, with and without PTH treatment. The same upward trends were observed in JNK and NFATc1, both of which play key roles in osteoclast differentiation. Moreover, the expression of β-catenin reduced in cells under fluoride with and without PTH treatment, and PTH administration aggravated reduction. In short, fluoride the stimulated RANK-JNK-NFAtc1 pathway to mediate the osteoclastic differentiation induced by co-culture with osteocytes. Fluoride impeded OPG expression in osteocytes by reciprocal inhibition of β-catenin expression in co-cultured osteoclasts. Meanwhile, PTH played a key role in osteocyte-modulated osteoclastic differentiation under fluoride treatment.

Authors: Ning-Ning Jiang, Hui Xu, Feng-Yang Guo, Xiu-Yun Zhang. School of Pharmaceutical Sciences, Jilin University, Changchun 130021, People's Republic of China.
Correspondence: Hui Xu; E-mail: xu_hui@jlu.edu.cn.
Keywords: Osteocyte-modulated osteoclastic differentiation; PTH.

SKELETAL FEATURES OF CHILDREN LIVING IN THE AREA OF COAL-BURNING TYPE OF ENDEMIC FLUOROSIS DETECTED BY X-RAY IMAGING

Qing-Hong Duan, Ying Li, Ping-Gui Lei, Xu-Guang Chen, Zhi-Zhong Guan
Guiyang, People's Republic of China.

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The aim of the study was to reveal the skeletal features of children living in the area of the coal-burning type of endemic fluorosis by employing X-ray imaging. The X-ray CR images on the pelvis, left calf (knee joint), and left forearm (including the elbow joint) were examined in 135 children who were born in Shui-Cheng County, Guizhou Province of China, a typical area with the coal-burning type of endemic fluorosis. According to the diagnostic criteria of WS192-2008 on endemic skeletal fluorosis combined with reference to the data in the literature data, two experienced radiologists in our group carefully read the imaging results of CR in the 135 children with dental fluorosis and they made the imaging diagnosis after reaching agreement. The changes of skeletal fluorosis were considered to be the occurrence of two abnormal characteristics in bone trabeculae in two or more sites. Among all of the children studied, 24 cases were diagnosed as having skeletal fluorosis. In the children with skeletal fluorosis, 22 cases were considered to have mild skeletal fluorosis, a rate in the total number studied was 16.3% (22/135). There were 2 cases of moderate skeletal fluorosis, a the rate of 1.5% (2/135). No case of severe skeletal fluorosis was found in the children examined. In the children with mild skeletal fluorosis, slightly increased bone density, increased bone trabeculae, and thickened bone trabeculae were observed. The changes found were the granular change in 4 cases, thick reticular in 9 cases, fine bone trabeculae in 5 cases, bony spot formation in 9 cases, decreased trabeculae in 4 cases, and the blurred change in 4 cases. In addition, there were carelessly changed trabeculae in 4 cases, a decreased epiphysis in the proximal end of radius in 7 cases, epiphyseal hypertrophy in 2 cases, and high density in 8 cases. On the other hand, no periosteal change and joint degeneration were found. In the children with the moderate skeletal fluorosis, there was increased density of bone mineral in 1 case, significantly thickened trabeculae of bone in 1 case, formation of bone plaque in 1 case, decreased density and trabeculae of bone in 1 case, and rough trabeculae of bone in 1 case. No periosteal and degenerative changes were observed. Our study indicated that when skeletal fluorosis was detected by X-ray in children living in areas of coal-burning type of endemic fluorosis, it was usually of a mild degree but a few children had moderate skeletal fluorosis.

Authors: Qing-Hong Duan,[a] Ying Li,[a] Ping-Gui Lei,[a] Xu-Guang Chen,[a] Zhi-Zhong Guan.[b]
[a]Department of Medical Imaging and [b]Department of Pathology, the Affiliated Hospital of Guizhou Medical University, Guiyang 550004, People's Republic of China.
Correspondence: Qing-Hong Duan; Email: duanqinghong2002@163.com
Key words: Fluorosis; Skeletal fluorosis; Radiography; Children.

MITOCHONDRIAL FISSION INHIBITION INDUCES DEFECTIVE AUTOPHAGY AND EXCESSIVE APOPTOSIS CONTRIBUTING TO DEVELOPMENTAL FLUORIDE NEUROTOXICITY

Qian Zhao, Qiang Niu, Jing-Wen Chen, Tao Xia, Guo-Yu Zhou, Pei Li, Li-Xin Dong, Chun-Yan Xu, Zhi-Yuan Tian, Chen Luo, Lu-Ming Liu, Shun Zhang, Ai-Guo Wang
Wuhan, Hubei, People' s Republic of China

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The aim of the study was to investigate how mitochondrial fusion/fission contributes to fluoride-induced developmental

