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To cite this version:
Victoria Akpambang, Giorgia Purcaro, Labunmi Lajide, Isiaka Amoo, Lanfranco Conte, et al.. Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat. Food Additives and Contaminants, 2009, 26 (07), pp.1096-1103. 10.1080/02652030902855406 . hal-00573864

HAL Id: hal-00573864
https://hal.archives-ouvertes.fr/hal-00573864
Submitted on 5 Mar 2011

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| Methods/Techniques: | Chromatography - HPLC, Clean-up, Extraction |
| Additives/Contaminants: | Process contaminants - PAH’s |
| Food Types: | Fish, Meat |
Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat

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Abstract
Smoking and/or grilling, when carried out with traditional methods involving direct contact with wood combustion fumes, is responsible for high contamination levels with carcinogenic polycyclic aromatic hydrocarbons (PAHs). The aim of this work was to investigate the PAH content of different smoked or grilled meat and fish products, commonly consumed in Nigeria. A rapid method involving microwave assisted saponification and simultaneous extraction followed by solid phase extraction (SPE), HPLC separation and spectrofluorometric detection was employed. Samples which were smoked or grilled using traditional systems, which use a wood fire, were heavily contaminated with benzo[a]pyrene (BaP) at levels ranging from 2.4 to 31.2 µg kg⁻¹ wet weight. Considerably lower contamination levels were found in samples smoked or grilled in the laboratory using a charcoal fire (BaP from 0.7 to 2.8 µg kg⁻¹ wet weight). The health risk associated to a daily consumption of 100 g of these products was also evaluated using the margin of exposure (MOE) approach. MOE values lower than 10,000 were obtained for all smoked/grilled commercial samples, indicating a potential concern for consumer health.

Keywords: polycyclic aromatic hydrocarbons (PAHs), microwave assisted extraction (MAE), HPLC, fish, meat, smoking, grilling, risk assessment.
Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental contaminants, originating from incomplete combustion of organic matter, which have received much attention over the years because of their ability to cause cancer. It is well known that raw meat (from mammals) does not contain appreciable levels of carcinogenic PAHs and no accumulation along the food chain has been observed for these contaminants in animal fat tissue (EFSA, 2008). More controversial is the presence of PAHs in vertebrate fish. According to most of the authors, due to their ability to rapidly metabolize PAHs, fish generally contain very low PAH concentrations, even when they come from heavily contaminated areas (Oost et al., 2003). Nevertheless, some researchers found high PAH concentrations in raw fish from contaminated waters (Visciano et al., 2006). Some authors (Akpan et al., 1994) reported benzo[a]pyrene (BaP) concentrations ranging from 5.4 to 44.0 µg kg\(^{-1}\) dry weight for raw fish from 3 Nigerian cities. Amounts found in fish from the same contaminated area showed a correlation with their lipid content indicating the ability to bio-accumulate PAHs in their fatty tissues. More recently, Anyakora et al. (2008) reported BaP concentrations between 1.5 and 10.5 µg kg\(^{-1}\) in 4 different fish samples from the Niger delta, a highly contaminated site due to the extensive petroleum production activities.

All food processing involving thermal treatments at high temperature and/or direct contact with combustion gases, such as smoking, toasting, roasting or grilling may be responsible for high PAH levels in processed foodstuffs. The amount of PAHs generated during the thermal food processing depends on several parameters such as temperature, duration of the treatment, distance from the source of heating, oxygen accessibility, fat content, and type of combustible used (Alonge, 1988, Visciano et al., 2006). Very high contamination levels are expected when food is smoked over an open flame, while charcoal grilling usually yields small amounts of PAHs (Larsson et al., 1983).

Smoking is an age-old technique which brings desired flavors, improves color and appearance, has a tenderizing action and preservatives foods (Ihekoronye and Ngoddy, 1985). Traditional direct smoking, in which the smoke is generated in the same chamber where the product is processed, exposes it to higher PAH content than indirect smoking which uses a separate chamber for smoke generation. In the latter case, it is possible to lower PAH load by passing the smoke through filters and washers or by cooling it before it comes in contact with the food. While in developed countries computerized smoking chambers with external smoke generators, and emission and temperature control systems, able
to minimize PAH production, have generally replaced direct smoking systems using traditional kilns (Karl and Leinemann 1996, Hattula et al., 2001), in other countries such as Nigeria, traditional direct smoking systems are almost exclusively used even today. The most popular fish smoking method in Nigeria utilizes wire gauze on steel drums fuelled by wood.

