Polycyclic Aromatic Hydrocarbons in Fresh and Smoked *Clupea heregus* and *Hake* Fish Consumed in Ekiti State, Nigeria and their Health Implications

Adeolu J. Adesina[1], Samuel S. Asaolu[2], Samuel O. Adefemi[3], Abdul A. Olaleye[4], Abiodun F. Akinsola[5], Kolade A. Idowu[6]

[1]Senior Lecturer, [2]Professor, [3]Professor, [4]Lecturer,Chemistry Department, Federal University Dutse, Jigawa State Nigeria, [5]Senior Lecturer, [6]Post graduate Student, Department of Chemistry, Ekiti State University, P.M.B 5363, Ado- Ekiti, Nigeria

Abstract

Levels of polycyclic aromatic hydrocarbons (PAHs) in fresh and smoked *Clupea herengus* (*shawa*) and *Hake* (*panla*) fish commonly consumed in Ekiti State, Nigeria were investigated using gas chromatographic method. Effect of smoking on the PAHs level was also considered. The PAHs concentration ranged from 0.0001 µg/kg Acenapthene and Indeo (1,2,3-cd) pyrene to 0.240 µg/kg (Benzo (a) pyrene) for fresh unsmoked *Clupea herengus* (SF), 0.0001µg/kg Acenapthene and Anthracene to 0.638 µg/kg Benzo (b) fluoranthene for smoked *C. herengus* (SF), 0.0001 µg/kg Acenapthene, Anthracene, Fluoranthene and Benzo(ghi) perylene and Indeno(1,2,3-cd)pyrene to 0.171 µg/kg (Benzo (a) pyrene) for fresh Hake (panla) fish (PF) and 0.0001 µg/kg Indeno(1,2,3-cd)pyrene to 0.966 µg/kg pyrene for smoked *Hake* (*panla*) fish (PS) samples. Results also revealed TPAHs levels in the smoked fish were 62.3-62.5% times higher than the fresh. Values of PAHs levels in the fish samples were below EU-regulatory limits (30µg/kg) for PAHs. Hazard index and lifetime excess carcinogenic risk of the samples revealed no potential cancer and mutagenic risks.

Keywords*: Clupea herengus and Hake, hazard index, polycyclic aromatic hydrocarbons, gas chromatograph*

Introduction

Polycyclic aromatic hydrocarbon (PAHs) are wide spread organic pollutant in the environment[1]. They are known to be potent carcinogens[2]. They are also known for their mutagenic effects and bio-accumulate in animal and human tissues[1]. Ishizaki *et al*.[3] reported that PAHs are usually present in the various environments or supernatant i.e in water, air, soil and traces of them discovered in some food products. Many exposure routes have been reported for PAHs, among which is a thermal treatment that occurs during processing of food (drying and smoking) and cooking (roasting, baking and frying). PAHs are formed by incomplete combustion process which occurs whenever wood, coal or oil are burnt, the absorption and deposition of particulates during food processing such as smoking, grilling, boiling and toasting, the pyrolysis of fats and the incomplete combustion of charcoal[4].

In foods of animal origin, it was proposed that the lipophilic character of PAHs is responsible for the accumulation in the fat of animal which eat contaminated plants[5]. They have been reported to occur as contaminants in different food categories and beverages including water fresh and smoked fishes fruit, cereals and oil[6-7]. Non processed fish contain low PAHs concentration even when it comes from contaminated water because fishes rapidly metabolize PAHs resulting in low steady state level in the tissue[8]. Health effects of PAHs have been reported to include: growth retardation, low birth weight,

Corresponding Author:
Adeolu J. Adesina
E-mail: adeolu.adesina@eksu.edu.ng

small head circumference, low IQ, damaged DNA in unborn children and disruption of endocrine systems such as estrogens, thyroid, and steroid[8]. According to Devos[9], PAHs have been included in several priority pollutant Lists of the Agency of Toxic substances and Disease Register (ATSDR), the International Agency for Research on Cancer (IARC), the European Community (EC) and environmental protection agency (USEPA) and to this end, several studies have been done to determine the levels of exposure of humans to PAHs. Farhadian *et al*.[10] have identified diets as the major source of human exposure to PAHs as it accounts for 88% to 98% of such contamination. Processing of food at high temperature (grilling, roasting, frying and smoking) are major source producing PAHs. Gullen *et al.*[11] have reported various levels of individual PAHs in smoked fish and meat samples (up to 200µg/kg) and about 130µg/kg in barbecued meat whereas in an uncooked food samples a level range between 0.01 to 1µg/kg was reported.

Smoking as one of the oldest food preservation technologies have been used to achieve characteristics taste, colour and aroma for food (especially fish and fish products meat and meat products[12]. Smoking enhances preservation due to the dehydrating bactericidal and antioxidant properties of smoke such as phenol derivatives, carbonyls furan derivatives, organic acids and their esters[13]. The actual levels of PAHs in the smoked foods depend on several variables in the smoking process, including type of smoke generator, combustion, temperature and degree of smoking[14]. Considerable amount of PAHs can be produced by incomplete combustion of wood during smoking and these can penetrate through the surface of products[7, 15-17].

