PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 23 (7) 1349-1352 July 2019

Carcinogenic and Genotoxicity of some PAHs in commonly consumed smoked fish (*Parachanna obscura* and *Ethmalosa fimbriata*)

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ABSTRACT: The dietary exposure of Polycyclic Aromatic Hydrocarbon (PAHs) and potential risk to human health was instigated in two different traditionally smoked species of fish (*Parachanna obscura* and *Ethmalosa frimbriata)* purchased from three markets in Benin City. Identification and quantitative analysis of PAHs components were achieved by Gas Chromatography/High Performance Liquid Chromatography. The result obtained showed that, Benzo(a)pyrene had an occurrence of 83.33% in all samples analysed. Risk assessment conducted using benzo(a)pyrene carcinogenic and mutagenic toxicity equivalent factor (TEQ & MEQ) showed slight to high risk (7.44 $x10^{-5}$ -1.95 $x10^{-3}$) and exceeded the USEPA guideline (1.0 x 10^{-5}) for potential Cancer. Levels of PAHs present in smoked fish prepared using traditional method may pose elevated cancer risks if consumed at high rates over many years.

DOI: https://dx.doi.org/10.4314/jasem.v23i7.24

Dates: Received: 18 May 2019; Revised: 21 June 2019; Accepted 11 July 2019

Keyword: Health Risk, *Parachanna obscura, Ethmalosa fimbriata,* PAHs

When food particularly meat, meat products and fish is smoked, roasted, barbecued, or grilled; PAHs are formed as a result of incomplete combustion or thermal decomposition of the organic materials (WHO, 2005). Pyrolysis of the fats in the meat/fish generates PAH that become deposited on the meat/fish. PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the meat/fish and the proximity of the food to the heat source (Phillips, 1999). Several analyses of charcoal roasted/grilled common food items have proven the presence of PAHs such as benzo[a]pyrene, anthracene, chrysene, benzo[a]anthracene, indeno[1,2,3-c,d]pyrene (Camargo *et al*., 2011). Most of these PAHs have been found to be carcinogenic while some are not (Pikuda and Ilelaboye, 2009). Traditional smoking techniques involve treating of pre-salted, whole or filleted fish with wood smoke in which smoke from incomplete wood burning comes into direct contact with the product, this can lead to its contamination with PAHs if the process is not adequately controlled or if very intense smoking procedures are employed (Gómez-Estaca *et al.,* 2011). Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke; mainly PAHs, derivatives of PAHs, such as nitro-PAH or oxygenated PAH and to a lesser extent heterocyclic amines (Stołyhwo and Sikorski, 2005Among PAHs, the benzo[a]pyrene (BaP) concentration has received particular attention due to its higher contribution to overall burden of cancer in humans, being used as a marker for the occurrence and effect of carcinogenic PAHs in food (Rey-Salgueiro *et al.,* 2009).Smoked fish may contribute significantly to the intake of PAHs if such foods form a large part of the usual diet. The primary purpose of this study is to identify and quantify the concentration levels and distribution of PAHs in smoked fish consumed by people in Benin City, Nigeria.

MATERIALS AND METHODS

*Sampling and Analysis***:** Locally smoked fish (about 5 g) of two different species commonly consumed in Benin city, namely *Ethmolosa fimbriata* (bonga fish) and *Parachanna obscura* (Traditionally called Ewi), were purchased from three different market centres from local vendors in Benin city, Edo state. The selected markets are major sources of smoked fish for most markets in Edo State. Samples were wrapped in aluminium foil, packed in labelled polythene bags and transported to the laboratory for analysis

Extraction: Extraction of PAHs was carried out based on the method described by Pena *et al*., (2006). 10 g of the homogenized fish sample was thoroughly mixed with anhydrous Na_2SO_4 to dehydrate the sample. 20

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ml of the extraction solvent (di-chloromethane) was added to the sample. Samples were covered with aluminium foil to prevent evaporation and sonicated to separate supernatants of extracts. Extracts were concentrated using an evaporator. Extracts were then cleaned up using a chromatographic column, moderately packed at the bottom with 1 cm glass wool. 2 g of silica gel and 1 cm of anhydrous Na_2SO_4 was added to the column while the column was pre-eluted with 20 ml dichloromethane. Extracts were then concentrated and collected in 2 ml vials.

