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Dietary Exposure, Risk and Toxicological Evaluation of Polycyclic Aromatic Hydrocarbons in Some Grains (Beans, Soya Beans, Corn, Guinea Corn) from Markets in Nigeria

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Abstract: This study aimed at determining the; quantity of polycyclic aromatic hydrocarbons, PAHs in beans, soyabeans, corn and guinea corn consumed in South East Nigeria; source of emission; daily intake amount and health risks associated with their consumption among adult male and female individuals. Thirty six grain samples from markets in Eastern part of Nigeria were analyzed of 16 PAHs. The extraction and analysis were respectively by sonication and gas chromatography coupled flame ionization detector, GC-FID. The limif of detection, LOD and limit of quantification, LOQ obtained ranged from 0.03×10^{-3} µg/dm^3 - 0.09×10^{-3} µg/dm^3 and 0.09 x 10^{-3} µg/dm^3 - 0.25×10^{-3} µg/dm^3 respectively. The average recoveries ranged from 94.0% - 99.2%. The ∑16 PAHs concentrations ($\times 10^{-2}$µg/kg) detected ranged from 18.69±1.991 - 28.581±11.213. The mean total of HMW PAHs ranged from 12.673±5.554 - 20.792±2.437. The sum of eight probable carcinogenic PAHs detected ranged from 8.875±2.725 - 13.573±8.793. The mean dietary exposure to PAH for male adult individuals (90.95 µg/kg bw/day) was less than that of female (100.52 µg/kg bw/day) implying that adult female were more exposed. The margin of exposure, MOE values were greater than 10,000 indicating no health concern for risk management actions. The source determination revealed fuel combustion and petrogenic as PAHs emission sources. The TTEC for the cPAHs of the analyzed grains showed non toxicity of the samples. The PAHs detected in the samples were below 1.0 µg/kg which is the permissible limit established by EFSA and are considered safe for consumption.

Keywords: Margin of Exposure, Dietary Exposure, Grains, Polycyclic Aromatic Hydrocarbons, Gas Chromatography

1. Introduction

PAHs are a group of complex chemicals that are formed and released during incomplete burning of organic matter such as waste or food, during industrial processes and other human activities. They are also formed in natural processes such as carbonisation. Studies have shown toxicological, genotoxic and carcinogenic effects of PAHs [1]. The most important property driving PAH remediation is toxicity (or carcinogenicity). PAHs ranked 9th on the 2015 Agency of Toxic Substances and Disease Registry. [2]

The SCF recommended that, in view of the non-threshold effects of these genotoxic substances, the levels of PAHs in foods should be reduced to as low as reasonably achievable. Sixteen PAHs were identified as a priority due to their potential genotoxicity and/or carcinogenicity in humans. The two biggest contributors to the dietary exposure were found to be cereals and cereal products such as rice, guinea corn [3], maize [4-6] roasted plantain, [7] and pasta products [8], legumes [9] seafood and seafood products [10, 11] it should be noted however, that little data were available on foods with potentially high PAH content such as barbecued, smoked and roast meat products. [12, 13]

Humans can be exposed to PAHs through different routes.

For the general population, the major routes of exposure are from food and inhaled air, while in smokers, the contributions from smoking and food may be of a similar magnitude.

In food, PAHs may be formed during processing and domestic food preparation such as barbecuing, smoking, drying, roasting, baking, frying or grilling. Vegetables may be contaminated by the deposition of airborne particles and through being grown in contaminated soil. [14, 15]

For different food categories and subcategories, the data on PAH8, PAH4 and PAH2 were then used for the exposure calculation as well as the estimation of margins of exposure (MOEs) based on the bench mark dose lower confidence limit for a 10% increase in the number of tumour bearing animals compared to control animals ($BMDL_{10}$).

Maize was introduced into Africa in the 16^{th} Century and has since become one of Africa's dominant food crops. Maize is processed and prepared in various forms depending on the country. Ground maize is prepared into porridge in Eastern and Southern Africa, while maize flour is prepared into porridge in West Africa. In all parts of Africa, green (fresh) maize is boiled or roasted on its cob and served as a snack. Popcorn is also a popular snack. Maize is the most important cereal crop in sub-Saharan Africa (SSA) and an important staple food for more than 1.2 billion people in SSA and Latin America [16]. In industrialized countries, maize is largely used as livestock feed and as a raw material for industrial products.

Sorghum (Guinea corn) has been, for Centuries, one of the most important staple foods for millions of poor rural people in the semi-arid tropics of Asia and Africa. Sorghum remains a principal source of energy, protein, vitamins and minerals. It is usually grown without application of any fertilizers or other inputs by a multitude of small-holder farmers in many countries. [17] Guinea corn is a source of starch and alcohol. The green leaves and grains are used for livestock food. Also, its dry stems are used for fuel, baskets, mats, roof and fences. It is typically ground into meal and made into bread.

Beans are among the most versatile and commonly eaten foods throughout the world, and many varieties are grown in the U.S. because of their nutritional composition. These economical foods have the potential to improve the diet quality and long-term health of those who consume beans regularly. Dry edible beans contain a variety of vitamins, minerals and other nutrients while providing a moderate amount of calories. Beans provide protein, fiber, folate, iron, potassium and magnesium while containing little or no total fat, trans-fat, sodium and cholesterol. The dietary fibre content of beans may play a role in reducing the risk of colorectal cancers.