Fish is known worldwide as a very important component of human diet because of its high nutritive value and significance in improving human health[18]. It contributes significantly to the survival and well being of a large number of the people around the world. Fish is an important source of essential nutrients which includes; protein, lipids, vitamins and minerals[19]. Fish is known to be efficient converter of food for human consumption and saving children from kwashiorkor due to low protein intake and unbalanced diet and there is little or no religious restriction on its consumption[20]. Fish is relatively cheaper and readily available, therefore making quality protein available to the poor people in most developing countries of the world including Nigeria[21]. FDF[22] noted that almost half of the total animal protein consumed in Nigeria is from fish and fish products and this makes it to occupy a unique position being the cheapest source of animal protein. In Nigeria, the most populous country in Africa, fish is an important part of the household diet. Fish makes up around **40%** of the country's protein intake, with fish consumption at **13.3 kg/person/per year**.Total fish production per year is close to **1 million metric tons** (313,231 metric tons from aquaculture and 759,828 metric tons from fisheries). The majority of this fish is consumed domestically, while around 10% is exported[23].

Among the marine fishes consumed in Nigeria are *Clupea heregus* and *Hake*. ***Clupea*** is genus of planktivorous bony fish belonging to the family Clupeidae, commonly known as herrings. They are found in the shallow, temperate waters of the North Pacific and the North Atlantic oceans, including the Baltic Sea. Three species of *Clupea* are recognized. The main taxa, the Atlantic herring (*Clupea harengus*) and the Pacific herring (*Clupea pallasii*) may each be divided into subspecies. Herrings are forage fish moving in vast schools, coming in spring to the shores of Europe and America, where they form important commercial fisheries. Adult herring are harvested for their meat and eggs, and they are often used as baitfish. The trade in herring is an important sector of many national economies. In Europe the fish has been called the "silver of the sea", and its trade has been so significant to many countries that it has been regarded as the most commercially important fishery in history[24]. Environmental Defense has suggested that the Atlantic herring (*Clupea harengus*) fishery is one of the more environmentally responsible fisheries[25]. Hake is in the same taxonomic order (Gadiformes) as cod and haddock. It is a medium-to-large fish averaging from 1 to 8 pounds (0.45 to 3.63 kg) in weight, with specimens as large as 60 pounds (27 kg)[26] The fish can grow up to 1 metre (3 ft 3 in) in length with a lifespan of as long as 14 years. Hake may be found in the Atlantic Ocean and Pacific Ocean in waters from 200 to 350 metres (656 to 1,148 ft) deep. The highest demand for hake has been in Europe. Hake has been primarily divided into three principal levels—fresh, frozen, and frozen fillet. Fresh hake is mainly supplied by European

production and imports. Frozen hake and frozen hake fillet are effectively supplied by imports and European processing companies. Hake is sold as frozen, fillets or steaks, fresh, smoked, or salted [26].

Considering the potential risk posed by PAHs to public health, the study seeks to determine the effects of smoking process on PAHs content in fresh and smoked samples of two major fishes *Clupea harengus* (Shawa fish) and Hake fish (*Panla*) consumed in south western part of Nigeria.

Materials and Methods

Sample collection and preparation

Fresh fish and commercially smoked fish of two different species commonly consumed in Nigeria, namely *Clupea herengus* (Shawa fish) and Hake (Panla fish) were purchased from ten different local fish vendors in Ado-Ekiti major markets. Fresh and commercially smoked fishes from these vendors were pooled together to obtain representative samples for each of two types of fish species. The trunk (muscle) part used for this analysis was carefully separated, composited, homogenized, packed in amber bottles and kept in the refrigerator prior to analysis.

Reagents used

The reagents used were of spectra purity. They included GC grade dichloromethane (DCM), n-hexane and alumina.

Extraction and clean-up procedure of the samples for PAHs analysis

The extraction method for the analysis of polycyclic aromatic profiles in the samples was by employing the modified methods of ASTM[27] and ASTM[28]. Fifty gramme of each sample was carefully taken and emptied into a 27 ml capacity McCartney bottle of borosilicate material and 10 ml of the ratio 3:1 n-hexane: dichloromethane was added. The bottle and its content were placed in the sonicator to extract the hydrocarbon for about 2 hours. The organic layer was filtered into 250 ml capacity borosilicate beaker[7].

The concentrated oil was separated into the aliphatic profile and polyaromatic hydrocarbons profiles by packing the glass column with activated alumina (neutral and activity/grade 1). 10 ml of the treated alumina was packed into the column and cleaned properly with n-hexane. The extract was poured onto the alumina and was allowed to run with the aid of the n-hexane to remove the aliphatic profiles into the pre-cleaned 20 ml capacity glass container. The mixture was concentrated to 1.0 ml by stream of nitrogen gas before the gas chromatography analysis[7].

Gas chromatographic condition

The gas chromatography conditions for the analysis of PAHs were as follows: GC model: HP6890 powered with HP ChemStation Rev. A 09.01[1206]; the carrier gas flow rate was 2.0 ml/min; injector temperature: Split injection: 20:1; carrier gas: nitrogen; inlet temperature: 250 ^{o}C; column type: HP-1 ; column dimension: (30 m x 0.25 µm x 0.25 mm; oven programme: initial temperature at 60 ^{o}C for 5 minutes, first ramping 15 ^{o}C/ min for 14 min (180 ^{o}C); maintained for 3 min; second ramping at 10 ^{o}C/min for 5 min (300 ^{o}C); maintained for 4 min; detector: flame ionization detector (FID); detector temperature: 320 ^{o}C; hydrogen pressure: 28 psi; nitrogen column air: 30 psi; compressed air: 32 psi. The total run time was 31 minutes[7].

