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Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Some Fish Samples from Mushin Area of Lagos, Nigeria: Effects of Smoking

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ABSTRACT

Five different fish samples (mackerel (*Scomber scombrus*), tilapia (*Oreochromis niloticus*), croaker (*Micropogonias unduletus*), herring (*Clupea harengus*), horse mackerel (*Trachurus trachurus*) and blue whitting (*Micromesistus pontassou*)), fresh and smoked, bought from Mushin market in Lagos State, Nigeria, were sampled for polycyclic aromatic hydrocarbon (PAH) contaminants. The levels of PAHs ranged from 0.004 µg/kg for acenaphthene in dry *M. pontassou* to 2.275 µg/kg for phenathrene in dry *S. scombrus*. Florene, phenathrene, anthracene and fluoranthene were detected in all the fish samples. Indeno (123) perylene, dibenzo (ah) anthracene and benzo (ghi) perylene were not found in any of the fish samples. Smoking increased the levels of PAHs in the fish samples. These results show that PAH levels in the fish samples are higher than the permissible limit set by the World Health Organization (WHO) and the Agency for Toxic Substances and Disease Registry (ATSDR). The implications of these contaminants in the environment together with their associated health hazards are also examined.

Keywords: carcinogenic substance, environment, PAHs, smoked fish, toxicity

INTRODUCTION

Polycyclic aromatic hydrocarbons (also called PAH, PAHs, PAH'S, polyaromates, polyaromatic hydrocarbons) are a group of over 100 different chemicals that are formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco or charbroiled meat (Amoako *et al*. 2011; Ni *et al*. 2011; Halek *et al*. 2010). PAHs are also manufactured for use in the pesticide, pharmaceutical and dye making industries; they are ubiquitous being present in soil, water air and food (Zhu *et al*. 2004; Zohair 2006) and pose a serious concern to the health of aquatic life and humans through bioaccumulation (Okafor and Opuene 2007; Fagbote and Olaufekum 2010; Lee and Byeon 2010). They are hydrophobic and can be readily adsorbed onto particulate matter, thus bio-accumulate in the environment; hence, coastal and marine sediments become the ultimate sinks for such compounds (Yu *et al.* 2005; Osuji and Ezeburio 2006). They are an important class of environmental pollutants that are potent mutagens and carcinogens (Oleszczuk 2006; Peruguni *et al.* 2007; Yusuf *et al*. 2007) and immunotoxic (Sokhn *et al*. 2001; Reynaud and Deschaux 2006). When in contact with the skin of mice, several carcinogenic PAHs have also been shown to interact with the immune system, stimulating immune responses and resulting in the development of antigen-specific T-cell-mediated immunity (Yusuf *et al*. 2007). However, the effects of PAHs on both specific and non-specific immunity are contradictory and depend on the mode of exposure, the dose used or the species studied (Reynaud and Deschaux 2006).

PAHs are produced by burning jet fuel and are also found in emissions from generators and motor vehicles (Kumar and Kothiyal 2011). It is well known that PAHs are formed in large quantities as the result of secondary thermochemical reactions at temperatures over 700°C (Ledesma *et al.* 2002). Also, the formation of these compounds during gasification and combustion have been reported (Mastral and Callean 2000; Richter and Howard 2000). They generally occur as complex mixtures and some PAHs are manufactured. When pure, PAHs usually exist as colourless, white or pale yellow-green solids (Ames *et al*. 1990). PAHs are found in coal tar, crude oil, creosote and roofing tar but a few are used in medicines or to make dyes, plastics and pesticides (Ames *et al*. 1990). Others are contained in asphalt used in road construction. They can also be found in the environment, in the air, water and soil, hence are said to be ubiquitous and are found in almost all foods (RAS 2004; Zhu *et al.* 2004). Polycyclic aromatic hydrocarbons are produced when any incomplete combustion occurs (Lijinsky 1991). Thus, they are found in polluted air, cooking oil fumes, tobacco smoke, smoked foods (Gomaa *et al*. 1993), and foods cooked at high temperature (Ledesma *et al.* 2002). It has been noted that even air pollution usually contains many fewer PAHs than what a smoker breathes, and infact much less than the average level of PAHs in food (Wickstrom 1986; Lodovici *et al*. 1995). Even raw food can contain PAHs, due to air pollution (ATSDR 1995; Lin *et al.* 2005)

