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Blood Retention Pattern of Polycyclic Aromatic Hydrocarbons in Guinea-Pigs Treated with Crude Oil

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Abstract: ***Exposures to crude oil are common among oil workers and rural populace and contribute a global health burden. The aim of this study is to investigate the blood retention patterns of polycyclic aromatic hydrocarbons (PAHs) in guinea pigs treated with bonny light crude oil (BLCO). The objective of the study was to find out if there is a peculiar pattern of retention of PAH after exposure to crude oil. Fifteen adult male guinea pigs were used for the research and separated into five groups of three animals. All animals were fed with freshly cut elephant grass daily ad libitum. Group 1 served as the control (untreated) group. Group 2-6 were treated liberally with crude oil by skin application (dorsal side of ear) once. The sacrifices were done at 1, 2, 3 and 4 hours interval. Blood samples were collected and extracted with dichloromethane (DCM) and concentrated before GC-MS analysis. The chromatogram shows 14 PAHs absorbed and retained in the blood after 1hr exposure. 4 out of the 14 absorbed PAHs were still retained in the blood up to 2hrs after skin exposure. The chromatogram for 3 & 4 hrs show no retained PAHs but contained peaks which might be indicative of other PAHs. The results obtained from this study reveal that PAHs have a clearance time of approximately < 4hrs in the blood and that its metabolites may cause carcinogenesis if it forms conjugate with DNA at site critical for cell growth.***

Keywords: PAHs, Crude oil, GC/MS**,**

1. Introduction

Crude oil is a complex mixture of many different components. Its exploration and transportation has generated a lot of environmental problems. It is noteworthy, that the devastating consequences of crude oil spill in the Niger Delta of Nigeria poses great hazards on both human and animals.

Since crude oil is a complex liquid, there is potential occupational exposure to a variety of substances: various hydrocarbons and other organic compounds, dissolved gases and metal compounds. Exposure is possible in all operations involving the product, including drilling, pumping and treating steps; transport by pipeline, ships or rail cars; storage and refinery processing. The primary route of exposure is through skin contact.

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of chemicals that occur naturally in coal, crude oil and gasoline. Incomplete combustion of organic material results in emission of PAHs [1]. These molecules consist of two or more aromatic rings fused in linear, angular or cluster arrangements and by definition are composed of hydrogen and carbon. As pure chemicals, these compounds are colourless, white or pale yellow solids. Their physicochemical properties, vapour pressure and solubility vary according to their molecular weight. PAHs possess a highly characteristic UV absorbance spectrum although some may be fluorescent [2]. PAHs are ubiquitous and persistent as a consequence of natural (forest fires and volcanic eruptions) and human activities [3].

The main objective of this research work is to determine the blood retention pattern of PAHs in guinea-pigs treated with BLCO by skin application. And to ascertain whether or not, PAHs exposure could serve as an indicator of genomic toxicity.

Although several works have been done in this field of research, there is limited information on the retention patterns of various PAHs in the blood.

2. Materials and Methods

2.1 Test Substance

Fresh Nigerian (Bonny) Light Crude Oil (BLCO) was obtained from the Nigerian National Petroleum Corporation (NNPC) here in Port Harcourt, Rivers State, Nigeria, and brought to the laboratory in an amber sample bottle.

2.2 Laboratory animals

Fifteen adult male guinea pigs (8-12 weeks old) were purchased from the animal farm at University of Port Harcourt. The animals were brought to the biochemistry animal room and were fed with freshly cut elephant grass daily *ad libitum*.

2.3 Treatment of animals

The animals used in this research work were fifteen in number. They were divided into six groups. Group 1 consisted of two animals and served as the control (untreated) group. Group 2-5 consisted of two animals each, these groups was treated with liberally with BLCO once by skin application and the blood samples were collected after 1, 2, 3, and 4hours intervals. Group 6 consisted of three animals; this group was treated daily for 10 consecutive days, after the tenth day the urine and faeces were pooled for nickel and vanadium analysis.

2.4 Blood collection, processing and storage

3 ml of blood was taken from each research animal and was transferred into labeled heparin sample bottles. The sample bottles were placed into a covered ice container immediately after collection to protect the samples from heat and ambient light. The blood samples were then centrifuged at 10,000 rpm for 30 min on RP-1000 Centrifuge to obtain the plasma. The plasma from each sample was then separated from the whole blood and stored in tightly capped vials at - 4^0C until ready for use.

