



Original research article

Polycyclic aromatic hydrocarbons in imported *Sardinops sagax*: Levels and health risk assessments through dietary exposure in Nigeria

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) occurrence and assessment of dietary exposure from imported canned sardines (*Sardinops sagax*) commercially marketed in local stores and supermarkets in Nigeria were evaluated for the first time. PAHs determinations were performed using high performance liquid chromatography (HPLC) (Agilent 1290 model) equipped with UV-VIS diodes array detector (DAD) at $\lambda = 210$ nm and 214 nm. The percentage recoveries were higher than 96%. The degree of contamination expressed as total concentration of PAH congeners ranged between 2.53 and 35.55 $\mu\text{g kg}^{-1}$ dry weight (d. w.) at $\lambda = 210$ nm, and 1.30 and 27.93 $\mu\text{g kg}^{-1}$ (d.w.) at $\lambda = 214$ nm. The carcinogenic (TEQ_{BaP}) and mutagenic toxicities (MEQ_{BaP}) of eight priority PAHs were evaluated. Benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene and indeno[1,2,3-c,d]pyrene contributed significantly to the total carcinogenic equivalents of PAHs. The mutagenic equivalents were largely dominated by chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene and indeno[1,2,3-c,d]pyrene equivalence factors. The estimated lifetime average daily dose (LADD), average annual excess risk (A_R), excess cancer rate (ECR), and hazard quotient risk (HQ) were evaluated for adults, children and preteens exposure related risks. The LADD, ECR, A_R and HQ of PAHs for carcinogenic and non-carcinogenic risks are relatively higher in preteens than children and adults.

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1. Introduction

In most developing countries, fish and processed fish products constitute a dominant portion of daily human diet and are widely consumed as a cheap and ready source of protein, vitamins and essential minerals. According to Food and Agriculture Organization

of the United Nations (FAO) balance sheet of fish and fishery products in live weight and fish contribution to protein supply estimates, fish consumption rate in Nigeria has increased by about 22% since the early nineties with current supply quantity estimated at 63.76 g/capita/day (FAO, 2016). With a national fish demand of about 2.1 million metric tonnes per annum, it has been reported that an estimated 1.9 million tonnes of fish and fish products are imported into Nigeria annually (Agbo, 2015). About 60% of imported fish and fish products come in canned and

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prepackaged tins steeped in preservative oil, sometimes from sources with known cases of serious pollution challenges. In view of unconfirmed quality of imports and speculated sharp practices by importers, contamination risk assessment of imported food products in Nigeria should be routinely carried out to ascertain the presence and concentrations of cancer causing substances such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Traditionally, these substances are largely introduced into fish and fish products through the various stages of production, which includes smoking, roasting, boiling, grilling, amongst other methods (Adeyeye et al., 2015; Dunn and Fee, 2008; Karl and Leinemann, 2006; Olatunji et al., 2015). Fish is usually thermally treated to different degrees and with different techniques before consumption. PAHs can be present in seafood as a result of bioaccumulation in fatty tissues, introduction from overhead dispersion of wood smoke, pyrolysis of oils dripping into flame, and pyrolysis of nutritional elements (EFSA, 2008; Muyela et al., 2012).

Studies have shown that PAHs are introduced into the food matrix by direct pyrolysis of food nutrients during thermal treatments such as frying, grilling and smoking (Essumang et al., 2012; Karl and Leinemann, 2006; Wretling et al., 2010; Silva et al., 2011). Essentially, mono-unsaturated hydrocarbons in oils, fats and other nutrients undergo aromatization and dehydrocyclization reactions forming PAHs in the process (Olatunji et al., 2014). PAHs can also enter food matrices during thermal treatment from the combustion of fuels such as coal, wood and petroleum products (EFSA, 2008). Fish and fish products in Nigeria are considered as complements of major food group like carbohydrates because they are regarded as good sources of proteins and lipids. They however contribute significantly to the daily dietary intake of PAHs by humans. Several studies have highlighted enhanced levels of PAHs in food and relate subsequent consumption as an important exposure pathway (Essumang et al., 2012; Duedahl-Olesen et al., 2006; Drábová et al., 2010; EC, 2005a, 2005b; Jira, 2004; Reinik et al., 2007; Wretling et al., 2010). Humans are exposed to PAHs and other organic pollutants through three principal routes: respiration, dermal contact and dietary intake of contaminated food and water (Bandowe et al., 2014; Benson and Olufunke, 2011; Duedahl-Olesen et al., 2006). In recent times, several researchers have reported enhanced levels of PAHs in processed fish products (Ciecierska and Obiedzinski, 2007; Oluseyi et al., 2011; Yusuf et al., 2015). Generally, the presence of PAHs in human diet could pose serious health problems that may be associated with carcinogenic and mutagenic toxicities.

