

## ORIGINAL ARTICLE

### Haematological Parameters of Rats Fed on Prolonged Crude oil Contaminated Cassava-Based Diet.

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#### ABSTRACT

The toxic effect of consumption of crude oil contaminated cassava based rations was evaluated in an animal model experiment. The contents of some cassava based foods commonly consumed in the sampled locations ranged between 0.62+0.28 to 0.76+0.12 for total cyanide and 0.11+0.02 to 0.48+0.01mg /CN/100g DM for free cyanide. The total hydrocarbon content of raw cassava (700.36 x 10<sup>-3</sup>) ppm was high (in relation to standard normally encountered in cassava) and observed to reduce with processing. However, values obtained for the polyaromatic hydrocarbon (PAH) contents of raw cassava and gari were not significantly different. Only three major peaks: naphthalene, 2-methyl naphthalene and acenaphthene were identified in all feed samples. Serum parameters, which included glucose, cholesterol, protein, and creatinine levels were observed to be high in animals fed the contaminated diet in spite of the level of processing. Serum alkaline phosphatase levels obtained from blood of rats fed on the diets were elevated except in the control. Histopathological lesions were observed in the liver of rats that died during the course of the experiment. The most significant finding of this work is that the hydrocarbon probably suppressed the immune response of the rats which resulted in increased serum alkaline phosphatase levels. This may aid diagnosis when crude oil contaminated foods are consumed.

**Key words:** Serum Enzymes; Poly aromatic Hydrocarbons.

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#### Introduction

The effluent from crude oil exploration and processing consists of oil and an assortment of chemicals that include acids, alkalis, phenols, sulphides, hydrocarbons, heavy metals, mercaptan and other toxic components (Boon *et al*, 1992). These are substances that have been proven from studies to be carcinogenic in nature (Chu *et. al*, 1992). The release of these contaminants has polluted water sources, contaminated agricultural raw materials and destroyed fishery resources (NDDC Report, 2004).

During transpiration, these chemical pollutants get transferred from the soil through xylem cells to the leaves where the process of food manufacture (photosynthesis) takes place. Following photosynthesis, manufactured food and chemical contaminants are transported via the phloem cells to the various storage organs, which in the case of cassava are the roots, and in the case of yam, it is the stem. Where the storage site is the leaf, fruits are formed. These primary food items are at the bottom of the food chain, which later serve as food for other members of the food chain like rodents, fish and bigger mammals such as man and other carnivores. In this manner, pollutants get transferred ultimately into human bodies where they could bioaccumulate with attendant consequences. Such consequences could manifest as debilitating health symptoms with concomitant reduction in average life expectancy (NDDC Report, 2004).

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Information on the extent of environmental degradation appears scanty in literature. The few ones that are available relate to situations in climates other than the tropics (Langston, 1990; Leborans, 1998) and hence are not directly applicable to the Nigerian situation. The effect of large scale consumption of food processed from raw materials harvested from a crude oil polluted environment in Nigeria has not been previously studied.

Consequently it was the objective of this study to evaluate the extent to which chemical contaminants identified above have impacted on the food sources available in the endemic areas. The effects of these contaminants on the level of some serum parameters including possible effects on some internal organs are also investigated.

## Material and methods

Raw and processed food samples (fish, cassava, gari, fufu) were obtained from the sampling locations not too far from Kokori and Ogulagha, both locations in Delta State of Nigeria. Sample handling procedures were as contained in the Directorate of Petroleum (DPR) guidelines (1992). Where applicable, samples were processed into ready to eat foods using food processing technologies available locally. The processed food items were used as rations in an animal model experiment. The cyanide content of raw cassava and of cassava-based rations was as previously determined (Oke, 1987). All proximate determinations were carried out in line with AOAC procedure (2006).

Feed formulation, determination of feeding regime, conditions of treatment conformed to standard requirements of the American Institute of Nutrition (AIN, 1980), and the American Society for Animal Care. Collection of blood samples, determination of serum biochemical parameters and histopathological examination of sectioned slides were as described (Fouchecourt *et. al*, 1999). Each histological slide was assessed based on the perceived effect of toxicant chemical on each 'very mild' (0.1) through 'moderate' (0.4) to 'extremely severe' 1.0. The summaries of the observed pathological findings are reported. For each determination, mean  $\pm$  standard deviation were calculated. Means were later separated using LSD and tested statistically at 5% significant level.

