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Cooked Rice Products (*Kwenkwen, Jollof, Fried-rice, Angwamo* and *Kanzo*) as Sources of Polyaromatic Hydrocarbons and a Potential Public Health Concern

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Abstract Cooking methods for rice products may also present contaminating polycyclic aromatic hydrocarbons (PAHs), which are known to be carcinogenic. This study collected a total of 54 different cooked rice products from the study area and extracts were made using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuECheRS) method. After quantifying the PAH's concentrations using HPLC, standard protocols were used to determine the exposure of key PAHs (total benzo [a] pyrene (BaP), ΣPAH4, ΣPAH8) and the risks of consumers based on the rice consumption pattern from 760 participants. The results indicated 11 congeners, presenting 2-methylnaphthalene as the highly contaminating PAH (61.11%) and total benzo [a] pyrene (BaP) as the lowest (18.52%) contaminating PAH. The ΣPAH4 and the ΣPAH8 types presented 5^{th}-95^{th} percentiles concentrations ranging between 0.302 and 9.275 mg/kg for ΣPAH4 and between 0.304 and 9.315 mg/kg for ΣPAH8 respectively. However, benzo [a] pyrene (BaP)s concentration ranged between 0.050 and 1.526 mg/kg. The 95^{th} percentile exposure (1.28-8.04 mg/kg (bw)-day), margin of exposure (MoEs) ($<10^4$), and incremental life time cancer risk (ILTCR) (1×10^{-3} $>10^{-4}$) indicated high risk among the consumers. All eight non-carcinogenic PAHs showed significantly high hazard indices (HI >1) except for outliers which were isolated cases. Thus, the study suggests a high-risk predisposition of the top 5% of the consumer population of such cooked rice products. Constant monitoring is therefore warranted to maintain safety.

Keywords: *cooked rice product, food safety, toxicology, hazard index, margin of exposure, incremental lifetime cancer risks; risk assessment*

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1. Introduction

Polyaromatic hydrocarbons (PAHs) are organic compounds usually formed from incomplete combustion [1]. Several congeners or forms of these PAHs are composed of two or more fused aromatic rings. The PAHs having between two to three rings are described as light, while those with between 4 and 6 rings are said to be heavy. PAHs are derived from three sources; biogenic, pyrogenic and petrogenic origins. While living organisms produce the biogenic PAHs, the pyrogenic sources are derived from the thermal decomposition of organic matter (fat, carbohydrate, protein), including those made during processing foods at high temperatures [2]. The precise mechanism leading to the formation of PAHs is not clear; however, experts have proposed some steps. Temperatures above 200°C are favorable conditions for PAHs to form. More ringed-PAHs are produced at temperatures above 500°C [3]. Existing literature further indicates that some PAHs form when lipids drip directly into open flames at intense heat. This condition favors the formation of volatile PAHs, which deposit on foods that hang over the flames as the smoke rises. Incomplete combustion of fuel charcoal has also been documented to generate PAHs that adhere to the surfaces of the food [3]. Petrogenic sources of PAH are caused by geological processes, including natural gas leakages and fossil fuel release.

PAHs are lipophilic, making them readily absorbed as they travel along the alimentary canal and eventually, accumulating in vital organs and tissues. The toxicity of

PAHs may be influenced by their molecular structures, including their physical and chemical properties. Heavy PAHs are more stable and relatively more toxic than lighter PAHs [4]. The International Agency for Research on Cancer (IARC) classifies benzo(a)pyrene as a human carcinogen. Also, anthracene, chrysene and benzo(b)fluoranthene are classified as possibly carcinogenic to humans [5]. The oxidative hydrolyses of PAHs involving phase I and phase II enzymes generate metabolites such as epoxides and radical cations that facilitate adduct formation with proteins, DNA, RNA and glutathione, setting the tone for their adverse health effects [6].

Since 2008, European Food Safety Authority (EFSA) experts have questioned why benzo(a)pyrene is the only PAH marker used to indicate carcinogenicity. In 2011, benzo[a]pyrene as the only marker was revised to comprise the so-called sum of four PAHs (ΣPAH4), which include benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene. Again, a decision was also taken on the sum of eight PAHs (ΣPAH8) to involve benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, chrysene, benzo[k]fluoranthene, benzo[ghi]perylene, dibenz [a,h] anthracene, and indeno[1,2,3-cd]pyrene. These sums of PAHs (ΣPAH4 and ΣPAH8) were considered more suitable indicators for the occurrence of PAH in food [7]. Hazard characterization of these PAHs has delivered a trove of information on their dose-response indices. These include their benchmark doses, potency factors and reference doses, especially for individual congeners that may not be carcinogenic until they synergistically act with others. Exposure to PAHs from meat, fish, bakery products, coffee, and uncooked fresh produce such as rice, egg, vegetables, and vegetable oils has been reported [8].

Apart from their carcinogenic studies, PAHs have also been reported to cause other adverse effects. Animal model studies show a reference dose value of 0.3 µg/kg(bw)-day for neurobehavioral changes in developing animals [9]. Decreased ovarian follicles and ovary weight has also been reported in reproductive animal models with threshold reference dose value of 0.4 µg/kg(bw)-day. A reference dose value of 2 µg/kg(bw)-day has been reported as for studies on thymus weight and serum immunoglobulin in rats [10]. Exposure assessment of PAHs remains one fundamental item in the risk assessor's toolbox for risk quantification. PAHs occur as congeners; however, since BaP has been studied in detail, it is often used as a surrogate. Experimentally determined toxicity equivalency factors (TEF) are used to scale the individual PAHs' carcinogenic properties to indicate their potencies in the composite of BaP [11].

