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Characterization of Carcinogens in traditionally smoked fishes popularly sold in River State, Nigeria

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ABSTRACT

A study was carried out on chemical hazards associated with traditional smoked fish. A total of eighteen smoked samples of two species of *Clarias gariepinus* and *Micromesistus potassou* were randomly selected from three major markets (Mile1 market, Choba market and Igwuruta market) in River State, Nigeria. Fish samples were aseptically homogenized and subjected to GC Analysis using Buck 530GC machine. The Benzo[a]pyrene [B(a)P] equivalent was used to determine risks associated with toxicity and mutagenicity of Polycyclic Aromatic Hydrocarbons (PAHs) found from the smoked fish samples. Results showed eleven targeted PAHs compounds were found at varied concentrations from the smoked fish samples. Six of the PAHs are non-carcinogenic while five were carcinogenic. The concentrations of Acephthylene was between 0.49 and 24.82 µg/kg, 2.39 to 5.22 µg/kg Anthrancene, 0.90 to 6.26 µg/kg Naphthalene, 2.29 to 16.67 µg/kg pyrene, 0.69 to 24.82 µg/kg Benzo(g,h,i)pyrene, 1.70 to 16.40 µg/kg Benzo(a)pyrene, 14.57 to 58.64 µg/kg Dibenzo(a,h)anthracene, 0.96 to 58.31 µg/kg Benzo(k)fluoranthene, 2.22 to 15.59 µg/kg Benzo(b)fluoranthene and 0.06 µg/kg 1,2-Banzanthracene in the two species of smoked fish. In conclusion the consumers of smoked fish in the study areas are exposed to PAHs and may be predisposed to danger or health related diseases.

Keywords: Toxicity, Mutagenicity, Food safety, Polycyclic Aromatic Hydrocarbon, Smoked fish

INTRODUCTION

A hazard is defined as anything that could contaminate food and cause illness or injury or could otherwise violate established food safety program criteria if left unchecked or uncontrolled (Local Public Health Institute of Massachussett, 2016). Hazards could be categorized as biological e.g. bacteria, fungi or viral, or Physical (glass, sand, dust) or chemical hazards e.g. Pesticides, asbestos, heavy metals, packaging materials, solvents, Polycyclic Aromatic Hydrocarbons (PAHs) etc. Food can be contaminated from environmental sources, industrial food processing and from certain home cooking practices (EFSA, 2008).

Recently there was an increasing concern globally on food safety against environmental compounds such as polycyclic aromatic hydrocarbons (PAHs); these classes of compounds pose a great threat to public health by inducing carcinogenic effect (Ikeogu et al., 2023; Silva et al. 2011). Agerstad and Skog (2005) referred to PAHs as environmental pollutant which are ubiquitous but generated as a result of incomplete combustion in traditional smoking process of food or pyrolysis of organic compound and during many industrial processes (Silva et al, 2011). The presence of PAHs during smoking process becomes a danger to public health when residues in food is above recommended level. Though human is exposed to PAHs not only thorough food alone but also from the environment thereby attracted more attention of food, public health and environmental experts globally. PAHs upon ingestion into the body system above recommended level and over a period of time or exposure to PAHs could cause cancer of liver and as well as kidney. Other consequences of PAHs in human are reproductive abnormalities (Ravindraa et al., 2008)

Traditional smoking is one of ancient techniques that still in use for preservation of food such as meat, fish, pupuru etc in developing Africa and Asia countries (SFMP, 2016). Smoking remains a common preservation method in many households, particularly in riverine areas of Nigeria, for various perishable foods. Smoking affects the sensory quality, shelf life, and wholesomeness of the product, offers diversified quality that gives high marketing values to smoked fish compared to fresh fish or other methods such as frying, boiling, grilling etc. (Silva et al., 2011; Gómez et al., 2009). Stołyhwo and Sikorski, (2005) stated that the composition of smoke and types and concentration of PAHs compound generated depend on variables in smoking process such as type of woods (hardwood, softwood and bagasse) and combustion temperature. Broadly PAHs occur in mixtures and sixteen of them has been monitored by the United States Environmental Protection Agency (USEPA) and Benzo(a)pyrene (BaP) has been fingered as the most studied and has been described by the International Agency for Research on Cancer (IARC)

as probable human carcinogen in 1987 (IARC, 1999). Thus, the determination of BaP has been widely used in environmental analysis as marker for the entire PAH content (Jimah and Ogiedu, 2021). Therefore, the objectives of this research work were focused on the assessment of the levels of PAHs in and it safety against permissible limit of PAHs in traditional smoked fishes sold in some selected markets in Rivers State East Senatorial District.

