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A screen for polynuclear aromatic hydrocarbons in blood samples of occupationally exposed subjects

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ABSTRACT

Auto mechanics are occupational exposed to high doses of refined petroleum product which are known to contain considerable amount of polynuclear aromatic hydrocarbons. These compounds constitute great health hazard. In this study these exposed subjects and other unexposed subjects were screened for unmetablized PAHs. The study was carried out using Gas Chromatograph coupled with Mass Spectrometry Detector and the identification was based on retention time match and mass spectral match against those of standards. 25% of the exposed subjects were found to contain one polynuclear aromatic hydrocarbon or the other while one out of eleven unexposed subjects contains polynuclear aromatic hydrocarbon in their blood sample. The PAHs detected include anthracene, naphthalene and fluoranthene.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a widespread class of environmental chemical pollutants composed of two or more aromatic rings. They are a component of crude and refined petroleum and coal. Over a hundred PAHs have been identified and these usually are found as complex mixtures[1]. PAHs may also be generated as products of incomplete combustion processes such as forest fires and volcanic eruptions[2]. Anthropogenic sources such as industrial production, transportation and waste incineration generate significant levels of PAHs[3,4]. The effects of PAHs on human health have been of major concern[5,6]. PAHs are known to be toxic[7-10]. They are acutely lethal in concentrations of a few ppm and chronically lethal in sublethal concentrations in ppb[11,12]. Toxic effects observed due to PAHs include decreased body weight, enlarged liver with cell oedema and congestion of the liver parenchyma and inflammation of kidney cells[13]. PAHs have been found to cause reproductive toxicity. PAHs can affect female fertility by destruction of oocytes. Developmental toxicity such as embryolethality, reduced fetal weight and malformations have been reported in response to some PAHs[14]. A series of studies have been conducted on the reproductive and developmental toxicity in human. These were carried out in several countries such as Ukraine[15], United States[16], Poland[17], Czech Republic[18]. Several studies have also shown the carcinogenicity of PAHs[19-23]. They have been implicated in inducing lung, skin, stomach and breast cancer.

PAHs enter the body through a variety of ways. They enter through the lungs, when they are breathed in air that contains them(usually stuck to particles or dust). Drinking water and swallowing food, soil or dust par-

TABLE 1: Bio data of the volunteers

Sample	Sex	Age (years)	Marital status	Location	Occupation	Period of exposure(years)
a1	Male	32	Married	Satellite town	Panel beater	11
a2	Male	35	Married	Satellite town	Motor mechanic	18
a3	Male	44	Married	Satellite town	Motor mechanic	20
a4	Male	25	Married	Satellite town	Motor mechanic	2
a5	Male	27	Married	Satellite town	Motor mechanic	7
a6	Male	29	Married	Satellite town	Motor mechanic	7
a7	Male	55	Married	Satellite town	Motor mechanic	30
a8	Male	37	Married	Satellite town	Motor mechanic	20
a9	Male	25	Single	Satellite town	Motor mechanic	2
a10	Male	25	Single	Satellite town	Motor mechanic	12
a11	Male	29	Single	Satellite town	Motor mechanic	22
a12	Male	40	Married	Satellite town	Vulcaniser	25
a13	Male	20	Single	Satellite town	Motor mechanic	4
a14	Male	42	Married	Satellite town	Motor mechanic	23
a15	Male	38	Married	Satellite town	Motor mechanic	8
a16	Male	33	Single	Satellite town	Motor mechanic	14
a17	Male	21	Married	Satellite town	Motor mechanic	5
a18	Male	33	Married	Satellite town	Motor mechanic	15
a19	Male	17	Single	Satellite town	Motor mechanic	1
a20	Male	20	Single	Satellite town	Motor mechanic	1
b1	Male	25	Single	luth	student	0
b2	Male	23	Single	luth	student	0
b3	Female	21	Single	luth	student	0
b4	Female	22	Single	luth	student	0
b5	Female	22	Single	luth	student	0
b6	Female	26	Single	luth	student	0
b7	Male	25	Single	luth	student	0
b8	Male	22	Single	luth	student	0
b9	Female	26	Married	luth	student	0
b10	Male	22	Single	luth	student	0
b11	Female	20	Single	luth	student	0

ticles that contain PAHs are other routes for these chemicals to enter the body, but absorption is generally slow when PAHs are swallowed. PAH can enter the body if the skin comes in contact with soil that contains high levels of PAHs or with products that contain high level of PAHs such as crude oil and refined petroleum products. The rate at which PAHs can enter the body can be influenced by the presence of other compounds. PAHs can enter all the tissues of the body that contain fat. They tend to be stored mostly in the kidney, liver and fats. Smaller amount are stored in the spleen, adrenal gland and ovaries. PAHs are changed by the body into different substances some of which are more harmful than the original PAHs.