## Results and discussion

### Cyanide Content of Cassava -Based Experimental Diet

Table 1 presents the analysis of total, bound and free cyanide contents of cassava-marsh, used for formulation of the experimental diets. The cassava-marsh in the control diet (Diet1) had less of total cyanide in relation to other diets. Diet IV had the highest value of 0.76 mg/100g dry matter). The concentration in diet III was close to that of diet IV. The trend in free cyanide contents of the diets was slightly different. Though diet I had the least value and diet IV the highest, value in diet III was more than that in diet II. The increase in cyanide content from diet I to diet IV had to do with both the native cyanide content in the different samples as well as the method of processing applied.

**Table 1:** TOTAL, BOUND AND FREE CYANIDE CONTENT OF CASSAVA-BASED EXPERIMENTAL DIET

Treatment	CYANIDE CONTENT (MgCN/100gDM)		
	TOTAL	FREE	BOUND
DIET I	0.60 $\pm$ 0.10a	0.11 $\pm$ 0.02a	0.49 $\pm$ 0.18a
DIET II	0.69 $\pm$ 0.08b	0.19 $\pm$ 0.08 b	0.50 $\pm$ 0.08b
DIET III	0.72 $\pm$ 0.12b	0.22 $\pm$ 0.11b	0.50 $\pm$ 0.12b
DIET IV	0.76 $\pm$ 0.12c	0.48 $\pm$ 0.01c	0.28 $\pm$ 0.02c

Values are means of 4 replicates  $\pm$  SD. Means with same letters in a column are not significantly different at  $p < 0.05$ .

Diets II and III had the highest concentrations (obtained by difference) of bound cyanide. This value was followed by that of diet I with diet IV being the least. The amount of free cyanide obtained was low for diet I and highest for diet IV. Osuntokun *et. al*, (1969) remarked that this amount was incapable of disturbing both the physiological and the biochemical functions of the body.

The results obtained from the cyanide content analysis of the different marsh samples were observed to be consistent based on the point of view of the suitability of the tuber when used as food. Normally, cyanide is a constituent of human blood, albeit at low concentrations 120mol/L (Osuntokun, *et. al*, 1969). It has been suggested that at least some of the cyanide present in the body comes from defense processes continuously occurring in tissues. The lethal, dose range for humans taken by mouth, is 0.53-3.5 mg/ kg body weight for a 60kg adult.

The inference from here is that the cyanide level, apart from being very low, did not contribute any inhibitory property to diet utilization by the animals.

*Hydrocarbon Profile of Raw and Processed Cassava Products*

Table 2 provides information on the hydrocarbon content of the different cassava samples used for the analysis. Values were obtained in each case for both the control and two other locations depicting the level of severity of the contamination. In all the three samples from nine different locations including control, location two gave the highest concentrations for total hydrocarbon. Total aliphatics and total polyaromatics follow the same trend. The observed decrease in concentration was in this order: Cassava porridge < gari < raw cassava, this order is in agreement with the processing methods for the production of each sample. The total hydrocarbon contents for both garri (0.443) and cassava porridge (0.192) were above the minimal level recommended for ruminant nutrition (Oke, 1987).

**Table 2: HYDROCARBON PROFILE RAW AND PROCESSED CASSAVA PRODUCTS**

Cassava	Location	Total Hydrocarbon	Total Aliphatics PPM x 10 <sup>-3</sup>	Poly Aromatics Ppm x 10 <sup>-4</sup>					Total Products Poly Aromatics 10 <sup>-3</sup>
				1	2	3	4	others	
RAW	C	-							
	1	675.80	669.61	1.04	2.34	1.56	0.26	5.19	
	2	700.36	690.82	1.89	4.25	2.83	0.47	9.44	
GARRI	C	-							
	1	443.31	436.86	1.29	2.90	1.94	0.32	6.45	
	2	475.84	467.28	1.71	3.85	2.57	0.43	8.56	
PORRIDGE	C	-							
	1	177.80	168.08	1.94	4.37	2.92	0.49	9.72	
	2	192.34	181.74	2.12	4.77	3.18	0.53	10.60	

Legend  
 Naphthalene  
 2- methyl naphthalene  
 Acenaphthalene  
 Flourene

*Blood Chemistry*

Table 3 presents data of some blood parameters for the animals placed on different diets. Elevated levels of glucose, cholesterol, and inorganic phosphate were observed in animals sacrificed by the end of the feeding period. This observation is consistent with work of Poon *et al*, (1981). Administration of Prudhoe Bay Crude oil (PBCO) on mice for 2 days resulted in an increase in total lipids and individual lipids such as cholesterol, triglycerides and phospholipids (Khan *et. al*, 1981). Increase in blood glucose was the most prominent serum biochemical change observed in sera of rats put on the various diets and might be due to the activity of some hepatic enzymes (Poon *et al*, 1994). Elevated serum cholesterol observed in the present study was also seen in a 28-day dermal exposure study to chemical toxicants (Poon *et. al*, 1994). Protein concentration (Table 3) showed a progressive increase for each diet II – IV) considered. Animal in the control group, (diet I) demonstrated a lower protein concentration.