Polycyclic aromatic hydrocarbons have been a source of growing public concern because of their non-degradability and pervasiveness in the environment (Benson et al., 2014). They are a group of persistent organic pollutants formed during incomplete combustion or pyrolysis of carbonaceous materials, either by natural or anthropogenic agents (Benson and Essien, 2009; Benson et al., 2014; Huang et al., 2015; Lee and Vu, 2010; Maliszewska-Kordybach, 1999; Shukla et al., 2013). Due to the multiplicity of PAHs and their complex existence, several regulatory bodies including the European Food Safety Authority (EFSA) have prioritised some PAH compounds as representatives of the lot based on factors such as availability of information about them compared to others, degree of toxicity, chance of exposure, and degree of concentration (Ravindra et al., 2008). The Commission however, approved the use of benzo[a]pyrene as a marker for PAH contamination but stressed the need for continued data collection on the whole PAH profile to avoid being blindsided by changes in this profile (Alomirah et al., 2011; EFSA, 2008). Because they are practically unavoidable by human beings, regulatory bodies such as the USEPA and WHO have set

maximum tolerable limits beyond which exposed persons are at risk. These standards seek to control and monitor PAH exposure especially through food consumption. Fish and seafood form an essential part of the average human being's diet. Although a lot of research efforts have been channeled towards assessment of PAH contamination levels in smoked fish, little has been done to exclusively evaluate canned fish to ensure that their processing techniques do not expose the public to health risk. Therefore, the present study was designed to (i) determine and evaluate the PAHs content of canned fish products in Nigeria, (ii) conduct the health risk assessment of PAHs exposure using the carcinogenic (TEQ_{BaP}) and mutagenic (MEQ_{BaP}) quotients.

2. Materials and method

2.1. Chemicals and reagents

Sixteen priority PAH Standard Calibration Mix recommended by EPA610 method was purchased from Accustandard (New Haven, CT, United States) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-c,d]pyrene. The dichloromethane and *n*-hexane used were GC grade and of highest purity purchased from Merck (KGaA, Darmstadt, Germany). Activated amorphous silica powder was purchased from Loba Chemie (Mumbai, India), while anhydrous magnesium sulphate (Certified ACS reagent grade) used was sourced from Fisher Chemicals (Pittsburgh, PA, USA). Deionised water was used all through the experiments.

2.2. Sample collection, preparation and extraction

Five (5) brands of commercially imported canned samples of *Sardinops sagax* were purchased from local retail outlets in Ota, Nigeria. Products considered in this study were designated using codes TTS, SNV, NVY, MLO, and SAR for each branded *Sardinops sagax*. Five samples for each brand of sardine were purchased as representatives of each product, and were composited into twenty-five independent samples. The samples were carefully removed from their cans and the preservative oil discarded. They were then shredded to increase surface area and dried in an oven for about 12 h after which they were homogenized by grinding. 10 g of each sample was collected and further homogenized with 10 g anhydrous magnesium sulphate to remove moisture. Thereafter, 5 g of the sample was weighed and loaded into a column built by stuffing a burette with cotton wool, and 20 mL of dichloromethane (DCM) was added and the column allowed to stand for 30 min before draining. This procedure was followed by adding another 20 mL of dichloromethane to the burette and allowed to stand for 15 min before draining. The extracted liquid was allowed to evaporate to about 1 mL, and reconstituted with 3 mL of *n*-hexane, evaporated to about 2 mL, and was later subjected to sample clean-up.

2.3. Sample clean-up and analysis

A glass column was stuffed with glass wool up to the 3 mL mark, followed by activated amorphous silica powder up to the 10 mL mark. The sides of the column were tapped to properly settle and level the silica bed. The column was conditioned with 20 mL of *n*-hexane and 2 mL of the extract was loaded into it. The sample was then eluted with 20 mL of DCM for aromatics and collected into a glass amber bottle. It was evaporated to about 1 mL and reconstituted with 3 mL of *n*-hexane. It was allowed to evaporate

to about 2 mL before it was transferred into vials for HPLC analysis. The quantification of PAHs (naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLO), phenanthrene (PHA), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[a]anthracene (B[a]A), chrysene (ChY), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), dibenzo[a,h]anthracene (D[ah]A), benzo[g,h,i]perylene (B[ghi]P), and indeno[1,2,3-cd]pyrene (IP) was performed using an Agilent 1290 Model HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a G-4226A HiP auto-sampler, a G-4220A binary pump, a G-1316C column thermostat, a vacuum degasser, and a G-4212A UV diode-array detector (DAD). The HPLC with fluorescence detection is an analytical method that is most frequently used for determination of the carcinogenic PAHs in food samples (Barranco et al., 2004; Fontcuberta et al., 2006). In this study, the detection was carried out at two wavelengths, 210 nm and 214 nm. Prior to analytical chromatography, the C-18 column was stabilized at 25 °C for about 60 min. The HPLC analysis was carried out with a flow rate of 0.03 mL/min and the injection volume was 3.0 µL. The mobile phase was a gradient prepared from water (solvent A) and acetonitrile (solvent B). The details of the gradient, flow rate and pressure limit are presented in Table 1. The chromatogram from the UV diode-array detector (DAD) was displayed after each completed run on the computer and automated printout of results were made. The number of independent composite samples (n) analysed is twenty-five (n = 25). However, triplicate measurements were carried out for each extracted composite sample, totaling seventy-five (75) analytical replicates, and the results were averaged and recorded.