2.5 Sample preparation for PAHs assay

The plasma samples were thawed at room temperature. PAHs were extracted from the plasma by liquid-liquid extraction using high purity Dichloromethane (DCM). The apparatus for this consisted of a 25 ml volume separating funnel mounted on a retort stand. The separating funnel was thoroughly washed and dried over night in a muffle furnace at an elevated temperature. Prior to use the funnel was rinsed vigorously with dichloromethane for several minutes. This was removed and allowed to drain and dry completely in fume cupboard. The plasma sample (of less than 3 ml) was mixed with 5 ml of dichloromethane. This was shaken vigorously for 2 min and allowed to separate and settle. After 10 min, the organic layer was removed and the process was repeated with the aqueous layer twice. The three portions of the organic phase were combined and evaporated to 1 ml volume using a rotary evaporator.

2.6 Chromatographic instrumentation and condition

The analysis was carried out on a Hewlet Packard Gas Chromatograph coupled to a mass spectrometer with an autosampler and a 30 m 0.33 id DB-5 MS fused silica capillary column. Chromatographic analysis was run and interpreted with Chemstation for GC/MS for Agilent Technologies running on a Del computer with Windows NT. Helium was used as the carrier gas and the column head pressure was maintained at 10 psi to give an approximate flow rate of 1 ml/min. The injector and transfer line were maintained at 290 and 250^{o}C, respectively. All injection volumes were 1 µl in the split less mode. The column temperature was initially held at 70^{o}C for 4 min, ramped to 300^{o}C at a rate of 10^{o}C/min, and then temperature was held at 300^{o}C for 10 min. The mass spectrometer was used in electron ionization mode and all spectra were acquired using a mass range of m/z 50 – 400 and automatic gain control (AGC).

3. Results

Table 1: Estimation of PAHs in blood of untreated (controls) animals

Compound	R.T.	QIon	Response	Conc Units	Dev(Min)
Target Compounds					Qvalue
1) Naphthalene	0.000		0	N.D.	
2) 2-Methyl Naphthalene	0.000		0	N.D.	
3) Acenaphthene	0.000		0	N.D.	
4) Acenaphthylene	0.000		0	N.D.	
5) Fluorene	0.000		0	N.D.	
6) Phenanthrene	0.000		0	N.D.	
7) Anthracene	0.000		0	N.D.	
8) Fluoranthene	0.000		0	N.D.	
9) Pyrene	0.000		0	N.D.	
10) Benz (a) anthracene	0.000		0	N.D.	
11) Chrysene	0.000		0	N.D.	
12) Benzo (b) fluoranthene	0.000		0	N.D.	
13) Benzo (k) fluoranthene	0.000		0	N.D.	
14) Benzo (a) pyrene	0.000		0	N.D.	
15) Indeno (1,2,3-cd) pyrene	0.000		0	N.D.	
16) Dibenz (a,h) anthracene	0.000		0	N.D.	
17) Benzo (g,h,i) perylene	0.000		0	N.D.	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

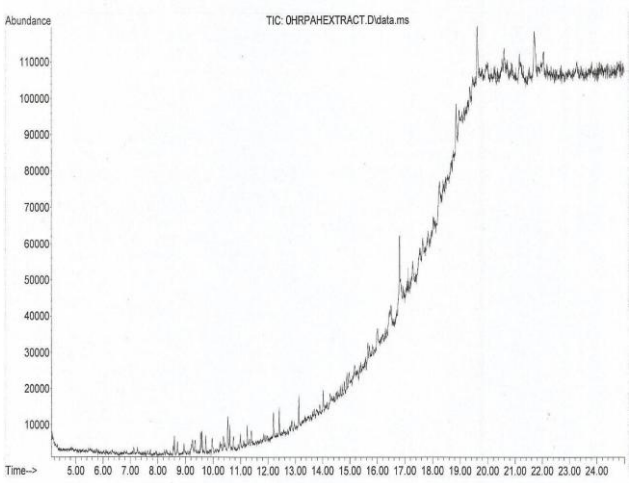

Figure 1: Chromatograph of PAHs in the blood at 0 hours.