MATERIALS AND METHODS

Study area and samples procurement

Rivers East senatorial district is one of the three senatorial districts in Rivers State, with a population of 2,670,903 individuals at 2014. It has eight (8) local government areas which includes Emohua, Etche, Ikwerre, Obio-Akpor, Ogu Bolo, Okrika, Omuma and Port Harcourt Local Government Areas To ensure randomization, fish samples were selected using a stratified random sampling method. Each of the three markets was divided into sections based on vendor clusters. From each section, vendors were assigned numbers and selected randomly using a random number table. Two samples of each fish species were obtained from each selected vendor, ensuring replicates and minimizing bias. The selected traditional smoked fish species: Blue Whiting, locally known as Panla (*Micromesistus poutassous*) and African catfish (*Clarias gariepinus*) were procured from three (3) notable markets; Mile 1 in Portharcourt L.G.A, Choba in Obio/Akpor L.G.A and Igwuruta market in Ikwerre L.G.A, of Rivers East Senatorial District. Fish samples were placed in already labeled aseptic containers immediately after procurement.

Sample preparation

The smoked fish Sample was prepared in two stages: The first stage involved macerating the fish samples using laboratory porcelain while the second stage involved the use of chemical reagents for the dissolution of homogenized samples prior analysis.

Preparation of samples for GC analysis by soxhlet extraction method

Twenty grams (20g) of the homogenized sample was mixed with 60g of anhydrous sodium sulphate in agate mortar to absorb moisture. The homogenized sample was place in a 500ml beaker. Extraction with 300ml of n-hexane was carried out for 24hours. The crude extract that was obtained was evaporated using a rotary vacuum evaporator at 40°C in order to dry well. The residue that was obtained after evaporation was transferred with n-hexane into a 5ml florisil column to clean up.

Analysis of PAHs Extraction

The procedure described by Amos-Tautua et al, (2013) was used for extraction and identification of PAHs in smoked fish samples. The procedure involved pulverizing the flesh of the fish sample using a manual grinding machine and homogenized. Five grams (5g) of the pulverized sample was thoroughly mixed with 10g of anhydrous sodium sulphate in a mortar to absorb moisture. The homogenate was placed into an extraction thimble and covered with a Whatman filter paper (125 mm diameter).This was then inserted into a Soxhlet extraction chamber of the Soxhlet extraction unit. Extractions were then carried out with 50mL mixture of redistilled n-hexane and dichloromethane in the ratio 3:1 for effective recovery. Then crude extract was filtered through a layer of anhydrous sodium sulphate. The filtrate was evaporated to near dryness.

Clean up

The clean-up process of the extract was carried out using activated silica gel and anhydrous Na2SO. The silica gel column was prepared by loading an activated silica gel (12g) onto a chromatographic column (id=1cm). A 1g of anhydrous Na2SO4 was added to the top of the silica gel in the column. After conditioning the columns with 20mL hexane the sample was applied and eluted with 200ml of mixture of Dichloromethane: Hexane (3:1). The eluate was collected into an evaporating flask and rotary evaporated to near dryness. The dry eluate was then dissolved in 1mL n-hexane for Gas Chromatographic analysis.

Instrumental analysis

The polycyclic aromatic hydrocarbon analysis was carried out using gas chromatograph system. The system consisted of a Hewlett Packard Model 5890 gas chromatography (GC) equipped with a flame ionization detector (FID) and a data processor (Hewlett Packard, Wilmington, DE, USA). The column used was HP-1932530, a non-polar, fused-silica capillary column (30m length × 25µm inner diameter × 0.25µm film thickness). The oven temperature was set initially at 60°C (5min hold), increased to 250°C at 15°C/min. (14min hold), and a final temperature of 320°C at10ºC/min (4min hold). Nitrogen gas was used as the carrier gas at a flow rate of 1mL/min at a pressure of 30psi. The injector temperature was set at 250°C, injection volume was 1mL and the detector temperature was set at 320°C. Verification of peaks was carried out based on retention times compared to those of external PAHs standards.