neurotoxicity and to illuminate the potential mechanism. SH-SY5Y cells were treated with different concentrations of sodium fluoride (NaF, 20, 40, and 60 mg/L) for 24 hr. Meanwhile, a Sprague Dawley (SD) rat model of developmental fluoride exposure from pre-pregnancy until 2 months after delivery was constructed and four groups (n=10) were set up: control group, three NaF-treated groups (NaF was administered at 10, 50, and 100 mg/L via dissolving with tap water). Mdivi-1 and Fis1 overexpression were employed to further explore the role of mitochondrial fission in fluoride-induced mitochondrial dysfunction and cell death. Wortmannin and AC-DEVD-CHO were used to test the influence of autophagy and apoptosis on the ultimate cellular viability, respectively. The mitochondrial morphology and function, representative mitochondrial fusion/fission, autophagy and apoptosis proteins, apoptotic rate, and cellular viability were detected. We found that NaF induced dysregulated mitochondrial fission/fusion which was manifested by inhibited fission and accelerated fusion, and was accompanied by mitochondrial heterogeneity and dysfunction such as mitochondrial membrane potential loss and mitochondrial superoxide overproduction in the SH-SY5Y cells. Mechanically, suppressing mitochondrial fission with Mdivi-1 exacerbated the NaF-induced mitochondrial injury and the resultant neuronal death was via excessive apoptosis and promoted autophagy. However, targeting promoting mitochondrial fission by Fis1 overexpression alleviated the NaF-induced detrimental outcomes by inhibiting apoptosis and enhancing autophagy. Furthermore, the promoted autophagy and excessive apoptosis after mitochondrial fission interference were validated to be beneficial and harmful for cellular survival, respectively. Mitochondrial fission reduction and fusion accession occurred consistently in the hippocampus in the NaF-exposed offspring rats with cognitive deficits and neuron damage, accompanied by defective autophagy and excessive apoptosis. Taken together, our results suggest that mitochondrial fission inhibition and the subsequent mitochondrial dysfunction trigger defective autophagy and excessive apoptosis contributing to developmental fluoride neurotoxicity.

Authors: Qian Zhao, Qiang Niu, Jing-Wen Chen, Tao Xia, Guo-Yu Zhou, Pei Li, Li-Xin Dong, Chun-Yan Xu, Zhi-Yuan Tian, Chen Luo, Lu-Ming Liu, Shun Zhang, Ai-Guo Wang. Department of Environmental Health and MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, People' s Republic of China.
Correspondence: Ai-Guo Wang; E-mail: wangaiguo@mails.tjmu.edu.cn
Keywords: Apoptosis; Autophagy; Developmental neurotoxicity; Fluoride; Mitochondrial fusion/fission.

FLUORIDE-INDUCED GLIAL CELL CHANGES IN THE MYENTERIC PLEXUS OF NEONATAL RATS

Saba Sarwar, Javed A Quadri, Seema Singh, Prasenjit Das, Tapas Chand Nag, Tata Sankar Roy, Ahmadulla Shariff
New Delhi, India

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The enteric nervous system (ENS) is embedded in the gut wall and consists of aggregates of neurons and glia called enteric ganglia. Glia within enteric ganglia are essential for homeostatic control. Disruption of glial functions could alter the gastrointestinal physiology by altering enteric neurotransmission or by permitting enteric neuronal death. Alterations in the number of enteric glial cells are linked with the inflammatory bowel diseases (IBDs) Crohn's and ulcerative colitis, chronic idiopathic intestinal pseudo-obstruction (CIIP), intractable slow-transit constipation (STC), Chagas disease, type II diabetes, and Parkinson's disease. In fluorosis endemic areas, gastrointestinal complications, including constipation, infrequent

diarrhea, loss of appetite, and abdominal pain, which mimic Irritable Bowel Syndrome (IBS) are very common. The aim of the study was to evaluate the fluoride-induced changes in the expression of GFAP, Vimentin, HuC/D, through immunofluorescence and real time PCR, and in the histological and ultrastructural changes in the glial cells in the myenteric plexus of the hind-gut. Female and male Wistar rats were randomly selected and allowed to mate. They were administered 50 and 100 ppm of fluoride in drinking water during the pregnancy and lactation (up to post-natal day 20). After birth, the pups were allowed to be on mother milk for 20 days, kept in the same cage and allowed to drink the same water. The pups were euthanized on the 20th post-natal day and the gut tissues were collected for the different types of investigations. In the fluoride-administered groups, the glial cells showed histological changes, increased glial cell numbers in the 50 ppm group, and decreased glial cell numbers in the 100 ppm group. The GFAP, Vimentin, and HuC/D expression decreased in both of the fluoride-treated groups. Various cellular and sub-cellular ultrastructural changes were also seen. Fluoride-induced ultrastructural alterations were observed in the glial cells including heterochromatic nuclei, dilated rER, ER fragmentation, mitochondrial disintegration, and nuclear membrane fragmentation. Fluoride-induced functional abnormalities of the gut may be due to alterations in the glial cells, neuronal cell injury, and apoptosis.

Authors: Saba Sarwar,[a] Javed A Quadri,[a] Seema Singh,[a] Prasenjit Das,[b] Tapas Chand Nag,[a] Tata Sankar Roy,[a] Ahmadulla Shariff.[a]
[a]Department of Anatomy; [b]Department of Pathology, All India Institute of Medical Sciences, New Delhi, 110029, India.
Correspondence: Javed Ahsan Quadri; E-mail: javedaiims@gmail.com
Keywords: Apoptosis; Enteric glia; Enteric nervous system (ENS); Fluoride toxicity; Ganglia; HuC/D; Inflammatory bowel diseases (IBD); Myenteric plexus Vimentin; Ultrastructural changes.

EFFECTS OF DRINKING WATER FLUOROSIS ON L-TYPE CALCIUM CHANNEL OF HIPPOCAMPAL NEURONS IN MICE

Qiu-Li Yu, Dan-Dan Shao, Wei OuYang, Zi-Gui Zhang
Jinhua, People's Republic of China