The agency for toxic substances and disease registry (ATSDR 1995) in a paper; Toxicological profile for PAHs, summarized as shown below what happens to polycyclic aromatic hydrocarbons when they enter the environment.

- PAHs enter the air mostly as releases from volcanoes, forest fires, burning coal, and automobile exhaust.
- PAHs can occur in air attached to dust particles.
- Some PAH particles can readily evaporate into the air from soil or surface waters.
- PAHs can break down by reacting with sunlight and other chemicals in the air, over a period of days to

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weeks.

- PAHs enter water through discharges from industrial and wastewater treatment plants.
- Most PAHs do not easily dissolve in water. They stick to solid particles and settle to the bottoms of lakes or rivers.
- Microorganisms can break down PAHs in soil or water after a period of weeks to months.
- In soils, PAHs are most likely to stick tightly to particles; certain PAHs move through soil to contaminate underground water.
- PAHs contents of plants and animals may be much higher than PAHs contents of soil or water in which they live.

This means that, there is the tendency of PAH bioaccumulation in tissues of animal and plants. Thus, as we go up the food pyramid, the concentration of PAHs increases. Some PAHs were chosen by U.S EPA (Luch 2005) to be analyzed in the environment (water, soil, air and food) because; more information is available on these PAHs than on the others; they are suspected to be more harmful than some of the other PAHs; they exhibit harmful effects that are representative of the PAHs; there is a greater chance that we are exposed to these PAHs than to the others; and of all the PAHs analyzed, these were the PAHs identified at the highest concentrations at hazardous waste sites. The common PAHs are acenaphthylene (Ace Naph), acenaphthene (Ace Nap), fluorene (Flu), phenanthrene (Phen), anthracene (Anth), fluoranthene (Flan), pyrene (Pyr), benzo (a) anthracene (B(a)A), chrysene (Chry), benzo (b) fluoranthene (B(b)F), benzo (k) fluoranthene (B(k)F), benzo (a) pyrene (B(a)P), dibenzo (ah) anthracene (Dib(ah)A), benzo (ghi) perylene (B(ghi)P), indeno (123-cd) pyrene (IP) (ATSDR 1995; Kumar and Kothiyal 2011). Animal studies have also shown that polycyclic aromatic hydrocarbons can cause harmful effects on the skin, body fluids and ability to fight disease (Knasmüller *et al*. 1992; Murphy *et al.* 2003; Morgan *et al*. 2006). Some PAHs are also known to be carcinogenic (Beier and Nigg 1994; ATSDR 1995), by interacting with the genetic material in the cell (Elmets *et al.* 1998; Takeda *et al.* 2003). Hence, as a result of some PAHs being declared priority pollutants in some countries (Atagana 2009), it is important to understand the fate of these compounds in the environment.

Mushin is a thickly populated area (of course, just like most other areas) in Lagos Nigeria, with an average population that lives below the poverty level. The residents are mostly petty traders, drivers and job seekers, who depend largely on the cheap and abundant seafood, one of which is fish, smoked and sold by the roadside. This gave rise to this study, to ascertain the levels of PAHs in both the fresh and smoked fish samples found in the area. This work also helps to find out the effect of smoking fish samples and the risk of exposure on these residents as they consume these fishes almost on daily basis.