**Table 3: CONCENTRATION OF SERUM EBZYMES**

		SCOT	SGPT	ALKALINE P <sup>i</sup> ASE (ALP)	CREATININE
DIET I	SHORT TERM	187.78 ± 3.87	102.68 ± 6.35	244.17 ± 4.57	1.01 ± 0.01
CONTROL	LONG TERM	210.41 ± 4.56	128.08 ± 6.42	280.80 ± 12.26	1.17 ± 0.05
DIET II	SHORT TERM	277.53 ± 6.15	258.04 ± 11.60	391.81 ± 12.10	2.19 ± 0.44
	LONG TERM	316.38 ± 10.22	291.64 ± 6.21	450.58 ± 14.36	2.54 ± 0.21
DIET III	SHORT TERM	2.60.72 ± 5.69	156.77 ± 5.32	452.51 ± 11.51	1.78 ± 0.11
	LONG TERM	309.60 ± 7.34	180.29 ± 7.34	524.91 ± 12.52	2.06 ± 0.00
DIET IV	SHORT TERM	452.14 ± 0.08	318.24 ± 6.14	455.84 ± 14.94	2.39 ± 0.02
	LONG TERM	519.96 ± 11.07	369.16 ± 8.45	528.77 ± 10.26	2.77 ± 0.00

Values are means of 4 determinations SD

Legend  
 Short Tern 4 weeks  
 Long Term 13 weeks  
 SGOT Serum glutamate oxaloacetate transaminase (IU)  
 SGPT Serum glutamate pyruvate transaminase (IU)  
 ALP Alkaline Phosphate (u/L)  
 CREATININE (mg/d).

The hydrocarbon toxicants might have affected the liver to stimulate synthesis of and excretion of protein into the serum. Chu *et. al.*, (1992) reported similar effect for heavy boiling coal co-processing products. In several organs, cell damage was followed by release of a number of cytoplasmic enzymes into the blood, a phenomenon that provides the basis for clinical diagnosis (Sundberg *et. al.*, 1994). The marked haemolytic effect of crude oil contaminated diet observed in this study are similar to that produced in rats following exposure to the heavy gas oil fraction of coal liquefaction products (Poon *et. al.*, 1996). The increased toxicity observed in diet IV are probably related to their content of higher molecular weight hydrocarbons (Poon *et al.*, 1994, Feuston *et. al.*, 1994) studied the effects of 13 Refinery products in rats following subchronic dermal exposure and reported decreased body weight; decreased red cells and platelet counts, increased kidney and liver weight and increased serum cholesterol and concluded that systemic and developmental effects were dependent upon the level of polycyclic aromatic hydrocarbons.

#### *Serum glutamate oxaloacetate transaminase and pyruvate transaminase (SGOT AND SGPT)*

In order to monitor the pathological events associated with the chemically polluted feeds, four enzymes generally associated with pathological derangement were chosen for analysis with a view to studying the sequence of cell damage. These enzymes were: SGOT, SGPT, alkaline phosphatase, and creatinine (Table 4). The basis of the choice of these enzyme markers (Wright and Plummer, 1974) was to assess the extent of injury and consequently determine their level in blood serum.

**Table 4:** GLUCOSE -6-PHOSPHATASE ACTIVITY

DIET	PERIOD (WEEKS)		
	4 WEEKS	7 WEEKS	13 WEEKS
I	11.53 ± 0.07	19.22 ± 0.10	24.57 ± 0.82
II	12.55 ± 0.04	18.16 ± 0.12	14.42 ± 0.06
III	11.75 ± 0.02	18.78 ± 0.12	13.62 ± 0.14
IV	12.20 ± 0.08	16.20 ± 0.19	14.62 ± 0.20

\* Activity per gram fresh wt of liver.

(Micromoles Pi produced/min/gram fresh wt of liver)

Values are means of 4 determinations S.D.

