

### **APPENDIX 3. Kenya study abstract [Neurath 2005a]**

#### **Correlation between the incidence of dental fluorosis and osteosarcoma in provinces of Kenya.**

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## FLUORIDE THERAPY FOR OSTEOPOROSIS REVISITED

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

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

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Keywords: Arterial thrombosis; Fluoride therapy; Osteoporosis treatment.

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

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

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

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

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

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

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

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

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

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

## EFFECT OF FLUORIDE ON THE PROLIFERATION OF OSTEOBLASTS

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**33**

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

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

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Keywords: Osteoblasts; Osteoblast proliferation.

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

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

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