Different approaches have been proposed for risk characterization of the PAH mixture in food, the most popular being the use of BaP as a marker and the toxic equivalency factor (TEF) approach. Based on examination of PAH profiles in food and on evaluation of a carcinogenicity study of two coal tar mixtures in mice, both the Scientific Committee on Food (SCF, 2002) and the Joint FAO/WHO Expert Committee on Food Additives Expert Committee on Food Additives (JECFA, 2005) suggested that BaP should be used as a marker of the occurrence and effect of PAHs in food. However, latterly the TEF approach was re-assessed and it was deemed as not scientifically valid because of the lack of data from oral carcinogenicity studies on individual PAHs, their different modes of action and the evidence of poor predictivity of the carcinogenic potency of PAH mixtures based on the currently proposed TEF values (SCF, 2002; EFSA, 2008).

In 2005, the European Commission (EC) introduced for BaP (chosen as a marker of the occurrence and carcinogenic potency of the entire class of carcinogenic and genotoxic PAHs) a maximum levels of 5 µg kg\(^{-1}\) in smoked fish and meat (Commission Regulation 2005/208/EC). Before the introduction of this Regulation, lower legal limits for BaP existed in a number of EU Member States such as Belgium (2 µg kg\(^{-1}\)), Slovak Republic and Germany (1 µg kg\(^{-1}\)) (Wenzl et al., 2006). Up to now no maximum allowable levels for PAHs and/or BaP have been established by the government of Nigeria.

Based on new available data, in 2008 the EFSA CONTAM Panel concluded that BaP is not a suitable indicator for the occurrence of PAHs in food and that 8 high molecular weight PAHs (PAH8), and a subgroup of 4 PAHs (PAH4), are the most suitable indicators of PAHs in foods, and suggested to use the margin of exposure (MOE) approach for the risk assessment (EFSA, 2008).

According to Kazerouni et al. (2001), smoked and grilled foods may contribute significantly to PAH dietary intake if such foods are part of the usual diet. The consumption of traditionally smoked products could be responsible for the higher incidence of primary liver and stomach cancer in Nigeria compared with that in Europe and in the USA (Alonge, 1988). To our knowledge there are no recent consumption data for fish and meat in Nigeria. Yeh et al. (1996) calculated for 394 Nigerians (Benin...
City) of different socioeconomic status an average daily meat and fish consumption of 43.5 and 70.5 g, respectively.

Limited information on the levels of PAHs in smoked fish and meat in Nigeria is available from the literature. Afolabi (1983) and Akpan (1994) found BaP concentrations ranging from 11.1 to 66.9 and from 35.5 to 139 µg kg\(^{-1}\) dry weight, respectively, in Nigerian traditionally smoked fish. Alonge (1988) reported BaP contents from 10.5 to 66.9 µg kg\(^{-1}\) in traditionally smoked meat from Nigeria, while Bababunmi et al. (1982) reported 8.5 µg kg\(^{-1}\) of BaP in suya meat (a spiced skewered beef very popular in West Africa, usually grilled over an open fire). More recently, Duke and Albert (2007) found BaP contents ranging from 6.5 to 21.5 µg kg\(^{-1}\) in suya meat from 4 different selling points.

PAH determination in meat and fish samples, when carried out by means of traditional sample preparation method, is solvent- and time-consuming and can be cause of analyte losses and source of spurious contamination. To overcome these disadvantages, new extraction techniques, such as microwave assisted extraction (MAE), able to perform simultaneous rapid extraction of a number of samples depending on the kind of apparatus, have been recently introduced and applied by different authors for PAH extraction from food matrices (García Falcón et al., 2000; Hernández-Borges et al., 2006; Pena et al., 2006).