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14
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This study investigated the effects of drinking water fluorosis on L-type calcium channels in mice hippocampal neurons. A total of 120 newly weaned ICR male mice were randomly divided into a control group (CON), a low fluoride group (LF), and a high fluoride group (HF), whose drinking water was tap water, water with 5 mg/L sodium fluoride (NaF), and water with 30 mg NaF/L, respectively. After 3 and 6 months exposure to the fluoride, based on the successful replication of subchronic and chronic fluorosis animal models, the patch clamp technique was used to detect the peak value and relative value (I/Imax), steady-state activation curve ratio (G/Gmax), decay time constant, and tail current time constant of L-type calcium channel currents in the hippocampal CA1 region of mice brain slices. The results showed that (i) exposure to fluoride significantly reduced the serum calcium and urinary calcium concentrations ($p<0.05$ and $p<0.01$) in the hippocampal CA1 region in the mice; and that (ii) the effects in the hippocampal CA1 region of the mice of chronic fluoride exposure were greater than that of subchronic exposure to fluoride. The peak value of the L-type calcium channel current in the pyramidal neurons was significantly increased ($p<0.05$ or $p<0.01$) with the prolonged exposure time and the relative values of the current and the steady-state coefficient were significantly changed; The decay time and tail current time increased significantly ($p<0.01$), and the higher the fluorine

concentration, the greater was the peak value and open time of the LTCC opening in the pyramidal neurons in the hippocampal CA1 region. It is suggested that the L-type calcium channels are sensitive to fluoride exposure, the activation voltage of calcium channels induced by fluoride exposure is decreased, the opening time of calcium channels is prolonged, and the calcium influx per unit time is increased. These changes overload the calcium concentration in the neurons and the increased calcium triggers cell apoptosis and eventually causes neuronal damage. The imbalance of calcium metabolism caused by fluorosis may be one of the mechanisms involved in the pathogenesis of brain injury induced by fluoride. It also suggests that the risk of brain damage from low-fluorine exposure cannot be ignored.

Authors: Qiu-Li Yu,[a] Dan-Dan Shao,[a] Wei OuYang,[b] Zi-Gui Zhang[a,c]
[a]College of Chemistry and Life Science at Zhejiang Normal University; [b]Physical Education College and Health Science of Zhejiang Normal University; [c]Zhejiang Normal University Xingzhi college -Jinhua 321004, People's Republic of China.
Correspondence: Zhi-Gui Zhang; E-mail: zzg@zjnu.cn
Keywords: Calcium imbalance; Fluorosis; Hippocampal CA1 region; L-type calcium channel; Patch clamp.

RNA DEEP SEQUENCING ANALYSIS OF THE RAT HEART EXPOSED TO FLUORIDE

Xiao-Lin Tian, Ni-Sha Dong, Jing Feng, Xiao-Yan Yan,
Taiyuan, Shanxi, People's Republic of China

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Fluorosis, caused by the long-term intake of fluoride, has a damaging effect on the cardiac tissue according to many studies. However, the mechanisms have not been elucidated completely. High-throughput mRNA sequencing (RNA-Seq) of cardiac tissue was performed in this study to explore the gene alterations of rats exposed to NaF. The results showed that some differentially expressed genes (DEGs) were identified by comparing the control group and the fluoride-exposed groups, who received NaF in the drinking water for 6 months in doses of 30 mg/L, the low fluoride (LF) group, or 90 mg/L, the high fluoride (HF) group. Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and Protein-Protein Interaction (PPI) network analysis were implemented subsequently. Additionally, the result of qRT-PCR and Western Blotting showed that the transcriptome level and the protein expression level of inflammatory related genes were decreased in both the LF and HF groups. These results add to the knowledge about the underlying mechanism of fluoride-induced cardiotoxicity.

Authors: Xiao-Lin Tian,[a] Ni-Sha Dong,[b] Jing Feng,[a] Xiao-Yan Yan,[b]
[a]Shanxi Key Laboratory of Experimental Animal and Human Disease Animal Models, Shanxi Medical University, Taiyuan, Shanxi, 030001, People's Republic of China.
[b]School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, 030001, People's Republic of China;
Correspondence: Xiao-Yan Yan; E-mail: yanxiaoyan@sxmu.edu.cn.
Keywords: Fluoride; Inflammatory injury; RNA deep Sequencing Analysis; Toll-like pathway

FLUOROSIS MANAGEMENT THROUGH PLANTS IN SOME ENDEMIC VILLAGES OF BIHAR

Rajesh Kumar, Virender Kumar
Patna, India

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The excessive consumption of fluorides for a long period in various forms may lead to the development of the different forms of fluorosis: dental, skeletal and non-skeletal. It is a well-established fact that, due to universal presence of fluoride in earth's crust, all water sources contain fluoride in varying concentrations. Our studies are mainly confined to the three villages of the Nawada district of Bihar, India, (Kachhariadih,

Hardia and Muslimtola) which are severely affected by fluorosis. In these villages hand pumps are the main source of drinking water which has a fluoride concentration range of 1.9–6.4 ppm, with the upper part of the range being much above the maximum permissible limit of 1.5 ppm. Our research team is working for the "Integrated Fluorosis Mitigation Programme" (IFM) in collaboration with (CSIR-NEERI) Nagpur, (NIRTH) Jabalpur, UNICEF, Patna, and PHED (Public Health Engineering Department), Government of Bihar, Patna. The main aim is to provide fluoride-safe water and to make the villagers aware of the need to consume specific nutritional foods because these two interventions are said to be the best approach to mitigate fluorosis. We are mainly doing our research work to mitigate fluorosis through plants since medicinal plants play an important role in our social health system. They are easily available in the wild and cultivated forms and can be used as a good source of dietary supplements. Considering the above facts and on the basis of our research observations, we have selected four medicinal plants for the amelioration of fluorosis due to a high fluoride concentration in the drinking water. The plants are: (i) *Sida cordifolia* of the family Malvaceae; (ii) *Ocimum sanctum* of the family Lamiaceae; (iii) *Tamarindus indica* of the family Fabaceae; and (iv) *Moringa oleifera* of the family Moringaceae. We are getting positive results which we could discuss during our oral presentation. Secondly our research work is focused on a miracle plant named *Cassia tora* of the family Caesalpinaceae for fluorosis patients in the lower income group. This plant is a very good source of dietary supplements as it contains substantial amounts of calcium, vitamin C, vitamin D_3, and Iron. They are available even in the remote areas and easily edible in the form of vegetables only. We are working extensively on the cytogenetical aspect of *Cassia tora* to induce polyploidy (tetraploid 4n) with the help of colchicine treatment. Diploid chromosome number 2n=26 is reported in this plant and we are trying to obtain the tetraploid plant having 4n=52. The chemical environment within the tetraploid cell will be changed leading to a doubling of calcium, iron, vitamin C, and vitamin D_3. This will solve the problems related to providing nutritional supplements of the plant for fluorosis patients by enhancing the size of the leaf, the thickness of the stem and the floral parts, and seed production. The resistance capacity of the plant will also be enhanced which could be exploited for other beneficial purposes in several ways.

