ABSTRACTS

ROLE OF FLUORIDE IN METABOLIC BONE DISEASE IN FROGS
FLUOROSIS AS A PROBABLE FACTOR IN METABOLIC BONE DISEASE IN CAPTIVE NEW ZEALAND NATIVE FROGS (LEIOPELMA SPECIES)

This report describes the investigations into the cause and treatment of metabolic bone disease (MBD) in captive native New Zealand frogs (Leiopelma spp.) and the role of fluoride in the disease. MBD was diagnosed in Leiopelma archeyi and Leiopelma hochstetteri in 2008 at three institutions: Auckland Zoo, Hamilton Zoo, and the University of Otago. Most of these frogs had originally been held at the University of Canterbury for several years (2000–2004) but some were collected directly from the wild. Radiographs on archived and live frogs showed that MBD had been present at Canterbury, but at a lower rate (3%) than in the current institutions (38–67%). Microcomputed tomography showed that the femoral diaphyses of the captive frogs at Auckland Zoo had greater bone volume, bone surface, cross-sectional thickness, and mean total cross-sectional bone perimeter, which is consistent with osteofluorosis. On histology of the same femurs, there was hyperplasia, periosteal growth, and thickening of trabeculae, which are also consistent with skeletal fluorosis. An increase in fluoride levels in the water supply preceded the rise in the incidence of the above pathology, further supporting the diagnosis of osteofluorosis. Analysis of long-standing husbandry practices showed that ultraviolet B (UVB) exposure and the dietary calcium:phosphorus ratio were deficient when compared with wild conditions—likely causing chronic underlying MBD. To prevent multifactorial MBD in captive Leiopelma, the authors recommend increasing dietary calcium by incorporating into the captive diet inherently calcium-rich invertebrates; increasing exposure to natural or artificial (UVB) light; and using defluoridated water. Addressing these three factors at Auckland Zoo reduced morbidity, bone fractures, and mortality rates.

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Keywords: Amphibian; Bone fluorosis; Calcium deficiency; Leiopelma; Metabolic bone disease; New Zealand Frogs; Osteodystrophy; Ultraviolet B exposure.

Editor’s Note: For a related comment, see Bruce Spittle: “Fluorosis in frogs: a red flag from New Zealand” [editorial] in this issue of Fluoride. pp.231-3.

META-ANALYSIS REVIEW OF FLUORIDE NEUROTOXICITY AND IQ
DEVELOPMENTAL FLUORIDE NEUROTOXICITY: A SYSTEMATIC REVIEW AND META-ANALYSIS

Although fluoride may cause neurotoxicity in animal models as well as in human adults, very little is known about long-term effects on children’s neurodevelopment. In order to investigate the effects of increased fluoride exposure and delayed neurobehavioral development, we performed a systematic review and meta-analysis of published studies. We searched the MEDLINE, EMBASE, Water Resources Abstracts, and TOXNET databases through 2011 for eligible studies. We also searched the China National Knowledge Infrastructure (CNKI) database, as many studies on fluoride neurotoxicity have been published
in Chinese journals only. In total, we identified 27 eligible epidemiological studies with high and reference exposures, endpoints of IQ scores or related cognitive function measures with means and variances for the two exposure groups. We estimated the standardized mean difference (SMD) between exposed and reference groups across all studies using random effects models. We conducted sensitivity analyses restricted to studies using the same outcome assessment and having drinking water fluoride as the only exposure. Cochran test for heterogeneity between studies, Begg’s funnel plot and Egger test to assess publication bias were performed. Meta-regressions to explore sources of variation in mean differences among the studies were conducted. The standardized weighted mean difference in IQ score between exposed and reference populations was \(-0.45\) (95% CI \(-0.56\) to \(-0.35\)) using a random-effects model. Thus, children in high fluoride areas had significantly lower IQ scores than those who lived in low fluoride areas. Subgroup and sensitivity analyses also indicated inverse associations, although the substantial heterogeneity did not appear to decrease. The results support the possibility of an adverse effect of high fluoride exposure on children’s neurodevelopment. Future research should include detailed individual-level information on prenatal exposure, neurobehavioral performance, and covariates for adjustment.

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Keywords: Fluoride Neurotoxicity; Intelligence.