The mechanism by which these enzymes are present in high concentrations in the blood has also been attributed to one in which their membrane pockets were damaged thereby leading to abnormal amounts being released into the blood (Connors, 1983). It is suggested that hydrocarbon (aliphatic or polyaromatic) interacts via hydrogen bond formation with the phospholipids bi-layers of the fluid mosaic structure of the membrane, thereby disrupting it.

A general increase was observed in the levels of SGOT and SGPT (Table 4) for diets II, III, & IV by the end of the experiment. When these values were compared with values obtained for the control (Diet I) these increases were observed to be significant ( $P < 0.05$ ). Diet IV had the highest value while diet II the least. This trend was slightly different for SGPT, where diet III had the lowest value. The mechanisms by which these enzymes are present in high concentrations in the blood has also been attributed to one in which their membrane pockets are damaged thereby leading to abnormal amounts being released into the blood (Mitoma *et. al.* 1985).

#### *Alkaline Phosphatase (ALP)*

Significant increases ( $P < 0.05$ ) in serum alkaline phosphatase activity were observed in diets II, III and IV while control diet I gave relatively low value (Table 4). Margin of increase for diet IV was the highest. Similar results were also observed in other animals with food restrictions and were probably caused cellatrophy. Lower rates were also observed in bone metabolism and in phosphorylation and dephosphorylation reactions. Since these animals were fed *ad libitum* the effects observed could not have been due to under feeding.

Colwich and Kaplan (1995) reported that fat ingestion; vitamin D deficiency and dietary Zn have been shown to correlate with increase in intestinal alkaline phosphatase activity. This enzyme functions also in the small intestine for absorption of phosphate from dietary phosphate. Alkaline phosphatase is a marker enzyme (Wright and Plummer, 1974). It is frequently employed to detect kidney damage (Wright and Plummer, 1974) and is the most sensitive and earliest indicator of changes in liver function (Kaplan, 1972). The increases observed were consistent with these observations.

The increase in serum alkaline phosphatase may be an indication of mild hepatic cholestasis (Mitoma, 1985b). However, the possibility that the increase in alkaline phosphatase originated from the bone or intestine could not be excluded.

### Creatinine

Values obtained for creatinine, though not significantly different, ( $P < 0.05$ ) were still indicative of malfunctioning of the liver (Table 4). Increased serum creatinine and lower creatinine clearances were related to diagnosis of chronic renal failure (Sesso *et. al*, 1996). Based on this, increased creatinine (Table 4) reflects renal damage. Creatinine is the anhydride of creatine. It is formed largely in the muscle by the irreversible and non enzymatic removal of water from creatine phosphate. Formation of creatinine is apparently a preliminary step required for the excretion of most of the creatine.

On the other hand, excretion of protein is currently one of the more sensitive indices of renal dysfunction (Bernard *et. al*, 1995). Thus, hyper proteinemia is in agreement with renal damage, Prasad and Nath (1995) showed that development of cadmium induced lesion in kidney is characterized by hyper proteinemia.

### Glucose -6- Phosphatase activity

Table 5 presents data on liver glucose - 6- phosphatase nativity. A progressive increase in specific activity of the enzyme was observed with diet I as the control. However, it was observed that for animals on the experimental diets (diet II- IV) the activity of the enzyme per gram fresh weight of liver increase sharply in the early stages up to the 7<sup>th</sup> week. For the control diet, this increase amounted to 60.68% and 116.85% in the 7<sup>th</sup> and 14<sup>th</sup> week respectively. For the test diets, the increases observed by the 7<sup>th</sup> week were 44.70% (diet II), and 59.82% (diet III). These reductions ran counter to the sustained increase experienced by the animals on the control diet.

**Table 5:** HISTORICAL CHANGES IN RATS FED POLLUTANT-CONTAMINATED DIETS.

	Short Term (Four weeks) <sup>a</sup>			Long Term (13 weeks) <sup>b</sup>		
	Kidney	Liver	Small Intestine	Kidney	Liver	Small Intestine
Diet I	0 <sup>c</sup>	0	0	3.0 (0.2) <sup>d</sup>	4.0 (0.2)	4.0 (0.2)
Diet II	5.0 (0.4)	4.0 (0.3)	6.0 (0.4)	4.0 (0.4)	4.0 (0.8)	3.0 (0.8)
Diet III	3.0 (0.4)	3.0 (0.5)	3.0 (0.7)	4.0 (0.8)	5.0 (0.7)	2.0 (0.8)
Diet IV	6.0 (0.6)	6.0 (0.8)	2.0 (0.7)	4.0 (0.8)	4.0 (0.8)	6.0 (0.7)

.a = Six animals per group

.b = Four animals per group

.c = Number of animals with histological changes

.d = Number in brackets denotes severity where 0.1 = very mild, 0.2= mild, 0.3 = not moderate

0.4 = moderate 0.5 = very moderate, 0.6 = severe, 0.7 = very severe, 0.8 = extremely severe.