The rapid industrialization worldwide has made PAHs ubiquitous in the environment, thus, driving their re-emergence as contaminants on a global scale. The Ghana government indicates that annual per capita rice consumption will reach 40 kg in 2020 [12]. The methods used in cooking rice in Ghana, involving excessively high temperature and long cooking periods, have the potential to contribute the total PAH exposure and risk through many degradative reactions. This study investigated the types and quantities of PAHs present in randomly sampled cooked ready-to-eat rice products. It also includes the evaluation of the exposure and risks of adverse health outcomes they pose to regular consumers.

2. Materials and Methods

Sixteen standard mixes of PAHs in methylene chloride: methanol (1:1) were purchased from Sigma Aldrich (Darmstadt, Germany). The concentrations were as follows; 2.5 µg/kg for naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, and acenaphthene; 5 µg/kg for anthracene, pyrene, fluorene, benzo(b)fluoranthene, and benzo(k)fluoranthene; 10 µg/kg for fluoranthene and benzo[a]pyrene. Samples of cooked rice products were collected around Kwame Nkrumah University of Science and Technology (KNUST) and its immediate environs; Ayigya, Ayeduase, and Bomso. These areas have high food vendor concentration because of the large number of consumers from diverse cultural background present.

The cooking methods of the sampled rice products under study had one underlying fact. There was detectable slightly burnt to smoky flavors possibly derived from the thermally induced Maillard reaction arising from the recipes of the cooked rice products [13]. Other thermally induced pathways involving Diel-Alder reactions could have contributed in generating these PAHs [14]. The cooked rice products were randomly sampled from vendors over two weeks in April 2019, giving a total of 54 samples, while surveys were run to collect demographic and rice consumption data from 760 participants using Google forms. The samples were then stored in at -18°C pending further analyses.

2.1. Extraction and the Determination of PAH Using HPLC

A 5 g portion of each rice sample was treated with 10 mL of HPLC grade acetonitrile and vortexed for 1 min. Using the QuECheRS method [15], 6.0 mL of an aliquot from the upper acetonitrile layer was transferred into AOAC dispersive SPE 15.0 mL tubes containing already packed 400 mg of PSA, 40 mg of carbon-18 EC and 1200 mg anhydrous $MgSO_4$. After vortexing for 1 min, they were then centrifuged at 4000 rpm for 5 min, after which 4 mL aliquot was filtered through 0.45 µm PVDF (Polyvinylladine fluoride) syringe filter then 100 µL of the extract was injected into the HPLC system [16].

The analysis was based on the method employed in other studies [17], where the Cecil-Adept binary pump HPLC was coupled with Shimadzu 10AxL fluorescence detector (excitation wavelength 254 nm and emission wavelength 290 nm) with Phenomenex HyperClone BDS C-18 column (150 × 4.6 mm, 5 µm). The mobile phase (acetonitrile) and deionized water were delivered individually by pumps A and B at 0.8 mL/min. The gradient elusion was calibrated to follow the scheme; 0-5 min = 60%A, 40%B; 5-15 min = 90%A, 10%B; 15-28 min= 100%A, 0%B; 28-40 min = 60%A, 40%B. Individual PAH were specifically identified using their retention times matched with the corresponding standards (Chromatogram-PAH) and subsequently quantified using the calibration curve.

A system quality was assured using a 5-point internal standard calibration which gave an r^2>0.98. The detection limit was determined as 3 × SD/s, where SD is standard deviation of five replicate measurements and **s,** the slope

of the calibration curve. The limit of detection (LOD) of the PAH ranged between 0.05 to 6.46 ng/g. Recovery assessment performed by spiking a food matrix with naphthalene (100 ng/g), fluorene (40 ng/g), pyrene (20 ng/g) and BaP (20 ng/g), gave an average recovery of 114±1.42%.

2.2. Data Analysis

To a large extent, a considerable sum of PAHs (ΣPAH4 and ΣPAH8) together with total benzo [*a*] pyrene (total BaP), considered to be more suitable indicators for the occurrence of PAHs in foods, were identified in the cooked rice products studied. Out of the eight ΣPAH8 types expected, only three were obtained (BbF, BkF, and BaP). Again, of the four ΣPAH4 types that were expected, only two (BbF and BaP) were found. Using the USEPA's method (Equation 1), the chronic exposure (ExP_c) was determined as;

$$ExP_c = \frac{C_{PAH} \times MF}{BW} \times \frac{EF \times ED}{AT}, \quad (1)$$

where C_{PAH} is the concentration of the type of PAH. All the other elements for quantifying the chronic exposure were secondary data. Mass of food (MF) is the mass of cooked rice products consumed (209.91 g/day) derived from food cluster diets in Ghana [18]. Exposure frequency (EF) in days of the consumption of rice in Ghana and exposure duration (ED) for the computation of exposure leading to cancers were taken as 365 days and 43 years, respectively [19]. Averaging time (AT) in days was taken as 70 years [20], and consumers' body weights (bw) were obtained from the survey conducted. To characterize the hazard index (HI) of all the non-carcinogenic PAHs, the hazard quotients of the individual PAH congeners (PAH_i) were determined by computing their chronic exposure separately and dividing by their specific reference doses [21]. The sum of all the individual hazard quotients yielded the hazard index according to Equation 2, where R_fD_i is the reference dose of individual PAH congener.