The aim of this study was to investigate on PAH levels in some smoked/grilled fish and meat products commonly consumed in Nigeria and to estimate the potential risk associated with consumption of such traditionally processed foodstuffs. Unprocessed samples were analyzed, too. For this purpose a quick and efficient microwave assisted extraction (MAE) method, involving simultaneous saponification and solvent extraction, was used for sample preparation before HPLC determination and spectrofluorometric detection of 15 EPA-priority PAHs. The attention was focused on the EPA priority PAHs as they include the 8 PAHs (PAH8) recently indicated (together with a subgroup of 4 PAHs) as the most suitable indicators of the presence of carcinogenic and genotoxic PAHs in foodstuffs (EFSA, 2008).

**Materials and methods**

**Reagents and standards**
All the solvents used were of HPLC grade (Sigma, St. Louis, MO, USA). Water was purified with a Milli-Q System (Millipore, Bedford, MA, USA). The standard PAH mixture (610M) in 1 ml of methanol/dichlorometane (Supelco, Bellefonte, PA, USA) consisted of: acenaphthene (Ac) (1000 µg/ml), fluoranthene (Fl) (200 µg/ml), naphthalene (Na) (1000 µg/ml), benzo[a]anthracene (BaA) (100 µg/ml), benzo[b]fluoranthene (BbF) (200 µg/ml), BaP (100 µg/ml), benzo[k]fluoranthene (BkF) (100 µg/ml), chrysene (Ch) (100 µg/ml), acenaphthylene (Ap) (2000 µg/ml), anthracene (A) (100 µg/ml), benzo[g,h,i]perylene (BghiP) (200 µg/ml), fluorene (F) (200 µg/ml), phenanthrene (Pa) (100 µg/ml), dibenz[a,h]anthracene (DBahA) (200 µg/ml), indeno[1,2,3-cd]pyrene (IP) (100 µg/ml), pyrene (P) (100 µg/ml). All the glassware was carefully washed and rinsed with distilled solvent (acetone and hexane) before use.

Samples

Raw fish (about 2.5 kg) and commercially smoked fish (about 1.5 kg) of four different species commonly consumed in Nigeria, namely mudfish (*Clarias gariepinus*, Claridae), croaker (*Pseudotolithus senegalensis*, Sciaenidae), mackerel (*Scomber scombrus*, Scombridae) and jackfish (*Selar crumenophthalmus*, Carangidae), were purchased from 4 different local fish vendors in Akure (Nigeria). The raw fish was gutted, cleaned and a part (about half) was placed over a wire gauze that was on burning hardwood charcoal (15 cm away from the hot hardwood charcoal ember). The mackerel and the jackfish were allowed to cook for 40 min on both sides, while the croaker and the mudfish were cooked for 90 min on both sides to obtain a greater level of drying. The usual way of commercial fish-smoking is done on wire gauze placed over steel drums fuelled by burning wood. This produces a lot of smoke which meets the products directly, without any filtering or washing of the smoke.

About 1 kg of suya meat and 2.5 kg of smoked dried antelope (“bush meat”) were purchased from each of three “suya spots” in Akure (Nigeria) and from two local vendors in Oja-Oba (Akure, Nigeria), respectively. Raw beef (about 1 kg) and antelope meat (about 2 kg) were purchased from 3 and 4 vendors in Akure, respectively. About half of the raw antelope meat and of the thinly-sliced raw beef meat (the latter brushed lightly with vegetable oil) was placed on a wire gauze over a hardwood charcoal fire and cooked on both sides slowly for 2 hours and 30 min, respectively.
All raw (A), laboratory smoked/grilled (B) and commercially smoked/grilled (C) samples were deboned (if necessary) and cut in small pieces. Samples from different vendors were pooled together to obtain representative samples (for A, B, and C) for each of 4 types of fish and 2 types of meat products analyzed. An aliquot of the sample so obtained (about 100 g) was lyophilized, milled, packed in aluminum foil wraps and stored in the freezer at -20°C prior to analysis.

