The Presence of Polycyclic Aromatic Hydrocarbons in the Blood of Pregnant Women in Cotonou, Benin Republic

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Abstract: *Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous air pollutants generated by combustion sources that include motor vehicles, coal-fired power plants and residential heating and cooking. They are compound consisting of several aromatic rings of hydrocarbons. They are classified as toxic, mutagens and carcinogens by World Health Organization (WHO). The study investigate the presence of these hydrocarbons in the blood of pregnant women expose to high endemic region of hydrocarbons to elucidate the prevalent causes of malformation of fetus and newborns witnessed in pregnant women from the area. Blood were isolated intravenously from seventeen (17) pregnant women; 2.0 ml per person and the blood were tested for polycyclic aromatic hydrocarbons using Gas chromatography (GC). The result indicated that nine (9) had between 4.2- 4.73ng/kg, and seven (7) had between 5.1- 5.27ng/kg and only one (1) had 7.09ng/kg of polycyclic aromatic hydrocarbons, indicating that only one woman had PAHs in her blood above WHO safety threshold of 7.00ng/kg pack cells (PC).*

Keywords: Polycyclic aromatic hydrocarbons (PAHs), Pregnant women, fetus, air pollutants, Gas chromatography (GC)

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous air pollutants generated by incomplete combustion from motor vehicles, coal-fired power plants and fumes from residential heating and cooking. Also tobacco smoking and environmental tobacco smoke (ETS) are a major source of PAHs in the atmosphere (BAEK et. al, 1991). A number of PAHs are human mutagens and carcinogens and are potentially significant reproductive and developmental toxicants. Epidemiological studies indicated associations between PAHs or PAH-DNA damage and fetal growth reduction (Perera et. al, 1988).

PAHs present themselves in two groups. The light weight PAHs (naphthalene, acenaphthene, and anthracene) which differ from the Heavy PAHs (Benzo(a) pyrene, Benzo (ghi) andPerylene, and by the number of aromatic rings and their molecular weight (Perera et al, 2005). The differences are placed on their physico-chemical and toxicological properties. In the environment, PAHS compounds with 2 to 7 aromatic rings are more present and more mobile. Their number is estimated at over 1000 and only 16 of them are commonly analyzed for the different components in the environment. These are identified as Priority Pollutants by the Agency of Environmental Protection in the United State (USEPA) because of the major environmental risks and toxicological problems they posed (ATSDR, 1995).

Exposure to polycyclic aromatic hydrocarbons usually occurs by breathing air contaminated by wild fires or coal tar, or by eating foods that have been grilled. PAHs have been found in at least 600 of the 1,430 National Priorities List sites identified by the Environmental Protection Agency of the United State (EPA). PAHS can leach from soil into water, water contamination also occurs from industrial effluents and accidental spills during oil shipment at sea.

Concentration of benzo(a) pyrene in drinking water are generally lower than those untreated water and about 100 fold lower than the U.S environmental protection agency (EPA) drinking water standard. The U.S environmental protection agency's maximum contaminant level (MCL) for benzo (a) pyrene in drinking water is 0.2 parts per billon (ppb). Soil contains measurable amounts of PAHs, primarily from air borne fallout. Documented levels of PAHS in soil near oil refineries have been as high as 200 µg/kg micro grams per kilogram of fine soil. Their level in soil samples obtained near cities and areas with high traffic were typically less than 200,000 µg/kg (IARC 1993).

In non –occupational setting most PAH exposure by a non - smoking person can be associated with diet. Route of expose may include inhalation, ingestion and dermal contact. Toxicity of PAHs is recognized and classified as substances that are carcinogenic, mutagenic and lethal. In addition to their ubiquitous nature, their high toxicity justifies their classification as persistent organic pollutant (POPs) and their inclusion as priority substances on the toxicity lists of the European Community, the Agency for Environmental Protection of the United States (USEPA) and the World Health Organization (WHO).

Polycyclic aromatic hydrocarbons (PAHs) are also pollutants released from the incomplete combustion of fossil fuels and are always found as a mixture of individual compounds. Due to economic growth and sharp increasing energy consumption in recent years, large quantities of PAHs and their derivatives are prevalent in our environment. PAHs have been extensively studied in recent times. Many authors have reviewed the origin and distribution of PAHs in the atmosphere, soil and sediment in natural environments. They found out that PAHs represent a class of toxicological compounds that can create a variety of hazardous effect *in vivo* and *in vitro*, including genotoxicity, immunotoxicity,

developmental toxicity and lethality which are also described. Numerous polycyclic aromatic hydrocarbons (PAHs) are carcinogenic making their presence in foods and the environment a major health concern.

Regulations around the world, limit levels of different PAHs in drinking water, food additives, cosmetics, workplaces, and factory emissions. PAHs are common environmental contaminants. They are found in the air, in the soil, in water, in plants and also in food. PAHs are formed during pyrolysis and the incomplete combustion of organic materials. Numbers of these pollutants in the environment are very dangerous to human health. The polycyclic aromatic hydrocarbons in our environment pose a significant toxicological risk. Their concentrations are usually raised high following accidental spill of hydrocarbons in the environment.

Studies have shown that exposure to these pollutants (PAHs) during pregnancy increases the risk of malformation in fetus and newborns. For the present of these PAHs in the environment and their adverse effect especially on human health, principally their carcinogenic, mutagenic or toxic properties, it's become necessary to deepen their studies. This justifies the huge investment and time ploughed into this research. Thus this work seeks to turn the searchlight into these polycyclic aromatic hydrocarbons (PAH) and to examine their impact on fetal growth.