Estimation of toxic and mutagenic potency of PAHs determined

The Benzo[a]pyrene [B(a)P] equivalent estimation described by Yusuf (2015) was used to determine toxic equivalency (TEFs) and mutagenic equivalency factor (MEFs). The TEFs (Yusuf, 2015; Nisbet and LaGoy, 1992) (Table 1) developed for a number of individual PAHs was used to expresses its potency relative to benzo[a]pyrene. The concentration of each of the individual PAH is multiplied by its TEF proposed and these values are summed to yield benzo[a]pyrene equivalent concentrations, TEQBaP (Yusuf, 2015). The mutagenicity of individual PAHs relative to B(a)P had also been computed using MEF (Table 1) proposed by Durant et al., (1999). The summed concentration of each individual PAH multiplied by the corresponding MEF is equal to mutagenic equivalents (MEQBaP).

$$TEQBaP = \Sigma(TEFi \times Ci) \quad \text{equation 1}$$

$$MEQBaP = \Sigma(MEFi \times Ci) \quad \text{equation 1}$$

Ci=measured individual PAHs concentrations for the 'ith' compound with the assigned TEFi or MEFi.

Statistical Analysis

Data collected was subjected to One Way Analysis of Variance (ANOVA) procedure using SPSS 20. 0 for windows and difference between mean value of treatment determined at $p<0.05$ level of significance using New Duncan's multiple range test.

RESULTS

Carcinogenic and non-carcinogenic PAHs content of smoked *Clarias gariepinus* from three markets is shown in Table 2**.** Ten (10) PAHs were found from smoked *Clarias gariepinus*, Moreover, the PAHs found in smoked *Clarias gariepinus* samples across the three markets were significantly difference ($p<0.05$). PAHs found include Acenaphthylene which ranged between 0.49 µg/kg (Choba market) and 24.82 µg/kg (Igwuruta market), 5.22 µg/kg Anthracene on Igwuruta fish sample, 0.90 – 6.26 µg/kg of Naphthalene, 2.29 µg/kg (Choba sample) to 16.67 µg/kg (Mile sample) of pyrene were found. Benzo(a)pyrene 1.70 µg/kg Igwuruta smoked fish sample and 0.96 to 4.98 µg/kg of Benzo(k)fluoranthene were found within the market studied. The concentration 2.22 – 2.72 µg/kg of Benzo(b)fluoranthene, 0.69 µg/kg Benzo(g,h,i)pyrene and 19.21 to 58.64 µg/kg Dibenzyl(a,h)Anhracene were found on the smoked *Clarias gariepinus* fish samples from Igwuruta, Mile 1and Choba markets respectively.

Table 1. Benzo[a]pyrene equivalent factors for carcinogenic (TEF) and mutagenic toxicity (MEF)

PAH COMPOUND	TEF	MEF
Naphthalene	0.001	-
Acenaphthylene	0.001	-
Acenaphthene	0.001	-
Fluorene	0.001	-
Phenanthrene	0.001	-
Anthracene	0.01	-
Fluoranthene	0.001	-
Pyrene	0.001	-
Benzo(a)anthracene	0.1	0.082
Crysene	0.001	0.017
Benzo(b)Fluoranthene	0.1	0.25
Benzo(k)Fluoranthene	0.01	0.11
Benzo(a)pyrene	1.0	1.0
Dibenzo(a,h)anthracene	1.0	0.29
Indeno(1,2,2-cd)pyrene	0.1	0.31
Benzo(g,h,i)perylene	0.01	-

Table 2. Carcinogenic and non-carcinogenic PAHs content of smoked *Clarias gariepinus* sold in some major markets in Rivers East Senatorial Zone

PAHs compounds Detected Conc(µg/kg)	Igwurut Market Area	Mile 1 Market Area	Choba Market Area	Mean Value	Standard Deviation	Least Significant Difference
Anthracene	5.22^{A}	ND	ND	1.74	3.01377	0.02041
Naphthalene	0.90^{C}	0.93^{B}	6.26^{A}	2.69	3.08597	0.05745
Pyrene	ND	16.67^{A}	2.29^{B}	6.32	9.03620	0.03512
Benzo(g,h,i)pyrene	0.69^{A}	ND	ND	0.23	0.39837	0.02449
Benzo(a)pyrene	1.70^{A}	ND	ND	0.57	0.98150	0.04082
Dibenzo(a,h)Anthra	ND	58.64^{A}	19.21^{B}	25.95	29.89537	0.05802
Benzo(k)\fluoranth	2.29^{B}	0.96^{C}	4.98^{A}	2.76	2.08078	0.043416
Benzo(b)fluoranthe	2.22^{B}	ND	2.72^{A}	1.65	1.44780	0.04619
1,2-Banzanthracene	ND	ND	0.06	0.06	0.0	0.0