Soya beans are considered by many Agencies to be a source of complete protein for vegetarians and vegans or for people who want to reduce the amount of meat they eat. According to the US Food and Drug Administration [18], soy protein products can be good substitutes for animal products because, unlike some other beans, soy offers a 'complete' protein profile.

The majority of the soya bean crop is processed into oil and meal. Oil extracted from soya beans is made into shortening, margarine, cooking oil, and salad dressings. Soy oil is also used in industrial paint, varnishes, caulking compounds, linoleum, printing inks and other products. It is also used in pharmaceuticals and protective coatings. Different studies have reported PAHs contamination in various foods such as rice, maize, beans, soya beans, bambara groundnut, guinea corn, pigeon peas, edible oil, vegetables, meat, fish, soil, air, water. These grains (beans, soya beans, corn and guinea corn) being staple foods that are commonly consumed in Nigeria supposed to be regularly analyzed to ensure its safety from PAHs contamination, hence this study.

2. Materials and Methods

2.1. Materials and Reagents

The reagents which included; hexane, dichloromethane, activated alumina were of analytical grade. Four PAHs surrogate standard mixtures-acenaphthalene d_{10}, chrysene d_{12}, phenathrene d_{10} and perylene d_{12} were purchased from Sigma Aldrich U S A.

Different types of beans, soya beans, corn and guinea corn were bought from some markets in Anambra and Enugu states, Nigeria.

2.2. Equipment and Instruments

Gas chromatography/flame ionization detector (HP 6890 Powered with HP Chem Station), rotary evaporator, borosilicate beaker, glass column, sonicator.

2.3. Sampling

Thirty six grain samples which included different types of beans (Potiskum, Nija red, Iron beans (white) Iron beans (brown), Gausau), soya beans (White and Black), corn (Yellow, White, Pop corn) and guinea corn (Red and White) were purchased from some major markets in Enugu and Anambra states of Nigeria. The markets included New market, Gariki market and Ogbete main market in Enugu East, Enugu South and Enugu North Local Government Areas of Enugu State respectively, Nsukka main market in Igboetiti Local Government Area, Awka central market in Awka South Local Government Area of Anambra State, Umunze main market in Orumba South L. G. A. of Anambra State. The samples were picked to remove sand and other impurities, ground and put in labeled amber sample bottles ready for extraction. The locations study area Anambra and Enugu states are shown in Figure 1.

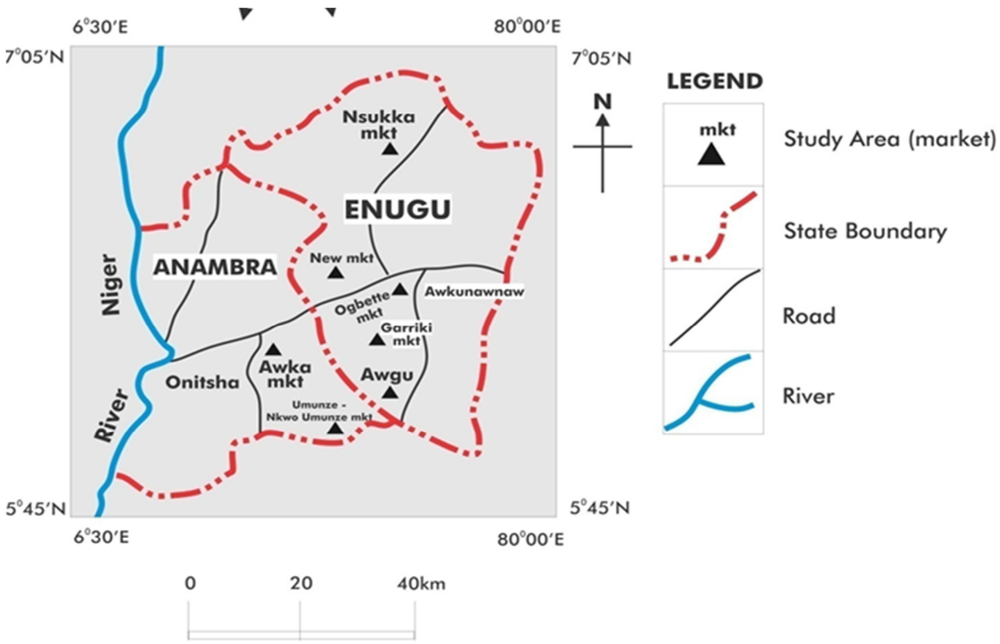

Figure 1. Map of the Area for Sample collection.

2.4. PAHs Determination in the Samples

All samples were each analyzed using GC/FID under the described instrument parameters. The initial oven temperature programme was 60°C for 5 min, raised to 250°C, first at a rate of 15°C/min and maintained at this temperature for 14 min and at second rate of 10°C/min for 5 min.

Nitrogen gas was used as the carrier gas at the flow rate of 1 cm^3/min at a pressure of 30 psi.

Retention time was used to characterize each PAH.

2.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD was determined by continuous dilution and analysis of standard solution until the least concentration was obtained at the signal to noise ratio of 3. Likewise LOQ was determined by continuous dilution and analysis of standard solution until the least concentration was obtained at signal to noise ratio of 10.

2.6. Statistical Analysis

The statistical analysis of the data obtained in different samples was carried out using SPSS version 16.00 to calculate analysis of variance, homogeneity of studied samples, level of significance at 95% confidence and Pearson correlation coefficient.