2. Materials and Methods

Many methods are used for qualitative and quantitative analysis of polycyclic aromatic hydrocarbons PAHs. The analysis of PAHs can be done by high pressure liquid chromatography (HPLC) coupled with ultraviolet spectrometer or/and Fluorescence Detections or by gas chromatography coupled with mass spectrometer GC/MS or flame ionization detector GC/FID.

Traditionally, PAHs have been separated using HPLC, but the method detection limits (MDLs) of HPLC techniques employing direct injection of samples were too high for the detection of the low concentrations in real samples that are near the regulated limit. Therefore, the analyses of these samples require pre-concentration before analysis. However, before the analysis of the samples many steps were taken and executed to obtain a pure sample concentrate of PAH.

In the case of our study, blood samples were collected from pregnant women at LABORATOIRE DE SURETE D EXPERTISE ET D'ANALYSE of Regional Institute of Industrial Engineering, Biotechnologies and Applied Sciences (IRGIB-AFRICA). Gas Chromatograph coupled with Mass Spectrometer *(GC/MS)* with high resolution was the equipment used for good analysis of PAHs analysis in the blood of pregnant women.

The first step consists of the extraction of PAHs using dichloromethane / hexane on which were added the standard solution. Finally, the extract was concentrated and analyzed by GC-MS working on selective acquisition mode (SAM)

The determination of the concentration of PAHs was based on two steps: Purification of the samples and the analysis of the samples by GC-MS. The concentration of PAHs was determined by comparing the chromatographic surface obtained at a specific retention time between a sample and the standard of PAHS.

3. Results

Sample	Cities	ng/kg of PC
S1	Abomey	4.8
S2	Sainte-Rita	4.31
S3	Avotrou	5.4
S4	Segbeya	5.3
S5	Djeregbe	4.51
S6	Ekpe	4.69
S7	Agbalangada	4.28
S8	Allada	5.11
S9	Hougbo	4.43
S10	Dogbo-tota	5.27
S11	Lokossa	5.1
S12	Bohicon	4.73
S13	Dantokpa	7.09
S14	Agbagnizon	5.1
S15	Abomey	5.1
S16	Segbeya	4.3
S17	Avotrou	4.2

ng/kg of PC	Number
(4-7)	16
(7-8)	1

4. Discussion

From the results obtained, it was discovered that, of the 17 samples analyzed, the average concentration was in the range from 4.0 - 7.09 ng/kg. Comparing these results with the standard that gives the required safety threshold concentration of between 4 - 7 ng/kg PC, one (1) of the pregnant women had excess concentration which allows us to conclude that the unborn child is at high risk of malformation. However, the risks can varied over time following the concentration of PAHs, the type, time and the extent of exposure of this woman to PAHs.

High prenatal exposure to PAHs is associated with lower IQ and asthmatic condition at childhood. The center for children's Environment health reported in one of their studies that the exposure to PAH pollution during pregnancy is related to adverse birth outcomes including low birth weight, premature delivery, and heart or internal organs malformations. Exposed babies umbilical cord blood cells showed DNA damage that was linked to cancer. In another instances regarded as a- follow-up studies, it showed a higher level of developmental delays at the age three, lower score on IQ tests and increase behavioral problems at age six and eight.

In addition, the 2012 Columbia university studies in Environment Health perspectives linked prenatal exposure to pollutants and eventual child behavioral outcomes. The study found that exposure to higher levels of PAHs was associated with a 24% higher score of anxiety and attitudinal

depression for children ages of 6 to 7 than those with low level of exposure. Babies found to have elevated PAHs levels in their umbilical cord blood were 46% more likely to have high score on the anxiety / depression scale than those with low PAH levels in umbilical cord blood (Perera et. al., 1988; Choi and Rauh, 20087).

Table 1: List of 16 Priority PAHs classes by US. EPA

Names	No of cycles	Chemical Formula
Naphtlene	2	$C_{10}H_{18}$
Acenaphtene	3	$C_{12}H_{18}$
Flourene		$C_{12}H_{10}$
Anthracene		$C_{14}H_{10}$
Phenanthrene		$C_{14}H_{10}$
Fluoranthene	4	$C_{16}H_{10}$
Pyrene		$C_{16}H_{10}$
Benzo(a) anthrcene		$C_{18}H_{12}$
Chrysene		$C_{18}H_{12}$
Benzo(a) pyrene	5	$C_{20}H_{12}$
Benzo(b) fluoranthene		$C_{20}H_{12}$
Dibenzo (a,h) anthracene		$C_{22}H_{14}$
Benzo(k) fluoranthene		$C_{20}H_{12}$

Table 2: List of 6 major PAHS WHO Propose to follow very closely

Name	No of Cycles	Chemical Formulae
Fluoranthene	4	$C_{16}H_{10}$
Benzo(a)pyrene Benzo(a) flupranthen Benzo(k)fluoranthene Benzo(ghi)perylene	5	$C_{20}H_{12}$ $C_{20}H_{12}$ $C_{2O}H_{12}$
Indeno (1,2,3-cd)pylene	6	$C_{2O}H_{12}$ $C_{22}H_{12}$

Table 4: Carcinogenic potential of PAHs (IARC, 1987_2002)

PAH	IARC Classification
Naphthalene Acenaphthene Acenaphthyene Fluorene	4
Phenanthrene Authracene Fluoranthene Pyrene Chrysene Benzo (ghi) perylene	3
Benzo(b) fluoranthene Benzo(K) fluoranthene Indeno (1,2,3-cd) pyrene	2B
Benzo(a) pyrene Dibenzo (a,h) anthracene Benzo (a) anthracene	2A

Legends:
2A: Probably Carcinogenic to humans;
2B: may be carcinogenic to human;
3: Not classable as to the carcinogenicity to humans (possible, but insufficiently studied)
4: Not studied.

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