INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS), VOL. 3, NOS.1& 2 (2008); P. 45 – 48, 1 TABLE.

# Mineral content of foetal femur following administration of Bonny light crude oil to pregnant wistar rats.

**O. E. Mesembe[*1], A. O. Igiri[1],V. A. Fischer[1], C. Eliakim-Ikechukwu[1], R. Asuquo[1] and E. U. Eyong.[2]**

**ABSTRACT**

The mineral concentrations of foetal femur following administration of Nigerian Bonny light crude oil to pregnant wistar rats was investigated. Twenty adult virgin female wistar rats weighing between 190 and 200g were randomly divided into 2 groups (designated A and B) of 10 rats each. They were mated with males of the same strain. The presence of sperms (tailed structures) in the vaginal smears obtained in the following morning confirmed coitus and the sperm positive day was designated as day zero of pregnancy. Group B animals were gavaged 9mls/kg body weight of Bonny light crude oil gestation day 10-14. Group A animals served as control and were gavaged 9mls/kg body weight of distilled water on corresponding days. Pregnancy was terminated on the 20$^{th}$ day of gestation, the foetuses obtained by uterectomy and the foetal femur dissected out. On analysis, the mineral concentration of foetal femur of Group B animals (Cu: 3.89 ± 1.9; Mg: 8.17 ± 3.1; Ca: 68.64 ± 3.4; Mn: 1.29 ± 2.3) were significantly ($p< 0.05$) lower than those from control group (Cu: 7.98 ± 2.2; Mg: 13.28 ± 2.8; Ca: 89. 42 ± 3.2; Mn: 1.94 ± 1.8). The results indicated a possible interference with the normal mineralization and developmental process in the foetal bones following maternal ingestion of crude oil.

## INTRODUCTION

Oil spillage and effluents generated by the activities of crude oil companies are toxic to marine life (Stubblefield *et. al.,* 1995; Shreiner *et. al.,* 1999). Marine animals ingest quantities of suspended and dissolved constituents; thus exposing the human population who depend on the animals for food source to a number of hazards.

Interestingly, inhabitants of the Niger Delta region ingest crude oil as an emetic; as an antidote to poison; as a treatment for arthritis; and as anticonvulsants (Dede *et. al.,* 2002; Didia *et. al.,* 2002). Whether as raw crude oil or bioaccumuated in marine life, ingestion of crude oil is hazardous to life (Shore and Douben, 1994; Feuston and Markerer, 1996; Sherry *et. al.,* 1997). Literature abounds with evidence of toxicity of crude oil in laboratory animals (Safer 1998; Dede *et. al.,* 2002; Didia *et. al.,* 2002; Didia *et. al.,* 2003). Furthermore, crude oil has been implicated as a developmental toxicant in laboratory animals (Feuston *et. al.,* 1997a; Feuston *et. al.,* 1997b; Fischer *et. al.,* 2006; Fischer *et. al.,* 2007).

Significantly, Feuston *et. al.,* 1997a; 1997b established that administration of crude oil to pregnant rate resulted in reduced ossification of skeletal elements in the foetus.

The present study was therefore designed to determine the effects of maternal ingestion of crude oil on the mineral concentration of foetal bones. The results may explain the reduced ossification of skeletal elements observed by Feuston *et. al.,* (1997a).

## MINERALS AND METHODS

Twenty adult virgin female Wistar rats weighing between 190 and 200g bred in the animal house of the Department of Anatomy, University of Calabar were used for this study.

The animals were randomly divided into 2 groups of 10 rats. Each group was housed in a separate cage and allowed normal daylight cycles. The temperature of the animal house was 28 ± 2 $^0$C. Commercial rat feed (Livestock feeds Nigeria limited, Lagos) and tap water were allowed *ad lithium* throughout the experimental period. The females were caged over night with sexually mature male rats of the same strain. The presence of sperms (tailed structures) in the vaginal smears obtained the following morning confirmed coitus and the sperm positive day was designed at day zero of pregnancy.

Bonny light crude oil used for this study was obtained from Shell Petroleum Company, port-Harcourt with permission from the Department of Petroleum Resources, NNPC, Lagos.

The treatment group (Group B) animals were gavaged 9ml/kg body weight of Bonny light crude oil from the 10$^{th}$ to the 14$^{th}$ day of gestation. The group A animals which served as control were gavaged 9ml/kg body weight of distilled water on corresponding days.