**Apparatus and HPLC conditions**

A microwave extractor (Mars, CEM Corporation, Matthews, North Carolina, USA) able to process up to 14 sample simultaneously was used to extract PAHs from lyophilized samples. A Varian model 9010 HPLC gradient pump (Varian, Palo Alto, CA, USA) equipped with a Rheodyne 7161 injector with a 20 µL loop was used for analytical determination. A mobile phase consisting of acetonitrile and water (flow rate of 1 ml/min) was used for HPLC determination. The gradient elution program started with 40% acetonitrile (isocratic for 5 min), going linearly to 100% acetonitrile (total run time: 40 min). The column was a C18 reversed phase (Supelcosil LC-PAH), 250 x 3 mm ID, 5μm particle size (Supelco, Bellefonte, PA, USA) thermostatted at 38°C. The detector was a programmable spectrofluorometer (Jasco, model FP 1520, Cremella, Como, Italy) whose wavelength settings are reported elsewhere (Moret and Conte 2002). A second spectrofluorometer set at different wavelengths settings was connected in series with the former and used to confirm analyte identity (Varian spectrofluorometer, model 9070). Quantification was carried out by the external standard method.

**Sample preparation**

PAH extraction was carried out by applying the method described by Pena et al. (2006), with little modifications. Briefly, 400 mg of lyophilized sample were weighed in a teflon lined vessel (Green Chem plus, CEM Corporation), added with 1.6 mL of water, 8 mL of saturated methanolic potassium hydroxide and 20 mL of n-hexane. A microwave assisted saponification/extraction was carried out at 120 °C for 20 min. Once cooled, vessels were opened and a measured amount (15 mL) of the organic extract, corresponding (with a relative uncertainty of less than 2%) to 3/4th of the added amount of n-hexane, was withdrawn with a volumetric pipette and concentrated to a few microlitres using a rotavapor and then a nitrogen flow. In order to minimize volatile PAH losses (which resulted higher when the last portion of solvent was evaporated to dryness under nitrogen flow or under vacuum), the residual solvent was allowed to evaporate spontaneously to dryness at room temperature, and the residue was then dissolved with 400 µL of n-hexane. Sample purification was performed by solid phase
extraction (SPE). To speed up sample preparation reducing sample manipulation (avoiding the necessity to wash the test tube with additional solvent in order to realise a quantitative transfer of the sample extract onto the cartridge), a measured part of the sample extract (200 µL) was directly loaded on a 500 mg silica cartridge (Supelco), previously conditioned with 2 mL of dichloromethane and 2 mL of n-hexane. After sample loading, the PAH fraction was eluted with 3 mL of n-hexane/dichloromethane (70/30). The fraction so obtained was gently concentrated to a few microlitres under a nitrogen flow and then left to evaporate spontaneously to dryness at room temperature. The sample residue was dissolved in 100 µL of acetonitrile and directly injected into the HLPC apparatus. Two different aliquots of each sample underwent the entire analytical procedure (two replicates) and each replicate was injected twice. Analytical blanks (prepared in duplicate by subjecting all reagents and solvents, except the sample, to the entire procedure) were analysed (in duplicate) every day together with the samples and average data obtained were subtracted to those obtained from the real sample.

Results and discussion

Microwave assisted saponification and extraction

The method proposed by Pena et al. (2006), and applied for this work with little modifications (introduced for practical reasons), allowed us to perform a simultaneous saponification and extraction with minimal sample manipulation and solvent consumption. With respect to the conditions optimised by Pena and co-workers, the amount of sample and all the solvent volumes were doubled in order to assure that the temperature probe was properly dipped into the liquid phase. Extraction temperature and extraction time were also adjusted to avoid that at higher temperature the pressure inside the vessel exceeded the maximum allowable value (when it happens the system is automatically stopped for safety).