$$\sum HI = \sum_{I=1}^{8} \left(\frac{Exposure(PAH_i)}{RfD_i} \right) \quad (2)$$

Standard procedures were applied to express the carcinogenic toxicity of all the PAHs. Firstly, the concentration of the surrogate, BaP, was determined by summing the weighted products of each PAH congener's concentrations and their corresponding toxic equivalence factors (TEF_L) [11] using Equation 3 as;

$$BaP_{TEF} = \sum_{i=1}^{11} (PAH_i) \times TEF_L \quad (3)$$

where; TEF_L indicates the toxic equivalency of the particular PAH congener published [11]. Secondly, the product of the chronic exposure of total BaP and its potency factor (1 (mg/kg (bw)-day)$^{-1}$) was computed according to standardized guidelines [22]. Subsequently, the margin of exposure (MoE) was calculated according to the EFSA's proposed Equation 4 [23] based on the exposure of total BaP, ΣPAH4 types, and ΣPAH8 types;

$$MoE = \frac{BMDL_{10}}{ExP_C}. \quad (4)$$

The $BMDL_{10}$ references used were; 0.34 mg/kg (bw)-day for ΣPAH4 [23], 0.49 mg/kg (bw)-day for ΣPAH8 [24], and 7.0×10^{-5} mg/kg (bw)-day for BaP [23]. The incremental lifetime cancer risk (ILTCR) due to total BaP was calculated based on the USEPA guidelines (Equation 5).

$$ILTCR = ExP_C \times PF, \quad (5)$$

where PF is the potency factor of BaP. Palisade @Risk software [25] was used as a Microsoft Excel add-on to fit the elements of exposure using Equation 1. All the other risk indices: HI, MoE, and ILTCR, were respectively determined using Equations 2, 4, and 5 and iterating at 100,000 times. The distribution patterns and the statistics (min, mean, mode, median, 5th, 95th percentile and max) of the concentrations of the various types of PAHs, their chronic exposure, and risk indices were then critically studied.

3. Results and Discussion

3.1. Types and Concentrations of PAHs

Table 1 presents the types and concentrations of PAHs (mg/kg) in the cooked rice samples characterized. It is evident that the eleven types of PAHs found in the cooked rice were fewer than the sixteen prioritized polycyclic aromatic hydrocarbons usually present in foods [26]. The eleven PAHs observed included naphthalene (Nap), 1-methylnaphthalene (1-M), 2-methylnaphthalene (2-M), acenaphthene (Ace), fluorene (Flu), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), and benzo[a]pyrene (BaP). Their concentrations ranged from non-detectable to the highest value of 112.58 mg/kg, registered as naphthalene. 2-methylnaphthalene was indicated to be the highest PAH congener (61.11%) present in the 54 characterized rice samples, and the lowest was BaP (18.52%).

Accordingly, those PAHs absent were acenaphthylene (Acy), phenanthrene (Phe), benz(a)anthracene (BaA), chrysene (Chr), dibenzo(a,h)anthracene (DahA), benzo(g,h,i)perylene(BghiP) and indeno(1,2,3–cd)pyrene (InP).

Out of the known sum of four PAHs (ΣPAH4) types, only two (BaP and BbF) were present relative to the sum of eight PAHs (ΣPAH8) types which presented only three (BaP, BbF and BkF). The concentration of this ΣPAH8 spread among the characterized cooked rice samples following a triangle statistical distribution (Triang 0,0,11.997) and showing a 95th percentile value of 9.315 mg/kg and a 5th percentile concentration at 0.34 mg/kg (Table 2). However, there was an outlier of maximum concentration at 11.71 mg/kg. Similarly, the concentration of ΣPAH4 distributed among the characterized rice samples as "Triang (0,0, 11.945)", showing a 95th percentile value of 9.275 mg/kg and presenting a maximum outlier concentration of

11.71 mg/kg. Using the additive relationship toxicity equivalency factors to sum the PAHs cumulatively [11], the concentration total of benzo [a] pyrene (total BaP) that were characterized, also spread as "Triang (0,0,1.9657)" showing a 95th percentile value of 1.526 mg/kg. The 95th percentile value of total BaP obtained in this study (1.526 mg/kg) was consistently higher than 0.005 mg/kg and 0.022-0.131 mg/kg reported in literature [4,27].