2.7. Daily Estimated Intake

The dietary intakes of the 16 PAHs were estimated, using a deterministic approach. A fixed value for the consumption of an individual food was multiplied by a fixed value for the contaminant concentration in that food [19] The total exposure was obtained by summing the intakes from all foods, using the following equation:

$$\text{Estimated daily Intake (EDI)} = \frac{\sum \text{Consumption rate} \times \text{Occurrence}}{\text{Body Weight}}$$

Body weight for adult female = 60 Kg and body weight for adult male = 70 Kg were assumed [20].

2.8. Risk Assessment

For risk assessment, the margin of exposure approach (MOE) as adopted by the EFSA Scientific Committee in the Opinion related to substances which are both genotoxic and carcinogenic [21].

The uncertainty in the assessment objectives is considered to be negligible. Margins of exposures (MOEs) were calculated by dividing the lowest $BMDL_{10}$ values among the models with acceptable fits by the mean and high level estimates of dietary exposure to benzo[*a*]pyrene, PAH2, PAH4 and PAH8. However, for high level consumers the MOEs are close to or less than 10,000, which as proposed by the EFSA Scientific Committee [21] indicates a potential concern for consumer health and a possible need for risk management action. But for lower level consumers, the MOEs of 10,000 or higher would be of low concern for human health and might be considered low priority for risk management actions [22].

The risk was estimated using the Margin of Exposure (MOE) approach according to the following equation [23]:

$$\text{MOE} = \frac{\text{BMDL}_{10}}{\text{EDI}}$$

Where BMDL10 is the benchmark dose lower confidence limit at 10% incidence level. Considering a BMDL10 of 0.07, 0.17, 0.34 and 0.49 all in mg/kg bw per day for BaP, PAH2, PAH4 and PAH8, respectively, for adult and children scenario, where:

BaP = Benzo[a] pyrene

PAH2 = Benzo [a]pyrene and chrysene

PAH4 = Benzo [a]anthracene, benzo[a] pyrene, benzo [b] fluoranthene and chrysene

PAH8 = The sum of eight carcinogenic PAHs: benzo [a] anthracene; benzo [b]fluoranthene; benzo [k] fluoranthene; benzo[g,h,i]perylene; benzo [a]pyrene; chrysene; dibenz[a,h]anthracene; and indeno[1,2,3-C,d] pyrene. [24]

2.9. Toxic Equivalency Factor (TEF)

The Total Toxic Equivalence Factor (TTEF) for each analyzed sample was calculated from the concentrations of cPAHs obtained. The mathematical expression to determine the toxicity equivalent concentration is given thus:

Total Toxicity Equivalence Concentration (TTEC) or TEQ $=\sum Cn \times TEFn$

Where; TTEC = Total Toxicity Equivalent Concentration,

TEQ = Total Toxicity Equivalence

C_n = Concentration of the individual cPAH in the mixture

TEF_n = Toxic equivalent factor of the individual cPAH associated with its respective mixture

PAH Diagnostic Ratios Analysis

The sources of the PAHs detected in this study will be calculated using PAH diagnostic ratios of Ant/(Phe+Ant), Fla/(Pyr+Fla), I[cd]P/(I[cd]P+B[ghi]P) and B[a]A/B[a]A +Chr.

3. Result and Discussion

3.1. Result

Table 1. *LOD and LOQ.*

PAH	LOD (μg/dm^3)	LOQ (μg/dm^3)
Naphthalene	0.00004	0.00011
Acenaphthylene	0.00003	0.0001
Acenaphthene	0.00004	0.00012
Fluorene	0.00003	0.00009
Phenanthrene	0.00005	0.00014
Anthracene	0.00004	0.00013
Fluoranthene	0.00005	0.00015
Pyrene	0.00006	0.00018
Benzo[a]anthracene	0.00008	0.00022
Chrysene	0.00007	0.00019
Benzo[b]fluoranthene	0.00007	0.0002
Benzo[k]fluoranthene	0.00006	0.00017
Benzo[a]pyrene	0.00009	0.00025
Indeno[1,2,3-cd]pyrene	0.00005	0.00014
Dibenzo[a,h]anthracene	0.00005	0.00013
Benzo[g,h,i]perylene	0.00003	0.00008

LOD= Limit of Detection; LOQ= Limit of Quantitation.

Table 2. *Mean concentration (× 10 μg/kg) of polycyclic aromatic hydrocarbons in types of beans.*