Estimation of Benzo(a)pyrene equivalent

In determining the carcinogenic risk from exposure to PAHs in fish, the USEPA guideline, as described by Cheung et al.[29] was employed. In this method, benzo(a) pyrene is used as a marker for the occurrence and effect of carcinogenic PAHs in food. The overall carcinogenic health risk from the measured PAHs was estimated based on toxic equivalence factors (TEFs) derived from the cancer potencies sof individual PAH compounds relative to the cancer potency of benzo(a) pyrene[30]. The benzo(a)pyrene equivalent concentrations TEQ_{Bap} is the sum of product of each individual PAH and its TEF [31]. The mutagenicity of individual PAH relative to BaP had also been computed using the mutagenic equivalent factor (MEF) proposed by Durant et al.[32-33]. The sum of the concentration of each individual PAH multiplied by the corresponding MEF gives the mutagenic equivalent (MEQ) (Table 5).

$$TEQ_{Bap} = \sum(TEF_i \times C_i) \quad \text{.....i}$$

$$MEQ_{Bap} = \sum(MEF_i \times C_i) \quad \text{.....ii}$$

Where C_i is the measured individual PAH concentration for the (i^{th}) compound with the assigned TEF_i or MEF_i.

Determination of dietary exposure to PAHs

Human dietary exposure doses express as (mg kg^{-1} BW day^{-1}) occurring over a lifetime were determined.

$$\text{Average daily dose} = \frac{\text{TEQ or MEQ x IR x CF}}{\text{BW}} \quad \ldots\ldots\ldots\ldots\ldots \text{iii}$$

Where, IR is the ingestion or intake rate of carcinogenic (mutagenic) PAHs based on average fish consumption rate set at 68.5 g day^{-1} per person from the annual per capital fish consumption of 25 kg for Nigeria[34]. CF is the conversion factor (0.001 $mgkg^{-1}$) and BW is the body weight which is set at 70kg.

Non-cancer hazard, carcinogenic and mutagenic risk calculations

The risk associated with the dietary exposure to non-carcinogenic PAHs was evaluated using hazard quotient approach. Hazard quotient represents a ratio of the exposure dose for each PAH divided by reference dose (RfD).

$$\text{Hazard quotient (HQ)} = \frac{\text{Average daily dose (ADD)}}{\text{Reference Dose (RfD)}} \quad \ldots\ldots\ldots\ldots\ldots \text{iv}$$

Summation of individual hazard quotients results gives the hazard index was calculated using the reference doses for non-carcinogenic PAHs.

$$\text{Hazard Index (HI)} = \sum(HQ_i + HQ_2 + \cdots HQ_n) \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{v}$$

The calculated TEQ_{Bap} and MEQ_{Bap} for the seven USEPA classified carcinogens (mutagens) were used to estimate carcinogenic and mutagenic risk involved in ingestion of fresh and smoked fish for a life time of 70 years[35]. The total risk due to exposure to mixtures of carcinogenic (or mutagenic) PAHs is the product of the dietary carcinogen exposure dose (mg kg^{-1} BW day^{-1}) and benzo (a) pyrene slope factor value are shown Table 5.

$$\text{Risk (carcinogenic or mutagenic)} = \text{Average daily dose x slope factor} \ldots\ldots\ldots\ldots \text{vi}$$

Statistical Analysis

Data obtained from the analysis were subjected to correlation analysis using IBM SPSS Statistics version 21 package.

Result and Discussion

The PAHs concentration µg /kg in non-smoked fresh *clupea herengus* (*Shawa*) and *Hake* (*Panla*) samples were presented in the Table 1. The PAHs levels were present in various concentrations ranging between 0.0001 and 0.996µg/kg in all the samples of fish analysed. PAH of significant concentrations in the samples were as follows: unsmoked fresh *clupea herengus* pyrene (0.124), Benzo(b)fluoranthene (0.190), Benzo(a)pyrene (0.240) and Dibenzo (a,h) anthracene (0.155); smoked *clupea herengus*: phenanthrene (0.929), fluoranthene (0.306), chrysene (0.467), Benzo(b) fluoranthene (0.638). The total PAHs (∑16 PAHs) were 1.05 and 2.80, ∑ non-carcinogenic PAHs (0.226 and 0.382) and ∑ carcinogenic PAHs were 0.752 and 1.39µg/kg respectively for unsmoked fresh and smoked *clupea herengus* (shawa fish) samples. Generally, the following PAHs were enchanced by smoking: naphthalene acenaphthylene, fluorene, and phenanthrene, fluoranthene, pyrene, benzo (a) anthracene chrysene, Benzo(b)fluoranthene and indeno (1, 2, 3, cd) pyrene with the highest % enchancement in phenanthrene (-0.9149).

In Hake fish (panla) samples the following PAHs were enchanced by smoking napthalene , acenaphthene fluorine, anthracene, fluoranthene, pyrene, benzo(K) fluoranthene and dibenzo (a,h)anthracene .however the PAHs wthh major concentration µg/kg in unsmoked fesh hake fish (panla) and smoked samples were :pyrene (0.156 and 0.966), bonzo(b) fluoranthene (0.156and 0.179) and dibenzo (a,h) anthracene (0.156 and 0.162) respectively.