MATERIALS AND METHODS

Collection of fish samples

Freshly caught fish samples (n=6) and smoked (or dried) fish samples (n=6) were bought randomly from the popular Mushin market Lagos Nigeria in July, 2006. These fish samples as explained by the sellers were obtained from fishermen from the Lagos lagoon. The following fish samples that were bought were properly identified at the Department of Animal and Environmental Biology, Abia State University, Uturu Nigeria, as mackerel (*Scomber scombrus*), tilapia (*Oreochromis niloticus*), croaker (*Micropogonias unduletus*), herring (*Clupea harengus*), horse mackerel (*Trachurus trachurus*) and blue whitting (*Micromesistus pontassou*). The samples were stored below 4°C in a refrigerator until use (within 5 days).

Extraction and analysis of samples

Each set of fish samples (2 kg) were dissected and the muscle tissue were removed and dried in an electric oven at 70-80°C for 3 days. After that the samples were powdered in a grinder and a homogenized powder was made (Anyakora *et al.* 2005). 10 g of homogenized sample were added to a 50 mL solvent of 50: 50 of acetone and methylene chloride mixture in a solvent rinsed beaker. The mixture was spiked with 1.0 mL of the surrogate mix. The sample was placed in a sonicator and sonicated at 20 kHz for about 10-15 min at about 70°C. 10 g of anhydrous sodium sulphate was added to sample until a clear extract developed. The extract solvent was poured into a round bottom flask. Additional 50 mL of solvent mix was used to sonicate and another round of clear solvent decanted into the same round bottom flask. The extract was concentrated, exchanged with hexane and re-concentrated to 1 to 3 mL. The sample was then fractionated into aliphatic and aromatic fractions using columns packed with 10 g of silica gel of 100-200 meshes, preconditioned (baked) at 105°C overnight. The silica gel was mixed with hexane to form slurry. Afterwards, the sample was analyzed (in duplicates) for PAHs using Gas Chromatography/mass spectrometer (GC/MS), model GI530A.

RESULTS AND DISCUSSION

The results of the concentrations of PAHs in fish samples bought from Mushin market that were analyzed are shown in **Table 1**. The results show that some PAHs were not detectable in some fish samples. Also, it could be seen that mostly the 3-4 rings PAHs were predominant in all the samples analyzed. From **Table 1**, the highest levels of PAHs were found for Phen, 2.275 µg/kg in *S. scombrus* dry; 0.594 µg/kg in *C. harengus* dry and for Chry 1.505 µg/kg in *S. scombrus* dry. Flu, Phen, Anth and Flan were found in all the fish samples analyzed. B(b)F was found only in *M. unduletus* dry and *C. harengus* dry; B(a)P was found in *S. scombrus* dry, *M. unduletus* dry, *M. pontassou* dry and *O. niloticus* dry. IP, Dib(ah)A and B(ghi)P were not dictated in any of the fish samples analyzed. From the results for the total PAHs in the fish samples, it could be seen that for the wet fish samples, *S. scombrus* had the highest value of 1.423 µg/kg and the trend for the occurrence of the PAHs in the wet fish samples is *S. scombrus* > *O. niloticus* > *M. pontassou* > *T. trachurus* > *M. unduletus* > *C. harengus*. Generally, the total values obtained were higher than the WHO guidelines using B(a)P as an example. The WHO standards for B(a)P in drinking water is 0.7 µg/L (WHO 2003).