Method Performance

Linearity was verified by injecting in duplicate 7 diluted standard solutions in acetonitrile (in the range 0.1- 39.6 µg/kg) and plotting the mean peak area against PAH standard concentration. Regression coefficients ≥ 0.998 were obtained for all the PAHs. Limits of detection (LOD, s/n =3) and quantification (LOQ, s/n =10) lower than 0.1 and 0.3 µg/kg dry weight, respectively, were found for all PAHs (which corresponded to lower LOD and LOQ values when expressed on wet weight basis, depending on sample moisture content).
The precision of the method was estimated by performing 6 replicate analyses of the same sample for each one of the relevant matrices: fish (laboratory smoked mackerel) and meat (laboratory smoked antelope). Relative standard deviations (RSD%) lower than 10%, except for F and Pa (<23%) were obtained for all PAHs in both matrices.

Since a suitable reference material with certified content of the target analytes (to test the accuracy of the method) was not available for meat and fish products, a spiking procedure was used to calculate recoveries. A 10 g- aliquot of lyophilised raw meat and fish with a low amount of naturally occurring PAHs, was weighed in a round flask and slurred with 20 mL of \( n \)-hexane containing a known amount of PAH standard mixture (to obtain a final BaP concentration of 2 µg/kg and other quantified PAH concentrations ranging from 2 to 4 µg/kg). The mixture was efficiently mixed using a rotary evaporator without using the vacuum supply. The solvent was left to evaporate slowly under continuous stirring for 2 hours. After solvent removal the sample was left to age for 3 days in the darkness.

The recoveries (mean of 3 replicate analyses) were calculated by comparing the difference between spiked and unspiked sample with the known amount of PAHs added. Mean recoveries ranging from 78 to 108% (higher than 96% for PAH8) and from 75 to 101% (higher than 93% for PAH8) were found for meat and fish sample, respectively.

**PAH content in raw and smoked/grilled samples**

Table I reports concentrations (µg kg\(^{-1}\) dry weight) of each single PAH in both raw and processed samples. The dry weight of the samples was determined by oven-drying at 103±2 °C until a constant weight was achieved. PAH amounts are the average of duplicate analyses (two replicates for sample). Data were not corrected for recoveries. Due to their high volatility with consequent low recoveries and high coefficients of variation, data concerning Na e Ac were not reported. The sum of 5 low molecular weight PAHs (LMW-PAHs) (F, Pa, A, Fl and P), the sum of 8 high molecular weight PAHs (PAH8, including BaA, Ch, BbF, BkF, BaP, DBahA, BghiP and IP), the total PAHs (T-PAHs) and the LMW-PAHs/T-PAHs percentage ratios are reported, too. Results reported in table I are expressed on the basis of dry matter to exclude the diverse concentration effect due to the different loss of water occurring during food processing and to compare the data obtained to those reported by other authors on meat and fish products from the Nigerian market. Table II reports also BaP and PAH8 concentrations in smoked products on wet weight basis.
Raw samples (A) had LMW-PAHs ranging from 20.5 to 84.8 \( \mu g \ kg^{-1} \) dry weight (table I). Considering the widespread diffusion of these contaminants in the ambient air, it can not be excluded that, at least part of the contamination originated from food manipulation and processing (cutting, transport and storage) before and after cooking. PAH8 and BaP content varied between 2.1 and 19.3 and 0.1 and 0.7 \( \mu g \ kg^{-1} \) dry weight, respectively. Data on fish samples were considerably lower than those reported by other authors for samples from the Nigerian market (Akpan et al, 1994; Anyakora et al., 2008). Anyway our samples did not come from recognized polluted areas such as the Niger delta.

All the fish samples smoked or grilled on hardwood charcoal in the laboratory (B) had LMW-PAHs ranging from 113.5 to 209.3 \( \mu g \ kg^{-1} \) dry weight (on average 161.1 \( \mu g \ kg^{-1} \) dry weight), while the fish samples smoked by commercial vendors and purchased from the local market (C) had LMW-PAHs comprised between 152.1 and 936.4 \( \mu g \ kg^{-1} \) dry weight (on average 603.7 \( \mu g \ kg^{-1} \) dry weight). LMW-PAHs accounted for 77.5-90.0% of the total contamination in smoked/grilled products. Pa was found to be the most abundant compound, ranging from 30.9% to 58.5% of LMW-PAHs in all samples. Among PAH8, Ch and BaA, were found to be the most abundant PAH compounds. BaP contents in the range 0.9-3.4 and 3.0-38.0 \( \mu g \ kg^{-1} \) dry weight were found in samples B and C, respectively, while PAH8 varied from 15.4 to 43.9 \( \mu g \ kg^{-1} \) dry weight for samples B and from 44.1 to 197.2 \( \mu g \ kg^{-1} \) dry weight for samples C. Among fish samples, the mudfish and the croaker, which had higher quantities of high molecular weight PAHs, were smoked dried to nearly constant level of moisture, while the mackerel and jackfish were partially dried.