ND = NOT DETECTED

PAHS compounds detected on some selected samples of smoked *Micromesistus potassou* sold in the study area is presented in Table 3. A total number of 10 PAHs were found from smoked *Micromesistus potassou,* However, the concentrations of PAHs found in smoked *Micromesistus potassou*

samples across the markets within the study areas were significantly difference ($p<0.05$). The PAHs content showed that the concentration of 0.67 to 1.17 µg/kg Acenaphthylene. 1.35 µg/kg Naphthylene, 0.96 to 4.30 µg/kg pyrene, 2.84 to 28.40 µg/kg of Benzo(g,h,i)perylene were found. In addition, 14.57µg/kg Dibenzyl(a,h)Anthracene, 1.70 to 58.31 µg/kg Benzo(k)fluoranthene and 6.31 to 16.40 µg/kg Benzo(a)pyrene were found from samples obtained from the three markets in the study area. Anthracene was between 2.39 µg/kg and 6.03 µg/kg while 0.52 µg/kg Phenanthrene was recorded from the samples collected from Igwuruta, Mile 1 and Choba markets respectively. The concentration of Benzo(b)fluoranthene was ranged from 2.49 µg/kg to 15.59 µg/kg,

Table 3. PAHs type and concentration found on some selected samples of smoked *Micromesistus potassou* sold in three selected major markets in Rivers East Senatorial Zone

PAHS compounds detected Conc. (µg/kg)	Igwuruta market area	Mile 1 market area	Choba market area	Mean Values	Standard Deviation	Least Significant Difference
Acenaphthylene	nd	1.17[a]	0.67[b]	0.61	0.58705	0.101732
Naphthylene	nd	nd	1.35[a]	0.45	0.77942	0.04082
Pyrene	4.30[a]	0.96[b]	nd	1.75	2.25711	0.02380
Benzo (g,h,i) perylene	28.40[a]	2.94[b]	nd	10.45	15.61738	0.04564
Phenanthrene	0.52[a]	nd	nd	0.17	0.30022	0.04899
Anthracene	6.03[b]	2.39[a]	nd	2.81	3.03652	0.03764
Dibenzo(a,h)anthracene	nd	nd	14.57[a]	4.86	8.41199	0.01225
Benzo(k)fluoranthene	nd	1.70[b]	58.31[a]	20.00	33.18543	0.04203
Benzo(a)pyrene	16.40[a]	6.31[b]	nd	7.57	8.27229	0.05686
Benzo(b)fluoranthene	15.59[a]	2.49[b]	nd	6.03	8.37514	0.03464

ND = NOT DETECTED

Toxicity assessment of PAHs of two species of smoked fish retail in some markets in Rivers East Senatorial Zone was presented in Table 4. The total toxicity equivalency (TEQ_{BaP}) for smoked *Clarias gariepinus* from three markets in the study areas ranged from 2.02972 to 58.6772 µg/kg. Sample collected from Mile 1 market had 58.6772 µg/kg of ΣBaP-TEQ followed by 19.5358 µg/kg Choba market and the least 2.02972 µg/kg ΣBaP-TEQ was smoked *Clarias gariepinus* fish sample from Igwuruta market. The total toxicity equivalency (TEQ_{BaP}) for smoked *Micromesistus potassuo* ranged between 6.61443 to 18.479 µg/kg. Smoked *Micromesistus potassuo* fish samples collected from Igwuruta, Mile 1 and Choba markets recorded 18.479, 6.61443 and 7.45 x 10-4 µg/kg ΣBaP-TEQ respectively.

Table 4. Toxicity assessment of PAHs on the basis of Benzo[a]pyrene equivalency of two species of smoked fish retail in some markets in Rivers East Senatorial Zone