Prior to injection of extracted samples contained in well-labelled vials, a stock standard solution of 16 US EPA priority PAHs mix standard was serially diluted with acetonitrile to prepare five separate calibration standard PAH solutions containing 50, 40, 30, 20, and 10 µg/L of stock solution. Blanks samples containing no PAH were also analysed before and after injection of samples. The recovery of the analytical method was evaluated using 30 µg/L of PAH mix stock solution as an internal standard, which was added to representative samples and subsequently analysed. Measurements conducted on spiked samples were repeated in duplicates and the recoveries were calculated. The recoveries were considered as acceptable, and were in the range between 96.40 and 100.16% for NAP, ACY, ACE, FLO, PHA, ANT, FLA, PYR, B[a]A, ChY, B[b]F, B[k]F, B[a]P, D[ah]A, B[ghi]P, and IP. In addition, the linearity of calibration plots was satisfactory with regression coefficients greater than 99% for all the PAH congeners.

2.4. Benzo(a)pyrene-equivalent carcinogenicity and mutagenicity assessments

In order to assess the possible human exposure risks associated with carcinogenic or mutagenic PAHs in canned fish samples, the toxic equivalence factors (TEFs) and mutagenic potency equivalent factors (MEFs) were calculated relative to a Reference standard, benzo[a]pyrene, B[a]P as reported by (Benson et al., 2014; Koh

et al., 2004; Rogula-Kozłowska et al., 2013; Zhang et al., 2012). The overall carcinogenicity or mutagenicity of nonvolatile PAHs were estimated based on the weighted sum of individual congener concentrations and equivalence factors (TEFs or MEFs) relative to the cancer or DNA altering potency to B[a]P. This implies that carcinogenic equivalents (TEQ_{BaP}) and mutagenic equivalents (MEQ_{BaP}) were calculated as a product of the observed concentrations of the individual PAH congeners with its TEF for cancer potency relative to B[a]P, and MEF for DNA modification capacity relative to B[a]P, respectively. TEQ_{BaP} and MEQ_{BaP} were calculated as shown in the equations below:

$$TEQ_{BaP} = \sum_i C_i \times TEF_i \quad (1)$$

$$MEQ_{BaP} = \sum_i C_i \times MEF_i \quad (2)$$

where C_i , TEF_i and MEF_i are the individual PAH concentration, toxic equivalence factor and mutagenic equivalence factor, respectively. Further expansion of this equation would yield the following:

$$TEQ_{BaP} = [BaA] \times 0.1 + [ChY] \times 0.01 + [BbF] \times 0.1 + [BkF] \times 0.1 + [BaP] \times 1 + [IP] \times 0.1 + [DahA] \times 0.1 + [BghiP] \times 0.01$$

and

$$MEQ_{BaP} = [BaA] \times 0.082 + [ChY] \times 0.017 + [BbF] \times 0.25 + [BkF] \times 0.11 + [BaP] \times 1 + [IP] \times 0.31 + [DahA] \times 0.29 + [BghiP] \times 0.19$$

The toxicity equivalency factor (TEF) methodology was developed by the U.S. Environmental Protection Agency (EPA) to evaluate the relative toxicity and assess the risks of a mixture of groups A (known human) and B (probable human) carcinogenic polycyclic aromatic hydrocarbons, especially considering their common mechanism of action when ingested or inhaled by humans. These include benzo(a)anthracene, benzo(a)pyrene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene. The US EPA suggests that these eight PAHs have significant carcinogenic potential (Węgrzyn et al., 2006). In the article, we describe the analysis and use of these eight PAHs for evaluating the relative toxicity using the toxicity equivalent factors. The TEF for these PAHs is an estimate of the relative toxicity of each PAH compared to benzo(a)pyrene, which is assigned a reference value of 1 (Benson et al., 2014; Collins et al., 1998; Nisbet and LaGoy, 1992).

2.5. Risk assessment for carcinogenic exposure

The excess cancer risk (ECR) for adults and children was calculated based on a lifetime consumption of *Sardinops sagax* using Eq. (3):

$$ECR = \frac{\sum Q \times TEQ_{BaP} \times IFR \times ED_{tot}}{BW_a \times AT_n} \quad (3)$$

where Q (mg/kg/d) is the carcinogenic potency of B[a]P, ED_{tot} (years) exposure duration, BW_a (kg) is the average adult or children body weight and AT_n is the averaging time of exposure (365 days/year × number of exposure years). The value of Q is 7.3 mg/kg/d (Ding et al., 2012; Xia et al., 2010; Yoon et al., 2007). This represents the geometric mean calculated from the oral cancer slope factor (4.5, 5.9, 9.0 and 11.7) of benzo[a]pyrene (US EPA, 1991, 2001). IFR (g/day) is the fish ingestion rate. According to Food and Agriculture Organization of the United Nations, the fish ingestion rate in Nigeria based on 2013 estimate is 63.76 g/capita/day (FAO, 2016). The average body weight for children (age range 1–6), (age range 6–18 years) and adults in Nigeria are 19, 48 and 70 kg, respectively.

Table 1
Timetable of the mobile phase gradient.

Time (min)	Solvent A	Solvent B	Flow (mL/min)	Pressure (bar)
	Water (%)	Acetonitrile (%)		
1.50	40.00	60.00	0.030	1200
7.00	10.00	90.00	0.030	1200
13.00	0.00	100.00	0.030	1200
17.00	0.00	100.00	0.030	1200
22.00	40.00	60.00	0.030	1200

The ED_{tot} is estimated at 52.8 years for adults (World Bank 2016 estimate for average life expectancy in Nigeria) (World Bank, 2016).