**Table 6:** OBSERVED CHANGES IN INTERNAL ORGANS OF RAT FEED

DIET	ORGAN	OBSERVED CHANGES
I	Kidney	Normal cells, Empty tubules and glomeruli
	Liver	Normal prominent vessels, healthy cells and normal circulation, no polling of blood.
	Small intestine	Mild congestion, Normal villi.
II	Kidney	Mild degenerative changes of proximal and distal tubules lower incidence of renal tubular changes.
	Liver	Swelling of individual hepatic cells, picnosis of nuclei and dense chromatin bodies which are indicative of hyperplastic reaction.
	Small intestine	Shrunken villi, slight mucosal Hypertrophy, oedemation of cells.
III	Kidney	Edema of glomeruli and tubules. Areas of tubular debris in glomerular space
	Liver	Mild central lobular vacuolation. Picnosis of kupffer cells. Areas of sinusoidal congestion, areas of cloudy swelling.
	Small intestine	Mucosal hypertrophy with Shrunken villi, Damaged epithelia surfaces with fragmented nuclei in some areas.
IV	Kidney	Pooling of Blood, inflammatory cells and degeneration of distal tubules especially about the descending loop.
	Liver	Picnosis of kupffer cells. Areas of sinusoidal congestion.
	Small intestine	Inflamed mucosal capillary-venous vasodilation.

On the other hand, for animals on diet IV the margin of increase by the 7<sup>th</sup> week was the least (32.78%). This increase was significantly different ( $P < 0.05$ ) from values obtained for the other diets. By the 13<sup>th</sup> week further reduction (19.83%) was observed. Halm and Klair (1986) observed similar increase in plasma enzymes 96 hours after exposure to crude oil contaminated diet. Thereafter a decline was observed until it reached a lowest by the 13<sup>th</sup> week.

The decrease in activity by the 11<sup>th</sup> week observed with the experimental animals may be due to damage or disruptions of microsomal membranes or situations of liver cell injury as in poisoning by carbon tetrachloride (Reynold and Ree, 1993) or damage arising as a result of the effects of detergent on liver.

The mechanism by which the decrease in activity occurred is likely due to the interaction of the chemical agents (in free radical or ionic forms) with membrane phospholipids leading to disruptions of the fluid mosaic nature. The resultant effect is the release of this enzyme into the intracellular milieu.

Since glucose-6-phosphatase is very sensitive to the action of hepato toxic chemicals, decreases in its activity are regarded as useful biochemical index of early toxic liver damage which *ipso facto* can also be used as a biomarker of hydrocarbon poisoning. Biochemical studies have equally shown that chemical contaminants cause initial changes in the phospholipid-protein structure of the liver cell endoplasmic reticulum.

In man, similar effects have been noticed. Changes in antiprene elimination kinetics consistent with induced metabolism, however, have been detected in man after occupational exposure to chlorinated hydrocarbon insecticides and inhalational anesthetics.

### Conclusions

Information so far obtained has shown that the present method of food processing in the area was not effective in ensuring complete reduction of hydrocarbon loads of the common food staples. For example, while it was observed that the total hydrocarbon content of cassava reduced with processing, the values obtained for the polyaromatic hydrocarbon content of raw cassava and garri were not significantly different ( $p < 0.05$ ). Hence there was the need to further improve on the present method of food processing in the area.

The identification of the residual concentrations of these hydrocarbons, in processed food from this area and their effect on serum biochemical parameters call for the development of an appropriate food processing technology that will eliminate this residual concentration without sacrificing the nutrient profile of the affected food sources.

Another significant finding is that the hydrocarbon probably suppressed the immune response of the animals, which resulted in increased serum alkaline phosphatase levels. This may aid diagnosis when crude oil contaminated foods are consumed. The long term bioaccumulation effects of some of these hydrocarbons that escaped food processing operations may constitute serious health problem.

Although dietary levels arising from the consumption of these toxicants are still uncertain, more especially with research still being extrapolated to humans, confirmation of these through inclusion of specific concentrations in the diets of higher mammals in monitoring programmes should be considered.

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