PAHS compounds detected Conc. (µg/kg)	*Clarias gariepinus* Toxicity Equivalency (TEQ)			Smoked *Micromesistus potassou* Toxicity Equivalency (TEQ)		
	Igwuruta Market Area	Mile 1 Market Area	Choba Market Area	Igwuruta Market Area	Mile 1 Market Area	Choba Market Area
Acenaphthylene	$2.482x10^{-2}$	$1.0x10^{-2}$	$4.9x10^{-4}$	-	$1.17x10^{-3}$	$6.1x10^{-4}$
Anthracene	$5.22\ x10^{-2}$	-	-	$6.03\ x10^{-2}$	$2.39\ x10^{-2}$	-
Naphthalene	$9.0x10^{-4}$	$9.3x10^{-4}$	$6.26x10^{-4}$	-	-	$1.35x10^{-4}$
Pyrene	-	$1.667\ x10^{-2}$	$2.29\ x10^{-3}$	$4.3\ x10^{-3}$	$9.6\ x10^{-4}$	-
Benzo(g,h,i)pyrene	$6.9\ x10^{-3}$	-	-	$2.84\ x10^{-1}$	$2.94\ x10^{-2}$	-
Benzo(a)pyrene	1.70	-	-	16.40	6.31	-
Benzo(k)\fluoranthene	$2.29\ x10^{-2}$	$9.6\ x10^{-3}$	$5.04\ x10^{-2}$	$1.714\ x10^{-1}$	-	-
Benzo(b)fluoranthene	$2.22\ x10^{-1}$		$2.72\ x10^{-1}$	1.559	$2.49\ x10^{-1}$	
Dibenzo(a,h) Anthracene	-	58.64	19.21	-	-	-
1,2-Banzanthracene	-	-	$6.0\ x\ 10^{-4}$			
ΣBaP-TEQ	2.02972	58.6772	19.535806	18.479	6.61443	$7.45x10^{-4}$

Table 5 presented the Mutagenicity estimation of PAHs on the basis of Benzo[a]pyrene equivalency of smoked retail *Clarias gariepinus* and *Micromesistus potassou* in some markets in Rivers East Senatorial Zone. The total Mutagenicity for smoked *Clarias gariepinus* from three markets in the study areas ranged from 2.5069 to 17.1112 µg/kg. The total Mutagenicity for smoked *Micromesistus potassuo* ranged between 13.3466 to 20.4845 µg/kg. 6.8053 µg/kg and 17.1112 µg/kg were recorded for smoked *Clarias gariepinus* from Choba market and Mile 1 market respectively, while the least 2.5069 was recorded from fish gotten at Igwuruta Market.

Table 5. Mutagenicity estimation of PAHs on the basis of Benzo[a]pyrene equivalency of two species of smoked fish retail in some markets in Rivers East Senatorial Zone

PAHS compounds detected Conc. (µg/kg)	*Clarias gariepinus* Mutagenicity Equivalency (MEQ)			Smoked *Micromesistus potassou* Mutagenicity Equivalency (MEQ)		
	Igwuruta Market Area	Mile 1 Market Area	Choba Market Area	Igwuruta Market Area	Mile 1 Market Area	Choba Market Area
Acenaphthylene	-	-	-	-	-	-
Anthracene	-	-	-	-	-	-
Naphthalene	-	-	-	-	-	-
Pyrene	-	-	-	-	-	-
Benzo(g,h,i)perylene	-	-	-	-	-	-
Benzo(a)pyrene	1.70	-	-	16.40	6.31	-
Benzo(k)\fluoranthene	0.2519	0.1056	0.5544	0.187	6.4141	-
Benzo(b)fluoranthene	0.555	-	0.68	3.8975	0.6225	-
Dibenzyl(a,h) Anthracene	-	17.0056	5.5709	-	-	-
1,2-Banzanthracene	-	-	0.006	-	-	-
ΣBaP-MEQ	2.5069	17.1112	6.8053	20.4845	13.3466	-

Comparative mean concentration of PAHs between smoked *Clarias gariepinus* and *Micromesistus potassou* studied in this work was presented in Table 6. total PAHs of 162.93 µg/kg in *Micromesistus potassou* samples and 155.17 µg/kg in *Clarias gariepinus* samples. Dibenzyl (a,h) Anthracene 77.85 µg/kg was the highest recorded for *Clarias gariepinus* while Benzo(a)pyrene was recorded for *Micromesistus potassou* at 60.01 µg/kg.