Rajesh Kumar,[a] Virender Kumar[b]
[a]Project Supervisor, Centre for Fluorosis Research A.N. College, Patna 800013, India; [b]Project Officer, Centre for Fluorosis Research A.N. College, Patna 800013, India.
Correspondence: Rajesh Kumar; E-mail: rajeshkumar1952@gmail.com.
Keywords: *Cassia tora*; Fluorosis management; Polyploidy.

THE ROLE OF MAPK SIGNAL TRANSDUCTION PATHWAY AND ECM IN PATHOGENESIS OF BRAIN DAMAGE OF RATS WITH CHRONIC FLUOROSIS

Yan-Jie Liu, Zhi-Zhong Guan
Guiyang, People's Republic of China

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17
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Chronic fluorosis can induce severe damages to many systems of the body. Many researchers have focused on the injury in the central nervous system (CNS) caused by chronic fluorosis. However, the mechanism of brain injury induced by fluoride toxication is still not clear. Interestingly, the function of the neurons, glia, and the extracellular matrix (ECM) is always connected to neuronal signaling transduction in the CNS. The

mitogen-activated protein kinases (MAPK) pathway is an evolutionarily conserved signaling cascade involved in both synaptic plasticity and memory formation. We aimed to study MAPK-dependent translation regulation in the neurons and ECM with prolonged overexposure to fluoride. We analyzed the role of family members of MAPK including ERK1/2, JNK, p38 and ERK5 in animal models and through the neuroblastoma cells SH-SY5Y and investigated the correlation between MAPK signal transduction and apoptosis. The results of our study, with the appearance of dental fluorosis in the fluoride-exposed animals and occurrence of increases in the fluoride content in the urine, blood, and bones, compared to the control, indicated that we established the animal model successfully. In the rats with chronic fluorosis, we found an impairment learning and memory and a number of neuropathological changes in the brain, including condensed cytoplasm, heightened acidophilia, decreased Nissl bodies, and an increased expression of ERK1/2, ERK5 and JNK in protein and the mRNA level. A change of some transcription factor CREB Elk-1 was also found in rat brain and in SH-SY5Y cells. No change was found after 3 months in the apoptotic death rate of rat brain but it was increased after 6 months. The increased apoptotic death rate was also found in the SH-SY5Y cells treated with fluoride together with a changed expression of c-jun mRNA and c-fos mRNA. We also found there was crosstalking between JNK, p38, and ERK1/2. The results showed that crosstalk between p38 and ERK1/2 promoted the expression of c-fos and the apoptotic death rate. Crosstalk between JNK and ERK1/2 did not influence the expression of c-fos. An increased phospho-ERK1/2, phospho-JNK, and p38 was found in the silenced nAChRα3 subunit of the SH-SY5Y cell treated with fluoride, and the expression level of the kinase was different to that expression in SH-SY5Y cells treated with fluoride at the same time point. The distribution of phospho-ERK1/2 was interesting, especially in the neuropil of hippocampus and cortex, so we set up the hypothesis that the MAPK pathway, ECM, and maybe glia were involved in the pathogenesis of the brain injury caused by fluorosis. We detected the MMP2, MMP9, GFAP, SYN in rat brain and SH-SY5Y cells overexposed to fluoride and we also treated the rats and SH-SY5Y cells with the key molecule chondroitin sulfate. The expression of phospho-Erk1/2 and Erk1/2 in the brain tissue of the rats with experimental fluorosis was significantly higher than that in control group and the chondroitin sulfate-treated group. Immunohistochemistry results showed that average gray value of phospho-Erk1/2, in the chondroitin sulfate-treated group was significantly higher than that of the control group and the fluoride group. The average gray value of MMP-2 in the fluoride group was significantly higher than that in the control group and in the chondroitin sulfate-treated group. The average gray value of the MMP-9 protein of the fluoride group and the chondroitin sulfate-treated group, was significantly higher than that of the control group, respectively. The survival time and the amount of SH-SY5Y cells treated with chondroitin sulfate was significantly higher than in the control group and in the fluoride group. The animal experiment results showed that most of the rats in the chondroitin sulfate-treated group performed better in the Morris water maze test, by showing a better search pattern significantly more often than the rats in the fluoride and control groups. In summary, in the pathogenesis of the damage to the nervous system resulting from chronic fluorosis, the MAPK pathway may be activated and participate in the procedure of neuronal apoptosis and in the changed ability of learning and memory. The Erk1/2 pathway possibly maintains the stability of cell survival by regulating the

expression of MMP-2 and MMP-9. To a certain extent, chondroitin sulfate can be protective of nerve cells and provide some resistance to the nervous system damage caused by fluorosis. The changed MAPK pathway might be one of the major mechanisms of brain damage induced by chronic fluorosis.