In this study we tried to look at a class of individuals who are occupationally exposed to PAHs to see if there are traces of unmetabolized PAHs in their plasma. The subjects in this study are auto mechanics in based in Lagos area of Nigeria. This set of people are very much exposed to petroleum product (which contain considerable amount of PAHs) as result of their work. The study was carried out using GC-MS with NIST library facility to enable the identification of PAH compounds beyond those whose standards are available.

EXPERIMENTAL

Subjects

The study was a qualitative screening of 31 healthy adult volunteers resident in the city of Lagos in Nigeria. The volunteers were of two categories. The first was made up of subjects thought to have little exposure to PAH contamination. These were students of the University of Lagos, whose ages ranged from 20 to 26 years. They were 11 in number made up of five male and six female. The second category was made up of subjects thought to have greater exposure to PAHs as

a result of their occupation. They mainly work as automobile mechanics or related profession. They have a lot of contact with petroleum products which are a good source of PAHs. They were all adult men of ages ranging from 17 to 55 years. Their years in the occupation and hence exposure to PAHs ranges from 1 to 30 years. They are 20 in number. TABLE 1 shows the subjects and some of their characteristics.

Blood collection, processing and storage

Three ml of blood were taken from an antecubital vein of each of the volunteers using 5ml syringes and were transferred into labeled heparinised sample bottles. The sample bottles were immediately after blood collection placed into a covered ice container to protect the samples from heat and ambient light. The blood samples were then centrifuged at 10,000rpm for 30 minutes on RP000 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^{o}C until ready for use.

Chemicals

All solvents used were of HPLC grade or reagent grade. HPLC grade Dichloromethane was obtained from Sigma-Aldrich laboratories GmbH, Seeize, Germany. Heparin 2500 I.U. was obtained from Choongwae Pharmaceuticals, China. Sodium Chloride I.V. Infusion B.P. was obtained from Unique Pharmaceuticals, Lagos. A PM-525A EPA method 525 PAH mixture(comprising of 13 PAHs namely: acenaphthylene, fluorene, phenanthrene, anthracene, pyrene, benz [a]anthracene, chrysene, benzo[b] fluoranthene, benzo [k]fluoranthene, benzo[a]pyrene, benzo[ghi] perylene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene) was obtained from Ultra Scientific North Kingstown, Rhodes Island. Methylated Spirit B.P. was obtained from Nino Pharmaceuticals and Chemical Company Ltd, Lagos.

Sample preparation

The plasma samples were thawed at room temperature. PAHs were extracted from the plasma by the means of liquid-liquid extraction using high purity Dichloromethane. The apparatus for this consisted of a 25ml 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 3ml) was mixed with 5ml of dichloromethane. This was shaken vigorously for 2minutes and allowed to separate and settle. After 10minutes the organic layer was removed and the process repeated with the aqueous layer twice. The three portions of the organic phase were combined and evaporated to 1ml volume using a rotary evaporator.

Chromatographic instrumentation and condition

The analysis was carried out on a Hewlet Packard Gas Chromatograph coupled to mass spectrometry equipped with an autosampler and a 30m 0.33 id DB-5 MS fused silica capillary column. Chromatographic analysis was run and interpreted with a 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 1ml/min. The injector and transfer line were maintained at 290^{0}C and 250^{0}C respectively. All injection volumes were 1µl in the splitless mode. The column temperature was initially held at 70^{0}C for 4 minutes, ramped to 300^{0}C at a rate of 10^{0}C/min, then temperature was held at 300^{0}C for 10 minutes. 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).

RESULTS AND DISCUSSION

Most of the PAHs in the list of 16 priority pollutants were not detected in the samples of these exposed subjects. The PAHs detected are shown in figure 1 and are anthracene, naphthalene and fluoranthene. These were identified in the case of anthracene and naphtha-

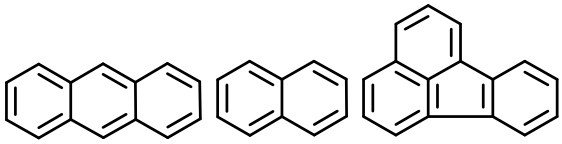

Figure 1: The chemical structures of anthracene, naphthalene and fluoranthene respectively (the detected compounds)

lene by a combination of retention time and mass spectral match which corroborated with the NIST library software attached to the system. For want of standards, fluoranthene was identified by the mass spectral match. There were a number of alkylated PAHs and very long chain aliphatic hydrocarbons detected too but this is not the focus of this study.