Editor’s Note: For a related review, see Bruce Spittle: “Neurotoxic effects of fluoride” [editorial] in Fluoride 2011;44(3):117-24. The overall standardized mean difference (SMD) in IQ score between the 27 exposed and reference populations found, using a random-effects model, by Choi et al. of \(-0.45\) (95% CI \(-0.56\) to \(-0.35\)) is a weighted measure which refers to an average difference of 0.45 standard deviations rather than to an average difference in the IQ score of 0.45. For example, the standardized mean difference of \(-0.41\) standard deviations (95% CI \(-0.77\) to \(-0.04\)) found for the study by Poureslami et al. corresponds to a difference in the IQ score of approximately 6.5. Poureslami et al. found, using a Persian version of Raven’s Progressive Matrices Intelligence Test, that the mean IQ score of the children in low F Baft (0.41 ppm F in water) was 97.80±15.95 and in high F Koohbanan (2.38 ppm F in water) it was significantly lower at 91.37±15.63 (Poureslami HR, Horri A, Garrusi B. A comparative study of the IQ of children age 7–9 in a high and a low fluoride water city in Iran. Fluoride 2011;44(3):163-7]. Thus the standardized mean difference (SMD) is a weighted mean difference which can be standardized across studies to give the average difference in standard deviations for the measures of a particular outcome.
LOWER IQ FROM HIGH WATER FLUORIDE IN MAKOO/IRAN

EFFECT OF HIGH WATER FLUORIDE CONCENTRATION ON THE INTELLECTUAL DEVELOPMENT OF CHILDREN IN MAKOO/IRAN

Prolonged excessive intake of fluoride during children’s growth and development stages has been reported to be associated with mental and physical impairment. The aim of this cross-sectional study was to investigate the effect of excessive fluoride intake on the intelligence quotient (IQ) of children living in five rural areas in Makoo/Iran. A total of 293 children aged 6–11 years were selected from five villages in Makoo with normal (low) fluoride (0.8±0.3 ppm), medium fluoride (3.1±0.9 ppm), and high fluoride (5.2±1.1 ppm) in their water supplies. The IQ of each child was measured by the Raven’s test. Educational and residential information and the medical history of each child were recorded by a questionnaire completed by the parents. Data were analyzed by ANOVA with a significance level of 0.05. The mean IQ scores decreased from 97.77±18.91 for the normal, low fluoride group, to 89.03±12.99 for the medium fluoride group, and to 88.58±16.01 for the high fluoride group (P=0.001). Thus, children residing in areas with higher than normal or medium water fluoride levels exhibited more impaired development of intelligence, indicating that children’s intelligence may be affected by high water fluoride levels.

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FLUORIDE ALTERATION OF SPERM CHROMATIN STRUCTURE IN MICE

ALTERED SPERM CHROMATIN STRUCTURE IN MICE EXPOSED TO SODIUM FLUORIDE THROUGH DRINKING WATER

This study investigated the effects of sodium fluoride (NaF) on sperm abnormality, sperm chromatin structure, protamine 1 and protamine 2 (P1 and P2) mRNA expression, and histones expression in sperm in male mice. NaF was orally administrated to male mice at 30, 70, and 150 mg/L for 49 days (more than one spermatogenic cycle). Sperm head and tail abnormalities were significantly enhanced at middle and high doses. Similarly, sperm chromatin structure was also adversely affected by NaF exposure, indicating DNA integrity damage. Furthermore, middle and high NaF significantly reduced the mRNA expressions of P1 and P2, and P1/P2 ratio, whereas the sperm histones level was increased, suggesting the abnormal histone-protamine replacement. Therefore, we concluded that the mechanism by which F induced mice sperm abnormality and DNA integrity damage may be involved in the alterations in P1, P2, and histones expression in sperm of mice.

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Keywords: Chromatin structure; Fluoride; Mice; Sperm.
**Chronic Pain From Waterborne Fluoride in Thailand**

**Association of Fluoride in Water for Consumption and Chronic Pain of Body Parts in Residents of San Kamphaeng District, Chiang Mai, Thailand**

In this study we assessed the dose response of fluoride exposure from water and chronic pain. Using a retrospective cohort design, the study was conducted in two sub-districts of San Kamphaeng district, Poo-kha and On-tai. Five hundred and thirty-four residents aged ≥50 years of age were interviewed about their sources of drinking water and assessed for chronic pain. Each water source was sampled for fluoride measurement, from which the average daily fluoride dose was estimated. Binary logistic regression with forward stepwise (likelihood ratio) model selection technique was used to examine the association between the average daily fluoride dose and chronic pain. We found associations between the average daily fluoride dose and lower back pain [odds ratio (OR) = 5.12; 95% confidence interval (CI), 1.59–16.98], and between the high fluoride area vs. the low fluoride area (OR = 1.58; 95% CI, 1.10–2.28; relative risk = 1.22 with 95% CI, 1.14–1.31) to lower back pain. Other risk factors, such as family history of body pain and a history of injury of the lower body, were also associated with lower back pain. However, there were no relationships between the average daily fluoride dose and leg and knee pains. To prevent further lower back pain, we recommend that the water in this area be treated to reduce its fluoride content.