Table 6. Comparative mean concentration of PAHs between fish samples from three markets

PAHs Compounds detected	*Clarias gariepinus* (µg/kg)	*Micromesistus potassou* (µg/kg)	T-test Value	Level of Significance
Acenaphthylene	35.71	0.67	22341.00	0.0
Anthracene	5.22	8.42	-238.77	0.003
Naphthalene	1.83	1.35	1446.16	0.0
Pyrene	18.96	5.26	3045.66	0.0
Benzo(g,h,i pyrene	0.69	31.34	-6812.33	0.0
Phenanthrene	-	0.52	-	-
Benzo(a)pyrene	1.7	60.01	-6999.00	0.0
Benzo(k)fluoranthene	8.21	22.71	-5901.00	0.0
Benzo(b)fluoranthene	4.94	18.08	-8759.00	0.0
Dibenzyl (a,h) Anthracene	77.85	14.57	24810.76	0.0
1,2-Banzanthracene	0.06	-	-	-
ΣPAHs	155.17	162.93	25883.66	0.0

DISCUSSION

Sixteen toxic chemical compounds contained in PAH standard mixture (95.9 to 99.9% purity) manufactured by AccuStandard Chemical Company USA that are major PAHs of concern in assessment of smoked food for public health safety (Yusuf, 2015) were used as target in this study. Ten (10) PAHs and 11 PAHs were found from smoked *Clarias gariepinus* and *Micromesistus potassou* respectively. The number of PAHs found in two species smoked samples from different market were closely related to eleven (11) PAHs smoked meat and fish samples reported by Sojinu *et al*. (2019) but rather lower than 15 PAHs compounds content of smoked snacks reported by Amos-Tautau *et al*., (2013). The number of PAHs found in the work corroborates the report of Yusuf (2015). Furthermore, six PAHs, including acenaphthylene, naphthylene, pyrene, Benzo(g,h,I) perylene, phenanthrene and anthrancene found in this work non-casinogenic while five (5) PAHs namely Benzo(a)pyrene, Benzo(k)fluoranthene, Benzo(b)fluoranthene, Dibenzo(a,h) anthrancene and 1,2-Banzanthracene were carcinogenic (Yusuf, 2015). Indication from the results suggested that the species of the fish, market location and handling style during sales may not necessarily contribute significantly on the number and concentration of PAHs found on smoked fish but heat source, flame intensity and temperature and particulate materials generated during smoking process could influence the types and concentration of PAHs in smoked foods (Silva *et al*, 2011, Wretling *et al*, 2010).

However, the concentrations of PAHs found in smoked fish samples across the markets within the study areas were significantly difference ($p<0.05$). PAHs found from smoked *Claria gariepinus* specie include Acenaphthylene which ranged between 0.49 µg/kg (Choba market) and 24.82 µg/kg (Igwuruta market), 5.22 µg/kg Anthracene on Igwuruta fish sample, 0.90 – 6.26 µg/kg of Naphthalene, 2.29 µg/kg (Choba sample) to 16.67 µg/kg (Mile sample) of pyrene were found. Benzo(a)pyrene 1.70 µg/kg Igwuruta smoked fish sample and 0.96 to 4.98 µg/kg of Benzo(k)fluoranthene were found within the market studied. The concentration 2.22 – 2.72 µg/kg of Benzo(b)fluoranthene, 0.69 µg/kg Benzo(g,h,i)pyrene and 19.21 to 58.64 µg/kg Dibenzyl(a,h)Anhracene were found on the smoked *Clarias gariepinus* fish samples from Igwuruta, Mile 1and Choba markets respectively.

The PAHs content in smoked *Micromesistus potassou* fish samples concentration range of 0.67 to 1.17 µg/kg Acenaphthylene. 1.35 µg/kg Naphthylene, 0.96 to 4.30 µg/kg pyrene, 2.84 to 28.40 µg/kg of Benzo(g,h,i) perylene were found. In addition, 14.57µg/kg Dibenzyl(a,h) Anthracene, 1.70 to 58.31 µg/kg Benzo(k)fluoranthene and 6.31 to 16.40 µg/kg Benzo(a)pyrene were found from samples obtained from the three markets studied in this work. Also, the concentration of Benzo(b)fluoranthene was ranged from 2.49 µg/kg to 15.59 µg/kg; Anthracene was between 2.39 µg/kg and 6.03 µg/kg while 0.52 µg/kg Phenanthrene was recorded from the samples collected from Igwuruta, Mile 1 and Choba markets respectively.