On the other hand, the exposure rate to carcinogenic PAHs during the lifetime of adults, children and preteens in Nigeria was also evaluated using the lifetime average daily dose, LADD (mg/kg/d). The PAHs exposure dose rate is computed following Eq. (4):

$$ADD = \frac{C_{PAHs} \times EF \times ED_{tot} \times IFR}{BW_a \times AT_n} \quad (4)$$

where C_{PAHs} represents the concentration of individual PAHs, EF is the exposure frequency, and IFR, ED_{tot} , BW_a and AT_n represent variables as previously defined. However, the average annual excess risk of cancer for an individual is calculated using Eq. (5):

$$A_R = \frac{1 - e^{-\frac{LADD}{SF}}}{AT_n} \quad (5)$$

where A_R is the average annual excess risk of cancer for an individual, dimensionless; SF is the level of intensity of carcinogenic chemicals (mg/kg/d); and AT_n is the number of an individual average lifetime presented in years.

2.6. Risk characterisation

The evaluation of exposure risk associated with threshold contaminants (i.e. acenaphthene, anthracene, fluoranthene, fluorene, and pyrene) was accomplished by calculating an exposure ratio called a hazard quotient (HQ) using the Hazard Quotient Risk Calculation model RISC 4.02 (USEPA, 1989). The HQ is usually calculated to characterise non-carcinogenic risks on the basis that at threshold level (HQ=0.2), exposure to contaminants would unlikely have effects on sensitive populations. For a calculated hazard quotient greater than 0.2, a risk to human health potentially exists. In the present study, risks associated with threshold contaminants were estimated for preteens (1–6years), children (6–18) years and adults.

2.7. Statistical analysis

All data were analysed using the XLSTAT-Pro software (AddinSoft, Inc., NY, USA) and Microsoft Excel 2011. Comparative and continuous summary descriptives were also performed. Fischer's two-tailed tests was used to test the method's precision, and the statistical significance was considered for $p < 0.05$.

3. Results and discussion

3.1. Levels of PAHs

Sixteen (16) PAHs were investigated in branded *Sardinops sagax*. However, fourteen (14) PAH concentrations were detected (Tables 2 and 3). The mean concentrations of PAH congeners in all the samples at the two wavelengths are presented. The PAH concentrations varied largely with individual branded sardines. The SAR brand indicated higher concentrations of PAHs with phenanthrene, pyrene and anthracene having concentrations higher than eleven other PAH congeners. Comparatively, the estimated total PAH concentrations (Σ PAHs) are significantly higher in SAR samples at both wavelengths with 35.54 and 27.92 $\mu\text{g}/\text{kg}$ at 210 and 240 nm, respectively. The concentration of each PAH congeners ranged between BDL – $15.27 \pm 0.68 \mu\text{g}/\text{kg}$. PAHs levels in food substances are generally known to be variable and in low concentrations, which are primarily attributed to metabolic residues of contaminated food ingestion via the web chain or dermal contact through exogenous exposure pathway in contaminated aquatic ecosystems (Bandowe et al., 2014; Duedahl-Olesen et al., 2006; Essumang et al., 2012; Olatunji et al., 2015). The high molecular weight PAHs, which are generally more potent carcinogens, contributed less to the total PAH concentration. Benzo [a]anthracene was not detected in any of the samples at any wavelength. In all the samples, B[a]P concentrations were well below the European market maximum permissible limit for processed fish of 2 $\mu\text{g}/\text{kg}$ (EFSA, 2008; Yusuf et al., 2015). However, since analysis using HPLC-DAD was carried out at two wavelengths (210 nm and 214 nm), it was observed that the measurements at 210 nm showed significant levels of PAH detection compared to 214 nm for the five canned fish products analysed, detecting all sixteen priority congeners, while 214 nm detected fifteen (excluding fluorene). Also, the detected concentrations of congeners at 210 nm were generally higher and more significant those detected at 214 nm. However, in order to test whether there is a statistical difference at 95% confidence level ($P > 0.05$) between PAHs concentrations obtained at 210 and 214 nm, a two-tailed F-test was employed. The F-test otherwise termed the Fischer's test is a mathematical tool that looks at the test of precision, and basically involves the comparison of the variances or standard deviations of two analytical methods with the sole objective of elucidating their differences that can be explained by indeterminate error. The results indicated that at 95% certainty, there is no difference

Table 2
Mean PAH concentrations ($\mu\text{g}/\text{kg}$) in imported sardines (*Sardinops sagax*) obtained at 210 nm.