Table 1. Concentrations of PAH in cooked rice (mg/kg)

Sample Code	Nap*	1-M*	2-M*	Ace*	Flu*^{0}	Ant*	Flt*^{0}	Pyr*^{0}	BbF^{2*†}	BkF^{2*†}	BaP^{2*††}
Sample 1	11.95	0.00	1.95	2.15	1.15	0.79	0.00	0.56	0.00	0.00	0.00
Sample 2	0.00	0.00	21.46	1.92	0.21	0.00	6.15	0.18	0.00	2.18	0.00
Sample 3	56.78	4.67	8.45	1.35	1.05	0.00	0.00	0.00	0.00	0.00	0.00
Sample 4	0.00	0.21	4.21	0.00	0.04	0.00	0.00	2.56	2.94	0.00	0.00
Sample 5	0.00	0.00	0.00	0.00	5.15	0.76	8.94	0.00	0.00	0.00	0.00
Sample 6	31.68	0.00	0.00	0.00	1.96	0.00	0.00	0.00	0.00	5.82	0.00
Sample 7	0.00	12.45	0.00	0.00	1.04	1.01	0.00	0.00	0.00	0.00	0.00
Sample 8	3.12	0.00	3.16	2.37	0.00	0.00	0.00	1.45	0.00	0.00	0.18
Sample 9	0.00	0.00	0.34	0.00	0.00	0.00	0.00	1.86	2.48	0.00	0.31
Sample 10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sample 11	0.00	56.54	3.56	0.00	0.00	1.56	1.56	1.67	0.00	0.67	0.00
Sample 12	7.04	0.00	6.95	4.09	0.00	2.01	2.05	2.19	3.12	1.34	0.00
Sample 13	1.34	0.00	2.18	3.52	2.07	2.87	2.67	4.64	0.00	0.46	0.00
Sample 14	2.70	0.00	0.93	1.61	0.00	3.76	2.17	0.00	0.00	2.41	0.00
Sample 15	0.89	0.32	5.36	2.35	0.00	5.82	1.65	5.02	0.00	3.05	0.00
Sample 16	0.00	0.18	5.18	2.04	7.05	1.46	3.98	1.56	1.14	0.31	0.00
Sample 17	0.00	0.31	10.64	0.00	0.00	2.68	4.32	0.00	0.00	1.35	0.00
Sample 18	0.00	0.42	7.25	2.45	0.00	1.87	3.09	3.50	0.00	0.00	0.00
Sample 19	5.89	0.05	3.29	5.43	0.00	4.03	4.89	2.46	0.00	6.46	0.00
Sample 20	0.00	0.47	3.07	1.59	0.00	3.36	1.86	0.00	0.00	0.00	0.00
Sample 21	0.00	0.04	1.45	1.01	0.56	0.40	7.89	0.68	0.47	0.00	0.00
Sample 22	0.00	0.21	1.69	1.34	0.00	0.14	0.00	0.46	1.34	0.00	0.00
Sample 23	75.92	0.18	2.01	0.53	1.49	0.24	8.59	0.08	3.56	0.00	0.00
Sample 24	0.00	0.13	0.68	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Sample 25	0.00	0.00	0.92	0.00	1.31	0.00	0.00	0.03	3.25	6.69	0.00
Sample 26	0.00	0.00	2.01	0.45	0.00	0.00	11.06	0.02	1.67	0.00	0.00
Sample 27	0.00	0.00	3.26	0.78	0.00	1.34	0.00	1.04	3.58	0.00	0.00
Sample 28	0.12	0.00	2.21	1.05	0.00	0.89	0.00	3.05	0.00	0.00	0.00
Sample 29	0.31	12.89	0.10	2.60	0.00	0.56	0.00	4.34	0.53	0.00	0.00
Sample 30	0.54	0.00	0.00	0.78	0.45	0.00	0.00	0.00	0.00	3.41	0.00
Sample 31	1.08	0.00	0.00	0.12	0.00	0.74	0.00	0.03	0.00	0.76	0.24
Sample 32	2.56	0.00	44.75	0.56	0.00	0.97	0.00	0.25	0.00	0.42	0.00
Sample 33	1.76	0.00	0.00	0.76	0.00	0.00	2.58	0.00	0.00	0.00	0.00
Sample 34	4.95	21.68	24.00	0.93	0.00	0.00	0.00	1.04	0.00	0.00	0.82
Sample 35	2.67	0.00	28.16	0.28	2.47	0.00	0.00	0.95	0.00	0.54	0.56
Sample 36	0.00	0.00	0.00	0.32	0.00	0.24	7.84	0.00	2.59	0.29	0.00
Sample 37	0.00	0.00	0.00	0.43	3.95	0.39	0.00	0.45	0.00	0.00	0.00
Sample 38	0.54	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00	1.97	0.00
Sample 39	0.23	0.00	23.85	0.51	2.59	0.00	0.00	0.86	3.67	0.00	0.84
Sample 40	0.00	0.00	0.67	0.00	0.00	0.46	3.68	0.00	0.00	0.00	0.00
Sample 41	0.00	0.00	0.00	0.00	5.86	0.00	0.00	0.00	0.00	0.30	0.81
Sample 42	0.00	11.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.33
Sample 43	12.96	0.00	34.21	2.93	0.00	0.00	4.69	0.00	0.00	0.00	0.00
Sample 44	0.00	0.00	0.00	0.00	3.17	0.00	0.00	1.45	0.00	0.00	0.00
Sample 45	0.00	0.00	0.00	0.00	0.00	1.56	0.00	0.00	11.47	0.00	0.24
Sample 46	56.84	15.78	0.00	8.35	0.00	0.00	3.51	0.00	0.00	0.00	0.00
Sample 47	0.00	0.00	22.43	0.00	4.07	2.05	0.00	0.19	0.00	0.42	0.00
Sample 48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sample 49	33.16	0.00	0.00	0.00	0.00	0.00	6.89	0.00	2.67	0.00	0.00
Sample 50	0.00	0.00	0.00	64.76	5.13	2.44	0.00	0.00	0.00	0.00	0.00
Sample 51	0.00	19.42	0.00	0.00	0.00	0.00	0.00	4.59	0.00	0.06	0.00
Sample 52	0.00	0.00	17.47	0.00	0.00	0.00	0.00	0.00	0.00	0.06	1.89
Sample 53	112.58	0.00	0.00	0.00	7.06	4.37	0.00	0.00	0.00	0.06	0.00
Sample 54	0.00	0.00	0.00	5.57	0.00	0.00	0.00	0.00	1.35	0.06	0.00

