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Induced Geophagy with Local Kaolin from Cameroon Market and Heavy Metals (Lead, Cadmium and Mercury) Profile of Rat Blood, Liver, Placentas and Litters

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Scientific findings revealed that local kaolin from the Cameroon market is contaminated with lead, mercury and cadmium. This study was carried out to assess the bioavailability of these heavy metals as well as their transplacental transport and their passage into rat litters. Eighty pregnant female albino rats were fed with kaolin for 21 consecutive days and on days 0, 7, 14 and 21, four of them were sacrificed from each group by cervical dislocation to obtain blood and liver samples. Kaolin dosage was calculated per body mass considering daily human consumption range (w/w). Whole blood and liver samples were digested with concentrated nitric acid. Based on results obtained in the first study, a second experimental study consisting of 10 pregnant albino rats verified the trans-placental transport of lead and the passage of lead to litters at birth and during breastfeeding. In the first study results, values of blood lead in the control group compared to the various test groups were statistically significant. No statistical significance occurred for cadmium and mercury. The values of liver lead in the control group compared to the other test groups on average bases were statistically significant. Results of the second study showed that transplacental transport of lead occurred only for high kaolin consumption and the passage of lead to litters occurred during breastfeeding. The study revealed that lead in kaolin is bioavailable. Cadmium and mercury are absorbed from the digestive tract but are both managed at the level of the liver.

Key words: Geophagy, kaolin, heavy metals, bioavailability, transplacental transport

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INTRODUCTION

Exposure to heavy metals is a major risk factor for several human and animal diseases and the structures of industrial ecological systems have made exposure to them common for most people in the world of today (Bellinger and Needleman, 2003; Farombi *et al*., 2007). The situation is even more disturbing because documented evidence shows that there has been an increase over the past decades of industrial use of these heavy metals leading to an inevitable increase in their flux in the environment and in the level of contamination of foods (Sahibin *et al*., 2002) or substances consumed by man and animals. An increased consumption of foods or clay (as in the case of geophagy) contaminated by these minerals (Bonglaisin *et al*., 2011) may result in their bioavailability with an eventual consequent defect on health.

For example, increasing concentration of these minerals in the body has been reported to trigger a variety of physical and cognitive disorders (Bellinger and Needleman, 2003). Under chronic exposure such as the case of lead, irreversible functional disorders may result and even cause death. Similar observations have been reported as in the case of cadmium (Rashed, 2001), mercury (ATSDR., 1999) etc. Given these deleterious effects the accumulation of heavy metals can have on health, the study of their bioavailability in foods (or substances consumed by man) and their influence on the foetus and neonate is necessary.

Lead is absorbed by ingestion and inhalation. Absorption varies from individual to individual and depends on the chemical form of lead and type of exposure. The alimentary and respiratory tracts are the main portals of entry for lead (Pb) into the body (Garrettson, 1990). For oral exposure, as will occur during the consumption of Pb contaminated local kaolin specifically, the liver and the blood are entry points into the system and the concentration of Pb in these organs can be assessed during Pb intoxication. Since, Pb concentration in the blood is known to be transient and only represents recent exposure of some days (Barltrop, 1969; Maugh, 1978), it can be exploited as a biomarker only within the exposure period. Assessing Pb bioavailability in rats submitted to a contaminated local kaolin based diet is therefore relevant. Pregnant women's Pb level can affect their foetuses as Pb easily traverses the placenta (Tellez-Rojo *et al*., 2006). Effects on litters of rats (including their Pb content) at birth and during breastfeeding will confirm or infirm the fact that foetuses and litters are intoxicated during the consumption of Pb contaminated kaolin.

Recent findings reveal that ingested cadmium (Cd) is poorly absorbed and only about 5% of the total Cd ingested in food or water is absorbed. This absorption is affirmed to increase with iron or calcium deficiency (ARL., 2014). Kaolin contains these two minerals (Gamiz *et al*., 1988; Talabi *et al*., 2012), indicating that there is more possibility of it forming complexes with cadmium (Hooda *et al*., 2004), instead of facilitating its bioavailability. If kaolin doesn't complex Cd, then it is absorbed and is distributed throughout the body with major portions found in the liver and kidney (ARL., 2014). About 50% of ingested or inhaled Cd is stored in these organs (ATSDR., 1999). Studying the level of Cd in rat liver and blood provides information regarding its bioavailability.