Phen has been reported as being one of the more water-soluble PAHs than the higher molecular weight PAHs (Zohair 2006). Thus, these more water soluble PAHs becomes more susceptible to uptake by the fishes as dissolution is much higher in water, as seen from the results of this study. PAHs have been reported in water samples of Lagos lagoon (Anyakora *et al.* 2004); and other marine samples (Anyakora *et al.* 2005). Also, PAHs have been investigated in other samples such as tea leaves (Lin *et al.* 2005); Italian vegetables (Lodovici *et al.* 1995); sediments (Yunker *et al* 1996; Benlahcen *et al.* 1997; Baumard *et al* 1998); mussels (Baumard *et al.* 1998) and Egyptian vegetables (Zohair 2006). The distribution behaviour of polycyclic aromatic hydrocarbons in roadside soils at traffic intercepts within developing cities such as Kota Bharu Malaysia (Fadzil *et al*. 2008), Delhi airport soil data India (Sharmila *et al*. 2007) and Jalandhar India (Kumar and Kothiyal 2011) have been reported. In all these reports, PAHs have been implicated in the contamination of the samples. Reports indicates that the Lagos lagoon is fairly contaminated with PCBs and DDTs, higher than what is obtained in most developed countries (Osibanjo 1994) and suggesting agricultural and industrial activities as sources of contamination in the lagoon (Adeyemi *et al*. 2009). The pollution of the Lagos lagoon by urban and industrial waste is a major problem as the large population depends on it for potable and recreational water, as well as a source of cheap and affordable protein in form of fish (Adeyemi *et al*. 2009).

Table 1 Concentration of polycyclic aromatic hydrocarbons in fish samples bought from Mushin market, Lagos.

COMPONENT	*S. scombrus* (wet)	*S. scombrus* (dry)	*M. unduletus* (dry)	*M. unduletus* (wet)	*C. harengus* (wet)	*C. harengus* (dry)	*M. pontassou* (dry)	*M. pontassou* (wet)	*O. niloticus* (wet)	*O. niloticus* (dry)	*T. trachurus* (dry)	*T. trachurus* (wet)
Naphthalene	0.308	ND	0.079	ND	ND	ND	0.059	ND	ND	ND	0.092	ND
2-Methy naphthalene	ND	ND	0.175	ND	ND	ND	0.068	ND	ND	0.254	0.194	ND
Acenaphthalene	ND	0.034	0.024	ND	ND	0.035	0.018	ND	ND	ND	0.100	ND
Acenaphthene	ND	0.591	0.037	ND	ND	0.065	0.004	ND	ND	0.116	0.183	0.072
Florene	0.315	0.618	0.078	0.220	0.150	0.047	0.215	0.269	0.225	0.028	0.106	0.142
Phenathrene	0.244	2.275	0.214	0.284	0.027	0.594	0.314	0.421	0.423	0.218	0.053	0.116
Anthracene	0.023	0.031	0.158	0.043	0.115	0.066	0.092	0.230	0.221	0.036	0.154	0.072
Fluoranthene	0.101	0.247	0.154	0.029	0.117	0.347	0.408	0.212	0.199	0.211	0.071	0.283
Pyrene	0.155	0.170	0.149	ND	ND	0.163	0.457	ND	ND	0.805	0.994	0.156
Benzo(a)anthracene	0.217	0.425	0.038	0.075	ND	0.157	ND	ND	ND	ND	0.485	0.165
Cyrsene	ND	1.505	0.029	ND	ND	0.124	ND	ND	ND	ND	0.215	ND
Benzo(b)fluoranthrene	ND	ND	0.035	ND	ND	0.079	ND	ND	ND	ND	ND	ND
Benzo(a)pyrene	ND	0.273	0.057	ND	ND	ND	0.161	ND	ND	0.155	ND	ND
Benzo(k)fluoranthrene	0.062	ND	0.237	0.129	ND	0.179	ND	0.068	0.188	0.063	0.030	0.116
Indeno (1,2,3-cd) pyrene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzo(a,h)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo(g,h,i) perylene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total PAHs (µg/Kg)	1.423	6.170	1.463	0.780	0.409	1.967	1.795	1.200	1.256	1.885	2.677	1.123