Mean Concentration (× 10^{-2} μg/kg) of Polycyclic Aromatic Hydrocarbons in Types of Beans from Markets in Eastern Nigeria					
	POTISKUM	WHITE IRON BEANS	BROWN IRON BEANS	GAUSAU	NIJA RED
PAH	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD	MEAN± SD
Naphthalene	0.031 ± 0.00	10.026 ± 0.008	0.0207 ± 0.012	0.024 ± 0.009	0.030 ± 0.006
Acenaphthylene	0.081 ± 0.05	70.095 ± 0.075	0.103 ± 0.047	0.154 ± 0.187	0.099 ± 0.059
Acenaphthene	1.314 ± 1.81	00.688 ± 0.711	2.661 ± 2.140	1.068 ± 1.630	0.602 ± 0.408
Fluroene	0.126 ± 0.18	30.107 ± 0.152	0.226 ± 0.214	0.171 ± 0.260	0.071 ± 0.087
Phenanthrene	4.889 ± 4.21	54.49 ± 3.020	5.097 ± 3.365	5.229 ± 3.823	3.945 ± 1.149
Anthracene	3.423 ± 2.88	53.437 ± 2.559	4.482 ± 3.095	4.415 ± 2.977	4.832 ± 1.185
Fluoranthene	0.713 ± 0.39	80.772 ± 0.439	1.946 ± 1.272	1.190 ± 1.067	0.954 ± 0.344
Pyrene	2.843 ± 2.83	63.026 ± 3.065	1.708 ± 0.348	3.022 ± 2.737	4.744 ± 1.781
Benzo[a]anthracene	2.611 ± 2.15	62.016 ± 2.070	2.229 ± 0.619	2.870 ± 2.407	3.747 ± 1.458
Chrysene	1.106 ± 1.12	61.255 ± 1.354	3.918 ± 2.747	1.729 ± 2.184	1.100 ± 0.788
Benzo[b]fluoranthene	0.389 ± 0.11	30.421 ± 0.145	0.724 ± 0.406	0.514 ± 0.204	0.523 ± 0.051
Benzo[k]fluoranthene	0.435 ± 0.07	10.421 ± 0.044	0.390 ± 0.039	0.397 ± 0.093	0.398 ± 0.043
Benzo[a]pyrene	4.463 ± 2.36	24.486 ± 2.243	4.046 ± 0.591	4.798 ± 1.728	5.071 ± 1.242
Indeno[1,2,3-cd]pyrene	0.051 ± 0.04	30.049 ± 0.049	0.133 ± 0.098	0.066 ± 0.069	0.101 ± 0.088
Dibenzo[a,h]anthracen e	0.032 ± 0.02	40.031 ± 0.020	0.051 ± 0.031	0.097 ± 0.107	0.062 ± 0.038
Benzo[g,h,i]perylene	0.228 ± 0.10	20.195 ± 0.092	0.305 ± 0.190	0.255± 0.124	0.297 ± 0.038
∑16 PAHs	22.735 ± 7.7	921.516 ± 7.42 9	28.04 ± 11.10	25.10 ± 9.845	26.576 ± 3.513
∑LMW PAHs	9.864 ± 6.69	48.842 ± 4.743	12.59 ± 8.068	11.061 ± 7.49 7	9.579 ± 1.486
∑HMW PAHs	12.871 ± 5.8	912.673 ± 5.55 4	15.45 ± 3.976	14.937 ± 5.09 5	16.998 ± 3.129
PAH2	5.569 ± 1.23	75.741 ± 0.903	7.964 ± 2.450	6.527 ± 0.721	6.172 ± 0.718
PAH4	8.568 ± 3.30	18.179 ± 2.637	10.918 ± 2.727	9.911 ± 2.518	10.442 ± 1.998
PAH8	9.315 ± 3.35	78.875 ± 2.725	11.796 ± 3.018	10.726 ± 2.77 9	11.300 ± 1.971

Table 3. *Mean concentration (× 10^{-2} μg/kg) levels of polycyclic aromatic hydrocarbons in types of soyabeans from markets in eastern Nigeria.*

PAHs	White Soyabean	Black Soyabean
Naphthalene	0.051±0.042	0.079±0.013
Acenaphthylene	0.08±0.112	0.195±0.048
Acenaphthene	0.615±0.654	0.651±0.016
Fluorene	0.086±0.120	0.162±0.035
Phenanthrene	2.024±1.372	1.943±0.0389
Anthracene	4.032±2.466	2.033±0.943
Fluoranthene	0.761±0.420	1.252±0.231

PAHs	White Soyabean	Black Soyabean
Pyrene	4.324±3.501	1.674±1.249
Benzo[a]anthracene	3.796±2.654	2.065±0.817
Chrysene	0.995±1.322	2.463±0.692
Benzo[b]fluoranthene	0.446±0.110	0.716±0.127
Benzo[k]fluoranthene	0.328±0.182	0.292±0.018
Benzo[a]pyrene	4.961±2.482	3.453±0.711
Indeno[1,2,3-cd]pyrene	0.017±0.007	0.375±0.168
Dibenzo[a,h]anthracene	0.027±0.004	0.135±0.051
Benzo[g,h,i]perylene	0.347±0.202	1.205±0.404
∑16 PAHs	22.893±6.389	18.69±1.991
∑LMW PAHs	6.889±1.522	5.062±0.869
∑HMW PAHs	16.004±6.633	13.629±1.122
PAH2	5.956±1.162	5.916±0.020
PAH4	10.198±3.756	8.697±0.709
PAH8	10.918±3.688	10.703±0.103

Table 4. *Mean concentration (× 10^{-2} µg/kg) levels of polycyclic aromatic hydrocarbons in types of corns from markets in eastern Nigeria.*