The level of individual PAHs in the two samples reported were in close agreement with what was reported for cat and sole fish subjected to traditional and modern smoking methods[6], *Arius heude loti* and *cynoglossus senegalensis* samples smoked by traditional method[2]. The individual PAHs of lower molecular weight found to increase in smoked samples could be attributed to the lower average wood temperature used in the smoking process[36]. This is an indication that smoking process contributed to the general increase in the percentage concentration of the PAHs. Of note is the concentration of benz(a)pyrene which is usually used as biomarker in

monitoring carcinogenic PAHs[6] had mean concentration much lower than the maximum tolerable limit of 5.0 and 20µg/kg in smoked fish established by the European Commission Regulation[37] and Turkish Codex Regulation[38] respectively. Hence the result obtained in this work therefore indicated that the smoked fish samples may not contribute to cancer and cancer –related health problems among the consumers because benzo(a) pyrene is commonly known for its carcinogenity and mutagenicity, although further studies may be needed to established this nevertheless, consumers and fish vendor may need to be careful of repeated smoking as a way of preserving these fishes to avoid buildup of PAHs levels thereby increasing the chances of causing health problem

The source characterization of PAHs in the unsmoked fresh and smoked samples of C*lupea herengus* and Hake (panla) were depicted in Table 2. Indicator of pollution and mechanism of PAHs distribution (source) are usually obtained from PAHs ratios of selected compounds. Adeyeye *et al*.[7] compiled standard values ratios on PAHs sources as shown in Table 3. The ratios of [Ant/(Ant+phe)] in this study was between 0.000108 and 0.569 an indicator of predominance of petrogenic source for PAHs (ratios <0.1) except for *Hake* (*Panla*) 0.569>0.1, showing that wood was the major source of contribution of PAHs in the samples. The [flu|(flu+chr)] ratio also ranged from 0.000633 to 0.805 which indicated both wood and petroleum as a source for the PAHs in the smoked fish samples [BaA/(BaA+Chr)] (Bap/BghiP) and [Icdp/(Icdp+Bghip)] ratios further confirmed that the PAHs levels were generally from both wood and petroleum sources.

Risk assessment based on non-carcinogenic equivalent, average daily dose and harzard index of the fish samples were shown in Table 3. The carcinogenic toxicity (TEQ_{BaP}) and mutagenic toxicity (MEQ_{Bap}) relative to B(a)p were calculated for the carcinogenic and mutagenic risk associated with ingestion of the smoked fish (Tables 1 and 8). Essumang *et al.* (2013) reported that while TEQ_{Bap} is directly associated with carcinogenicity, MEQ_{Bap}(mutagenic activity) may not be directly associated with cancer and may have implication for other non-cancerous adverse effect like pulmonary disease, birth defect, impotency, low intelligent quotient.

From the result in Table 3, the TEQ for the seven USEPA priority carcinogens were SF ($1.16e^{-3}$), SS ($2.06e^{-3}$), PF ($2.47e^{-4}$) and PS ($1.23e^{-3}$). The corresponding EQ_{Bap} daily dose and carcinogenic risk for an adult involved in life time of 70 years ingestion of the unsmoked fresh and smoked fish samples were calculated to be $1.14e^{-6}$, $1.99e^{-6}$, $2.42e^{-7}$ and $1.20e^{-6}$ mg /kg /day respectively for a risk of $2.43e^{-3}$,$5.14e^{-1}$, $6.24e^{-2}$ and $3.10e^{-3}$(hazard index) smoking 1 out of 1000000 adults is likely to suffer from cancer in their lifetime .this implied that the consumption of C*lupea herengus* (*Shawa*) and Hake (*Panla*) prepared by traditional smoking pose no risk because they were lower than the USEPA[39-40] carcinogenic unit risk $1x10^{-5}$ (carcinogenesis threshold).

Also the Table 3, the risk assessment based on carcinogenic equivalent, average daily dose and the risk associated with the fish samples. Based on the calculated life time excess carcinogenic (LECR) implied the consuming the samples would not pose any health risk to human. Provided a repeated smoking is not done for preservation.

The mutagenic equivalent for the PAHs calculated were: SF ($3.04e^{-1}$), SS ($1.35e^{-1}$) PF ($2.66e^{-1}$) and PS ($1.58e^{-1}$) for unsmoked fresh and smoked fish samples prepared by traditional smoking method (Table 3). The corresponding EQ_{Bap} daily doses were also calculated to be $2.97e^{-4}$ (SF), $1.33e^{-4}$(SS), $2.60e^{-4}$(PF) and $1.54e^{-4}$ (PS), (Tables 3 and 5) based on the mutagenic risks and LECR involved in ingestion of these smoked fish samples, people are likely to suffer from non-cancer and other cancer related disease in their life time if repeated smoking method is adopted for preservation. Generally, relatively lower $\sum MEQ_{Bap}$ and mutagenic risk values below the acceptable USEPA[35,39] unit risk of 10^{-2} and 10^{-4} respectively were recorded for *Clupea herengus* and Hake fish (Panla) prepared by traditional smoking method.