Congeners	TTS	SNV	NVY	MLO	SAR
Naphthalene	BDL	BDL	BDL	BDL	0.451 \pm 0.637
Acenaphthylene	0.017 \pm 0.024	BDL	BDL	BDL	0.004 \pm 0.0001
Acenaphthene	BDL	0.802 \pm 0.161	0.694 \pm 0.041	0.633 \pm 0.040	1.455 \pm 0.002
Fluorene	0.002 \pm 0.0001	0.001 \pm 0.0001	0.001 \pm 0.0001	0.001 \pm 0.0001	0.003 \pm 0.004
Phenanthrene	0.852 \pm 0.283	0.394 \pm 0.055	0.542 \pm 0.019	0.595 \pm 0.003	15.271 \pm 0.689
Anthracene	BDL	1.217 \pm 0.721	BDL	BDL	5.012 \pm 0.089
Fluoranthene	BDL	0.011 \pm 0.016	BDL	BDL	0.073 \pm 0.003
Pyrene	0.415 \pm 0.065	3.72 \pm 0.206	1.291 \pm 0.017	0.851 \pm 0.031	11.845 \pm 0.191
Benzo[a]anthracene	BDL	BDL	BDL	BDL	BDL
Chrysene	0.015 \pm 0.021	0.049 \pm 0.002	BDL	0.060 \pm 0.084	0.271 \pm 0.031
Benzo[b]fluoranthene	0.192 \pm 0.272	0.865 \pm 0.049	BDL	BDL	BDL
Benzo[k]fluoranthene	0.048 \pm 0.068	BDL	BDL	0.062 \pm 0.088	0.236 \pm 0.033
Benzo[a]pyrene	0.202 \pm 0.012	0.428 \pm 0.282	0.062 \pm 0.088	0.288 \pm 0.005	0.925 \pm 0.062
Dibenzo[a,h]anthracene	0.172 \pm 0.172	0.136 \pm 0.010	BDL	0.0363 \pm 0.051	BDL
Benzo[g,h,i]perylene	BDL	BDL	BDL	BDL	BDL
Indeno[1,2,3-cd]pyrene	3.72 \pm 0.267	0.405 \pm 0.223	BDL	BDL	BDL
Σ PAH content	5.639	8.033	2.590	2.526	35.546

BDL – Below detection limit; Limit of Detection = < 0.001 ; (number of independent samples, $n = 25$).

Table 3
Mean PAH concentrations ($\mu\text{g}/\text{kg}$) in imported sardines (*Sardinops sagax*) obtained at 214 nm.

Congeners	TTS	SNV	NVY	MLO	SAR
Naphthalene	BDL	BDL	BDL	BDL	1.676 \pm 0.370
Acenaphthylene	0.031 \pm 0.043	BDL	BDL	BDL	0.014 \pm 0.0002
Acenaphthene	1.461 \pm 0.451	0.253 \pm 0.344	0.316 \pm 0.004	0.321 \pm 0.002	0.777 \pm 0.001
Fluorene	BDL	BDL	BDL	BDL	BDL
Phenanthrene	0.234 \pm 0.108	0.116 \pm 0.160	0.151 \pm 0.090	0.233 \pm 0.014	12.645 \pm 1.532
Anthracene	0.026 \pm 0.007	0.009 \pm 0.002	BDL	BDL	3.665 \pm 0.183
Fluoranthene	BDL	0.006 \pm 0.001	BDL	BDL	0.147 \pm 0.003
Pyrene	0.254 \pm 0.031	1.163 \pm 0.637	0.833 \pm 0.007	0.528 \pm 0.023	7.935 \pm 0.113
Benzo[a]anthracene	BDL	BDL	BDL	BDL	BDL
Chrysene	BDL	0.054 \pm 0.001	BDL	0.091 \pm 0.032	0.272 \pm 0.041
Benzo[b]fluoranthene	BDL	1.793 \pm 0.535	BDL	BDL	BDL
Benzo[k]fluoranthene	0.041 \pm 0.058	BDL	BDL	BDL	0.112 \pm 0.015
Benzo[a]pyrene	0.119 \pm 0.048	0.191 \pm 0.012	BDL	0.117 \pm 0.010	0.686 \pm 0.074
Dibenzo[a,h]anthracene	BDL	BDL	BDL	BDL	BDL
Benzo[g,h,i]perylene	BDL	BDL	BDL	BDL	BDL
Indeno[1,2,3-cd]pyrene	0.750 \pm 0.060	3.328 \pm 0.706	BDL	BDL	BDL
Σ PAH content	2.916	6.913	1.299	1.289	27.929

BDL – Below detection limit; Limit of Detection = <0.001 ; (number of independent samples, $n=25$).

between the standard deviations of the two PAHs quantification wavelengths.

At wavelength 210 nm, TTS samples showed a total PAH content of 5.64 $\mu\text{g}/\text{kg}$, with the highest contributor being indeno[1,2,3-cd]pyrene (3.72 \pm 0.26 $\mu\text{g}/\text{kg}$) followed by phenanthrene (0.85 \pm 0.28 $\mu\text{g}/\text{kg}$). However, at 214 nm acenaphthene concentration was found to be 1.46 \pm 0.45 $\mu\text{g}/\text{kg}$ although it was not detected in TTS samples at 210 nm (Table 2). This discrepancy might be attributable to non-detectability of peak concentration or retention time above 210 nm maximum wavelength, or chromatographic conditions (Węgrzyn et al., 2006). The SNV samples showed high concentrations of pyrene (3.72 \pm 0.21 $\mu\text{g}/\text{kg}$) and anthracene (1.22 \pm 0.72 $\mu\text{g}/\text{kg}$) at 210 nm. At wavelength 214 nm, pyrene also recorded a relatively high concentration (1.16 \pm 0.64 $\mu\text{g}/\text{kg}$) although indeno[1,2,3-cd]pyrene contributed more to the total PAH content with 3.33 \pm 0.71 $\mu\text{g}/\text{g}$. Generally, NVY, MLO and SAR samples showed less presence of HMW PAHs. The NVY indicated very low and insignificant levels of PAHs. However, its pyrene content was consistently high at both wavelengths, impacting on the total PAH content, while MLO had the lowest total PAH content with 2.53 $\mu\text{g}/\text{kg}$ and 1.28 $\mu\text{g}/\text{kg}$ at 210 nm and 214 nm, respectively. SAR showed extremely high concentrations of phenanthrene and pyrene and these impacted on the total PAH contents, giving 35.54 $\mu\text{g}/\text{kg}$ and 27.93 $\mu\text{g}/\text{kg}$ at 210 and 214 nm, respectively. In the present study, the concentration of B[a]P in all brands of sardines investigated indicated levels that are lower than the threshold B[a]P level of 2.0 ng g^{-1} ww set by the European Union for fish muscle. The PAHs levels obtained in the present work are comparable to concentrations reported in other studies (Duedahl-Olesen et al., 2006; Bandowe et al., 2014; Essumang et al., 2012; Silva et al., 2011; Wretling et al., 2010).