*= Low molecular weight (2-4 aromatic rings), 2**= high molecular weight (5 or 6 aromatic rings) ††= Probably carcinogenic to humans, †= possibly carcinogenic to humans, 0=not classifiable as to their carcinogenicity to humans.

Table 2. Elements for the determination of PAH exposure

Elements	Statistical Distribution	Statistics						
		Min	Mean	Mode	Median	5th	95th	Max
ΣPAH8 (mg/kg)	Triang (0,0,11.997)	ND	3.999	ND	3.514	0.304	9.315	11.710
ΣPAH4 (mg/kg)	Triang (0,0, 11.945)	ND	3.982	ND	3.499	0.302	9.275	11.710
total BaP (mg/kg)	Triang (0,0,1.9657)	ND	0.655	ND	0.576	0.050	1.526	1.914
		Sources						
Mass of rice(g)	209.910	(WHO/GEM FOODS, 2013)						
BW_F	62.0878	*Loglogistic (17.651,42.092, 5.5386)						
BW_M	61.000	*Laplace (61, 14.7941)						

*Obtained from the survey, BW_F and BW_M are the body weights for female and male consumers, respectively.

Unlike in the EU [7], where limits are placed on total BaP (5.0 µg/kg) and ΣPAH4 (30.0 µg/kg) in some foods, there are no current regulations of PAHs levels of foods in Ghana, just like in some foods in the US [28]. However, these regulated concentrations of PAHs in the EU were higher than what was obtained in the current study for total BaP (1.526 mg/kg) and ΣPAH4 (9.275 mg/kg). While these PAH levels obtained in the current study may be permitted in EU member states, it suggests that such foods must be regulated on a case-by-case basis since total diets may reach action levels when least expected. Again, though concentrations of individual non-carcinogenic PAHs in the current study may be low, exposure in total food basket of consumers may exceed the levels permitted (30.0 µg/kg) by the EU [7]. This uncertainty should be of public health concern since non-carcinogenic toxicological end-points have also been associated with individual PAHs [29]. A surprisingly high total BaP, ranging between 12.8 mg/kg and 13.1 mg/kg, has been reported in China [30], thus, making the value of total BaP (1.914 mg/kg, max) obtained in the current research relatively low.

In a related study [17], comparatively low levels of naphthalene (6.5 mg/kg, 95th percentile) was obtained in all foods analyzed relative to the exceptionally high value (89.2 mg/kg) observed in the current research. However, the 95th percentile concentration of 1-methylnaphthalene obtained in Fufu (pounded cooked cassava and plantain) (0.095 mg/kg) for that study was lower than the concentration of naphthalene (44.75 mg/kg) obtained in this current study. Again, the concentration of fluorene (0.095 mg/kg) reported in the other study was relatively lower than that obtained in this current research (5.75 mg/kg). Though its final characterization has not been settled, the presence of naphthalene and its derivatives in foods is problematic because it is considered as either a probable or possible human carcinogen, depending on the route of exposure [31].

Besides rice, other food sources have also been contaminated with BaP, ΣPAH4, ΣPAH8, and other PAH congeners comparable to the cooked rice products under this study. The maximum level of total BaP reported in this study (1.914 mg/kg) was found to be greater than the total maximum value of BaP (0.566 mg/kg) reported in beef burgers and sausages presented by other experts [32]. The 95th percentile value of BaP in bakery, milk, and processed cocoa products, ranging from 0.04 to 3.5 µg/kg has been reported [33]. These values were significantly lower than the levels of BaP in the cooked rice products reported in this study. Some experts [34] reported the total BaP levels in grilled chicken (0.491 µg/kg) to be lower than those in the cooked rice products obtained in this current study. However, the concentrations of ΣPAH4 (21.3 mg/kg) and ΣPAH8 (36.8 mg/kg) reported in those studies were significantly higher than the ΣPAH4 (11.710 mg/kg) and ΣPAH8 (11.710 mg/kg) quantified in the cooked rice products reported in this study. Non-patterned concentrations of PAHs were observed in this study and this observation could be attributed to various contributing factors such as type of food, fat content, duration of cooking, type of fuel used, the proximity of heat source to food, cooking temperature, and cooking method employed. [3].