Table 4 presents the statistical analysis of the results based two-tailed correlation. The results were significant at $p_{=0.05}$.

Conclusion

This research has shown that smoked *Clupea herengus* and *Hake* were fit for human consumption.

The levels of PAHs, Hazard index and cancer risks obtained in the present report were within the safe levels specified by EU and USEPA regulations, however, smoked samples from commercial fish vendors showed elevated level of PAHs compared to the unsmoked fresh samples and this may result in health-related ailments with time if the processing method and consumption is not regulated. This therefore requires proper education of fish monger/vendor about safe smoking practices that would not enhance the level of toxic substances which may lead to health problems.

Table 1. Concentration (µg/kg) of PAHs in the fresh and smoked fish muscle samples

PAHs	SF	SS	Mean	CV%	Diff (%)	PF	PS	Mean	CV%	Diff (%)
Naphthalene+ (Nap)	0.0081	0.0439	0.026	97.5	0.0352(443)	0.0082	0.040	0.0241	93.0	-0.032(384)
Acenaphthylene+ (Acy)	0.0173	0.0336	0.0255	45.3	-0.016(94.2)	0.0153	0.0105	0.0129	26.3	0.0048(31.4)
Acenapthene+ (Ace)	0.0001	0.0001	0.0001	0.0	0.00(0.00)	0.0001	0.0323	0.0162	141	-0.032(32200)
Fluorene+ (Flu)	0.0184	0.0221	0.0203	12.9	-0.004(20.1)	0.0145	0.0309	0.0227	51.1	-0.0164(113)
Phenanthrene+ (Phen)	0.0142	0.929	0.472	137	-0.9148(6442)	0.0397	0.0088	0.0243	89.9	0.0309(77.7)
Anthracene+ (Ant)	0.0094	0.0001	0.0047	138	0.0093(98.9)	0.0001	0.0116	0.0059	139	-0.0115(11500)
Fluoranthene* (Flt)	0.0155	0.306	0.161	128	-0.2905(1874)	0.0001	0.0283	0.0142	140	-0.0282(28200)
Pyrene* (Pyr)	0.124	0.0741	0.0991	35.6	0.0499(40.2)	0.158	0.966	0.562	102	-0.808(511)
Benz(a)anthracene** (B(a)A	0.0221	0.0614	0.0418	66.6	-0.0393(178)	0.0173	0.0503	0.0338	69.0	-0.033(191)
Chrysene** (Cry)	0.0572	0.467	0.262	111	-0.4098(716)	0.0322	0.221	0.127	105	-0.189(586)
Benzo(b) fluoranthene** (B(a)F)	0.190r	0.638	0.414	76.5	-0.4480(236)	0.156	0.179	0.168	9.71	-0.023(14.7)
Benzo(k) fluoranthene** (B(k)F)	0.0877	0.088	0.0879	0.241	-0.0003(0.342)	0.0001	0.0964	0.0483	141	-0.096(96300)
Benzo(a)pyrene** (B(a)P)	0.240	0.0885	0.164	65.2	0.1515(63.1)	0.171	0.0964	0.134	39.5	0.075(43.6)
Dibenz(a,h) anthracene**(DB (ah)A)	0.155	0.0238	0.0894	104	0.1312(84.6)	0.115	0.162	0.139	24.0	-0.047(40.9)
Indeno(1,2,3-cd) pyrene** (IP)	0.0001	0.0210	0.0106	140	-0.021(21000)	0.0001	0.0001	0.0001	0.00	0.00(0.00)
Benzo(g,h,i)perylene* (B(ghi)P)	0.0868	0.0016	0.0442	136	0.0852(98.2)	0.0001	0.0001	0.0001	0.00	0.00(0.00)
∑ 16 PAHs	1.05	2.80	1.93	64.3	-1.75(167)	0.727	1.93	1.33	64.0	-1.20(165)
∑LMW –PAHs	0.0673	1.03	0.548	124	-0.9613(1427)	0.0777	0.134	0.106	37.6	-0.056(72.4)
∑HMW- PAHs	0.978	1.77	1.37	40.7	-0.7911(80.9)	0.650	1.80	1.22	66.4	-1.15(177)
∑nc –PAHs	0.226	0.382	0.304	36.1	-0.1554(68.7)	0.158	0.994	0.576	103	-0.84(529)
∑7c –PAHs	0.752	1.39	1.07	42.0	-0.6357(84.5)	0.492	0.805	0.648	34.2	-0.314(63.8)

PF= frsh Hake, PS= smoked Hake, SS= smoked *Clupea heregus*, SF= fresh *Clupea heregus*, SD= standard deviation; CV=coefficient of variation; $^{+}$**indicates PAHs classified as low molecular weight PAHs; * = high molecular weight and non carcinogenic PAHs; ** = high molecular weight and carcinogenic PAHs;** ∑7c-PAHs= sum of seven carcinogenic PAHs, ∑nc-PAHs= sum of non carcinogenic PAHs; ∑LMW-PAHs= sum of **low molecular weight** PAHs; ∑HMW-PAHs= sum of **high molecular weight** PAHs