Authors: Yan-Jie Liu,[a] Zhi-Zhong Guan[a,b]
[a]Department of Pathology, Guizhou Medical University affiliated hospital, Guiyang 550004, People's Republic of China; [b]The Key Lab of Molecular Biology in Guiyang Medical University, Guiyang 550004, People's Republic of China.
Correspondence: Yan-Jie Liu, Email: lyj_liuyanjie@hotmail.com
Keywords: Chronic fluorosis; ECM; MAPK pathway; Rats; SH-SY5Y cells.

EFFECT OF EXPOSURE TO FLUORIDE ON BONE DEVELOPMENT OF ZEBRAFISH

Jun-Jun Li, Qiao-Shi Zhao, Yan-Mei Yang, Dian-Jun Sun, Yan-Hui Gao
Harbin, People's Republic of China

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18
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The purpose of this study was to study the effect of fluoride exposure on skeletal development of zebrafish larvae, using an established zebrafish fluorosis model, in order to provide new ideas for further investigation of the exact pathogenesis of skeletal fluorosis. The zebrafish larvae at 3 days post fertilization (3 dpf) were exposed to the conventional fish water with 0, 0.5, 1.0, 4.0, 25.0, 50.0, and 100.0 mg/L of NaF for 5 days. The fluorine content of zebrafish larvae was detected by a F-ion selective electrode. The mineralization of the zebrafish skeleton was detected with alizarin red. The areas and IOD of the bone staining were quantitatively analyzed with a digital microscope. The activity of AKP and StrACP in the zebrafish larvae was detected with a microplate reader, Immunohistochemistry was used to detect the expression of the related proteins in the zebrafish larvae. Compared with the control group, the fluorine content of the zebrafish larvae in the fluoride-treated groups increased to various degrees ($p<0.01$). The larvae's fluorine content increased with the exposure dose. There was a strong positive correlation between the dose and the fluorine content ($r=0.889$, $p<0.05$). Compared with the control group, the mineralized area and mineralization of the skull increased in the low-dose (0.5–4.0 mg/L) groups ($p<0.05$), while it decreased in the high-dose (50.0–100.0 mg/L) groups ($p<0.05$). With the increasing dose of exposure, the density of the spinal bone decreased ($p<0.05$) and there was a strong negative correlation between fluoride exposure dose and the density of spinal bone ($r=0.737$, $p<0.05$). Compared with the control group, the AKP activity in the low-dose groups increased ($p<0.05$). There was a strong positive correlation between the fluorine exposure dose and the activity of StrACP ($r=0.588$, $p<0.05$). There was a strong positive protein expression of BMP2 in the fins and the basal bones of the skull of the zebrafish larvae. Compared with the control group, low-dose (1.0, 4.0 mg/L NaF) fluorine exposure increased the protein expression of BMP2 but high-dose (50.0 and 100.0 mg/L NaF) exposure decreased it. β-catenin protein is expressed on the surface of the whole body skin of zebrafish larvae, and symmetrically distributed from the head to the tail. The positive proportion of β-catenin protein in the low-dose (1.0, 4.0 mg/L NaF) groups was significantly higher than that in the control group. The total fluorine content of the zebrafish larvae in the fluoride-exposed groups increased significantly, and there was a strong positive correlation between the dose and the fluoride content, suggesting that the zebrafish larval fluorosis model was successfully established. Fluorine exposure can lead to osteoporosis and osteosclerosis in zebrafish larvae, and the damage at different

skeletal phases might depend on the fluoride exposure doses and the sites of the bone tissue: low-dose fluoride exposure leads to osteosclerosis of the skull, while high-dose exposure leads to osteoporosis; and fluorine exposure can lead to spinal osteoporosis. Exposure to fluorine enhanced the activity of osteogenesis and osteoclasts in zebrafish larvae. Exposure to 1.0 mg/L and 4.0 mg/L of NaF increased the protein expression of BMP2 and β-catenin in zebrafish larvae.

Authors: Jun-Jun Li, Qiao-Shi Zhao, Yan-Mei Yang, Dian-Jun Sun, Yan-Hui Gao; Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University; Key Lab of Etiology and Epidemiology, Education Bureau of Heilongjiang Province & Ministry of Health; and Heilongjiang Provincial Key Laboratory of Trace Elements and Human Health, Harbin Medical University, Harbin, People's Republic of China.
Correspondence: Yan-Hui Gao; E-mail: gaoyh411@163.com
Keywords: Osteoporosis; Osteosclerosis; Skeletal fluorosis; Zebrafish.

FALSE REPORTING OF WATER FLUORIDATION DATA AND FABRICATED STATEMENTS REGARDING FLUORIDATION SAFETY BY THE U.S. CENTERS FOR DISEASE CONTROL

Douglas Arthur Cragoe
North Hollywood, California, USA

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The U.S. Centers for Disease Control (CDC) is a federal agency that promotes fluoridation. It operates a website called "My Water's Fluoride" which has false data on water fluoride levels. Unknown to readers, the fluoride levels for all the water systems that add fluoride are automatically set to a "default" value so they are all the same, regardless of the actual tests or targets for fluoride levels. Fluoride levels for water systems with natural fluoride are frequently incorrect, or based on tests that are many years out of date. A CDC webpage used to contain fabricated statements about the safety of fluoride and fluoridation. The CDC was notified, but nothing happened for many months until an E-mail was sent to the CDC director who was about to leave the agency.

.Author: Douglas Arthur Cragoe; Fluoride Action Network, USA; North Hollywood, CA, USA.
Correspondence: E-mail: cragoe@sbcglobal.net.
Key words: Autism spectrum disorders; Aluminum; Aluminofluoride complexes; Fluoride; Glutamatergic neurotransmission; Immunoexcitotoxicity; Microglial activation; Neurodevelopment.