PAHs	White Corn	Yellow Corn	Pop Corn
Naphthalene	0.029±0.021	0.028±0.0170	0.027±0.0191
Acenaphthylene	0.033±0.026	0.023±0.0189	0.032±0.0276
Acenaphthene	1.845±3.587	1.807±3.676	0.269±0.248
Fluorene	0.097±0.178	0.072±0.138	0.022±0.0184
Phenanthrene	1.854±1.684	2.388±1.656	1.182±0.370
Anthracene	5.56±5.842	4.457±5.704	4.467±4.0418
Fluoranthene	0.799±0.865	0.794±0.844	0.553±0.393
Pyrene	4.483±0.854	3.79±0.783	6.461±5.192
Benzo[a]anthracene	3.757±1.782	5.281±1.924	3.787±1.665
Chrysene	2.54±4.863	2.023±3.764	0.231±0.136
Benzo[b]fluoranthene	0.387±0.195	0.338±0.197	0.609±0.363
Benzo[k]fluoranthene	0.452±0.334	0.342±0.233	0.441±0.328
Benzo[a]pyrene	5.095±2.413	5.193±2.268	5.508±3.453
Indeno[1,2,3-cd]pyrene	0.073±0.119	0.072±0.115	0.021±0.0106
Dibenzo[a,h]anthracene	0.098±0.175	0.093±0.167	0.029±0.0247
Benzo[g,h,i]perylene	0.249±0.167	0.233±0.124	0.169±0.203
∑16 PAHs	27.252±23.105	26.933±21.629	23.84±16.493
∑LMW PAHs	9.385±11.339	8.7753±11.209	5.998±4.725
∑HMW PAHs	17.867±11.766	18.153±10.421	17.84±11.768
PAH2	7.57±7.276	7.215±6.032	5.739±3.589
PAH4	11.713±9.253	12.833±8.153	10.16±5.617
PAH8	12.585±10.047	13.573±8.793	10.82±6.183

Table 5. *PAHs Concentration (× 10^{-2}µg/kg) in Guinea Corn.*

PAHs	Red	White
Naphthalene	0.026 ± 0.001	0.03 ± 0.008
Acenaphthylene	0.036 ± 0.008	0.036 ± 0.008
Acenaphthene	0.193 ± 0.069	0.166 ± 0.066
Fluorene	0.018 ± 0.01	0.027 ± 0.022
Phenanthrene	1.838 ± 1.768	1.891 ± 1.68
Anthracene	4.121 ± 2.142	5.636 ± 3.884
Fluoranthene	0.56 ± 0.159	0.724 ± 0.220
Pyrene	5.592 ± 0.652	6.679 ± 2.874
Benzo[a]anthracene	4.046 ± 2.397	4.915 ± 1.510
Chrysene	0.233 ± 0.051	0.294 ± 0.043
Benzo[b]fluoranthene	0.384 ± 0.120	0.51 ± 0.218
Benzo[k]fluoranthene	0.399 ± 0.127	0.515 ± 0.227
Benzo[a]pyrene	6.356 ± 0.045	6.741 ± 0.264
Indeno[1,2,3-cd]pyrene	0.02 ± 0.005	0.026 ± 0.001
Dibenzo[a,h]anthracene	0.026 ±0.006	0.04 ± 0.023
Benzo[g,h,i]perylene	0.222 ± 0.006	0.351 ± 0.164
∑16 PAHs	24.07±7.56	28.581±11.213
∑LMW PAHs	6.217±0.442	7.78±2.309
∑HMW PAHs	17.837±1.44	20.792±2.437
PAH2	6.59±0.0962	7.034±0.221
PAH4	11.019±2.374	12.459±1.072
PAH8	11.685±2.252	13.391±0.657

Table 6. *Estimated daily intake, EDI (× 10^{-2} µg/kg/bw/day) 0f adult male individuals on beans, soyabean, corn and guinea corn.*

GRAIN	BaP	PAH2	PAH4	PAH8
BEANS				
Potiskum	1.148	1.432	2.203	3.395
White Iron	1.154	1.476	2.103	2.282
Brown Iron	1.04	2.048	2.807	3.033
Gausau	1.234	1.678	2.549	2.758
Nija Red	1.304	1.587	2.685	2.906
SOYABEAN				
White Soyabean	1.134	1.361	2.331	2.496
Black Soyabean	0.789	1.352	1.988	2.446
CORN				
White Corn	1.165	1.947	3.012	3.236
Yellow Corn	1.335	1.855	3.3	3.49
Pop Corn	1.416	1.476	2.613	2.782
GUINEA CORN				
Red Guinea corn	0.726	0.753	1.259	1.335
White Guinea corn	0.77	0.804	1.424	1.53

Table 7. *Estimated daily intake, EDI (× 10^{-2} µg/kg/bw/day) 0f adult female individuals on beans, soyabean, corn and guinea corn.*

GRAIN	BaP	PAH2	PAH4	PAH8
BEANS				
Potiskum	1.265	1.578	2.428	2.639
White Iron	1.271	1.627	2.317	2.515
Brown Iron	1.146	2.256	3.093	3.342
Gausau	1.359	1.849	2.808	3.039
Nija Red	1.437	1.749	2.959	3.202
SOYABEAN				
White Soyabean	1.158	1.39	2.39	2.548
Black Soyabean	0.806	1.38	2.029	2.497
CORN				
White Corn	1.529	2.271	3.514	3.776
Yellow Corn	1.558	2.165	3.85	4.072
Pop Corn	1.652	1.722	3.048	3.246
GUINEA CORN				
Red Guinea corn	0.847	0.879	1.469	1.558
White Guinea corn	0.899	0.938	1.661	1.785

Table 8. *Margin of exposure, moe for adult male exposed individuals on beans, soyabean, corn and guinea corn.*

GRAIN	BaP	PAH2	PAH4	PAH8
BEANS				
Potiskum	60975	118715	154335	144329
White Iron	60659	115176	161674	214723
Brown Iron	67308	83007	121126	161556
Gausau	56726	101311	133386	177665
Nija Red	53681	107120	126629	168617
SOYABEAN				
White Soyabean	61728	124908	145860	196314
Black Soyabean	88720	125740	171026	200327
CORN				
W hite Corn	60086	87314	112882	151422
Yellow Corn	52434	91644	103030	140401
Pop Corn	49435	115176	130119	176132
GUINEA CORN				
Red Guinea corn	96419	225764	270056	367041
White Guinea corn	90909	211443	238764	320261