It is assume that most other priority PAHs have been metabolized since it is well known that PAHs never occur alone but occur as a mixture of individual PAHs[1]. Among the exposed subject only 25% of the population was found to contain one PAH or the other in its unmetabolized form in their blood. The samples from this group of subjects(ie auto mechanics) were coded 'A', the PAHs found in them include: fluoranthene, naphthalene, and anthracene. Fluoranthene was found in the blood sample of the subject $A_{3.}$ Naphthalene was found in the blood sample of the subject $A_{10.}$ Anthracene was found in blood samples of subjects $A_{16,}$ A_{17} and A_{18}. For the second group of subjects who are supposed to be unexposed anthracene was detected in the blood sample one of the subjects namely sample $B_{9,}$ even though this is least expected.

This result supports very much earlier findings that PAHs are metabolized in the body[24]. Due to the individual differences in genetic make up the rate of metabolism varies from person to person as there is no particular association (such as age or number of years of exposure) linked with the subjects that contain PAHs in their blood. TABLE 2 shows the distribution of unmetabolized PAHs in the blood samples of the subjects. It may be worth noting that in all the samples from the subject many PAHs metabolites were identified but to lesser extent in the samples from the unexposed group. Figure 2 and 3 compares the years of exposure with PAH detection and age of subject and PAH detected respectively.

A lot of epidemiological studies have been conducted on workers exposed to heavy load of PAHs especially those working in coal mine, asphalt works, foundries, aluminium smelting etc. Even though the concentrations to which these workers are exposed to are not clear, increased risk of lung cancer was observed among the workers[14]. Partanen and Boffetta[25] also observed increased risk of lung tumors in both pavers and roofers. Tumors of the stomach, bladder and skin and leukemia were also observed. Increased mortality from lung cancer has been observed consistently in many studies of foundry workers[26]. Increased risks for lung

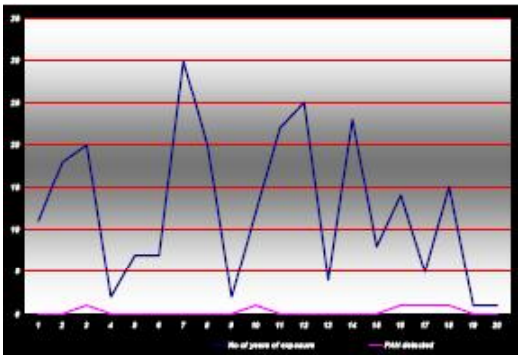

Figure 2: Comparison of years of exposure and PAHs detected

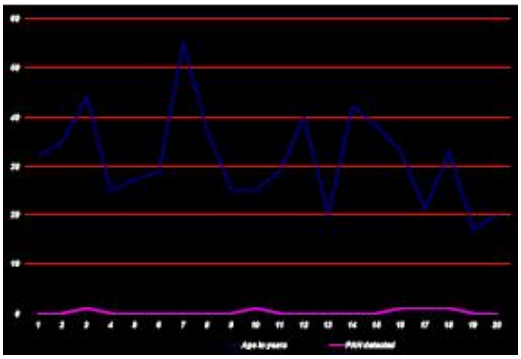

Figure 3: Comparison of age of subject and PAHs detected

TABLE 2: PAH distribution in samples

Sample	Age (years)	Period of exposure(years)	PAH present
A1	32	11	-
A2	35	18	-
A3	44	20	Fluoranthene
A4	25	2	-
A5	27	7	-
A6	29	7	-
A7	55	30	-
A8	37	20	-
A9	25	2	-
A10	25	12	Naphthalene
A11	29	22	-
A12	40	25	-
A13	20	4	-
A14	42	23	-
A15	38	8	-
A16	33	14	Anthracene
A17	21	5	Anthracene
A18	33	15	Anthracene
A19	17	1	-
A20	20	1	-

cancer were found in several studies of workers exposed to diesel exhaust[27]. There has been an increased risk seen for workers in several other occupations, which have exposure to PAH. In Xuan Wei, located in Yunnan province in China, mortality rate from lung cancer was found to be five times the Chinese national average especially among women. This was correlated to the use of 'smoky' coal as fuel (medium volatile bituminous coal with low sulphur and high ash). Further studies by Mumford et al.[28] attributed this to high level of PAHs in the atmosphere as a result of the use of this 'smoky' coal.

From this finding and further extrapolation based on numerous previous studies, auto mechanics could also be tagged occupationally exposed to PAHs which have adverse health effect. Future studies will look at the metabolites and many other cancer biomakers in the blood sample of these exposed subjects. It is worth noting that PAHs are ubiquitous[29] hence other source may contribute to the amount of PAHs intake, as the findings revealed in the case of occupationally unexposed subject which gave positive result for PAHs in the blood.

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