3.2. Health risk assessment

The carcinogenic toxicity (TEQ_{BaP}) and mutagenic toxicity (MEQ_{BaP}) relative to B[a]P were calculated to find the potential carcinogenic and mutagenic risk associated with ingestion of these canned fish. B[a]P is commonly used as a biomarker as well as an index chemical to estimate the health risks posed by exposure to Σ PAHs because of the paucity of experimental risk assessment values for each individual Σ PAH. While TEQ_{BaP} is used to evaluate potential carcinogenicity of a given PAH mixture, MEQ_{BaP} (mutagenic activity) provides information for a wider range of health issues other than cancer including pulmonary diseases, birth defects, impotency, low intelligent quotient, etc. (Li et al.,

2014; Hsu et al., 2014; Stolyhwo and Sikorsi, 2005). From the results in Table 4, the TEQ for the eight USEPA priority carcinogens were shown to be low compared to those of grilled and smoked items as analysed by Alomirah et al. (2011). Known carcinogenic PAHs were not found in NVY sample at wavelength 214 nm, hence the absence of TEQ and MEQ values. The following congeners B[b]F, B[k]F and IP made significant contributions to carcinogenic PAHs equivalents in nearly all investigated samples at both wavelengths and the mutagenic equivalents were also largely influenced by equivalence factors of B[a]P, ChY, B[b]F, B[k]F, and IP.

The exposure rate due to lifetime average daily dose is presented in Table 5, while the average annual excess risk of cancer for adults and children in Nigeria due to individual PAHs is shown in Table 6. According to the US EPA, one out of a million (1×10^{-6}) chance of developing cancer over a lifetime is the level of risk considered to be acceptable or inconsequential, whereas a lifetime cancer risk of one in ten thousand (1×10^{-4}) or greater is considered serious.

The estimated lifetime average daily dose (LADD) of PAHs for carcinogenic risks is relatively higher in the preteens aged 1–6 years old than in children (6–18 years) and adult Nigerians. Also, the estimated carcinogenic risks associated with PAHs exposure through oral ingestion of different brands of canned sardines considered in this study and commercially sold in Nigeria were calculated to be 3.4×10^{-6} , 4.57×10^{-7} , and 1.07×10^{-8} in preteens, children and adults, respectively, for PAHs quantification made at 210 nm. On the other hand, the estimates made at 214 nm indicated unit risks of 5.06×10^{-7} for preteens, 6.67×10^{-8} for children, and 1.57×10^{-8} for adults. Results indicate that preteens are the most vulnerable and sensitive group, with vulnerability trend showing preteens > children > adults. This means that approximately, 35 out of every 10,000,000 preteens in Nigeria may likely develop cancer related disease(s) in their lifetime as a result of exposure to PAHs associated with consumption of branded canned sardines

Table 4
Calculated carcinogenic and mutagenic equivalents in imported sardines (*Sardinops sagax*).

	210 nm		214 nm	
	TEQ_{BaP}	MEQ_{BaP}	TEQ_{BaP}	MEQ_{BaP}
TTS	0.7703	1.4599	0.1979	0.3558
SNV	0.6909	0.8100	0.7035	1.6716
NVY	0.0624	0.0624	0	0
MLO	0.3310	0.3062	0.1176	0.1182
SAR	0.9547	0.9590	0.6997	0.7027

Table 5
Lifetime average daily dose (mg/kg/day) of PAHs in adults and children for different brands of *Sardinops sagax*.