Chromatographic analysis: Chromatographic analysis was carried out based on the method described Tongo *et al.,* (2017). The cleaned up extracts were analysed for benzo[b]fluoranthene, dibenzo[a,h]anthracene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[a]anthracene and indeno[1,2,3-cd]pyrene. Corresponding results were obtained using Gas chromatography (GC, Hewlett-Packard HP-5890 Series II with flame ionization detection (GC-FID)). The GC was programmed as follows: initial temperature of 60 °C for 2 min and ramped at 25 °C/min to 300 °C for 5 min and allowed to stay for 15 min giving a total of run time of 22 mins. A 2 µL volume splitless injection mode was used and the injection port temperature was set at 250 °C, while 300 °C was maintained for the injection port of the FID detector. A standard mixture of 17 priority PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno(1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene) was obtained and used for the analysis. Compounds were identified by comparing the retention time of standards with that obtained from the extracts and individual analysis of PAHs were used for quantitation

*Human Health Risk Assessment***:** WHO (2014) defines human health risk assessment is a process intended to estimate the risk to a given target organism, system or (sub) population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system (IPCS, 2004). The carcinogenic toxic equivalents (TEQs) was then obtained by summing the carcinogenic potencies of individual PAHs. TEFs are used to calculate toxic equivalent (TEQ), i.e. the sum of all individual congener's TEF multiplied by each congener's concentration in the mixture (Fan, 2014).

$$TEQ_{Bap} = \sum(TEFi \times Ci) \text{ --------- (1)}$$

TEF (Nisbet & LaGoy 1992)

$$MEQ_{Bap} = \sum(MEFi \times Ci) \text{--------- (2)}$$

MEF (Durant *et al*., 1996 & 1999)

$$\text{ADDC} = TEQ \times IR \times \frac{CF}{BW} \text{ ---------- (3)}$$

Where ADDC = Average Daily Dose of Carcinogenic (Mutagenic PAH IR= Ingestion Rate (65.5g/day), CF= Conversion Factor (0.001mg/µg), BW= Body Weight (70kg)

$$\text{HQ} = \text{Average daily dose } \left(\frac{ADD}{RFD}\right) \quad .(4)$$

Where HQ = Hard Quotient

The hazard index, which estimates the total risk from multiple contaminant pathways, was obtained by summing the HQ of the contaminant pathway (Equation 4). Risk was evaluated for both carcinogenic risk and genotoxicity. Values of HQ and HI of contaminants under one (1) are considered as safe (USEPA, 1986). The RFD (mg/kg/day) value adopted from USEPA, 2004

$$(HI) = \sum(HQ_1 + HQ_2 \ldots HQ_n) \ldots (5)$$

Where HI = Hazard Index

Statistical Data Analysis: Data analysis were performed using Microsoft Excel 7.0 program. Individual PAHs, Total PAHs (∑PAHs) and total carcinogenic PAHs (∑CPAHs) concentrations were summarized separately for each fish species using descriptive statistics (means, range, standard deviation, standard error). Statistical differences between individual PAH concentrations, low and high molecular weight PAHs, ring types, estimated daily intake (EDI), and carcinogenic potencies of individual PAH concentrations (B (A) Pteq), between the species were performed using Analysis of variance (ANOVA) at 0.05 level of significance.