Fish smoked under controlled conditions contains generally about 0.1 \( \mu g \ kg^{-1} \) of BaP (Stolyhwo and Sikorsky, 2005). Compared to these data and those obtained from a number of surveys on smoked fish and meat products from the European market (Moret et al., 1999, Storelli et al., 2003, Watson et al., 2004, Yurchenko & Mölder, 2005, Duedahl-Olesen et al., 2006, EFSA 2008), which rarely reported BaP concentration exceeding 1 \( \mu g \ kg^{-1} \), samples from the Nigerian market resulted heavily contaminated. Nevertheless, BaP values found in commercial samples resulted in agreement or were slightly lower than those reported by Afolabi (1983), Alonge (1988) and Akpan et al. (1994) in Nigerian traditionally smoked fish and meat. Also data on suya meat well agree with those reported by Bababunmi et al. (1982) and more recently by Duke and Albert (2007).

The samples analyzed had all a similar PAH profile in agreement with the fact that the source of contamination was the same for all the samples. With the exception of jackfish (which had similar PAH content in both commercially and laboratory smoked samples), fish and meat samples from the market
(C) had considerably higher PAH load than the corresponding samples smoked or grilled in the laboratory (B). The reason for this could be the food smoking technique utilized for commercial samples which made use of wood fires instead of charcoal fire used for laboratory smoked samples. According to Larsson et al. (1983), the combustion of charcoal, being an already pyrolized material, gives a relatively clean smoke and, accordingly, lower PAH levels in the grilled sample.

Furthermore, in order to increase the shelf-life of the product, fish and meat vendors may re-smoke it many times until it is sold, thus contributing to increase PAH formation (Afolabi et al., 1983, Alonge, 1988). Histograms of figure 1 show average PAH concentrations as well as minimum and maximum values for both laboratory and commercially smoked/grilled fish and meat samples. It is interesting to note that in commercially processed samples PAH amounts vary within a wider range than laboratory processed products. This could be explained with the fact that, while laboratory samples were smoked under standardized conditions aiming to avoid the generation of high amount of PAHs, commercial samples were obtained by different vendors using different conditions which can result in different PAH load. Smoked/grilled samples had fat content, determined by the AOAC method (1990), ranging from 13 to 21%. No evident correlation between fat and PAH content was found, neither for commercial samples (C) nor for samples processed under the same conditions (B).

Figure 2 shows the HPLC traces of a raw fish sample (croaker) and the corresponding sample smoked in the laboratory.

*Compliance with the European legal limit and risk assessment*

To make a comparison with the legal limit settled for smoked fish and meat by the European Commission in 2005 and to calculate PAH intake, table II reports (for the only smoked/grilled products) BaP and PAH8 contents, expressed in µg kg⁻¹ wet weight. As we can see, 5 of the 6 commercially smoked/grilled samples contained BaP amounts exceeding the 5 µg kg⁻¹ limit, while none of the laboratory smoked samples exceeded it.

Table II reports also the percentage ratio of BaP to PAH8, as well as BaP and PAH8 intake (ng kg⁻¹ bw day⁻¹), calculated considering a daily consumption of 100 g of product (fish and/or meat) per person and assuming a reference body weight of 60 kg. We used the amount of 100 g product for risk assessment because it roughly accounts for total meat and fish consumption of Nigerian population (Yeh et al., 1996). Compared to the median European dietary exposure to PAHs for mean and high
dietary consumers, which varied between 3.9 and 6.5 ng kg\(^{-1}\) bw day\(^{-1}\) respectively for BaP alone, and between 28.8 and 51.3 ng kg\(^{-1}\) bw day\(^{-1}\), respectively for PAH8 (EFSA, 2008), results obtained for commercially smoked samples always lead to higher dietary exposure (without considering the possible contribution from other food items). Of course, higher health risk is associated to higher consumption levels.