Acute doses of mercury (Hg) or an increase in the chronic dose level can readily precipitate renal failure (Vimy *et al*., 1985), one of the classic symptoms of Hg poisoning. In its vapour form, Hg is easily absorbed by inhalation through the air passages and the mucosa into the blood system (Danscher *et al*., 1990) which spreads it throughout the body at high speed. This inhalation exposure is common in those female kaolin dealers who crack kaolin into smaller portions with a possibility of pulverizing its dust into the air thereby, facilitating its penetration into the nasal cavities. Also, the concentration of Hg exceeding the maximum permissible oral exposure limit of 0.23 μg g^{-1} in foods (FAO and WHO., 2006) causes serious health problems. A study of the bioavailability of Hg in rats through oral exposure can give a clue about its toxicity during kaolin consumption. Consumers are mostly exposed to Hg from kaolin through this pathway.

The objectives of this study were to:

- Determine the absorption of the heavy metals (Pb, Cd and Hg) during local kaolin consumption and to find out if their bioavailability is statistically significant enough to cause havoc in living systems
- Determine the risk of local kaolin consumption during pregnancy on rat foetus
- Determine the risk of local kaolin consumption during breastfeeding on rat litters

MATERIALS AND METHODS

The study designs were as described by Yang *et al*. (1997) and OECD (1998). Test item was kaolin while control item was placebo in normal diet. To ensure contamination, local kaolin from Nigeria, Balengou and Mbengwi were analyzed for their heavy metal load (Pb, Cd and Hg) as described in our previous study (Bonglaisin *et al*., 2011) prior to the experiment. They were subsequently blended into one sample by thorough mixing so as to simulate human consumption of local kaolin. It was then re-analyzed in quadruple to check uniform heavy metals repartition.

The study design segmented the experiment into parts; an initial investigation that studied the bioavailability of Pb, Cd and Hg and a secondary investigation that studied transplacental transport of the bioavailable heavy metal (Pb) as well as its passage into rat litters.

The test living system used initially was 120 albino rats of 12 weeks of age, with body weight ranging from: 210-260 g and sex consisting of 80 females and 40 males. The administration of kaolin doses were done following the principle of functioning of the Institute of Medical Research and Medicinal Plant Studies (IMPM) metabolism or animal laboratory. After assuring the acclimatization of rats in the study milieu for one week, kaolin in pellets of rat food was served every day to the rats after starvation overnight for 21 days, according to kaolin consumption (w/w) in human (Bonglaisin *et al*., 2011). The rats were segmented into five groups of 16 rats each. This included the control group, then the low, medium, high and very high groups. Except for the group with very high kaolin the range adopted was according to the range found in human. Kaolin dose was calculated as follows:

$$\text{Kaolin dose} = \frac{\text{Weight of rat in grams} \times \text{Quantity consumed by human in grams}}{\text{Average weight for women (70 kg) in grams (i.e., 70000 g)}}$$

An average weight of women consuming kaolin was obtained by weighing 1102 individuals from cosmopolitan cities during an administration of a questionnaire. In view of getting data that reflect the true image of these cities church settings such as Liberty church International (Douala), Etoug Ebe Baptist church (Yaounde), Hope/Menda Baptist churches (Bamenda) and Navigators women of Cameroon were used for data collection. Kaolin consumer women constituted 31.6% of the total number of women who filled the questionnaire. The mean weight of women consuming kaolin was found to be 69.8±13.3 kg, approximated to 70 kg to ease calculations (kaolin dose formula). Attention on the questionnaire was also focused on information concerning consumption rate and range at the level of the population. A consumption range in human was found to be 26.2, 63 and 120 g corresponding approximately to kaolin consumption of 0.094, 0.225 and 0.429 g in rats, respectively using the above kaolin dose formula.

In order to determine the bioavailability of Pb, Cd and Hg, two female rats were paired to one male in the rearing cage for 8 days (two rat cycles) to increase the chances of pregnancy. Spermatozoids were examined (through vaginal smear) in the microscope every two days within the above 8 days to situate approximately the days of giving birth by the rats. Four rats were sacrificed from each of the five groups on days 0, 7, 14 and 21 and the blood and liver samples were collected for heavy metal determination. All specimens collected were identified by a unique number.