ND: Not detectable

The use of the molecular ratios of specific PAHs has been reported in the investigation of the sources of PAHs into the environment (Lin *et al.* 2005). For example, a ratio of fluoranthene to pyrene concentrations (Flu/Pyr) greater than 1.0 were reported as characteristic of pyrolitic origin, whereas ratio less than 1.0 was characteristic of petroleum hydrocarbons (Baumard *et al.* 1998; Zohair 2006). On the other hand, a ratio of phenanthrene to anthracene (Phen/Anth) less than 10 suggested combustion sources, while Phen/Anth greater than 10 implied petrogenic sources (Yunker *et al.* 1996; Benlahcen *et al.* 1997; Zohair 2006). From our results, the calculation of Flu/Pyr and Phen/Anth in the fish samples indicated that PAHs resulted mainly from incomplete combustion products via pyrolytic processes and combustion processes in addition to atmospheric fall out of automobiles exhausts fumes. This is similar to the results of Zohair (2006), who reported that the contamination of Egyptian vegetables by PAHs also resulted mainly from incomplete combustion products via pyrolytic processes and combustion processes in addition to atmospheric fall out of automobiles exhausts fumes.

A lot of research have been carried out to ascertain the chemistry, reactivity, and toxicity of PAHs which include; the infrared spectra of PAHs (Hudgins and Sandford 1998; Langhoff *et al*. 1998; Allamandola *et al*. 1999); the chemistry of PAHs and PAHs in extraterrestrial materials (Allamandola *et al*. 1987; Bernstein *et al*. 1999); the ultraviolet/visible spectra of PAHs (Salama and Allamandola 1991; Du *et al*. 1993; Salama *et al*. 1994; Romanini *et al*. 1999; Salama 1999). In all these, researchers established the reactions, mutagenicity and carcinogenicity of PAHs. Also, the degradation of PAHs in the environment has been extensively studied and documented in the literature and a large diversity of microorganisms, including bacteria and fungi, are capable of oxidizing or mineralizing a range of PAH (Daane *et al*. 2002). It has been reported that the fate of PAHs in the environment is associated with both abiotic and biotic processes (Kumar and Kothiyal 2011), including volatilization, photooxidation, chemical oxidation, bioaccumulation and microbial transformation and that microbial activity has been deemed the most influential and significant cause of PAH removal (Haghighat *et al.* 2008; Nwuche and Ugoji 2008; Agbozu and Opuene 2009; Atlas and Bragg 2009; Arulazhagan *et al*. 2010).

Effect of smoking

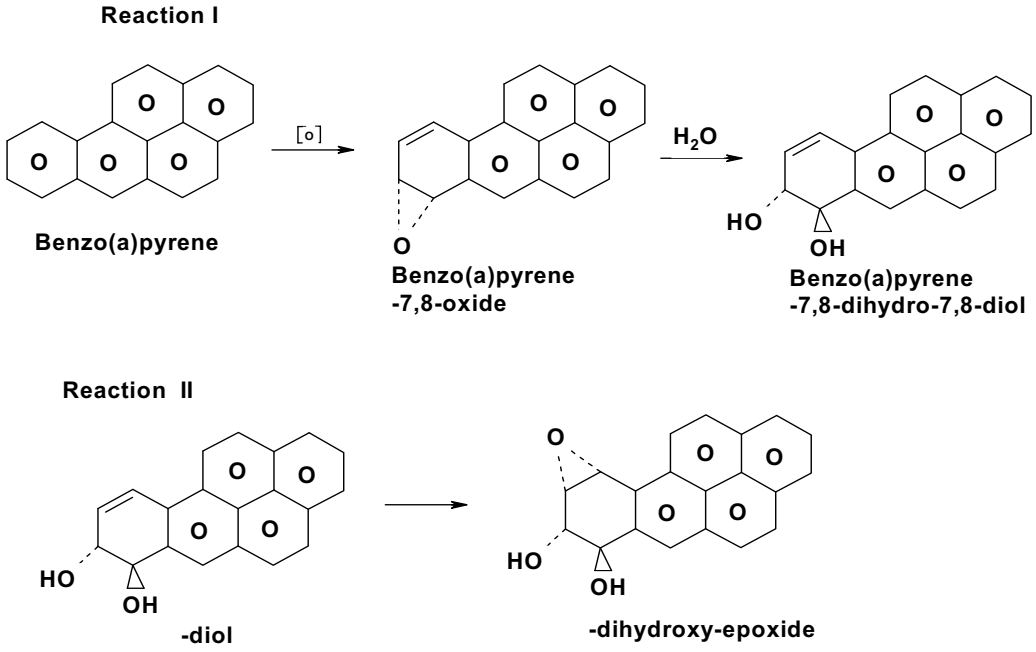
It has been established that there are two major sources of PAHs formation in foods. The first source is mainly due to the method of food preparation while the second major source of contamination of foodstuffs is by contact with either petroleum products or coal tar products (Zohair 2006). Hence, the method of smoking the fish samples with charcoal and kerosene fraction of petroleum products, either way will contribute and result to increase in the level of PAHs in the smoked fish samples.