Table 2: Estimation of PAHs in the blood after 1 hour of dermal application of crude oil

Compound	R.T.	QIon	Response	Conc Units	Dev(Min)
Target Compounds					Qvalue
1) Naphthalene	4.791	128	133588	0.02 PPM	# 69
2) 2-Methyl Naphthalene	5.657	142	1043740	1.11 PPM	94
3) Acenaphthene	7.053	152	197097	0.12 PPM	# 1
4) Acenaphthylene	7.382	153	354092	0.39 PPM	86
5) Fluorene	8.371	166	326879	0.34 PPM	# 63
6) Phenanthrene	10.397	178	2081477	1.74 PPM	# 90
7) Anthracene	10.476	178	394084	0.32 PPM	# 66
8) Fluoranthene	13.112	202	659854	0.56 PPM	# 94
9) Pyrene	13.627	202	599276	0.48 PPM	# 86
10) Benz (a) anthracene	16.496	228	115649	0.11 PPM	# 34
11) Chrysene	16.580	228	208449	0.18 PPM	# 2
12) Benzo (b) fluoranthene	18.911	252	47770	0.04 PPM	# 1
13) Benzo (k) fluoranthene	18.912	252	47909	0.02 PPM	# 1
14) Benzo (a) pyrene	19.814	252	71227	0.06 PPM	# 55
15) Indeno (1,2,3-cd) pyrene	0.000		0	N.D.	
16) Dibenz (a,h) anthracene	0.000		0	N.D.	
17) Benzo (g,h,i) perylene	0.000		0	N.D.	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

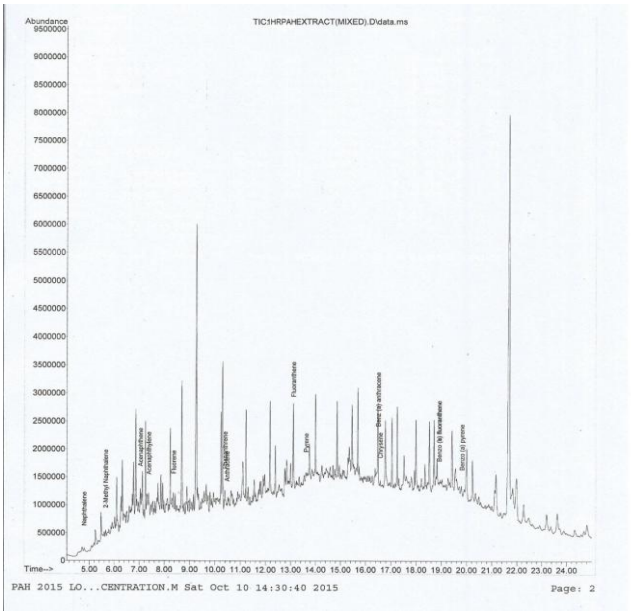

Figure 2: Chromatogram of PAHs in the blood after 1 hour of dermal application of crude oil.

Table 3: Estimation of PAHs in blood after 2 hours of dermal application of crude oil

Compound	R.T.	QIon	Response	Conc Units	Dev(Min)
Target Compounds					Qvalue
1) Naphthalene	0.000		0	N.D.	
2) 2-Methyl Naphthalene	0.000		0	N.D.	
3) Acenaphthene	0.000		0	N.D.	
4) Acenaphthylene	0.000		0	N.D.	
5) Fluorene	0.000		0	N.D.	
6) Phenanthrene	10.388	178	1796080	1.50 PPM #	76
7) Anthracene	10.479	178	743234	0.62 PPM #	63
8) Fluoranthene	13.125	202	275347	0.22 PPM #	60
9) Pyrene	13.636	202	192888	0.14 PPM #	58
10) Benz (a) anthracene	0.000		0	N.D.	
11) Chrysene	0.000		0	N.D.	
12) Benzo (b) fluoranthene	0.000		0	N.D.	
13) Benzo (k) fluoranthene	0.000		0	N.D.	
14) Benzo (a) pyrene	0.000		0	N.D.	
15) Indeno (1,2,3-cd) pyrene	0.000		0	N.D.	
16) Dibenz (a,h) anthracene	0.000		0	N.D.	
17) Benzo (g,h,i) perylene	0.000		0	N.D.	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

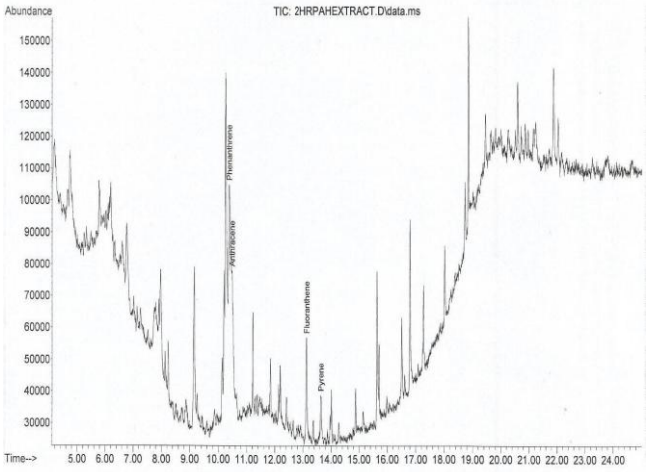

Figure 3: Chromatogram of PAHs in the blood after 2 hours of dermal application of crude oil.