Kaolin pellets were formed by mixing the above stipulated kaolin qualities with about 10 g of the following composition of rats' food: A 5 kg of dry fish mixed with 2 kg of 10% soya concentrate, 1.5 kg of palm-squirrel flour, 1 kg of cotton cake flour, 1 kg of bone flour, 5 kg of oats and barley flour, 30 kg of maize flour and a bit of multivitamin.

The second investigation that involved the transplacental transport of Pb as well as the passage of Pb into litters of albino rats at birth and during breastfeeding was carried out using a group of 15 albino rats. Their body weights ranged from 168-220 at the beginning (and 183.3-262.6 at the end of the study) and their sex consisted of 10 females and 5 males. Pregnancy was ensured as described above. During this study, the administration of kaolin dose was meticulously carried out as stipulated above. Modification of the dose was done after every 3 days at which time the weights of the rats were observed to change remarkably, ensuring that the dose was adjusted with respect to increase in weight. Food composition remained the same as specified in the above section.

Placentas were collected and analyzed for Pb to study its transplacental transport. While maintaining the rats in the metabolic cages according to their groups, litters were Killed and dried at 100°C (on days 0, 5 and 10 from the time of delivery) in order to assess the passage of Pb into the litters at birth and during breastfeeding.

Wet digestion of samples: Whole blood (1 mL) was digested with concentrated nitric acid (HNO_3) according to Welz's method (Welz, 1985). After cooling, the samples were transferred into 10 mL round bottom flasks with the help of sterile transfer pipettes and then topped to the mark after 5 rinsing of the capsules. The contents were each transferred into 20 mL test tubes (initially decontaminated by steeping in 10% nitric acid). Livers (1 g) were dried and ground to fine powder and digested as stipulated in the NIOSH (National Institute for Occupational Safety and Health, USA) manual of analytical methods (Eller and Cassinelli, 1994). After cooling the samples were transferred into 25 mL round bottom flasks with the help of sterile transfer pipettes and then topped to the mark after 5 rinsing of each capsule. The contents were each transferred into 30 mL test tubes (initially decontaminated by steeping in 10% nitric acid). The samples were thus ready for reading at the atomic absorption spectrophotometer.

In the subsequent experiment rats were maintained in the metabolic cages according to their groups. Litters (neonates) were killed and dried at 100°C for 24 h on

days 0, 5 and 10 in order to determine their lead content at birth and during breastfeeding. The dried litters were ground to fine particles. Liquid mineralization was done exactly like the liver samples (Eller and Cassinelli, 1994). After cooling the samples were transferred into 10 mL round bottom flasks with the help of sterile transfer pipettes and then topped to the mark after 3 rinsing of each capsule. The contents were each transferred into 20 mL test tubes (initially decontaminated by steeping in 10% nitric acid) for reading at the atomic absorption spectrophotometer.

All chemicals were of analytical reagent grade. De-ionized water was used to prepare all solutions. Lead (Pb), Cd and Hg stock solutions of 1000 ppm (Fisher chemicals) were used for the preparation of standard concentrations.

Reading at the atomic absorption spectrophotometer: The heavy metal analysis was done using Perkin 311 model Atomic Absorption Spectrophotometer, as described by Burtis and Ashwood (2001). From the stock solution of each element containing 1000 ppm, four different standard solutions were prepared. A blank was prepared using de-ionized water and concentrated nitric acid only.

The blank was first aspirated into the flame to give a reading of zero concentration. Thereafter, each of the four standards was aspirated in turn, starting from the solution with the lowest concentration. Each standard gave an absorbance value that corresponded to its concentration. Air-acetylene gas was used as fuel while the following wavelengths were used for the cationic estimations (Lead 283.3 nm, cadmium 228.8 nm and mercury 253.65 nm). The absorption signals were evaluated by subtracting the value of blank from the signal of the sample.

Sample concentrations were derived using a linear regression equation of the standards and their absorbance readings in Excel sheets. Considering the initial 1.0 g of whole blood, ground liver (dried) or litters (dried) and the total volume of 10 or 25 mL, concentrations of Pb were expressed conventionally in μg g^{-1} of whole blood, of liver or litter of rat. Similar calculations were done for cadmium and mercury in the respective samples.

Statistical analyses: The data obtained was analyzed by Statgraphic 5.0 and subjected to a one-way Analysis of Variance (ANOVA) according to the procedure of Steel and Torrie (1980). Significantly different means were separated using the methods of Duncan (1955). The values obtained were presented as Least Significance Differences (LSD) of means at ($p<0.05$) compared to those which did not differ significantly ($p>0.05$) from the value of Duncan. and SigmaPlot 11.0 was used for surface plot.