Table 9. *Margin of exposure, moe for adult female exposed individuals on beans, soyabean, corn and guinea corn.*

GRAIN	BaP	PAH2	PAH4	PAH8
BEANS				
Potiskum	55336	107731	140033	185676
White Iron	55075	104487	146741	194831
Brown Iron	61082	75355	109926	146619
Gausau	51508	91942	121083	123769
Nija Red	48713	97198	114904	153029

GRAIN	BaP	PAH2	PAH4	PAH8
SOYABEAN				
White Soyabean	60449	122302	142259	192308
Black Soyabean	86849	123188	167570	196235
CORN				
White Corn	45782	62731	96756	129767
Yellow Corn	44929	78522	88312	120334
Pop Corn	42373	98722	111549	218166
GUINEA CORN				
Red Guinea corn	82644	193402	231450	314506
White Guinea corn	77864	181237	204696	274510

Table 10. *Source determination.*

GRAIN	ANT/ANT + PHE	FLA/FLA + PYR	I[cd]P/I[cd]P +B[ghi]P	B[a]A/B[a]A + CHR
BEANS				
Potiskum	0.412	0.201	0.183	0.702
White Iron	0.434	0.203	0.201	0.616
Brown Iron	0.468	0.533	0.304	0.206
Gausau	0.458	0.283	0.206	0.624
Nija Red	0.551	0.167	0.254	0.773
SOYABEAN				
White Soyabean	0.666	0.15	0.047	0.792
Black Soyabean	0.511	0.428	0.237	0.456
CORN				
White Corn	0.75	0.151	0.227	0.597
Yellow Corn	0.651	0.173	0.236	0.723
Pop Corn	0.791	0.079	0.111	0.943
GUINEA CORN				
Red Guinea corn	0.692	0.1	0.083	0.946
White Guinea corn	0.749	0.098	0.069	0.944

Table 11. Total toxicity equivalence concentration of the grains, TTEC ($\times 10^{-2}$ μg/kg).

GRAIN	TTEC ($\times 10^{-2}$ μg/kg)
BEANS	
Potiskum	4.826
White Iron	4.793
Brown Iron	4.438
Gausau	5.21
Nija Red	5.582
SOYABEAN	
White Soyabean	5.433
Black Soyabean	3.836
CORN	
White Corn	5.597
Yellow Corn	5.826
Pop Corn	5100
GUINEA CORN	
Red Guinea corn	6.846
White Guinea corn	7.345

3.2. Discussion

Accuracy and precision of determination was tested by using three different spiking concentrations which enabled the plotting of calibration graphs that gave straight lines. The average recoveries ranged from 94.0% to 99.2% showing that the method applied was very efficient and precise since the recommended recoveries by United States Environmental Protection Agency, US EPA [25] ranges from 50% to 120%. From table 1, Limits of detection and limits of quantitation ranged from 0.03×10^{-3} μg/dm^3 - 0.09×10^{-3} μg/dm^3 and 0.09 x 10^{-3} μg/dm^3 - 0.25×10^{-3} μg/dm^3 respectively which means that the method showed adequate sensitivity regarding the target compounds.

The sixteen PAHs were detected in all the analyzed samples. Tables 2, 3, 4 & 5 showed the PAHs concentration levels obtained from the analysis of different types of beans (potaskium, white iron, brown iron, gausau and Nija red beans), soya beans (white and black soya beans), corn (white, yellow and pop corn) and guinea corn (red and white guinea corn) respectively. From table 2, the ∑16 PAHs obtained in analyzed beans samples ranged from 21.516±7.429 in white iron beans to 28.04±11.10 in brown iron beans. The total mean concentration levels of LMW PAHs in beans varied from 8.842±4.743 in white iron to 12.59±8.068 in brown iron beans. While the ∑HMW-PAHs ranged from 14.937±5.095 in gausau beans to 16.998±3.129 in Nija red beans. The mean concentration levels ($\times 10^{-2}$ μg/kg) of the eight probable carcinogenic PAHs, ∑8 PAHs ranged from 8.875±2.725 in white iron beans to 11.796±3.018 in brown iron beans. Table 3 revealed that the ∑16 PAHs obtained in soya beans varied from 18.69±1.991 in black soya bean to 22.893±6.389 in white soya beans. The total mean concentration levels of LMW PAHs in soya beans varied from 5.062±0.869 to 6.889±1.522 respectively in black and white soya beans and the ∑HMW-PAHs ranged from 13.629±1.122 to 16.00 4±6.633 respectively in black and white soya beans. The mean concentration levels ($\times 10^{-2}$ μg/kg) of the eight probable carcinogenic PAHs,

∑8 PAHs ranged from 10.703±0.103 to 10.918±3.68 respectively in black and white soya beans. The data from table 4 showed that the concentration levels of ∑16 PAHs gotten in corn varied from 23.84±16.493 in pop corn to 27.252±23.105 in white corn. The concentration of ∑LMW-PAHs ranged from 5.998±4.725 in pop corn to 9.385±11.339 in white corn while the ∑HMW-PAHs ranged from 17.84±11.768 to 18.153±10.421 in pop corn and yellow corn respectively. The ∑8 PAHs varied from 10.82±6.183 in pop corn to 13.573±8.793 in yellow corn. Table 5 reported the analysis of guinea corn. From table 5 the ∑16 PAHs concentration levels ranged from 24.07±7.56 in red to 28.581±11.213 in white guinea corn. The total mean concentration levels of LMW- PAHs of guinea corn varied from 6.217±0.442 to 7.78±2.309 in white and red guinea corn respectively and ∑HMW-PAHs from 17.837±1.44 in red guinea corn to 20.792±2.437 in white guinea corn. The mean concentration levels ($\times 10^{-2}$ µg/kg) of the eight probable carcinogenic PAHs, ∑8 PAHs ranged from 11.685±2.252 to 13.391±0.657 in red and white guinea corn respectively.