	TTS	SNV	NVY	MLO	SAR
Chrysene	4.14E-08 ^a	1.35E-07	0	1.65E-07	7.47E-07
	1.63E-08 ^b	5.35E-08	0	6.55E-08	2.76E-07
	1.22E-08 ^c	3.67E-08	0	4.49E-08	2.03E-07
	(0) ^{a*}	(1.49E-07)	(0)	(2.51E-07)	(7.50E-07)
	(0) ^{b*}	(5.89E-08)	(0)	(9.94E-08)	(2.97E-07)
	(0) ^{c*}	(4.04E-08)	(0)	(6.81E-08)	(2.04E-07)
Benzo[b]fluoranthene	5.29E-07 ^a	2.38E-06	0	0	0
	2.09E-07 ^b	9.44E-07	0	0	0
	1.44E-07 ^c	6.48E-07	0	0	0
	(0) ^{a*}	(4.95E-06)	(0)	(0)	(0)
	(0) ^{b*}	(1.96E-06)	(0)	(0)	(0)
	(0) ^{c*}	(1.34E-06)	(0)	(0)	(0)
Benzo[k]fluoranthene	1.32E-07 ^a	0	0	1.71E-07	6.51E-07
	5.24E-08 ^b	0	0	6.77E-08	2.58E-07
	3.59E-08 ^c	0	0	4.64E-08	1.77E-07
	(1.13E-07) ^{a*}	(0)	(0)	(0)	(3.09E-07)
	(4.48E-07) ^{b*}	(0)	(0)	(0)	(1.22E-07)
	(3.07E-08) ^{c*}	(0)	(0)	(0)	(8.38E-08)
Benzo[a]pyrene	5.57E-07 ^a	1.19E-06	1.71E-07	7.94E-07	2.55E-06
	2.21E-07 ^b	4.67E-07	6.76E-08	3.14E-07	1.01E-06
	1.51E-08 ^c	3.20E-07	4.64E-08	2.16E-07	6.93E-07
	(3.28E-07) ^{a*}	(5.27E-07)	(0)	(3.23E-07)	(1.89E-06)
	(1.29E-07) ^{b*}	(2.09E-07)	(0)	(1.28E-07)	(7.49E-07)
	(8.91E-08) ^{c*}	(1.43E-07)	(0)	(8.76E-08)	(5.14E-07)
Dibenzo[a,h]anthracene	4.74E-07 ^a	3.75E-07	0	1.00E-07	0
	1.88E-07 ^b	1.48E-07	0	3.96E-08	0
	1.29E-07 ^c	1.02E-07	0	2.72E-08	0
	(0) ^{a*}	(0)	(0)	(0)	(0)
	(0) ^{b*}	(0)	(0)	(0)	(0)
	(0) ^{c*}	(0)	(0)	(0)	(0)
Indeno[1,2,3-cd]pyrene	1.03E-05 ^a	1.12E-06	0	0	0
	4.06E-06 ^b	4.42E-07	0	0	0
	2.78E-06 ^c	3.03E-07	0	0	0
	(2.07E-06) ^{a*}	(9.18E-06)	(0)	(0)	(0)
	(8.19E-07) ^{b*}	(3.63E-06)	(0)	(0)	(0)
	(5.61E-07) ^{c*}	(2.49E-06)	(0)	(0)	(0)

a = LADD for preteens (1–6yrs) at 210 nm, b = LADD for children (6–18yrs) at 210 nm, c = LADD for adults at 210 nm.

a* = LADD for preteens (1–6yrs) at 214 nm, b* = LADD for children (6–18yrs) at 214 nm, c* = LADD for adults at 214 nm.

Table 6
Annual exposure rate for preteens, children and adults in Nigeria.

	1–6 years		6–18 years		Adult	
	210 nm	214 nm	210 nm	214 nm	210 nm	214 nm
Chrysene	9.45E-07	0	1.25E-07	0	2.93E-08	0
Benzo[b]fluoranthene	1.21E-07	0	1.59E-08	0	3.75E-09	0
Benzo[k]fluoranthene	3.02E-08	2.58E-08	3.98E-09	3.40E-09	9.37E-10	8.01E-10
Benzo[a]pyrene	1.27E-08	7.49E-09	1.68E-09	9.88E-10	3.94E-10	2.32E-10
Dibenzo[a,h]anthracene	1.08E-08	0	1.43E-09	0	3.36E-10	0
Indeno[1,2,3-cd]pyrene	2.34E-06	4.72E-07	3.09E-07	6.23E-08	7.27E-08	1.46E-08
Total	3.46E-06	5.06E-07	4.57E-07	6.67E-08	1.07E-08	1.57E-08

imported into the country. In the same vein, about 5 out of every 10,000,000 children aged 6–18 years may likely develop cancer or cancer related disease via ingestion of sardines. The most notable endpoint of PAH toxicity includes liver, skin, lung, bladder, and gastrointestinal cancers (ATSDR, 2013). In addition, risk assessment calculations using excess cancer risk model corroborate this finding (Fig. 1). The calculated excess cancer risk (ECR) from dietary exposure to the investigated brands of sardines is less than 1.0×10^{-6} , therefore the level of risk is acceptable or might be considered to be inconsequential.

In this study, the health risk associated with threshold contaminants estimated for preteens (1–6years), children (6–18) years and adults using the Hazard Quotient Risk Calculation model RISC 4.02, is presented in Table 7. Results suggest that calculated hazard quotients for PAH concentrations in the SAR brand of *Sardinops sagax* are greater than 0.2 for preteens, children and adults (Table 7), while SNV brand indicated an hazard quotient of 0.48 for preteens only. Therefore, dietary exposure to PAHs through these brands could potentially pose serious health risks to these populations. Likely non-carcinogenic effects or risks associated