RESULT AND DISCUSSION

In total, 60 samples prepared using traditional smoking methods were chemically analysed. Of the 16 PAHs analysed, 10 were consistently above WHO/EU limits in both fishes. These included Dibenzo(a,h)anthracene, Benzo(a)pyrene and Indeno(1,2,3-cd)pyrene, Fluorene, Benzo(g,h,i)perylene. PAHs with high molecular weights occurred more than the low molecular weight in all samples. Individual PAH levels ranged from < 1 –93 µg kg^{-1}. Benzo (a)pyrene was the most abundant

PAH found in all fish samples, this was followed by Benzo(b)fluoranthene, fluorene, Benzo(g,h,i)perylene and Benzo(k)fluoranthene . The summation of 5 congeners accounted for 75–80% of the total mass of PAHs measured across all smoked *Ethmalosa fimbriata* while 6 accounted for 87-89% of the ΣPAHs in *Parachanna obscura*. Risk values for the studied fishes prepared by traditional smoking reveals that 2 out of 200,000 adults are likely to suffer cancer in their 70 years life time. This implies that daily consumption of traditional smoked *Ethmalosa fimbriata* and *Parachanna obscura* for 70 years is likely to pose risk, because it is higher than USEPA (1993, 2009) carcinogenic limit of 1.0 x 10^{-5}. The Carcinogenic and Mutagenic equivalents recorded for both species of fish ranged (Carcinogenic: 1.96-14.2 and Mutagenic 3.91-8.47) respectively, these high levels of risk assessment lead to higher EQ_{BaP} daily dose in both fishes. Therefore the Mutagenic and carcinogenic risk involved in daily consumption of traditional smoked *Ethmalosa fimbriata* and *Parachanna obscura* for 70 years was calculated to be far >1.The result further reveals that 2 out 200,000 and 2 out of 2000 adults are likely to suffer cancer and non-cancer related diseases if they exposed to oral ingestion of traditional smoked *Ethmalosa fimbriata* and *Parachanna obscura* for 70 years on a daily bases. Non carcinogenic PAHs produced hazard >1, a level that can trigger the development of non-cancer health effects through oral ingestion.

Table 1: Risk Assessment (Carcinogenic Equivalent)

	Parachanna obscura			*Ethmalosa fimbriata*		
Carcinogenic Equivalency	A	B	C	A	B	C
Benzo(a)anthracence	0.31	0.15	0.03	0.15	ND	ND
Benzo(b)fluoranthene	0.08	ND	0.05	0.07	ND	0.27
Benzo(k)fluoranthene	0.04	0.07	0.05	0.08	0.05	0.07
Benzo(a)pyrene	4.20	2.61	2.40	2.29	2.30	ND
Indeno(1,2,3-cd)pyrene	0.21	0.27	0.81	0.96	0.65	0.72
Dibenzo(a,h)anthracene	7.92	1.35	7.26	10.3	11.2	0.90
?BaP-TEQ	12.8	4.45	10.6	14.0	14.2	1.96

Table 2: Risk Assessment (Mutagenic Equivalent)

Mutagenic Equivalency	*Parachanna obscura*			*Ethmalosa fimbriata*		
	A	B	C	A	B	C
Benzo(a)anthracence	0.25	0.13	0.03	0.13	ND	ND
Benzo(b)fluoranthene	0.63	0.35	ND	0.19	ND	0.68
Benzo(k)fluoranthene	0.41	0.82	0.53	0.96	0.59	0.73
Benzo(a)pyrene	4.23	2.61	2.40	2.29	2.30	ND
Indeno(1,2,3-cd)pyrene	0.65	0.84	2.51	3.02	2.03	2.24
Dibenzo(a,h)anthracene	2.30	0.27	2.11	2.98	3.24	0.26
ΣBaP-TEQ	8.47	5.02	7.57	8.16	8.16	3.91

Conclusion: The present study showed varying levels of PAHs in two smoked Fish in Edo State. Levels of PAHs present in smoked fish prepared using traditional method may pose elevated cancer risks if consumed at high rates over many years.

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