RESULTS AND DISCUSSION

Level of Pb, Cd and Hg in the blood samples of albino rats: The blood concentrations in micrograms per milliliter (μg mL^{-1}) of blood of Pb, Cd and Hg are presented in Table 1. The differences between the values of blood Pb in the control group with zero quantity of kaolin and the various test groups, with different kaolin quantities are statistically significant ($p<0.05$ in all cases). On the contrary, the concentrations of Hg and Cd in the control group and the various test groups with different kaolin quantities are not statistically significant ($p>0.05$ in all cases). Figure 1 illustrates gradual increase of lead in blood as the experiment progresses. Cd and Hg are rapidly managed by the albino rats and their concentration

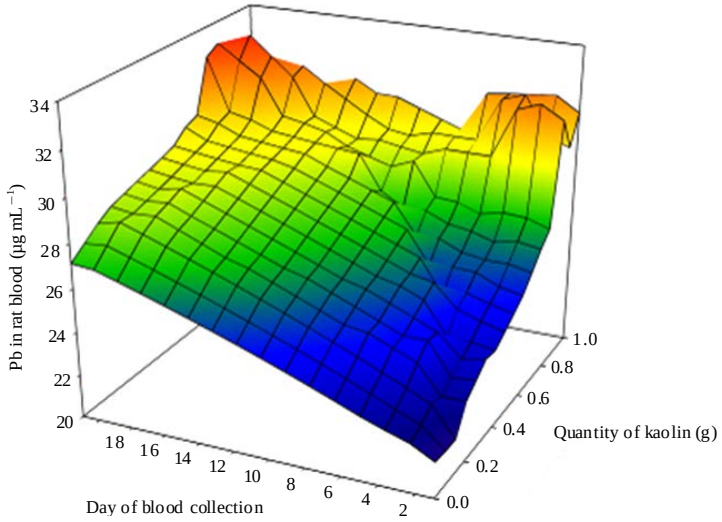

Fig. 1: Lead in rat blood versus day of experiment

Table 1: Pb content of blood samples of rats fed with kaolin

Kaolin quantity (g)	Pb (μg mL^{-1} of blood)	Cd (μg mL^{-1} of blood)	Hg (μg mL^{-1} of blood)
0	0.61±0.5^{a}	0.97±0.71^{a}	0.24±0.13^{a}
0.094	4.18±3.03^{b}	0.93±0.89^{a}	0.33±0.17^{a}
0.225	4.33±3.51^{b}	1.16±0.64^{a}	0.31±0.14^{a}
0.429	6.33±5.27^{bc}	1.23±0.86^{a}	0.33±0.16^{a}
1.0	8.69±4.71^{c}	1.29±0.91^{a}	0.35±0.16^{a}

Values in the same column having the same superscripts are not significantly different (p > 0.05), Values are Mean±STD

Table 2: Pb, Cd and Hg content of liver samples of rats fed with kaolin

Kaolin quantity (g)	Pb (μg g^{-1} of liver)	Cd (μg g^{-1} of liver)	Hg (μg g^{-1} of liver)
0	22.60±14.26^{a}	2.46±0.65^{a}	0.33±0.12^{a}
0.094	37.72±12.48^{b}	3.16±1.52^{ab}	0.67±0.17^{b}
0.225	18.81±10.64^{a}	3.56±0.93^{ab}	0.52±0.18^{ab}
0.429	31.61±7.7^{ab}	4.11±2.11^{b}	0.52±0.29^{ab}
1.0	29.83±14.01^{ab}	2.45±1.94^{a}	0.55±0.26^{b}

Values in the same column having the same superscripts are not significantly different (p > 0.05), Values are Mean±STD

Table 3: Pb content of litters of rats (at birth) fed with kaolin

	Pb (μg g^{-1} of litter)	
Kaolin quantity (g)	Mean±STD	Range
0	1.1±0.66^{a}	0.6-2.0
0.094	1.75±0.50^{a}	1.0-2.0
0.225	0.15±0.30^{a}	0.0-0.6
0.429	7.43±3.51^{b}	4.0-12.0
1.0	13.75±4.35^{c}	10.0-18.0

Values in the same column having the same superscripts are not significantly different (p>0.05)

is also rapidly checked to curb down their toxicity. May *et al*. (2007), also found similar increased concentrations of lead in hellbender blood and fish fillets during the Missouri conservation monitoring programs. Their results were on the contrary not the same for Cd and Hg, as high blood Cd and Hg were found during these programs. Similar results were obtained for blood Pb after oral administration of 1000, 1500 and 2000 ppm of Pb solution to albino rats for 7 consecutive days (Babalola *et al*., 2010), indicating evidence of Pb bioavailability when its soluble form is released in the intestines.