EFFECTS OF PHYSICAL EXERCISE ON BONE REMODELING AND NEUROGENESIS IN MICE EXPOSED TO FLUORIDE

Rui-Yan Niu, Zi-Long Sun, Ji-Xiang Wang, Rui Li, Zhen-Zhen Guo, Jin-Ming Wang, Jian-Hai Zhang, Jun-Dong Wang
Taigu, Shanxi, People's Republic of China

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In order to study on the effects of physical exercise (PE) on bone remodeling and neurogenesis in developing mice treated with fluoride, mouse pups were exposed to 100 mg NaF/L through drinking water from postnatal days 21 to 70. Meanwhile, the mice also exercised at the speed of 5 m/min for 2 hr/day. The results showed that exposure to 100 mg NaF/L significantly enhanced the serum alkaline phosphatase activity and the ratio of the receptor activator of nuclear factor kappa B ligand and osteoprotegerin (RANKL: OPG), while the expression of osterix (OSX), runt-related transcription factor 2-I (RUNX2-1) in femur, and postsynaptic density-95 (PDS-95) in hippocampus were reduced. In addition, the daily PE reversed the changes in the indexes mentioned above. These findings suggest that physical exercise can weaken

the impact of F on bone and nervous system by preventing the F-induced changes in the expression of bone and synaptic markers.

Author: Rui-Yan Niu, Zi-Long Sun, Ji-Xiang Wang, Rui Li, Zhen-Zhen Guo, Jin-Ming Wang, Jian-Hai Zhang, Jun-Dong Wang; Shanxi Key Laboratory of Ecological Animal Science and Environmental Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi, People's Republic of China.
Correspondence: Rui-Yan Niu; E-mail: niuruiyan2000@163.com.
Key words: Alkaline phosphatase; Bone remodelling; Hippocampus; Mice; Neurogenesis; Physical exercise; Postsynaptic density-95 in hippocampus; RANKL:OPG ratio; RUNX2-1; Osterix (OSG).

FLUORIDE INDUCES ALTERATION IN GUT CHEMISTRY

Javed Ahsan Quadria, Saba Sarwara, A Shariffa
New Delhi, India.

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An increased prevalence of malnutrition and gastrointestinal complications has been reported in fluorosis endemic areas. Fluoride induces injuries to soft tissue due to multiple changes in cellular metabolism and physiology including oxidative stress, protein damages, lipid peroxidation (LPO), and gut mucosal injury. Previously it has been reported that fluoride induced mucosal injury and retarded the absorption of ingested nutrients. In the present study, it was hypothesized that the chronic ingestion of fluoride may alter gut chemistry due to the fluoride ion having the highest electro negativity. This study was planned to examine the effects of fluoride ingestion on gut chemistry. After approval of the protocol by the Institutional Ethics Committee, AIIMS, New Delhi, India, fifteen-weeks-old Wistar rats (n=28) were randomly divided in to three groups. Group-1 was continuously administered 50 ppm of fluoride and Group-2 was administered 100 ppm of fluoride (equivalent to doses in humans of 10 and 20 ppm of fluoride, respectively) *ad libitum*, for two months. Group-3 was kept on normal drinking water and considered as a control group. Following two months of fluoride administration, the animals were sacrificed and their gut tissue and gut contents were harvested and subjected to various chemical and molecular evaluations. Histological and ultrastructural analyses were done. In the fluoride-exposed groups, a significant ($p<0.05$) change in the chemical nature of gut content was observed. Many histopathological and ultrastructural injuries in the colon were also seen due to the chronic fluoride toxicity. These changes lead to malnutrition in fluoride-exposed groups.

Authors: Javed Ahsan Quadria, Saba Sarwara, A Shariffa; Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi, India.
Correspondence: Javed Ahsan Quadri; E-mail: javedaiims@gmail.com
Keywords: Apoptosis; Fecal chemistry; Fluoride toxicity; Gut; Inflammatory bowel diseases (IBD); Ultrastructural changes.

THE HISTORICAL REVIEW AND DEVELOPMENT STRATEGIES ON PREVENTION AND CONTROL OF COAL-BURNING TYPE OF ENDEMIC FLUOROSIS IN LIUPANSHUI, GUIZHOU OF CHINA

Tin-Xu Jin, Hua Zhang, Zhi-Zhong Guan
Guiyang, Guizhou Province, People's Republic of China

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To aim of the study was to introduce the historical review development strategies on the prevention and control of coal-burning fluorosis in Liupanshui, Guizhou. Studies were conducted to investigate the prevention and control of coal-burning type of endemic fluorosis (CBF) situation and compare the effects from 2004 to 2015 in Liupannshui, Guizhou. It showed that in 2004, the concentration of fluoride in indoor and outdoor

air was 4.3 to 5.6 times over the national standard. Fluoride in drinking water of the 4 towns did not exceed the national standard (≤1.0 mg/L). The foods with a higher fluorine content are corn and chili. In CBF areas, all intakes are higher than the national standard (2.0 mg/L). The main intake pathway is food. The dental fluorosis prevalence rate in children is 63% (Jinpen), 43% (Shuangga), 56% (Douqin), and 0 (Huaga). The highest urine fluoride concentration of children was in Jinpen town. The dental fluorosis prevalence rates and the urinary fluoride in children increased as the total fluoride intake increased. In 2012, after comprehensive prevention and control, the rate of awareness of the prevention and treatment of fluorosis among primary school students in 37 counties of Guizhou Province increased from 45.03% to 89.79%, a rise of 44.76 percentage points. The results among women of childbearing age showed the awareness rate increased from 38.97% to 76.55%, a rise of 37.58 percentage points. In 2015, the dental fluorosis prevalence rate was 27.0% and the detection rate, the defect rate, the rate of medium dental fluorosis, and the dental fluorosis index in children between the ages of 8 and 12 years in Douqin were lower than the corresponding values in 2001 ($p<0.05$). In order to lower or eliminate the coal-burning type of endemic fluorosis, comprehensive and sustainable prevention and control system is necessary. After years of work, the coal-burning fluorosis in Liupanshui has been effectively controlled.