3.2. Exposure of PAHs in Cooked Rice Products

Table 3. Chronic exposure to cooked rice products among participants

Chronic Exposure (mg/kg-day)	Statistics						
	Min (10^{-8})	Mean (10^{-3})	Mode (10^{-4})	Median (10^{-3})	5th (10^{-4})	95th (10^{-3})	Max
$ΣPAH8_F$	1.620	3.196	1.140	2.698	2.320	7.843	0.021
$ΣPAH8_M$	0.000	3.110	2.520	2.730	2.300	8.080	4.775
$ΣPAH4_F$	0.600	3.187	0.223	2.690	2.280	7.832	0.018
$ΣPAH4_M$	0.000	3.220	1.540	2.720	2.270	8.040	2.590
total $BaP_{(F)}$	0.460	0.524	0.490	0.443	0.380	1.280	0.003
total $BaP_{(M)}$	0.000	0.444	0.180	0.448	0.370	1.320	0.734

The estimates of chronic exposure to cooked rice products of dietary ΣPAH4 and ΣPAH8, types, and total BaP among male and female participants are presented in Table 3. The frequently occurring (modal) chronic exposure of total BaP ranged from a low of non-detected up to 0.734 mg/kg(bw)-day in males, relative to the female consumers (0.46×10^{-3}-0.003 mg/kg(bw)-day). However, the most frequently occurring (modal) exposure of total BaP in the female consumers (0.049 µg/kg(bw)-day) was higher relative to the male consumers (0.18×10^{-4} mg/kg(bw)-day). Again, for the ΣPAH4 type, the frequently occurring (modal) exposure was higher in the male consumers (0.154 µg/kg(bw)-day) relative to the female consumers (0.022 µg/kg(bw)-day), though the ΣPAH4 type ranged up to 2.59 mg/kg(bw)-day in male consumers. A similar trend was observed between the male and female consumers in the exposure of ΣPAH8 type. The consistently high maximum values of total BaP, ΣPAH4, and ΣPAH8, recorded in this study, only show isolated cases of very high exposure in the sampled cooked rice products.

In a similar study, the exposure of total BaP for male and female consumers in China were reported to range between 4.41 and 4.53 ng/kg (bw)-day [30]. These same researchers reported levels of total BaP ingestion (3.2 ng/kg (bw)-day) in the diets of Koreans, which is higher than those observed in this current study at the 95th percentile (0.37-1.320 ng/kg (bw)-day). From a previous study at the 95th percentile, the exposure of naphthalene (0.2 µg/kg(bw)-day), 1-methylnaphthalene (0.7 µg/kg(bw)-day), and fluorene (0.4 µg/kg(bw)-day), were relatively lower [17]. This observation is in sharp contrast to what has been indicated in the current study, where naphthalene ranged from 0.748 to 0.774 µg/kg(bw)-day). A closely related study [17] indicated additional PAHs in cooked rice products as 2-methylnaphthalene, acenaphthene, anthracene, fluoranthene, and pyrene. However, the remaining PAHs; 1-methylnaphthalene (0.375-0.388 µg/kg-day) and fluorene (0.0484-0.0498 µg/kg-day), were comparatively higher. Thus, the exposure characterized in the present study is a cause for concern.

Apart from rice, another study also reported the presence of PAHs in Fufu and soup, a popular staple in Ghana [17]. At the 95th percentile, some of the major PAHs reported for Fufu, included naphthalene (0.10 mg/kg (bw)-day), 1-methylnaphthalene (0.21 µg/kg(bw)-day), 2-methyl naphthalene (0.012 µg/kg(bw)-day), acenaphthene (0.52 µg/kg(bw)-day), and fluorene (0.30 µg/kg(bw)-day). Fufu is usually consumed with soup which is also prepared using fish or meat (or a mixture thereof) and other vegetables and cooked until it is done. Thus, the final levels of exposure would be higher than the reported PAHs in Fufu alone. The presence of similar non-carcinogenic PAHs in both studies suggests that the formation of these PAHs is prevalent in our staple foods. Thus, these PAHs could have arisen through conventional thermal manufacturing processes that influence their formations or from contaminated environmental sources [28].

Though information on chronic exposure of PAH from the consumption of cooked rice is limited in literature, exposure of PAH in other foods are ubiquitous. They abound in meat, fish, milk, egg, vegetables, sesame oil, olive oil, and beverages including tea leaves, coffee and mate. For instance, studies have reported of total BaP of 4.12 µg/kg in coffee beans and indicated that exposure of total BaP in coffee beans, tea leaves and mate was 1.94×10^{-3} µg/kg (bw)-day, for a standardized cup of coffee and average body weight of a man (70 kg) [35].

A similar report of total BaP in Sesame oil was made, indicating a 95th percentile exposure of total BaP amounting to 3.06×10^{-2} ng/kg (bw)-day, while ΣPAH4 was reported to be 0.158 ng/kg(bw)-day [36]. A study in Turkey indicated exposure of ΣPAH4, ΣPAH8, and total BaP in meat and chicken, respectively, as ranging between 1.24 and 1.78 ng/kg (bw)-day [34]. Comparatively, the 95th percentile exposure of ΣPAH4, ΣPAH8, and total BaP obtained in this current study were significantly higher than those obtained in the meat products reported [34]. Similarly, these researchers reported of the exposure of PAHs in fish: ΣPAH4 (0.8 ng/kg(bw)-day), ΣPAH8(1.25 ng/kg(bw)-day), and total BaP (1.77 ng/kg(bw)-day) to be significantly lower. Comparatively, the PAH content in the meat products they reported was lower than those characterized in the cooked rice products in this current study (Table 3). The consistently high exposure of PAH observed in this present study, relative to other studies, suggest a possibly neglected, though significant contamination of PAHs from cooked rice products. Whether high or low, the concentration of PAHs in diets may not matter since a multivariate regression analysis of intake of BaP and the corresponding risk of esophageal cancer suggests an independent effect [37]. Thus, ingestion of dietary PAHs may precipitate adverse health based on some confounding risk factors.