In all PAHs compounds content of smoked fish samples found in this work 10.04 to 28.82 µg/kg Acenaphthylene, 5.22 µg/kg Anthracene, 6.26 µg/kg Naphthalene and 16.67 µg/kg pyrene (non-carcinogenic) in smoked *Clarias gariepinus* fish samples were relatively above 5 µg/kg safety limit regulation recommended by European Commission (EU) (Sojinu *et al*., 2019; EU, 2006; EFSA, 2008; USEPA, 2000). However, those PAHs above the EU regulation are class of non-carcinogenic PAHs (Yusuf, 2015) this may allay public health concerns. The concentration between 19.21 µg/kg and 58.64 µg/kg of Dibenzyl(a,h) Anthracene was the only carcinogenic PAH found above regulation limit of EU. These results agreed with the findings of Sojinu et al., (2019) who reported range between 0.01ppm and 0.03ppm from smoked *Caria gariepinus* and *Tilapia guineensis* samples from different market in Abeokuta, Ogun State. Sojinu et al. (2019) further explained that high PAHs is associated with thick black carbon deposit on the skin of smoked food. Moreso, the types and concentrations of PAHs compounds that above EU regulation found from smoked *Micromesistus potassou* samples differ when compared with *Clarias gariepinus*. Benzo(g,h,i) perylene (28.40 µg/kg) and 6.03 µg/kg Anthracene were the two PAHs among non-carcinogenic that concentrations above 5.0 µg/kg (Euorean Union regulation). Four PAHs 14.57 µg/kg of Dibenzyl(a,h) Anthrancene, 58.31 µg/kg Benzo(k) fluoranthene, 16.40 µg/kg Benzo(a) pyrene and 15.59 µg/kg Benzo(b) fluoranthene were

concentration above regulation limit. These results could be attributed to the nature of fatty composition of the species of fish used in this work.

These findings are consistent with global reports on PAH contamination in smoked fish. In Sri Lanka, Jinadasa et al. (2020) found BaP concentrations in fish smoked with cinnamon wood reaching 2033.87 µg per 100 g—orders of magnitude above international limits. Similarly, Malika et al. (2017) reported Σ16 PAH concentrations in traditionally smoked fish from Sri Lanka ranging from 35.3 to 1489.63 µg/kg. In Ghana, Essumang et al. (2014) observed total PAHs in smoked mackerel, sardine, tuna, and cigar minnows ranging from 212.56 to 472.98 µg/kg (dry weight basis). In another study, smoked sardines (*Sardinella aurita*) from 12 coastal Ghanaian communities contained PAH levels as high as 1461.79 µg/kg, with BaP averaging 73.78 µg/kg (Essumang et al., 2012). These elevated values have been associated with rising cancer rates in those regions.

Gómez-Estaca et al. (2011) found similar trends in the Mediterranean, with sardines showing PAH levels of 618 µg/kg—much higher than the 107 µg/kg recorded in dolphinfish. Even in Europe, where regulations are stricter, Světlíková and Dobríková (2007) reported that average BaP concentrations over a ten-year period were highest in smoked fish (1.15 µg/kg), exceeding those found in smoked meats and poultry.

These international findings affirm the trend observed in this study: traditional smoking methods, particularly those lacking temperature control and regulated combustion conditions, produce smoked fish with high PAH concentrations. The use of open fires and untreated wood increases the formation of carcinogenic PAHs, as documented across Africa, Asia, and South America.

There is increasing public health safety concern worldwide on the danger pose by PAHs compounds in smoked foods when the concentration of occurrence is above recommended level (Ikeogu et al., 2023; Chen and Chen, 2001). Most of the cases of adverse effect of PAHs in humans were through smoked foods consumption. Exposure to PAHs over a period of time can affect the brain system, induce tumor development (cancer) in body organs such as liver and kidney (Ikeogu et al., 2023). It can also result to mutation, reproductive abnormalities and subsequently growth retardation. The findings in this work suggest that there is possibility of prevalent cancer and cancer related diseases that may affect public health in the study areas and by extension River State over a long period of exposure to PAHs without precautionary measures to eliminate or prevent future challenges.

The total toxicity equivalency (TEQ_{BaP}) for smoked *Clarias gariepinus* from three markets in the study areas ranged from 2.02972 to 58.6772 µg/kg while between 6.61443 to 18.479 µg/kg were estimated for *Micromesistus potassuo* specie of the smoked fish. Sample collected from Mile 1 market had 58.6772 µg/kg of ΣBaP-TEQ followed by 19.5358 µg/kg Choba market and the least

