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TISSUE AND SUBCELLULAR CONCENTRATIONS OF TOTAL HYDROCARBONS, AND LEVEL OF HEPATIC SUCCINIC DEHYDROGENASE ACTIVITY AFTER TREATMENT OF GUINEA-PIGS WITH BONNY LIGHT CRUDE OIL.

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ABSTRACT

The concentration of total hydrocarbons derived from Bonny Light Crude Oil (Crude Oil Total Hydrocarbons – COTH) in heart, lungs, liver and kidneys; liver (hepatic) subcellular fractions and the effect to mitochondrial *marker* enzyme, succinic dehyrogenase activity were determined in guinea-pigs after the intraperitoneal treatment with 2.5 ml and 5.0 ml/kg body weight of Bonny Light Crude Oil (BLCO). Treatment was for one week in alternate days while controls were not treated. Heart, Lungs, Liver and Kidneys were excised following sacrifice of all animals on the eighth day, and 10% homogenate of each tissue was prepared in 0.05M potassium phosphate buffer at pH 7.4. Liver homogenate was fractionated to subcellular components (nuclear and cytoplasmic) by centrifugation. All homogenates and subcellular fractions were extracted with toluene and total COTH contents were measured spectrophotometrically. Succinic dehydrogenase (SDH) activity was measured colorimetrically in liver homogenates by the formazan method. Results show that the concentration of COTH was increased in all tissues in the treated guinea-pigs over the untreated controls. The pattern of this increase was dose-related: at the lower dose of 2.5 ml/kg bw, the order was: lungs > liver = kidney > heart, while at the higher dose of 5.0 ml/kg bw, order somewhat reversed: liver = lungs > kidney > heart.
Concentration of COTH to hepatic subcellular components however show a near-10 fold increase in the nuclear fraction over the cytoplasmic fraction at both dose levels and over the controls. Similarly, the specific activity of SDH increased markedly at 5.0ml/kg bw BLCO over untreated controls. These results may shed more light on the possible dynamics of the potential toxicity of BLCO.

KEYWORDS: Crude oil, Total Hydrocarbons; Succinic dehydrogenase.

INTRODUCTION

Crude oil has been defined as a highly complex mixture of paraffinic, cycloparaffinic (naphthenic) and aromatic hydrocarbons, containing low percentage of sulfur and trace amounts of nitrogen and oxygen compounds; the bulk of the compounds found in crude oil therefore are hydrocarbons (IARC, 1989).

Bonny light crude oil (BLCO) is the major petroleum produced in Nigeria from the Niger Delta region, and its popularity abroad especially in the high fuel consuming countries such as United States, is due to its near –zero sulfur content ('sweet') and the high content of the low molecular weight aliphatic hydrocarbons ('light': NNPC – personal communication). BLCO is known to contain a 20:80 ratio of polyaromatics ('heavy fraction') to aliphatics ('light fraction' NNPC- personal communication).

Oil spillages unfortunately are chronic in the Niger Delta region of Nigeria which threaten both aerial, terrestrial and aquatic life and human health (Akpofure, et. al., 1998-2000).
The toxic effects of crude oil in laboratory animals have been reported widely (Rahimtula, et. al., 1987 and Khan, et. al, 1986); for example, the oral administration of Prudhoe Bay crude oil at 5.0ml/kg body weight daily for two days to male Charles River CD-1 mice resulted in increases in liver weight, hepatic proteins, RNA, glycogen, total lipids, cholesterol, triglycerides and phospholipids; and an increase (15-20 fold maximal activity) 12h following the intraperitoneal administration of 4.0ml/kg bw crude oil in hepatic ornithine decarboxylase activity and a 34-fold increase in hepatic putrescine levels over those in controls (Khan, et. al., 1987).
Although Nigerian (Bonny) Light Crude Oil (BLCO) is the country's major foreign exchange earner and there are frequent oil spillages in the Niger- Delta region of Nigeria, the potential toxicities of BLCO to man have not been adequately reported in international journals as extensive internet search showed no references to such studies.

Recently, in a pioneer study we showed that the pattern of urinary excretion of nickel in guinea- pigs treated with a single dose (5.0ml/kg bw) of BLCO by skin application was duration-dependent, as the highest urinary nickel level occurred at four days (Oruambo, 2004).