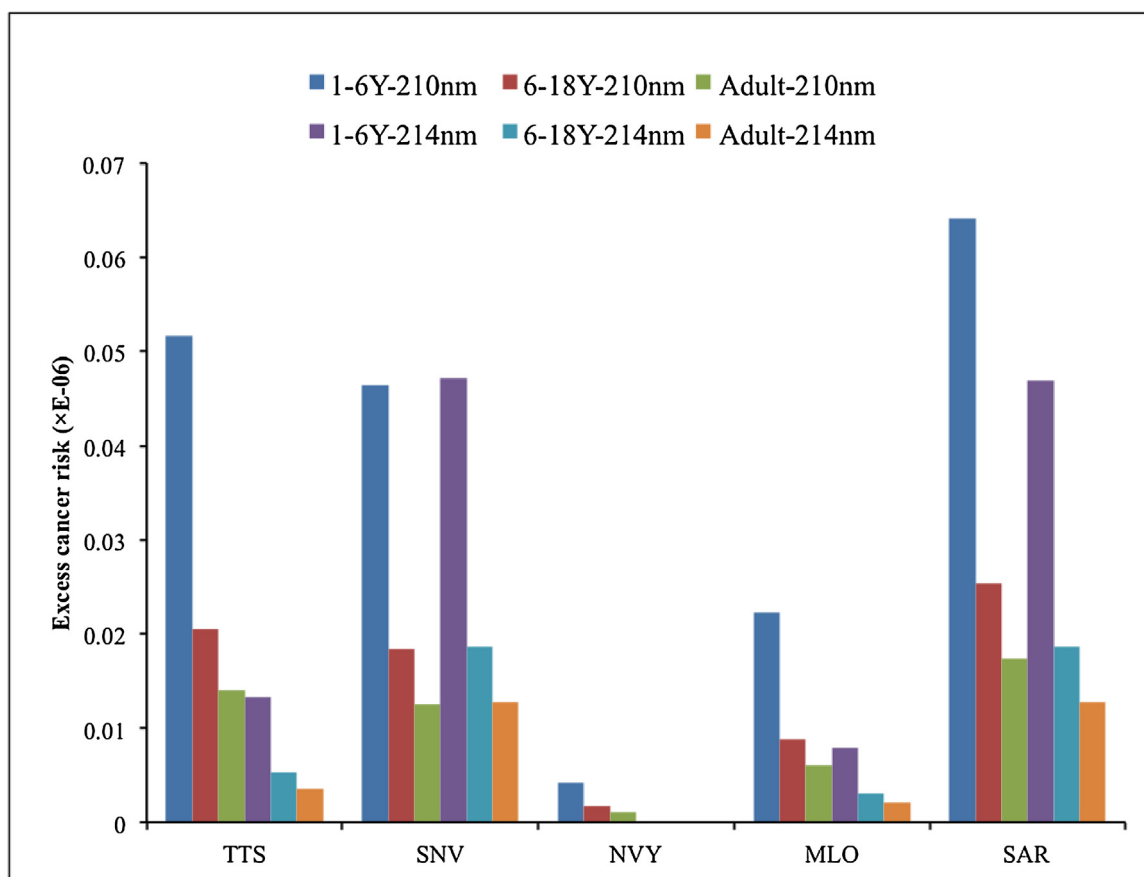


Fig. 1. Calculated excess cancer risk for different brands of *Sardinops sagax*.

Table 7
Summary of hazard quotient risk estimates for preteens, children and adults.

		TTS	SNV	NVY	MLO	SAR
210 nm	HQ _{PT}	0.047	0.475	0.183	0.131	1.544
	HQ _{CH}	0.018	0.188	0.073	0.052	0.611
	HQ _{AD}	0.013	0.129	0.049	0.035	0.419
214 nm	HQ _{PT}	0.837	0.145	0.110	0.077	1.266
	HQ _{CH}	0.331	0.057	0.043	0.030	0.501
	HQ _{AD}	0.227	0.039	0.030	0.021	0.343

(HQ = acenaphthene, anthracene, fluoranthene, fluorene, pyrene); HQ_{PT} = Hazard Quotient for preteens; HQ_{CH} = Hazard Quotient for children; HQ_{AD} = Hazard Quotient for adults.

with PAHs exposure involve primarily the gastrointestinal, pulmonary, dermatologic and renal systems (ATSDR, 2013).

4. Conclusions

The results of the present study show moderate to elevated concentrations of PAHs in various brands of canned sardines commercially sold in Nigeria. However, considering the relatively high rate of consumption of these imported sardines by Nigerians, potential health assessment estimates indicate carcinogenic and non-carcinogenic associated risks. The lifetime average daily dose and excess cancer risk associated with consumption of canned sardines was relatively higher in preteen than children and adults, with ECR value below the 1×10^{-6} threshold (guideline) value. The concentration of B[a]P in all investigated brands of sardines indicate levels lower than the European Union threshold B[a]P level. However, B[b]F, B[k]F and IP made significant contributions

to carcinogenic PAHs equivalents, while the mutagenic equivalents are largely influenced by B[a]P, ChY, B[b]F, B[k]F, and IP equivalence factors.

The outcome of this study has provided useful information about the level of PAHs in most of the commercially imported canned sardine. This study will serve as a baseline for future research work to determine the level of PAHs in most imported canned foods. Having considered the findings and the observations made in this research, it is imperative that a continuous monitoring and assessment of PAHs in imported and local food products be instituted to avoid PAHs exposures.

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Conflicts of interest

The authors declare no conflict of interest.

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