Comparing the analyzed grains, the ∑16 PAHs concentration dominated in white guinea corn and brown iron beans and were at lower concentrations in soya beans and white iron beans. The ∑8 PAHs concentrations recorded high occurrence in brown iron and Nija red beans, white and yellow corn, red and white guinea corn. The LMW-PAHs occurred high in brown iron and Gausau beans and pop corn, soya beans on the other hand recorded low concentration. While HMW-PAHs occurred high in white guinea corn and yellow corn and low in potasikum and white iron beans.

Although literature has not reported much studies on bean, soya bean and guinea corn grains, the total mean PAHs concentration levels obtained in this study compared very low to some reported studies on grains. However, the values of PAH concentrations obtained in this work were very low in comparison with those reports [25, 26] in soya bean oil. They reported non detection of B[g,h,i]P in soya bean oil.

The PAH concentrations of maize obtained in this study were far below that obtained by the research [6] in analysis of imported canned maize. This might be as a result of contribution of more PAHs from preservatives added in the canned maize. Also Olabemiwo, O. M. et al. [5] in their study on PAHs determination in three local snacks which included pop corn (guguru) collected from Ogbomoso in Nigeria, obtained the total 16 PAHs concentration levels in roasted pop corn as 3.835 µg/kg but in this study, the total 16 PAHs concentration obtained in pop corn was 0.024 µg/kg. This is comparably very low, probably because the pop corn analyzed in this present work were not exposed to a high temperature.

The total 16 PAHs detected in maize, 26.027 $\times 10^{-2}$ µg/kg in this study compared low to that reported by Olabemiwo in roasted maize from road side vendor in Ogbomoso, Nigeria. This is because maize grains analyzed in this study were not exposed to a very high temperature.

Tables 6 and 7 showed the daily estimated intakes of adult male and female individuals respectively for all the analyzed grains using the indicators BaP, PAH2, PAH4 & PAH8. Table 6 showed that the total dietary exposure of adult male individuals ($\times 10^{-2}$ µg/kg bw/day) for BaP in the analyzed grains ranged from 0.726 in red guinea corn to 1.416 in pop corn, PAH2 varied from 0.753 red guinea corn to 2.048 in brown iron beans. PAH4 ranged from 1.259 also in red guinea corn to 3.012 in white corn while PAH8 ranged from 1.335 in red guinea corn to 3.49 in yellow corn. There was dominance of the indicators in corn and beans.

Table 7 reported that the total dietary exposure of adult female individuals ($\times 10^{-2}$ µg/kg bw/day) for BaP in all the analyzed grains varied from 0.806 to 1.652 black soya beans and pop corn respectively and PAH2 from 0.879 to 2.271 respectively in red guinea corn and white corn. PAH4 ranged from 1.469 to 3.85 in red guinea corn and yellow corn respectively while PAH8 from 1.558 to 4.072 respectively in red guinea corn and yellow corn. The total dietary exposure of male (90.947 µg/kg bw/day) was less than that of female (100.516 µg/kg bw/day). Comparing tables 6 and 7, adult female individuals have higher intakes, in other words they are more exposed to health risk when compared to adult male individuals. The result [28, 29] showed that the mean (range) of CDI (PAH13) for adults who consumed corn, sunflower, blended oils, and frying was calculated as 0.49–0.54 (0.14–0.80) ng BaPeq/kg bw day, and that for children was calculated as 2.53–2.81 (0.73–4.13) ng BaPeq/kg bw day. The results here were also much lower than the study [30] which reported that in Brazil, the consumption of soybean oils revealed the exposure level to PAH4 to be 7.3 ng BaPeq/kg bw day. The result of this study varied from the study [15] which reported that the dietary exposure of male (9064ng/day) in Nanjing China was more than that of female (8308ng/day).

Tables 8 and 9 show cased the margin of exposure, MOE values calculated using the 95th percentile dietary risk lowest-case for male and female exposed individuals respectively. From table 8, the MOE of the indicators obtained from the analyzed grains in male exposed individuals for BaP varied from 49435 in pop corn to 96419 in red guinea corn and PAH2 from 83007 in brown iron beans to 225761 in red guinea corn. The PAH4 varied from 103030 to 270056 respectively in yellow corn and red guinea corn and PAH8 from 140401 in yellow corn to 367041 in red guinea corn. Table 9 reported the MOE of female exposed individual showing that BaP varied from 43373 in pop corn to 86849 in black soya beans while PAH2 varied from 62731 to 193402 in white corn and red guinea corn ($\times 10^{-2}$ µg/kg bw/day) respectively. The PAH4 ranged from 88,312 in yellow corn to 231450 in red guinea corn and PAH8 from 120334 to 314506 respectively in yellow corn and red guinea corn. The indicators in this case dominated in guinea corn. The total value of MOE obtained in adult male individuals was more (6,594,073) than that obtained in adult female individuals (5,974,450). However the values of margin of exposure, MOE obtained for all the indicators were much higher than 10000 which according to EFSA indicate low concern for human health and considered low priority for risk management actions. The values of MOE in this study compared well with the values obtained by the findings [8, 28-32]. The study [29] on the Levels and Health Risk

Assessment of Polycyclic Aromatic Hydrocarbons in Vegetable Oils and Frying Oils by Using the Margin of Exposure (MOE) and the Incremental.