In the present study, it was of basic interest to know the pattern of the distribution and concentration of the hydrocarbons contained in BLCO to four key tissues, i.e. liver, lungs, kidneys and heart and to hepatic nuclear and cytoplasmic fractions after guinea-pigs were treated by intraperitoneal injection with two doses of BLCO. Also we needed to determine its effect on a specific molecular marker, in this case, mitochondrial succcnic dehydrogenase.

MATERIALS AND METHODS

Fresh Bonny Light Crude Oil (BLCO) was obtained from the Nigerian National Petroleum Company, Port Harcourt, Rivers State, Nigeria and brought to the laboratory of the Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt in an amber container. Fifteen (15) adult female guinea-pigs were grouped into three of five animals per group. Each animal in two groups received 2.5 ml/kg bw BLCO by intraperitoneal (i.p.) injection and 5.0 ml/kg bw, respectively; i.e. ten (10) animals in all were treated with two varying doses of BLCO.

The third group of five (5) animals were the untreated controls. Treatment was semi-chronic that is in alternate days for seven days.
All animals were given commercial rodent chow and drinking water *ad libitum* through out the treatment.
On the eighth day, all animals including the controls were sacrificed and four key organs (heart, liver, lungs and kidneys) were excised, grouped, pooled and 10% homogenate was prepared in 0.05M potassium phosphate buffer, pH 7.4.

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Liver homogenate was divided into two portions for subcellular fractionation. The COTH were extracted in all tissue homogenates by groups with toluene at 1:2 (v/v) ratio by vigorous shaking for five minutes. After settling, the upper toluene phase (layer) was decanted, left at room temperature for twenty minutes and the spectrophotometric absorbance was read at 420nm. The concentration of COTH in each tissue sample was derived from a calibration curve of known concentrations of n-hexane.

The split liver homogenate sample was centrifuged at 2000 rpm at room temperature for ten minutes. The resultant pellet (sediment) and the supernatant were collected, representing nuclear and cytoplasmic subcellular fractions, respectively. These were washed twice in 0.05M potassium phosphate buffer, pH 7.4 by re-suspension and re-configuration at 2000 rpm.
Each fraction was then extracted with toluene and the COTH concentration was determined as already described.

Enzyme Assay: Succcnic dehydrogenase (SDH) activity was measured calorimetrically by the formazan method (Oruambo, 1989) in liver homogenates from untreated controls and the guinea-pigs treated intraperitoneally with 5.0 ml/kg bw BLCO.
A mixture consisting of 50mM potassium phosphate buffer, pH 7.4; 0.1% INT (2-p-iodophenyl -3-p- nitrophenyl-5- phenyl tetrazolium chloride); 50mM sodium succinate (omitted for blanks); and 250mM sucrose in a volume of 1.0ml was added to 0.1ml enzyme (as mg homogenate protein) and incubated at 37^0C for 30 minutes. The reaction was stopped with the addition of 1.0ml 10% trichloroacetic acid, and the formazan produced on reduction of INT was extracted with 4.0 ml ethyl acetate.
All tubes were centrifuged, and the absorbance of the upper layer was measured at 490nm. Results are expressed as change in Absorbance x 10^3/30 minutes /mg protein.

RESULTS AND DISCUSSION

Table I shows the concentrations of COTH in the four key organs studied at 2.5ml/kg bw and 5.0ml/kg bw BLCO; clearly at 2.5 ml/kg bw, the liver and the heart concentrated over 3-fold more crude oil hydrocarbons when compared to the untreated controls. However, the increase of COTH at this lower dose level in the lungs and kidneys were marginal over the controls at 35% and 25%, respectively.
Inter-tissue distribution showed that at 2.5 ml/kg bw BLCO, the order of COTH concentration seems to be: lungs > liver = kidney > heart.

On the other hand, at the higher dose of 5.0ml/kg bw BLCO, inter- tissue concentration of COTH somewhat reversed; liver = lungs > kidney > heart. Again, all four tissues liver, heart, lungs and kidneys concentrated higher COTH at 5.0ml/kg bw than the controls, at 63%, 53%, 33% and 25%, respectively; therefore, a dose –related distribution of COTH is suggested.
These results suggest the liver and lungs to perhaps be the primary target organs for potential BLCO systemic toxicity. In the case of the liver, the higher COTH concentration at 5.0ml/kg bw BLCO may reflect an increased amount of polyaromatic hydrocarbons which would be expected to be bio-transformed more readily by liver enzymes.