The MOE approach was used for risk assessment. MOE is the ratio between a defined point on the dose-response curve (reference point) for the adverse effect of the compound in the animal carcinogenicity study and the estimated human intake of the compound. The reference point is calculated using the benchmark dose (BMD) approach, based on a mathematical modelling being fitted to the experimental tumour data within the observed range. It estimates the dose that causes a low but measurable response. The use of benchmark dose lower limit (BMDL\(_{10}\)) representing the lower bound of a 95% confidence interval on the BMD corresponding to a 10% tumour incidence was chosen as reference point on the dose-response curve (Larsen, 2006).

The ESFA Contaminant Panel calculated for BaP and PAH8 a BMDL\(_{10}\) of 0.07 and 0.49 mg kg bw\(^{-1}\), respectively (EFSA, 2008). As we can see in table II, using these BMDL\(_{10}\) values (considering a daily consumption of 100 g per person), MOE values lower than 10,000, were found in all commercially smoked samples for both BaP (except for the jackfish sample) and PAH8. The lowest values were found for the mudfish sample which presented MOE of 1,346 and 1,816 for BaP and PAH8, respectively.

**Conclusions**

Traditionally smoked and/or grilled fish and meat from the Nigerian market was found to be heavily contaminated, with BaP amounts often exceeding by far the limit of 5 µg kg\(^{-1}\) settled in 2005 by the European Commission. MOE values lower than 10,000, which according to EFSA (2008) indicate a potential concern for consumer health, were generally found in commercially smoked samples for both BaP and PAH8. This demonstrates the need for legal limits in traditionally smoked foodstuffs in Nigeria and possible risk management action. In agreement with previous works, it has been demonstrated that, the use of charcoal smoke instead of smoke from burning wood can help in lowering the final PAH load at levels around 2 µg kg\(^{-1}\).
References


Figure 1. Average PAH concentration (µg kg\(^{-1}\)) for laboratory (B) and commercially smoked/grilled (C) fish and meat samples. Bars indicate minimum and maximum values. For abbreviations see table I.

Figure 2. HPLC traces of a raw sample of croaker and of the corresponding sample smoked in the laboratory (using charcoal fire). For peak abbreviations see table I.
Table I. PAH content ($\mu$g kg$^{-1}$ dry weight) in raw, laboratory and commercially smoked/grilled fish and meat samples from the Nigerian market

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</table>

Data are mean of two replicate analyses (each one injected twice). A = raw samples; B = laboratory smoked/grilled samples; C = commercially smoked/grilled samples; n.q. = not quantifiable for the presence of interference. Abbreviations: fluorene (F), phenanthrene (Pa), anthracene (A), fluoranthene (Fl), pyrene (P), benz[a]anthracene (BaA), chrysene (Ch), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DBahA), benzo[g,h,i]perylene (BghiP), indeno[1,2,3-cd]pyrene (IP), LMW-PAHs = low molecular weight PAHs (F+Pa+A+Fl+P), PAH8 = BaA+Ch+BbF+BkF+BaP+DBahA+BghiP+IP; T-PAHs = total PAHs (LMW-PAHs + PAH8).
Table II. Risk assessment

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<th></th>
<th>Mudfish</th>
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<td>C</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>C</td>
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<tr>
<td><strong>BaP (µg kg⁻¹ wet weight)</strong></td>
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<td><strong>PAH8 (µg kg⁻¹ wet weight)</strong></td>
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<td>8,379</td>
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<td>23,386</td>
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</table>

Data are mean of two replicates analyses (each one injected twice). B = laboratory smoked/grilled samples; C= commercially smoked/grilled samples.

*Daily human exposure was calculated on the basis of amounts of 100 g fish or meat consumed daily.

**Assuming a reference body weight of 60 kg.