Lifetime Cancer Risk (ILCR) Approach in China reported the values of MOE were above 1.0×10^6 for PAH4 and PAH8 in adults, which were 104,649 and 115,378 for males and 103,737 and 114,122 for females, respectively. The study [31] on Polycyclic aromatic hydrocarbon concentrations on commercially available infant formulae in Nigeria reported that the values of all the indicators-BaP, PAH2, PAH4, PAH8 were greater than 10,000 and that of the research [8] on Risk assessment of polycyclic aromatic hydrocarbons in pasta products consumed in Nigeria revealed that the MOE values for adult consumers were far higher than 10,000 indicating no health risk from consumption of the products. Also the study [32] on the Occurrence and Risk characterization of PAHs of edible oils reported the values of MOEs (between 66094 and 1729776) were over 1.0×10^4 indicating that the risk of 4 PAHs in edible oils were of low concern from a public health point of view. Kang, B.; Lee et al. [30] reported that MOEs of PAHs from vegetable oils in Korea were 4,000,000 for BaP and 137,000 for PAH4, and thus concluded that the concern was negligible. Also Mojtaba, Y. et al. [28] reported similar results that MOE values of vegetable oils in both children and adults were in a safe range (MOE ≥ 10,000) in Iran.

From Table 10, for Ant/Ant+Phe all the sample concentrations ranged from 0.412 in potaskium to 0.791 in popcorn. They were all > 0.1 indicating fuel combustion source; Fla/Fla + Pyr ratio ranged from 0.079 in popcorn to 0.533 in brown iron beans. Some values were < 0.4 while some fell within 0.4 -0.5 indicating both petrogenic and fuel combustion source. B[a]A /B[a]A + Chr for all the samples varied from 0.206 in brown iron beans to 0.946 in red guinea corn. They were all > 0.35 except brown iron beans (0.206) indicating fuel combustion source. This indicate petrogenic and fuel combustion source. While for I[c,d]P/I[c,d]P + B[g,h,i]P ratio, the values of all the analyzed grains ranged from 0.047 in white soya beans to 0.304 in brown iron beans indicating petrogenic and fuel combustion source. Conclusively the sources of PAHs contamination in the samples analyzed in this study were fuel combustion and petrogenic..

From Table 11, the TTEC for the cPAHs of the analyzed grains ranged from $3.84 - 7.34 \times 10^{-2}$ µg/kg which was very low when compared with the Method B clean up level for benzo[a]pyrene which was 0.137 mg/kg. So the analyzed grains were not toxic.

From the analysis of variance, p>0.05 proving no significant difference between the PAHs concentration of the analyzed grains. It was also ascertained from the analysis that the PAHs concentrations of grains were homogeneous. The Pearson correlation coefficient analysis indicated strong positive correlation among the PAH concentrations of all the analyzed grain. Figure 2 gives the summary of the present study.

4. Conclusion

The sixteen PAHs contamination detected in the samples were from fuel combustion and petrogenic emission. The concentration levels were in two orders of magnitude below the permissible limit of 1.0 µg/kg established by EFSA but long time accumulation of these PAHs can be hazardous to human health. The MOE values for adult male and female consumers indicated low concern for human health and considered low priority for risk management action. The dietary exposure of female is higher than that of male consumers. The total toxicity equivalence concentration of the grains and other results of this study proved to high extent the safety of consuming these analyzed grains from markets in South East Nigeria. The results from this study can serve as a useful baseline for continuous monitoring of PAHs in the Nigerian grains. I am recommending regular analysis of all the grains commonly consumed in Nigeria to ensure protection of human health in the country.

Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

Authors Contributions

All authors contributed to the study conception and design. Sampling, extraction and analysis were performed by Odika Ifeoma, Okoye Chuma, Odidika Collins and Okafor Chidimma. The first draft of the manuscript was written by Odika Ifeoma Maryrose and all authors commented on drafted manuscript. All authors read and approved the final manuscript.

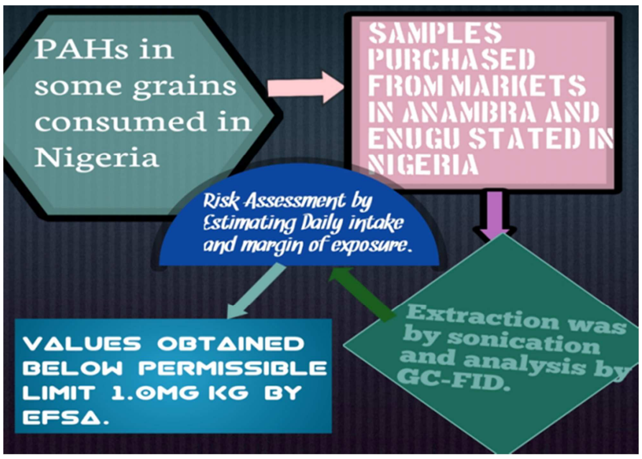

Figure 2. *Graphic table of content.*

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