Table 1; Tissue concentrations of hydrocarbons from the intraperitoneal treatment of adult female guinea pigs with 2.5ml/kg bw and 5.0 ml/kg BLCO

Treatment (Group)	Tissue	mg/ml
Control - untreated		
(n = 5)	Liver	0.0006
	Heart	0.0006
	Lungs	0.0012
	Kidney	0.0012
2.5ml/kg bw		
(n = 5)	Liver	0.0016
	Heart	0.0012
	Lungs	0.0019
	Kidney	0.0016
5.0ml/kg bw		
(n = 5)	Liver	0.0018
	Heart	0.0013
	Lungs	0.0018
	Kidney	0.0016

n = number of animals per group.

In Table 2, the nuclear fraction overwhelmingly concentrated COTH than the cytoplasmic fraction when compared with their controls in a more dose-response manner; *2-fold: little change* and *2.5-fold: : little change at* 2.5 ml/kg bw and 5.0ml/kg BLCO, respectively. However, since the bio-transformation enzymes are in the cytoplasm, it is not clear what the dynamics of the interaction of the BLCO hydrocarbons with subcellular macromolecules might have been to result in such quantitatively higher level of COTH in the nuclear component over the cytoplasmic component.

Table 2: Hepatic subcellular concentrations of total hydrocarbons by the intraperitoneal treatment of adult female guinea pigs with 2.5ml/kg bw and 5.0ml/kg bw BLCO.

Treatment (Group)	Fraction	mg/ml
Control - untreated		
(n = 3)	Nuclear	0.00060
	Cytoplasmic	0.00020
2.5ml/kg bw		
(n = 5)	Nuclear	0.00100
	Cytoplasmic	0.00021
5.0ml/kgbw		
(n = 5)	Nuclear	0.00160
	Cytoplasmic	0.00024

The implication of this is not totally clear, but there is the potential of damage to the DNA as the nuclear fraction (i.e. cellular nucleus) contains the chromosomes (Nelson, et. al., 2000). Accumulation of crude oil-derived hydrocarbons in the nucleus in close proximity to the chromosomes therefore could pose a potential genotoxic risk. Moreso, as all crude oils including BLCO, contain known and suspected human carcinogens, such as benzo(a)pyrene which like most chemical carcinogens, induce carcinogenicity by interacting with (covalent binding to) genomic DNA (Baneriee, et. al., 1980). Indeed, some geological crude oils have been shown to

induce tumors in laboratory animals (IARC, 1989). BLCO may therefore have such carcinogenic potential.

Table 3 shows a near-4 fold increase in hepatic SDH activity in guinea- pigs treated with 5.0 ml/kg bw BLCO over untreated controls.

Table 3: Hepatic Succinic dehydrogenase (SDH) Activity in adult female guinea-pigs treated by intra-peritoneal injection with 5.0 ml/kg bw BLCO.

Treatment	Specific Activity (SDH) (Change in Absorbance/30 mins./mg protein)
Untreated Controls (n = 5)	3.15
5.0 ml/kg bw BLCO Treated (n = 5)	11.84

n = number of animals per group

This induction of an enzyme, SDH, by BLCO is consistent with the findings of Khan and Rahimtula who reported a 15-fold increase in hepatic ornithine decarboxylase activity by Prudhoe Bay Crude Oil (3). Similarly, in another study we found a significant increase in Glucose-6-phosphatase activity by BLCO (unpublished data). It is therefore not entirely surprising to obtain this result with SDH. However, we are mindful that there may be differences in the patterns of xenobiotic interactions with enzyme-proteins due in part to structure differences, conformational differences, function differences and differences in accessibility. Therefore, the similar pattern of effect seen here as with our unpublished data on Glucose-6-phosphate may be purely co-incidental. Nonetheless, the induction of SDH activity well above control/normal level by BLCO may have implications for the mitochondria where SDH is membrane-bound and marker enzyme and where cellular energy, i.e. ATP, is produced; more specifically, this may modify the normal reactions of the Krebs cycle where SDH is a key enzyme (8), and consequently may adversely affect cellular ATP-energy production. The modification of this crucial pathway may be a link in the chain of events that could lead to tissue toxicity by BLCO.

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