Table 2. Source characterization of PAHs in the muscle of the fish samples

PAH ratios	SF	SS	PF	PS	Standard Value ratios 7	Remark
LMW PAHs/ HMW PAHs	0.069	0.581	0.120	0.0745	>1 <1	Petrogenic Pyrogenic
[Ant /(Ant + Phe)]	0.398	1.08e-4	2.50e-3	0.569	<0.1 >0.1	Petrogenic Wood
[Flu/(Flu + Cry)]	0.111	0.805	6.33e-4	0.0284	<0.4 >0.5	Petrogenic Wood
[B(a)A /(B(a)A + Cry)]	0.279	0.112	0.349	0.187	<0.2 1.2 – 50	Petrogenic Wood
[B(a)P/B(ghi)P]	2.76	55.3	1710	964	>0.6 1.2 – 5.0	Petrogenic wood
[IP/(IP + B(ghi)P]	0.0012	0.929	0.5	0.5	<0.5 >0.5	Petrogenic Wood

Ant = anthracene, Phe = phenanthrene, Fla = Fluoranthene, Py = Pyrene, BaA= benzo(a)anthracene, Cry = chryene, BaP= benzo(a)pyrene, BghiP= benzo(g,h,i)perylene, IcdP= Indo(1,2,3-cd)pyrene

Table 3. Calculated risk assessment based on non-carcinogenic, carcinogenic, mutagenic equivalent, average daily dose and hazard index for the muscle of fish samples

PAHs	Non-carcinogenic Equivalent[7]				PAHs	Carcinogenic equivalent[7]				Mutagenic equivalent[7]			
	SF	SS	PF	PS		SF	SS	PF	PS	SF	SS	PF	PS
Nap	8.10e-6	4.39 e-6	8.20 e-6	3.99 e-5	B(a)A	2.21 e-3	6.14 e-3	1.73 e-3	5.03 e-3	1.81e-3	5.03 e-3	1.42 e-3	4.12 e-3
Acy	1.73 e-5	3.36 e-5	1.53 e-5	1.05 e-5	B(b)F	1.92 e-2	6.38 e-2	1.56 e-2	1.79 e-2	3.23 e-3	1.08 e-2	2.65 e-3	3.04 e-3
Ace	1.00 e-7	1.00 e-7	1.00 e-7	3.23 e-5	B(k)F	8.77 e-4	8.80 e-3	1.00 e-6	9.64 e-4	9.65 e-3	9.68 e-3	1.10 e-5	1.10e-5
Flu	1.84 e-5	2.21 e-5	1.45 e-5	3.09 e-5	B(a)P	0.240	8.85 e-2	0.171	0.096	2.40 e-1	0.0885	0.171	0.0964
Phen	1.42 e-5	9.29 e-4	3.97 e-5	8.80 e-7	DB(a,h)A	1.55 e-2	2.38 e-3	0.0115	1.62 e-2	4.81 e-2	7.38 e-3	3.57 e-2	5.02 e-2
Ant	9.40 e-5	1.00 e-5	1.00 e-6	1.16 e-4	Cry	5.72 e-3	4.67 e-2	3.22 e-5	2.21 e-1	9.72 e-4	7.95 e-3	5.47 e-2	3.76 e-3
Flt	1.55 e-5	3.06 e-4	1.00 e-7	2.83 e-5	IP	1.00 e-4	1.60 e-3	1.00e-4	1.00e-4	2.90 e-5	6.09 e-3	2.90 e-5	2.95 e-5
Pyr	1.24 e-4	7.41 e-4	1.58 e-4	9.66 e-4	∑BaP TEQ	2.84 e-1	2.18 e-1	2.00 e-1	3.58 e-1	3.04 e-1	1.35 e-1	2.66 e-1	1.58 e-1
B(ghi)P	8.68 e-4	1.60 e-5	1.00 e-5	1.00 e-5	-	-	-	-	-	-	-	-	-
∑BaP TEQ	1.16 e-3	2.06 e-3	2.47 e-4	1.23 e-3	LECR	0.043	0.033	0.0304	0.0543	0.0461	0.0206	0.0403	0.0239
BaP TEQ daily dose mg kg^{-1} day^{-1}	1.14 e-6	1.99 e-6	2.42 e-7	1.20 e-6	-	2.77 e-4	2.13 e-4	1.96 e-4	3.50 e-4	2.97 e-4	1.33 e-4	2.60 e-4	1.54 e-4
HI	2.43 e-3	5.14 e-1	6.25 e-2	3.10 e-3	-	-	-	-	-	-	-	-	-

HI=Hazard index

Table 4. Statistical analysis of the results from Table 1

	Nap	**Acy**	**Ace**	**Fl**	**Phe**	**Ant**	**Flu**	**Py**	**BaA**	**Chr**	**BbF**	**BkF**	**BaP**	**DahA**	**IcdP**	**BghiP**
Nap	1															
Acy	.406	1														
Ace	.509	-.577	1													
Flt	.783	-.189	.897	1												
Phe	.627	.962*	-.351	.036	1											
Ant	.040	-.641	.692	.625	-.595	1										
Flu	.690	.941	-.270	.133	.994**	-.507	1									
Py	.444	-.638	.997**	.858	-.418	.696	-.342	1								
BaA	.987*	.528	.389	.718	.719	-.004	.780	.317	1							
Cry	.901	.762	.089	.469	.899	-.266	.935	.013	.951*	1						
B(b)F	.650	.958*	-.321	.085	.996**	-.526	.998**	-.392	.747	.915	1					
B(k)F	.603	.196	.416	.725	.264	.614	.361	.355	.653	.532	.346	1				
B(a)P	-.919	-.314	-.493	-.646	-.562	.187	-.596	-.446	-.866	-.795	-.551	-.248	1			
DB(ah)A	-.454	-.940	.504	.197	-.955*	.807	-.918	.555	-.536	-.762	-.926	.034	.493	1		
IP	.642	.959*	-.333	.059	1.000**	-.571	.997**	-.402	.734	.908	.998**	.292	-.567	-.945	1	
B(ghi)P	-.569	-.109	-.341	-.293	-.328	.443	-.313	-.333	-.477	-.444	-.274	.295	.848	.417	-.318	1