Pregnant animals were sacrificed on the 20$^{th}$ day of gestation following chloroform anesthesia foetuses were collected by uterectomy and anesthetized using chloroform. With the aid of dissecting forceps, dissecting needles and surgical blades, the fermurs were dissected out and the adhering muscle and fat were removed. The foetal bones were dried in an Astell Hearson hot air dryer oven at 60 $^0$C for 24hr. The bones were later ground with laboratory mortar and the powdery form was digested in 5 ml concentrated nitric acid and 1ml perchloric acid.

[*] Corresponding author. Email: Mesembe_otu@yahoo.com
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[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria

Statistical Analysis was performed using student's t test. Experimental data were presented as mean ± standard error of mean (SEM). Values of <0.05 were regarded as being statistically significant.

## RESULTS

The results obtained are shown in Table 1. The copper concentration of the foetal femur of the treatment group (Group B) pups (3.89 + 1.9 ppm) was significantly ($p< 0.05$) lower that of the control (Group A) pups (7. 98 + 2.2 ppm).

The Magnesium concentration of the foetal femur of the treatment group (8.17 + 3.1 ppm) was significantly ($p<0.05$) lower than that of the control (13.28+2.8 ppm)

The Calcium concentration of the foetal femur of the treatment group (68.64+3.4 ppm) was significantly ($p<0.05$) lower than that of the control (89.42 +3.2 ppm).

The Manganese concentration of the foetal femur of the treatment group (1.26 + 2.3 ppm) was significantly ($p<0.05$) lower than that of the control (1.94 + 1.8 ppm).

## DISCUSSION

Bone, the vascularized calcified supporting tissue of vertebrates constitutes the matrix made up of glycosaminoglycans and type 1 collagen of the fibres and impregnating hydroxy–apatites (Hall, 1988; Sembulinggam and Sembulingam, 2002). Impregnation of minerals in the bone matrix is vital process in the development of bones (Singh, 2002). Any disruption in the process of mineralization will usually reflect in low bone minerals and hence defective bone development.

Literature is replete with evidence of an intimate relationship between mineral concentration and abnormal bone development (Volozhin *et. al.,* 1980; (Akpan *et. al.,* 1991; Adebisi 2003; Mesembe *et. al.,* 2004). Pups from the treatment group recorded significantly lower concentration of Copper, Magnesium, Calcium, and Manganese in the foetal femur, this may be due to poor nutrition of the dams (Gudehithlu and Ramkrishnam, 1990b); probably as a consequence of malabsorption resulting from the presence of crude oil in the intestines of the dams.

Another probable reason for the reduced mineral concentration may be due to a direct toxic effect of crude oil hydrocarbons on the cells (osteoblast and chondroblast) that determine calcification. Toxicity of crude oil hydrocarbons particularly the Polynuclear Aromatic Compounds (PAC) have been reported in several studies. Feuston *et. al.,* (1997a) and (Feuston *et. al.,* 1997b) reported that administration of crude oil to pregnant rats resulted in reduced ossification of skeletal elements and delayed ossification of the palate. The authors suggested the possible toxicity of PAC on osteoblast and chondroblast. The resultant suppression of their function may cause a reduction in the deposition of matrix and calcium salts (Natelson and Natelson, 1975). This is of interest because any substance that increases matrix production, increases mineralization (Baron, 1973) and vice versa.

Copper, Magnesium, Calcium, Manganese play crucial roles in bone development and their levels in bones is usually reflective of adequate bone development. The lowered levels of these minerals in foetal femur therefore implies interactions between crude oil components and the mechanism of bone mineralization. Martin (1985) had long observed that Copper plays a crucial role in cross-linking bone collagen. Magnesium, Calcium and Manganese respectively play important roles in bone matrix formation and deficiency of either of these minerals is often reflective of defective bone formation and growth (Leach, 1976; Gunther, 1981 and Martin, 1985). The results obtained in the present study indicates a possible interference with the normal mineralization and developmental process in the foetal bones following maternal ingestion of Bonny light crude oil.

**Table 1. Minerals (Cu, Mg, Ca, Mn) concentration of foetal femur following administration of bonny light crude oil (BLCO)to pregnant Wistar rats. Values are mean+SEM. n=10**

| Group | Treatment | Copper (ppm) | Magnesium (ppm) | Calcium (ppm) | Manganese (ppm) |
|---|---|---|---|---|---|
| A | Control; distilled water | 7.98±2.2 | 13.28±2.8 | 89.42±3.2 | 1.94±1.8 |
| B | 9ml/kg body weight of BLCO | 3.89±1.9$^{xx}$ | 8.17±3.1$^{xx}$ | 68.64±3.4$^{xx}$ | 1.29±2.3$^{xx}$ |

$^{xx}$ Statistically significant ($p< 0.05$) compared to control

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