The chronic exposure of the non-cancer-causing PAHs, ranged from 0.01 mg/kg(bw)-day at the 5th percentile for pyrene (female consumers) to 0.774 mg/kg(bw)-day of naphthalene at the 95th percentile (male consumers). However, frequently occurring (modal) chronic exposure varied from 0.01 mg/kg(bw)-day for pyrene (female consumers) to naphthalene (1.05 mg/kg(bw)-day male consumers). Though there were high maximum exposure recorded for acenaphthalene (1.96 mg/kg(bw)-day, male consumers), 1-methylnaphthalene (3.078 mg/kg(bw)-day male consumers), and naphthalene (2.13 mg/kg(bw)-day, male consumers), they remain isolated cases and thus, prone to outliers [38].

3.3. Risk Indices

From the current studies, the risk indices characterized included hazard quotient (HQ), hazard index (HI), margin of exposure (MoE) and incremental lifetime cancer risks (ILTCR). Though all PAHs have additive effects and, summed together, have a significant carcinogenic impact, some individual PAHs have specific non-carcinogenic effects; thus, the risk output was also categorized as such (Table 4).

The risk output of dietary PAHs among participants in the study area are presented in Figure 1, which illustrates the HQ for the eight non-carcinogenic PAHs. These PAHs' frequently occurring (modal) toxic outcomes show that 1-methylnaphthalene and specifically pyrene (for only female consumers) presented high risk (HQ>1). At the 95th percentile (showing the top 5% heaviest consumers),

it was realized that fluoranthene and fluorene would not present adverse effects (HQ <1). However, the HI of all the eight non-carcinogenic PAHs presented high risk (HI>1) for all the statistics determined apart from isolated cases of male consumers at minimum exposure (Table 5). The 95th percentile values of the MoE for ΣPAH4, ΣPAH8, and total BaP ranged between 0.008 for the male consumers in terms of ΣPAH8 to over 2×10^3 for female consumers. Only the maximum values obtained showed isolated cases of high values of MoE, ranging from 5.76×10^3 for male consumers in the case of ΣPAH4 to the highest of 3×10^7 for female consumers in the case of ΣPAH8. Since values of MoE, less than 10^4, indicate higher public health concerns, it means there were only a few isolated cases in the study area that presented low carcinogenic risk in terms of the consumption of cooked rice products. Thus, the frequently occurring (modal) MoE values for cancer risks (0.0-82.7) show that cancer risks are of a high public health concern for the consumption of cooked rice products for both male or female consumers.

The high public health concern observed for the modal MoE values, reinforce the high ILTCR (female; 0.001-0.003, male; 0.001-0.734) indices in this current study. Related research in China, where rice is also a staple, presented high ILTCR among adolescent male (2.87×10^{-5}-4.43×10^{-5}) and female (2.85×10^{-5}-3.98×10^{-5}) consumers [30]. These ILTCR values, which were lower than what was obtained in this current study for the male (3.81×10^{-5}-0.001) and female (3.72×10^{-5}-0.001) consumers, show the extent of the risk by consuming such cooked rice products in Ghana. Though the most frequently occurring (modal) ILTCR values for male and female consumers recorded in this current study (1.7×10^{-5}-4.9×10^{-5}) were significantly higher, they ranged between the deminimis (10^{-6}) threshold and the priority risk level (10^{-4}) [39]. However, the 95th percentile ILTCR values, representing the heaviest consumers of cooked rice products, presented a more severe risk (1×10^{-3}). It is of grave concern that rice consumption is only a fraction of the total diet in the study area. Therefore, the ILTCR values based on the total diet is even bound to be higher in total diet.

Table 4. Chronic exposure of individual non-carcinogenic PAHs

Chronic Exposure (mg/kg-day)	Statistics						
	Min (10^{-3})	Mean (10^{-2})	Mode (10^{-3})	Median (10^{-2})	5th (10^{-2})	95th (10^{-3})	Max (10^{-1})
Pyr(Male)	0.0000	0.140	0.235	0.119	0.0099	0.035	0.0715
Pyr(Female)	0.0061	0.139	0.010	0.117	0.0100	0.034	0.0009
Flt(male)	0.0000	0.292	0.104	0.259	0.0216	0.077	0.1158
Flt(Female)	0.0248	0.303	0.329	0.256	0.0219	0.071	0.0018
Ant(Male)	0.0000	0.160	0.054	0.136	0.0114	0.040	0.1040
Ant(Female)	0.0108	0.159	0.057	0.134	0.0114	0.039	0.0009
Flu(male)	0.0000	2.060	0.039	0.169	0.0140	0.049	0.2700
Flu(Female)	0.0189	0.197	0.157	0.167	0.0143	0.048	0.0012
Ace(Male)	0.0000	1.710	0.854	1.510	0.1240	0.444	1.9600
Ace(Female)	0.1460	1.760	0.114	1.480	0.1260	0.430	0.0105
2-M(Male)	0.0000	1.240	0.239	1.040	0.0866	0.307	0.4840
2-M(Female)	0.0792	1.220	0.435	1.030	0.0883	0.298	0.0074
1-M(Male)	0.0000	1.570	0.527	1.310	0.1090	0.388	3.0780
1-M(Female)	0.2170	1.530	0.552	1.300	0.1110	0.375	0.0090
Nap(Male)	0.0000	3.090	1.050	2.620	0.2170	0.774	2.1390