The effect of smoking is clearly seen from **Table 1**. For example, whereas some of the PAHs are non-detectable in the wet samples, they were found in the dry (smoked) samples of the fish species. Also, the total amount of the PAHs levels in the dry or smoked fish samples increased more than that for the wet samples. This means that smoking the fish samples definitely was responsible for the introduction of the various PAHs in the fish samples. Therefore, PAHs, which ordinarily would not have been contacted by eating the wet or fresh fish, now contains PAHs as a result of smoking. This implies that the residents of Mushin, who are fond of eating smoked fish sold along the streets, are at risk of the problems associated with PAHs. *S. scombrus* still had the highest level of PAHs with the trend for the dry or smoked fish samples as *S. scombrus* > *T. trachurus* > *C. harengus* > *O. niloticus* > *M. pontassou* > *M. unduletus.*

The effect of smoking on different types of food and food products have been investigated by several workers; these includes; polycyclic aromatic hydrocarbons in smoked food products and commercial liquid smoke flavorings (Gomaa *et al*. 1993); the effect of cooking time and analysis of cooked muscle meat (Knize 1994; Knize *et al*. 1997); the influence of fried meat and fiber on cytochrome P-450 mediated activity of mutagens in rats (Lindeskog *et al*. 1988); mutagens and carcinogens formed by cooking meat and fish (Sugimura 1990). It was found that the method of cooking and length of time of smoking is a factor in the PAHs content. Also our results show that smoking increased the level of PAHs in fish.

Toxic potential

Studies have shown that the effects of polyaromatic hydrocarbons cannot be overemphasized (Lijinsky 1991; Gomaa *et al*. 1993; Lodovici *et al*. 1995). They do not degrade easily and are widespread in the environment, including at



low levels in food. Some PAHs, in particular B(a)P, and 7,12-dimethylbenz(a) anthracene (DMBA), have a potential to cause cancer by interacting with the genetic material in the cell (Jägerstad and Skog 1991). PAHs toxicity is very structurally dependent, with isomers (PAHs with the same formula and number of rings) varying from being nontoxic to being extremely toxic. The number of benzene rings present in the structure of a PAH affects its physical and chemical properties such as solubility, which is very important for bioavailability. The larger the number of rings, the more recalcitrant the compound becomes (Atagana 2009). One PAH compound, B(a)P, is notable for being the first chemical carcinogen to be discovered (and is one of many carcinogens found in cigarette smoke). PAHs known for their carcinogenic, mutagenic and teratogenic properties are B(a)A, Chry, B(b)F, B(j)F, B(k)F, B(a)P , B(ghi)P, coronene, Dib(ah)A, IP and ovalene (Luch 2005) Several of the PAHs, including B(a)A, B(a)P, B(b)F, B(j)F, B(k)F, Chry, Dib(ah)A, and IP, have caused tumors in laboratory animals when they breathed these substances in the air, when they ate them, or when they had long periods of skin contact with them (ATSDR 1995). Studies of people show that individuals exposed by breathing or skin contact for long periods to mixtures that contain PAHs and other compounds can also develop cancer. Skin tumors induced by carcinogenic PAHs express tumor specific antigens that elicit a cell-mediated immune response. Immunotherapeutic approaches that target these tumor antigens have been effective at producing tumor regression in experimental animal systems, and clinical trials evaluating tumor vaccines in melanoma and other human malignancies have been effective at controlling the growth of these tumors (Morgan *et al.* 2006).