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

Table 5. Proposed benzo(a)pyrene equivalent factors for carcinogenic (TEF)[41], mutagenic toxicity (MEF)[32-33], reference dose (RFD) and cancer slope factor (CSF)[42]

PAHs	TEF	MEF	RfD (mg kg-1 day-1)	CSF (mg kg-1 day-1)
Nap	0.001		2.00 × 10-2	
Acy	0.001		2.00 × 10-2	
Ace	0.001		6.00 × 10-2	
Flu	0.001		4.00 × 10-2	
Phen	0.001		-	
Ant	0.01		3.00 × 10-2	
Flt	0.001		4.00 × 10-2	
Pyr	0.001		3.00 × 10-2	
B(a)A	0.1	0.082		7.30 × 10-1
Cry	0.001	0.017		7.30 × 10-3
B(b)F	0.1	0.25		7.30 × 10-1
B(k)F	0.01	0.11		7.30 × 10-2
B(a)P	1	1		7.3
IP	1	0.29		7.30 × 10-1
DB(a,h)A	0.1	0.31		7.3
B(g,h,i)P	0.01		4.00 × 10-2	

Source: Adeyeye *et al*[7]

Conflict of Interest: Nil

Source of Funding: Self-funding

Ethical Clearance: Not applicable

References

1. Ajiboye OO, Yakubu AF, Adams TE. A review of PAHs and heavy metals contamination of fish from fishfarms. J Applied Environment, 2011; 15(1): 235-238.
2. Silva BO, Adetunde OT, Oluseyi TO, Olayinka KO, Alo BI. Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. African Journal of Food Science, 2011; 5(7): 384-391.
3. Ishizaki A, Saitoa K, Haiokab N, Narimatsub S, Kataokaa H. Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection. Journal of Chromatography, 2010; 1217: 5555 5563.
4. Larson BK, Sahlberg GP, Erickson AJ, Busk LA. PAHs in grilled food. J. Agric and Food Chem., 1983; 31: 867-873.
5. Guillen MD, Sopelana P, Cid C. Presence of PAH in the foods that form part of the diet of different

Europen countries. Alimentaria, 1996; 34 (278): 41-47.

6. Yusuf KA, Ezechukwu LN, Fakoya KA, Akintola SL, Agboola J, Omoleye TO. Influence of fish smoking methods on polycyclic aromatic hydrocarbons content and possible risks to human health. African Journal of Food Science, 2015; 9(3): 126-135.
7. Adeyeye EI, Ibigbami OA, Adesina AJ, Olatoye RA, Gbolagade AY. Polycyclic Aromatic Hydrocarbons in Smoked Body Parts of *Clarias gariepinus* (Burchell, 1822) and the Possible Risks to Human Health. Journal of Health and Environmental Studies, 2018; 2(1): 227-238.
8. Essumang DK, Dodoo DK, ADjei JK. Polycyclic aromatic hydrocarbon (PAH) contamination in smoked-cured fish products. Journal of Food Composition and Analysis, 2012; 27: 128-138.
9. De Vos RH. PAHs in Dutch total diet samples (1984-1986). Food Chem. Toxicol., 1990; 28:263-268.
10. Farhedian A, Jinaps S, Hamtah HN, Zaiidu IS. Effects of meat, preheating and wrapping on the levels of PAHs in the charcoal- grilled meat. Food Chem., 2011; 141-146.
11. Guillen, MD and Sopelana, P. Ploycyclic aromatic hydrocarbons in diverse foods. In: D'Mello JPF (Ed.), Food Safety: Contaminants and Toxins CABI, UK, 2003.
12. Djinovic J, Popovic A, Jira W. PAHs in different smoked meat products from Serbia. Meat Science, 2008; 80:449-456.
13. .Simko P. Determination of PAHs in smoked meat products and smoked flavouring food additives. Journal of Chromatography, 2002; 770: 3-8.
14. Moret S, Piani B, Bortolomeazzi R, Conte LS. HPLC determination of polycyclic hydrocarbons in olive oils. Zeitschrift fu r Lebensmittel-Untersuchung und- Forshung A, 1997; 205: 116 – 120.
15. Gomma EA, Gray JI, Rabie S, Lopez –Bote C, Booren AM. PAHs in smoked food products and commercial liquid smoke flavourings. Food Additives and Contaminants, 1993; 10:503-521.
16. Jira W, Ziegenhals K, Speeer K. PAHs in smokedmeatproducts according to EU standards. Fleishwirtschaft International, 2006; 4:11-17.
17. Reinik M, Tamme T, Roasto M, Juhkam K, Tenno K, Us A. PAHs in maetproducts and estimated PAH intake by children and the general population in Estonia. Food Additives and Contaminant, 2007;24(4): 429-437.
18. Ladu BMB. Special Report of National Institute for Fresh Water Fisheries Research (NIFFR). Nigeria Agricultural Managing Jan/Feb 2001 Vol. 5 No1.
19. Tsado J H, Adeniji O B, Ojo M A, Adebayo C O, Abdulazzeez R. Perception of women knowledge on the nutritive value of fish in kaduna north local government area of kaduna state, Nigeria. Journal of Agriculture and Social Research, 2012; 12(1): 162-169.
20. FAO. Aquaculture Production (1984-1986). Fisheries and its Importance in Green Revolution Programme. Proceedings of the Annual Conference of Fishery Society of Nigeria. Vol. 2 pp. 91-96, 1989.
21. Akinbode SO, Dipeolu AO. DoubleHurdle Model of Fresh Fish Consumption among Urban Households in South-West Nigeria. Current Research Journal of Social Sciences, 2012; 4(6): 431-439
22. Federal Department of Fisheries (FDF). Nigeria National Aquaculture Strategy. Assisted by FAO. Formally approved by Government. 18 p, 2009.
23. The world fish centre (https://www.worldfishcenter.org/country).
24. Herring Archived. A the Wayback Machine, from Census of Marine Life Archived 2010-08-04 at the Wayback Machine, 2010.
25. Eco-Best Fish. Eco-Best Fish Safe for the environment, from Environmental Defense Fund, 2010.
26. "Hake - all about fish on The Worldwide Gourmet". Theworldwidegourmet.com. Retrieved 2019-09-15.
27. ASTM. Standard methods for comparison of waterborne petroleum oils by gas chromatography. In: Annual book of ASTM standards, American Society for Test Materials: 31, (pp. 2153 – 2164). Philadelphia, 1978.
28. ASTM. Standard practice for identification of waterborne oils. Annual book of ASTM Standards,