Table 4: Estimation of PAHs in the blood after 3 hours of dermal application of crude oil

Compound	R.T.	QIon	Response	Conc Units	Dev(Min)
Target Compounds					Qvalue
1) Naphthalene	0.000		0	N.D.	
2) 2-Methyl Naphthalene	0.000		0	N.D.	
3) Acenaphthene	0.000		0	N.D.	
4) Acenaphthylene	0.000		0	N.D.	
5) Fluorene	0.000		0	N.D.	
6) Phenanthrene	0.000		0	N.D.	
7) Anthracene	0.000		0	N.D.	
8) Fluoranthene	0.000		0	N.D.	
9) Pyrene	0.000		0	N.D.	
10) Benz (a) anthracene	0.000		0	N.D.	
11) Chrysene	0.000		0	N.D.	
12) Benzo (b) fluoranthene	0.000		0	N.D.	
13) Benzo (k) fluoranthene	0.000		0	N.D.	
14) Benzo (a) pyrene	0.000		0	N.D.	
15) Indeno (1,2,3-cd) pyrene	0.000		0	N.D.	
16) Dibenz (a,h) anthracene	0.000		0	N.D.	
17) Benzo (g,h,i) perylene	0.000		0	N.D.	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

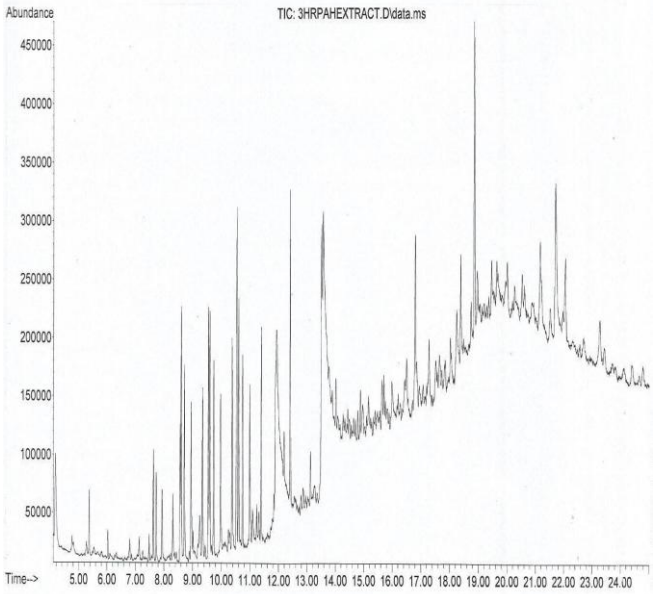

Figure 4: Chromatograph of PAHs in the blood after 3 hours of dermal application of crude oil.

Table 5: Table of estimation of PAHs in the blood after 4 hours of dermal application of crude oil

Compound	R.T.	QIon	Response	Conc Units	Dev(Min)
Target Compounds					Qvalue
1) Naphthalene	0.000		0	N.D.	
2) 2-Methyl Naphthalene	0.000		0	N.D.	
3) Acenaphthene	0.000		0	N.D.	
4) Acenaphthylene	0.000		0	N.D.	
5) Fluorene	0.000		0	N.D.	
6) Phenanthrene	0.000		0	N.D.	
7) Anthracene	0.000		0	N.D.	
8) Fluoranthene	0.000		0	N.D.	
9) Pyrene	0.000		0	N.D.	
10) Benz (a) anthracene	0.000		0	N.D.	
11) Chrysene	0.000		0	N.D.	
12) Benzo (b) fluoranthene	0.000		0	N.D.	
13) Benzo (k) fluoranthene	0.000		0	N.D.	
14) Benzo (a) pyrene	0.000		0	N.D.	
15) Indeno (1,2,3-cd) pyrene	0.000		0	N.D.	
16) Dibenz (a,h) anthracene	0.000		0	N.D.	
17) Benzo (g,h,i) perylene	0.000		0	N.D.	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