The primary human contact with PAHs is through ingestion and inhalation. Although dermal contact with PAHs occurs, it is relatively unimportant under normal circumstances (Skog 1993). Just like other lipid-soluble compounds, PAHs are generally well absorbed, but are briefly stored in the body, primarily in the kidney, liver and spleen. Most of the absorbed dose is then excreted into the bile and eventually into the faeces and to a much lesser extent the urine. Most of the PAHs are excreted in their metabolized forms and only very small amounts of the parent compounds find their way into the faeces and urine.

PAHs are relatively inert compounds that are metabolized via cytochrome P 450-dependent mono-oxygenases (Mukhtar 1984). Reactive intermediates, rather than the parent compound are the agents that bind to DNA (Rohr and Scheele 1983; Mukhtar 1984), and it is a diol expoxide metabolite that is the actual mutagenic and carcinogenic moiety. For example, B(a)P is converted to the diol through **Reaction I**. The intermediate, an epoxide (benzo(a)pyrene-7,8 oxide) undergoes nucleophilic substitution, a reaction that opens the epoxide ring to give a product that contains two functional groups. Since this reaction is in aqueous solution, then the product is a diol (benzo(a)pyrene-7,8 dihydro-7,8-diol) (Mukhtar 1984). Then from there, some of the diols undergo further epoxidation in a regioselective and stereoselective way to yield dihydroxy epoxides. This is shown as **Reaction II**. It is these dihydroxy epoxides that are believed to be the actual carcinogens formed by metabolism of polycyclic aromatic hydrocarbons.

PAHs also have significant interactions with the immune system (Teel *et al*. 1985; Knasmüller *et al*. 1992). DMBA, B(a)P, and 3-methyl cholanthrene are all contact allergens when applied to the skin (Beier and Nigg 1994). Like several other contact allergens, induction of contact hypersensivity to DMBA requires langerhan cells and is mediated by $CD8^+$ cells (Knasmüller *et al*. 1992). Furthermore, the same enzymatic pathways that are required for the mutagenic and carcinogenic activities of PAHs must also be fully operational for contact sensitization to occur (Beier and Nigg 1994).

Again, some rodents exposed to high doses of PAHs have been reported to die after a short-term exposure. But no deaths have been reported from the short-term occupational exposures in humans (Skog 1993). It has also been believed that environmental levels are generally much lower than occupational exposures, so that short-term exposure may likely cause death in human going by the environmental fate. Workers occupationally exposed to PAHs are prone to eye irritation, photophobia, and skin toxicity such as dermatitis and keratosis (Bleumink 1970).

CONCLUSION

The results of this experiment show high levels of PAHs in fish samples from Mushin area of Lagos Nigeria. The results show that, although PAHs occur in the environment - which explains its presence in fresh fish samples-smoking the fish introduces PAHs where they were not detected originally and increases their levels where they occur already. There is no doubt that the exposure to PAHs can have adverse effect on humans by some of the PAHs being carcinogenic, causing skin and sight problems among others. They can also affect the male and female reproductive systems. Therefore, the residents of Mushin area of Lagos Nigeria are bound to be contaminated by PAHs and hence suffer the adverse effects in the long run. We would also suggest here

that some of the residents need to be sampled to ascertain the level of PAHs in their system. This will help to confirm the level of contamination on the residents as we believe they could also pick-up PAHs from consumption of smoked plantains which is also one of their favorite foods. Also, PAHs are known to be contained in other foods and fruits. We recommend here that further work need to be done on determination of polyaromatic hydrocarbons in bread, vegetable, fruits, meats, cereals, flour, etc, found in the area. Such results will help to quantify on a larger scale the extent of PAHs contaminated food around the area.

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