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**Water Fluoridation Contrary to Medical Ethics**

**Ethics of Artificial Water Fluoridation in Australia**

A recent decision by several Australian federal politicians to support a parliamentary review of artificial water fluoridation has intensified the debate on the public health intervention. While there is a majority agreement among Australian dentists and other health professionals that adequate enamel fluoride is essential for dental health, the ethics of artificial fluoridation of public water supplies as a contemporary vehicle for facilitating adequate supply of fluoride to teeth is highly contested. Opponents of artificial water fluoridation insist that there are many alternative sources of fluoride, that mandatory water fluoridation violates the ethical principle of autonomy, and that water fluoridation is not only expensive and unnecessary but also may endanger health by causing fluorosis and, potentially, hypothyroidism and pathological bone fractures. In contrast, proponents of water fluoridation posit that mandatory water fluoridation facilitates health equity and that the benefits accruing to society from prevention of dental caries (beneficence principle) outweighs impairment of individual autonomy. This article utilizes Childress’ ‘justificatory conditions’ to evaluate the ethical appropriateness of artificial water fluoridation in Australia. The author concludes that there is insufficient ethical justification for artificial water fluoridation in Australia.
Endemic fluorosis is a serious problem in public health. Previous studies have indicated that patients with goiters usually live in fluoride-affected areas. However, the mechanism of goitrogenesis caused independently by fluoride is still unclear. The principle objective of this study was to investigate the possible roles of nitric oxide (NO) and vascular endothelial growth factor (VEGF) in the genesis of fluoride-induced nodular goiters. Eighty SD rats (40 males and 40 females) at the age of 4 weeks were used to establish animal models via fluoride-supplemented drinking water. These rats were randomly divided into four groups of 20. Group 1 was used as the control and were given deionized water. Group 2 (LF), group 3 (MF), and group 4 (HF) were given deionized water containing 50 mg/L, 100 mg/L, and 200 mg/L of sodium fluoride (NaF), respectively. Thyroid samples were collected on day 150. Pathological observation was performed to evaluate structural changes in the thyroid gland. The expression of VEGF mRNA in the thyroid glands was assessed by reverse transcriptional polymerase chain reaction (PCR). The serum NO level was analyzed by spectrometric methods. In addition, immuno-histochemistry was conducted to evaluate expression and deposition of VEGF in the thyroid gland. The results showed that the average relative weight of the thyroid glands of rats in the fluoride-treated groups was significantly higher than that in control rats (p<0.05). The proliferation and dilatation of capillary blood vessels, enlarged follicles with excessive colloid, and obvious nodules were found in the thyroid glands of fluoride-treated rats. Compared to the control group, the expression of VEGF mRNA in the thyroid gland and the serum NO levels in the fluoride-treated groups were significantly increased (p<0.05). Furthermore, the deposition of VEGF in epithelial and follicular cells of the thyroid gland was significantly higher in fluoride-treated groups than in the control group. These results suggested that abnormal expression of VEGF induced by fluoride can lead to the proliferation of vascular endothelial cells in the thyroid gland. Accordingly, VEGF over-secreted locally by vascular endothelial cells might contribute to the proliferation of epithelial and follicular cells, resulting in the formation of hyperplastic nodules and enlargement of the thyroid gland. Furthermore, we proposed that there might be a positive feedback mechanism between NO and VEGF expression in fluoride-induced goiter formation. It was concluded that angiogenic and vasodilative factors such as VEGF and NO must be involved in fluoride-induced thyroid goitrogenesis.

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Keywords: Goiter; Goitrogenesis; Nitric oxide; Thyroid; Vascular endothelial growth factor.
WATER FLUORIDE AND PREVALENCE OF FLUOROSIS IN CHINA

A NATIONAL CROSS-SECTIONAL STUDY ON EFFECTS OF FLUORIDE-SAFE WATER SUPPLY ON THE PREVALENCE OF FLUOROSIS IN CHINA

In 1985, villages in 27 provinces (or cities and municipalities) in 5 geographic areas in China were randomly selected for a cross-sectional study of the effects of low-fluoride drinking water considered safe for the prevention and control of endemic fluorosis in China. A total of 81,786 children aged from 8 to 12 and 594,698 adults aged over 16 participated in this study. Assessed were the prevalences of dental fluorosis and clinical skeletal fluorosis, along with the fluoride concentrations in the drinking water in the study villages, and in urine of the subjects. The study showed that in the villages where the drinking water fluoride concentrations were higher than the government standard of 1.2 mg/L, but no low-fluoride drinking-water supply scheme was provided for these areas, the prevalence rate and index of dental fluorosis in children and the prevalence rate of clinical skeletal fluorosis in adults were all significantly higher than those in the historical endemic fluorosis villages after the low-fluoride drinking water was provided. Additionally, the prevalence rates of dental fluorosis as well as clinical skeletal fluorosis, and the concentrations of fluoride in urine were found to increase with the fluoride concentration in drinking water, with significant positive correlations in the endemic fluoride areas. The prevalence rates of dental fluorosis and clinical skeletal fluorosis in different age groups and their degrees of prevalence were significantly lower in the low-fluoride areas than those in the endemic fluoride areas. It is concluded that the provision of low-fluoride drinking water supply schemes had significant positive effects on the prevention and control of dental fluorosis and skeletal fluorosis. The study also indicated, however, that both dental and skeletal fluorosis are still prevalent in high-fluoride drinking water areas in China.

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