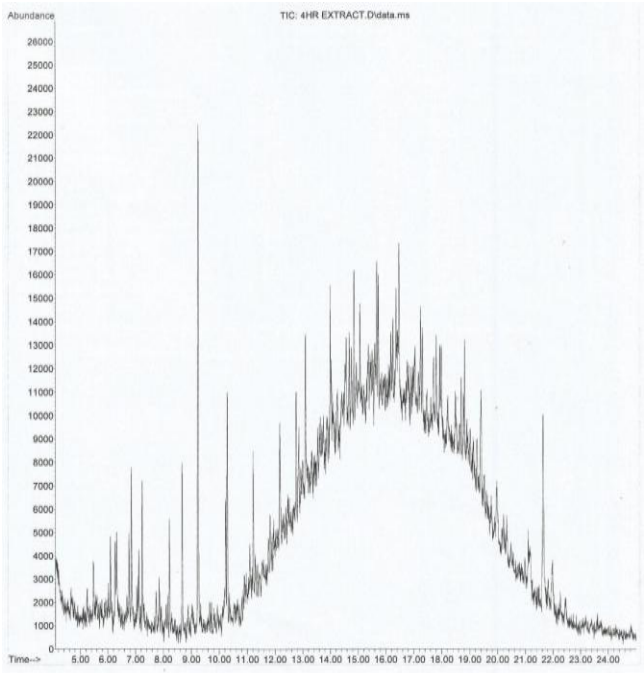

Figure 5: Chromatogram of PAHs in the blood after 4 hours of dermal application of crude oil.

4. Discussion

The chromatogram results show the appearance of PAH peaks, the source of which is the crude oil as the PAH peaks were not seen in the control (untreated) group (table 1 and figure 1). The results also show that 14 PAHs were retained in the blood after one hour of exposure (table 2 &figure 2). These 14 PAHs are: acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, fluoranthene, fluorene, 2-methyl naphthalene, naphthalene, phenanthrene and pyrene. Table 3 and figure3 shows that only 4 out of the 14 detected and absorbed PAHs were retained in the blood after two hours of exposure. These 4 PAHs are phenanthrene, anthracene, fluoranthene and pyrene. The 3 and 4 hours exposure analysis show no PAH retained (table 4&5, figure 4&5). This implies that 10 PAHs have a retention time of less than two hours (< 2hrs) and 4 PAHs have retention time of less than three hours (< 3hrs) in the blood. It was also observed that the concentration of anthracene was on a continual increase as opposed to the concentration of other PAHs after one hour of exposure. This might be as a result of differential diffusion, metabolic pathway of the research animals and other factors

Table 4 and 5 shows no retained PAHs, but figure 5 and 6 shows peaks which might be indicative of other PAHs that the instrument (GC-MS) could not identify because the instrument have no library for them..

Differential diffusion of PAHs through the intercellular spaces is considered to be the major pathway for dermal absorption into the blood stream because of the varying concentration of individual PAHs in the blood at various time intervals (table 1, 2 &3). This is in agreement with work done by Lenka Kotingová [4]

5. Conclusion

Differential diffusion of PAHs through the intercellular spaces is considered to be the major pathway for dermal absorption into the blood stream. PAHs have a varying retention pattern/time in the blood and in general have a retention time of less than four hours (<4hrs) in the blood.

References

[1] Agency for Toxic Substances and Disease Registry (1996). ToxFAQs™ for Polycyclic Aromatic Hydrocarbons (PAHs), In: Agency for Toxic Substances and Disease Registry, 16.01.2011, Available from http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=121&tid=25

[2] Fetzer, J. C. & Biggs, W. R. (1994). A Review of the Large Polycyclic Aromatic Hydrocarbons. *Polycyclic aromatic compounds*, Vol. 4, No.1, pp. 3 - 17, 1040-6638 March 18, 2011

[3] Jongeneelen, F. J. (2001). Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *Annals of Occupational Hygiene*, Vol. 45, No.1, (Jan), pp. 3-13, 0003-4878 (Print)

[4] Lenka Kotingová, (2014). Transdermal Absorption of Polycyclic Aromatic Hydrocarbons. Available from https://www.lfhk.cuni.cz/Studenti/Doktorske-studium-(Ph-D-)/Konference-studentu-DSP/Archiv-konferenci-studentu-DSP/FC-DSP-Abstracts-2014.aspx/