Authors: Tin-Xu Jin,[a] Hua Zhang,[a] Zhi-Zhong Guan[b]
[a]School of Public Health and [b]The Lab of Medical Molecular Biology, Guizhou Medical University, People's Republic of China.
Correspondence: Hua Zhang; E-mail: 616543807@qq.com
Keywords: Coal-burning fluorosis; Dental fluorosis; Historical review; Liupanshui; Prevention and control.

ASSESSMENT OF FLUORIDE CONTAMINATION IN GROUNDWATER OF DISTRICT MARDAN IN PAKISTAN

Zia ur Rahman, Bushra Khan, Hizbullah Khan, Mark L Brusseau, Iqbal Ahmad
Khyber Pakhtunkhwa, Pakistan, and Arizona, USA

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Consumption of groundwater with elevated levels of the fluoride ion (F^-) for drinking purpose is a major F^- exposure pathway for humans. Groundwater samples (n=100) were collected from 25 villages of district Mardan of the Khyber Pakhtunkhwa (KP) province and analyzed for anions including F^-, chloride (Cl^-), bromide (Br^-), phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) through ion chromatography. The concentrations of F^- in the groundwater samples ranged between 0.05 mg/L and 10.8 mg/L. The measured concentrations were within the permissible limits of the National Environmental Quality Standards (NEQS) of Pakistan for drinking water in 92% of the samples. The groundwater samples of three villages exhibited a F^- concentration greater than the permissible level of 1.5 mg/L. F^- has shown a positive correlation with electrical conductivity (EC), total dissolved solids (TDS), Br^-, PO_4^{3-} and SO_4^{2-}. To safeguard public health this study suggests further research in the area for possible sources and mitigation measures of F- contamination.

Authors: Zia ur Rahman,[a,b,c] Bushra Khan,[a] Hizbullah Khan,[a] Mark L Brusseau,[b] Iqbal Ahmad[a]
[a]Department of Environmental Science, University of Peshawar, Khyber Pakhtunkhwa, Pakistan; [b]Department of Soil, Water and Environmental Science, The University of Arizona, USA; [c]Department of Environmental Science, University of Haripur, Khyber Pakhtunkhwa, Pakistan.
Correspondence: Zia ur Rahman; E-mail: ziaesh@yahoo.com
Key words: Fluoride; Ground water; Pakistan.

DETERMINATION OF FLUORIDE CONTENTS IN COMMERCIALLY AVAILABLE BEVERAGES IN RAWALPINDI CITY, PAKISTAN

Sofia Khalid, Maria Shoukat, Saba Khurshid, Rahim Shah,
Muhammad Adrees, Muhammad Nauman Ahmad
Rawalpindi and Peshawar, Pakistan, and London, UK

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The increasing consumption of soft drinks, juices, and other beverages as a replacement for water have made the fluoride content in beverages an important issue. Fluoride intake greater than a certain limit may increase the risk for and development of dental fluorosis. This study was designed to evaluate the fluoride content in some commonly used brands of industrialized beverages consumed in Rawalpindi, a highly populated city of Pakistan. All the samples were chosen on the basis of their high public demand. an ion selective electrode (ISE) was used for the determination of the fluoride contents in juice and soft drink samples. The concentration of fluoride observed in all the analyzed samples ranged between 0.002 mg/L and 0.2 mg/L. The concentration of fluoride in most of the examined beverages was below 0.1 mg/L and lay within the values provided by the USDA database on the fluoride content of particular beverages. Therefore, it was concluded that ingestion of these beverages where concentration was below 0.1 mg/L at moderate level may not increase the human exposure to fluoride. However, a few brands were identified with greater concentrations of fluoride that may affect the teeth and skeleton.

Author: Sofia Khalid,[a] Maria Shoukat,[b] Saba Khurshid,[a] Rahim Shah,[b] Muhammad Adrees,[c] Muhammad Nauman Ahmad[d]
[a]Environmental Sciences Department, Fatima Jinnah Women University, Rawalpindi, Pakistan; [b]National Institute of Health, Islamabad, Pakistan; [c]Imperial College London UK; [d]Agriculture University, Peshawar, Pakistan.
Correspondence: Sofia Khalid; E-mail: sofiarahim@hotmail.com
Keywords: Beverages; Dental fluorosis; Fluoride contents; Ion Selective Electrode (ISE); Juice; Soft drink; USDA database.

SIMULTANEOUS REMOVAL FLUORIDE AND ARSENIC FROM DRINKING WATER USING A NEW NANO- MICRO CERAMIC MATERIALS

Gui-Fan Sun, Yong-Fang Li, Da Wang, Liang-Qi Dong, Xin Li, Bing Li,
Quan-Mei Zheng, Liang-Jie Dong
Shenyang and Suzhou, People's Republic of China, and Honolulu, Hawaii, USA

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Fluoride is widespread in the environment. Drinking water is the main source of the fluoride exposure. Long-term exposure to a high dose of fluoride can cause chronic fluorosis, with not only skeletal fluorosis, dental fluorosis, and other bone damages, but also damage of non-bone organs such as blood and the nervous system. The endemic fluorosis caused by drinking water is one of the most serious endemic diseases in China. Arsenic is another naturally occurring pollutant in ground water. On the global scale, drinking arsenic-contaminated water is the most common way for people to be exposed to arsenic. It is well known that long-term arsenic exposure can induce enormous health hazards, especially carcinogenicity. In China, residents exposed to these two chemicals have been found in many places. Therefore, developing technologies and materials for the simultaneous removal of fluoride and arsenic from water contaminated with these two elements could be very important for the people exposed to the two pollutants. A new ceramic adsorbent, which is synthesized with nano-scale microceramic materials, has been developed recently by our group. Three

kinds of products—microceramic granules, microceramic powder, and microceramic paper—have already been put on the market. The microceramic granules and the microceramic paper have recently been certified by NSF-International. Our findings have shown that these new nano-microceramic materials can effectively remove fluoride and arsenic contamination, not only from contaminated drinking water but also from industrially contaminated water. The microceramic material was highly effective for arsenic removal and did not cause secondary arsenic pollution. With the advantages of simple operation and strong practicability, we consider that the method can be applied generally in fluoride-contaminated areas, especially in those areas that are also polluted with arsenic.