Level of Pb, Cd and Hg in the liver samples of albino rats: The liver concentrations of Pb, Cd and Hg in micrograms per gram of liver samples are presented in Table 2. With the exception of 0.225 g kaolin concentration, the values of liver Pb in the control group compared to the other test groups (with different kaolin quantities) are statistically significant ($p<0.05$ in all cases). Apart from the group with 1.0 g of kaolin where there is no statistical difference with the control group for Cd ($p>0.05$), the values of liver Hg and Cd in the control group compared to the various test groups are statistically significant ($p<0.05$). Lead and cadmium accumulations were also found in the liver of some fish species exposed to these metals, but at sub lethal concentrations for periods of 32 days (Vinodhini and Narayanan, 2008). High values of Cd and Hg have been found in the livers of domestic animals such cows and goats (Asegbeloyin *et al*., 2010) exposed to these heavy metal toxicants.

Heavy metals concentrations in the blood and liver of experimental rats were expected to be proportionate to kaolin quantities in the experimental groups but the results obtained in this study did not agree with this view (Table 2). Kaolin quantities of 0.094 and 1.0 g are peculiar cases since, we expected that high quantity of kaolin would bring in more Pb and Cd in the liver compared to low quantity. This may be due to a common problem usually encountered in oral exposure methods. It is almost impossible to determine accurately the quantity of materials ingested by the animal, spillage and regurgitation cannot be overruled in many cases. The quantity of food and water taken by the animals also affects the amount of the material that they absorb. Similar results were obtained for blood and liver Pb after oral administration of 1000, 1500 and 2000 ppm of Pb solution to albino rats for 7 consecutive days (Babalola *et al*., 2010), indicating that Pb distribution in the organs is not proportionate to the quantity administered. However, the presence of Pb especially in the litters as shown on Table 3 indicates that Pb was absorbed in high quantities from the group of 1.0 g Kaolin. This shows that there is a command for rapid clearance of Pb when its concentration in blood rapidly increases, the placenta being one of the targets for this Pb orientation.

The exposure to lead possesses the potentials to induce hazardous biological effects in rats. Lead acetate in drinking water was found to induce a significant elevation of serum Alanine Transaminase (ALT), Aspirate Transaminase (AST), alkaline in Wistar rats (Moussa and Bashandy, 2008). These enzymes are generally used as clinical markers for liver disease or injury (Talmage *et al*., 1978). Administration of Pb has been observed to elevate plasma Low Density Lipoprotein (LDL) and reduce plasma High Density Lipoprotein

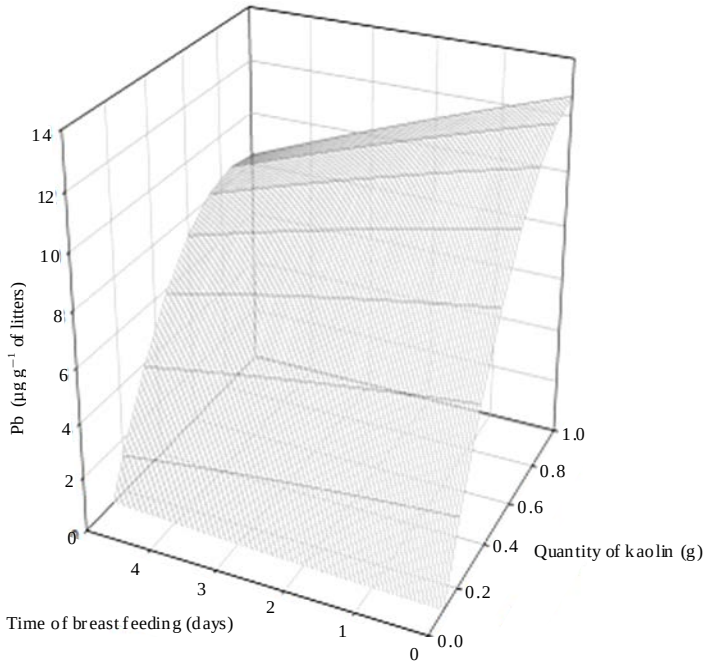

Fig. 2: Assessing the bioavailability of Pb in litters during breastfeeding

Table 4: Pb content of the placentas of rats fed with kaolin

	Pb (µg g^{-1} of placenta)	
Kaolin quantity (g)	Mean±STD	Range
0	0.35±0.41[a]	0.0-0.8
0.225	Not detected	0.0- 0.0
1.0	32.35±6.87[b]	24.0-38.6