American Society for Testing Materials: 31, (pp. 2096-2098). Philadelphia, 1979.

29. Cheung KC, Leung HM, Kong KY, Wong HM. Residual levels of DDTs and PAHs in freshwater and marine fish from Hong Kong markets and their health risk assessment. Chemosphere**,** 2007; 66: 460 – 468.
30. Nyarko E, Botwe BO, Klubi E. Polycyclic aromatic hydrocarbons (PAHs) levels in two commercially important fish species from the coastal waters of Ghana and their carcinogenic health risks. West African Journal of Appied Ecology, 2011; 19(1): 53-66.
31. AFSSA. Avis de l'AFSSA relative à une demande d'avis sur l'évaluation des risqué présentés par le benzo[a]pyrene B[a]P et par d'autres hydrocarbures aromatiques polycycliques (HAP), présents dans diverses denrées ou dans certaines huile végétales, ainsi que sur les iveaux de concentration en HAP dans les denrées au-delà desquels des problèmes de santé risquent de se poser. Agence Française de Sécurité Sanitaire des Aliments. Saisine n 2000-SA-005, 2003**.**
32. Durant J, Busby W, Lafleur A, Penman B, Crespi C. Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. Mutagenic Research and Genetic Toxicology**,** 1996; 371: 123-157.
33. Durant J, Lafleur A, Busby W, Donhoffner L, Penman B, Crespi C. Mutagenicity of C24H14 PAHs in human cells expressing CYPIA1. Mutagenic Research and Genetic Toxicology**,** 1999; 446: 1-14.
34. FAO. Fisheries- The State of world Fisheries and Aquaculture (SOFIA). PART 1. World Review of Fisheries and aquaculture; Fish consumption: Pp. 58-65, 2008.
35. USEPA. Risk assessment Guidance for superfund, human health evaluation manual, (Part E., Supplemental Guidance for Dermal Risk Assessment), 2004. Http://www.epa.gov/risk/risk-assessment-guidance-superfund-rags-part-e.
36. Nakamura T, Kawamoto H, Saka. Pyrolysis behaviour of the Japanese cedar wood lignin studied with various model dimmers. J. Anal Appl. Pyrol., 2008; 81: 173-182.
37. European Commission Regulation (ECR). No 333/2007 of march 2007,laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. Official Journal of the Europen Union, 724:1-14, 2007.
38. Turkish Food Codex. Turkish food codex communiqué on determining themaximum levels of certain contaminants in foodstuffs, the optical Gazzette.17.05.2008/26879, 2008.
39. USEPA. Provisional Guidance for Quantitative Risk Assessment of PAHs, EPA/600/R 93/089, United State Environmental Protection Agency, 1993.
40. USEPA. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, EPA/823/B-00/007, United States Environmental Protection Agency, 2000.
41. Nisbet ICT, LaGoy PK. Toxic equivalency factors (TEFs) for poly-cyclic aromatic hydrocarbons (PAHs). Regulatory Toxicology Pharm., 1992; 16: 290 -300.
42. USEPA. Risk assessment Guidance for superfund, human health evaluation manual, (Part E., Supplemental Guidance for Dermal Risk Assessment). Http://www.epa.gov/risk/risk-assessment-guidance-superfund-rags-part-e, 2009.