Authors: Gui-Fan Sun,[a,b] Yong-Fang Li,[a] Da Wang,[a] Liang-Qi Dong,[b] Xin Li,[a] Bing Li,[a] Quan-Mei Zheng,[b] Liang-Jie Dong[b,c]
[a]Research Center of Environment and Non-Communicable Disease, School of Public Health, China Medical University, Shenyang 110013, People's Republic of China; [b]Suzhou AFIO Institute of Environmental and Health, People's Republic of China; [c]Hawaii Institute of Interdisciplinary Research, Honolulu, HI, USA.
*Correspondence: Gui-Fan Sun, E-mail: sungf@mail.cmu.edu.cn
Keywords: Arsenic; Fluoride; Microceramic granules; Microceramic paper; Microceramic powder.

DATABASE MANAGEMENT OF DEFLUORIDATION WORK AS AN ESSENTIAL TOOL IN MINIMISING EXPOSURE TO FLUORIDE THROUGH WATER

Eli Dahi
Ngongongare, Tanzania and Helsinge, Denmark

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Since water wells may have different fluoride concentrations, even if they are dug or drilled within the same geographical area, and since the capacities of defluoride filters would further depend on the water use, defluoride filters may have different operation periods, even if the filters are of same design and installed at a household level. Institutional filters are normally larger and designed differently, so that the estimated operation periods are meant be ½, 1, or 2 years. The factual operation period may then show a higher multitude of variation. Proper recharge of filters, i.e., not much later or much earlier than the medium saturation and the fluoride breakthrough, is essential to avoid unintentional fluoride exposure or, on the other end, the waste of valuable defluoridation medium. Thus, a rational management of defluoridation works must include surveillance of defluoride filters, estimation of individual operation periods and on the time recharge of saturated filters. This is done best in a database, where all the necessary information is contained about the client, the site, the raw water, the filter, its recharges and, most importantly, its first coming date of recharge. Automatically, the database would produce up-to-date lists of installed filters that would need recharge and further generate E-mails to clients indicating to them the dated of the last filter installation and offering them a new recharge on time. Our experience shows that the database and the E-mail reminders contribute to a higher sustainability of defluoridation works. The paper explains further some of the principles and the setup of the Defluoridation Technology Project database.

Author: Eli Dahi, Defluoridation Technology Project, Tanzania/Denmark.
Correspondence: Eli Dahi; E-mail: elidahi@hotmail.com.
Key words: Database management; Defluoridation technology.

FABRICATION AND STUDY ON A DOMESTIC DEFLUORIDATOR USING BURNT BRICK CHIPS OF SPECIFIC COMPOSITION AS FILTER MATERIAL

Arvind Kumar Nag1* and Chiranjeev Kumar
Patna and Bodh Gaya, India

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Ground water forms the most common source of drinking water in the rural as well as the urban areas of Bihar, India. In some regions of the world, including India and in some rocky areas of Bihar, the earth crust is rich in fluoride-bearing minerals due to which the ground water is naturally fluoridated. Intake of fluoridated ground water for a long period causes fluorosis. Defluoridation of the available fluoride-rich water in fluorosis endemic areas for drinking and cooking purposes constitutes the main mitigation measure. Of the various defluoridation techniques available now, the one which is cheap, easy to operate, and which is associated with the least health hazard is the domestic defluoridator using burnt brick chips of specific composition as the filter material. The beneficiary families can operate, monitor, and change the filter material without any specialised training. This kind of domestic defluoridator based on burnt brick chips as adsorption material has been in use in fluorosis endemic regions of Sri Lanka for a long time. But the design of defluoridator developed in Sri Lanka and the type of filter material available there have their utility and suitability in Sri Lanka only. The climatic conditions available in Bihar, the level of fluoride concentration in groundwater here, the type of soil used for brick making in India, particularly in Bihar, etc. are quite different from those in Sri Lanka. Our team of researchers have designed and fabricated a model defluoridator using burnt brick pieces made of the red soil that is readily available in some areas of the Bihar and Jharkhand states. Fluoride-contaminated water samples collected from some fluorosis endemic areas of Bihar have been studied using the defluoridator designed and fabricated by our team. For this purpose fluoride-rich water samples were collected at the inlet and outlet points of the defluoridator and analysed for different physico-chemical parameters of drinking water including the fluoride concentration. The results obtained are encouraging, as the defluoridator fabricated by our team has been found to lower the fluoride concentration in fluoride rich water from 4–5 ppm to 1.0 ppm or even below, which is the safe limit for its use for drinking and cooking purposes.

Authors: Arvind Kumar Nag,[a] and Chiranjeev Kumar[b]
[a]Department of Chemistry, College of Commerce, Arts and Science, Patna-800020, India; [b]Faculty of Science (Environmental Sciences) Magadh University, Bodh Gaya, India.
Correspondence: Arvind Kumar Nag, E-mail: arvindkumar.nag@gmail.com
Keywords: Burnt brick chips; Domestic defluoridator.