Values in the same column having the same superscripts are not significantly different (p>0.05)

(HDL) in rat (Bashandy, 2006). In human, there is evidence that links increased serum cholesterol and LDL levels to a higher risk for developing coronary heart diseases (Glueck *et al*., 1986).

Liver and blood results for Cd and Hg revealed that much of these heavy metals were managed. This higher accumulation in liver may alter the levels of various biochemical parameters in liver and may also cause severe liver damage (Ferguson, 1989; Meyers and Hendricks, 1985).

Pb content of the placentas and litters of rats at birth: Lead content of litters shows no statistical difference (p>0.05) between the control group and the first two test groups (0.094 and 0.225 g kaolin). On the contrary, there exist statistical differences (p = 0.0 in both cases) between the control and last two test groups (0.429 and 1.0 g kaolin) with high and very high kaolin quantities (Table 3).

Also, the Pb contents of the placentas of rats fed with kaolin are shown on Table 4. Three groups (0, 0.094 and 1.0 g kaolin) gave birth to their litters in our presence while two groups 0.094 and 0.225 g gave birth against our vigilance and ate their placentas; this justifies the lack of data in these two groups. As for those groups available, there is no significant difference between the control and the second group that corresponds to 0.094 g kaolin (p>0.05). However, there exist a significant difference between the control and the test group of 1.0 g kaolin indicating the passage of Pb into the placenta for this kaolin quantity. The presence of Pb in the placenta is indicative of foetal intoxication by this heavy metal element. Some authors have found increased Pb concentrations in the placentas of rats exposed to lead acetate (Villeda-Hernandez *et al*., 2006). Significant increases of blood lead levels in the offspring of pregnant mice exposed to lead acetate through drinking water beginning at approximately day 15 of gestation as evidence of transplacental transfer of Pb have also been obtained (Snyder *et al*., 2000).

Pb content of litters during breastfeeding: The evolution of the passage of Pb into litters during breastfeeding (days 5 and 10) compared to day 0 is illustrated in Fig. 2. In comparison to the rat group of 0 g kaolin concentration, the passage of Pb through breast milk is statistically significant in all other groups (0.094, 0.225, 0.429 and 1.0 g). As time evolved in days, Pb concentration in the breast milk of rats was reducing, certainly because Pb stores in the organs and bones were depleting since rats were longer consuming kaolin. Mice in a continuous exposure to Pb beginning at

approximately 6 days prior to birth showed significant depleting of blood Pb levels 2 weeks after weaning, suggesting substantial transfer of Pb lactationally (Snyder *et al*., 2000).

Studies in human revealed that pregnant women's Pb level can affect their foetuses as Pb undergoes transplacental transport easily and foetal blood has almost the same Pb concentration as maternal blood (Lauyers *et al*., 1978; Carpenter, 1974; Ong and Lee, 1980). In geophagy, the situation is a bit different as Pb undergoes transplacental transport only at high or exaggerated quantities of kaolin (Table 4 and Fig. 2). However, findings on rat litters that showed evidence of the passage of Pb into litters (Table 3) during breastfeeding are consistent with those obtained in human (Jensen, 1983). Since rats were no longer consuming kaolin during breastfeeding, it seem reasonable to state that Pb was only stored (in the bones etc.) during prenatal Pb exposure from low or moderate kaolin intake.

CONCLUSION

The results of the present study revealed that Pb in kaolin is bioavailable and as such kaolin can be considered toxic to albino rats. Cadmium and Hg are absorbed from the digestive tract but are both eliminated at the level of the liver; their hazardous effects being limited only at that level.

The passage of Pb into litters was observed to occur during breast feeding where the incident of Pb in the litters was observed to increase with kaolin concentration. Lead doesn't undergo transplacental transport during low kaolin consumption but it does so when the quantity of kaolin consumed is either high or very high.

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