2.02972 µg/kg ΣBaP-TEQ was smoked *Clarias gariepinus* fish sample from Igwuruta market. Also, 18.479, 6.61443 and 7.45 x 10-4 µg/kg ΣBaP-TEQ were recorded from smoked *Micromesistus potassuo* fish samples collected from Igwuruta, Mile 1 and Choba markets respectively. The toxicity equivalency estimated from two species of smoked fish samples was higher than 1×10^{-5} carcinogenesis threshold risk unit recommended by USEPA (2009) except sample of *Micromesistus potassuo* collected from Choba market. This result aligned with the finding of Yusuf (2015) who reported higher toxicity equivalency (ΣBaP-TEQ) from traditional smoked fish sample above 1×10^{-5} carcinogenesis threshold safety limit set by USEPA (2000; 2009). The results of toxicity equivalency (TEQ) assessed of smoked fish samples further corroborates risk associated with PAHs, as their concentrations exceeded safety limits set by European Union (EU) (Sojinu et al., 2019, EFSA, 2008; European Union, 2006; USEPA, 2000).

The mutagenicity equivalency (MEQ) was estimated on PAHs that are carcinogenic compounds. The result showed that MEQ ranges between 2.5069 to 17.11 µg/kg ΣBaP-MEQ in smoked *Clarias gariepinus* and 13.3466 to 20.4845 µg/kg in *Micromesistus potassuo* fish samples from the three markets. Comparative mean concentration of PAHs between the two species of smoked studied revealed, a total PAHs of 162.93 µg/kg in *Micromesistus potassou* samples was slightly higher than 155.17 µg/kg in *Clarias gariepinus* samples. These values were relatively higher than 1×10^{-5} µg/kg limit for human safety. It could be concluded from these results that smoked fish samples sourced from the major three markets in Rivers East Senatorial district may pose moderate risk of toxicity and mutagenicity and may predispose consumer to chronic disease to the consumers over a long exposure to PAHs identified in this work.

PAHs are a group of toxic compounds known to have carcinogenic and mutagenic properties. The presence of PAHs above regulatory limits (such as those set by the European Union) in smoked fish samples raises concerns about public health risks associated with consuming these products.
Carcinogenic PAHs like Benzo(a)pyrene and Benzo(k)fluoranthene were found in concentrations exceeding safety thresholds, indicating a potential long-term health risk for consumers, including cancer and other adverse health effects. The study revealed that the concentrations of PAHs in both smoked fish species were significantly higher than recommended safety limits, posing potential health risks such as cancer, brain system effects, and reproductive abnormalities. The presence of carcinogenic PAHs at levels above regulatory limits underscores the need for improved monitoring and control measures in the smoking process.

The study observed differences in PAHs concentration between two fish species (*Clarias gariepinus* and *Micromesistus potassou*) and across different markets (Igwuruta, Mile 1, and Choba). These variations suggest that factors

such as fish species, market location, and smoking methods influence PAHs levels.

While fish species and market location did not significantly affect the number and types of PAHs detected, differences in smoking practices (heat source, flame intensity, temperature) did impact PAHs concentrations. This highlights the importance of monitoring and regulating smoking processes to mitigate PAHs formation. The study conducted toxicity equivalence (TEQ) and mutagenicity equivalency (MEQ) assessments to evaluate the overall health risks associated with PAHs in smoked fish. Results indicated that PAHs concentrations in some samples exceeded thresholds associated with increased carcinogenic and mutagenic risks, posing potential dangers to consumers.

To combat the challenges identified in this study regarding PAH contamination in traditionally smoked fish, several alternatives and measures can be implemented. One approach is to adopt safer smoking techniques, such as using indirect smoking methods or alternative fuels that produce fewer PAHs. Introducing modern smoking kilns with controlled temperatures and improved ventilation can significantly reduce PAH levels. Establishing stringent regulatory frameworks and continuous monitoring of PAH levels in smoked foods can ensure compliance with safety standards. Moreover, raising awareness among consumers about the risks of PAH exposure and promoting diversified preservation methods, such as drying or freezing, can further mitigate health risks. Investing in research and development to innovate and implement safer smoking technologies will also play a crucial role in building capacity and enhancing food safety in the region.

CONCLUSION

The findings of this study underscore a critical public health concern associated with traditional smoking methods used in food preservation in the Rivers East Senatorial District of Rivers State, Nigeria. The presence of high concentrations of PAHs, particularly carcinogenic ones, in smoked fish poses a significant risk to consumers, potentially leading to chronic diseases such as cancer over prolonged exposure. The concentrations of most of the PAHs found on the fish samples were relatively above the permissible carcinogenesis threshold level stipulated by European union and World Health Organization. Indication from toxicity and mutagenicity assessment also further reveals possibility of cancer or cancer related diseases over a long exposure to PAHs consumed through smoked fish.

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