Table 5. Risk output of dietary PAH among participants

Risk Indices	Statistics						
	Min	Mean	Mode	Median	5th	95th	Max
Hazard Index (HI)							
HI (Female)	3.11	189.83	42.00	162.67	30.25	439.10	9.87×10^2
HI (Male)	0.00	209.56	37.09	164.97	30.30	454.33	1.34×10^8
Margin of Exposure (MoE)							
MoE $_{SPAH4}$ (Female)	1.900	0.936	0.055	0.126	0.043	1.490	9.51×10^3
MoE $_{SPAH4}$ (Male)	0.000	0.869	0.061	0.125	0.042	1.450	5.76×10^3
MoE $_{SPAH8}$(Female)	229	149	82.7	182	6250	2110	3.00×10^7
MoE $_{SPAH8}$(Male)	0.000	0.003	0.000	0.003	0.000	0.008	5.00×10^0
MoE $_{(total\ BaP)}$(Female)	3.320	1.230	0.065	0.158	0.055	1.840	1.51×10^4
MoE $_{(total\ BaP)}$(Male)	0.000	1.200	0.074	0.156	0.053	1.830	1.67×10^4
Incremental Lifetime Cancer Risk (ILTCR)							
ILTCR$_{(total\ BaP)}$(Female)	4.6×10^{-9}	0.001	4.9×10^{-5}	4.43×10^{-4}	3.81×10^{-5}	0.001	0.003
ILTCR$_{(total\ BaP)}$(Male)	0.000	4.42×10^{-4}	1.7×10^{-5}	4.48×10^{-4}	3.72×10^{-5}	0.001	0.734

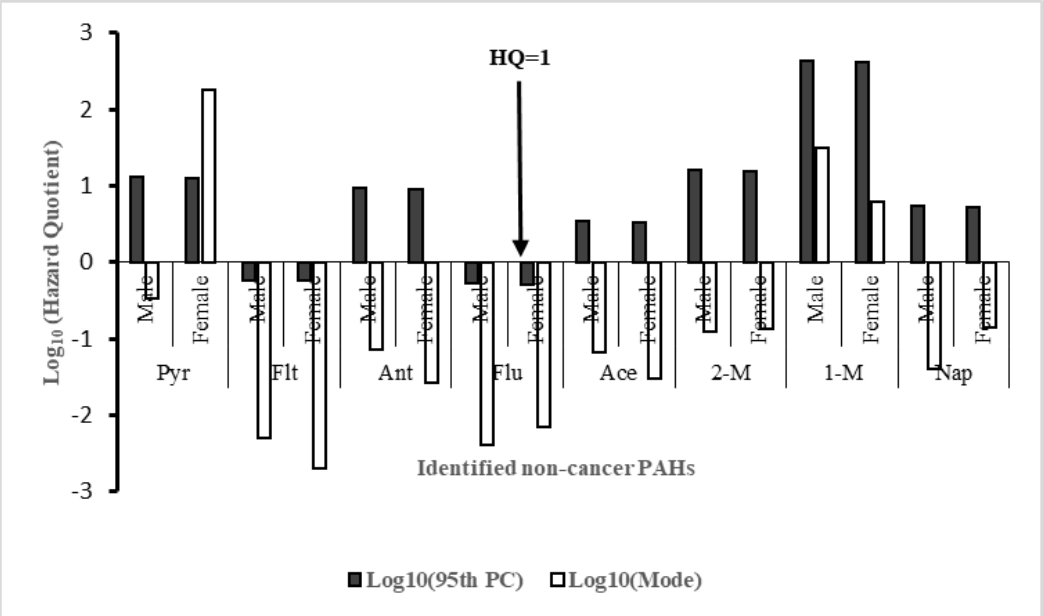

Figure 1. Hazard quotients of identified non-cancer PAHs

4. Conclusion

Out of the 54 characterized rice samples, 11 PAHs were identified. The single highest PAH congener, 2-methyl-naphthalene, was present in 61.11% of the samples analyzed relative to 18.52% attributed to benzo [a] pyrene contamination. The 11 PAHs identified included benzo[b]fluoranthene (BbF) and BaP as members of the ΣPAH4. Also included were BbF, benzo[k]fluoranthene (BkF) and BaP members of the ΣPAH8, which are all known to possess individual carcinogenic properties. Thus, the cooked rice products were more likely to be contaminated with fewer members of individual carcinogenic PAHs. Nonetheless, the concentrations of the total BaP, and the ΣPAH4 and ΣPAH8 groups, resulted in high consumer exposures. Such concentrations yielded low margin of exposure values (10^4, 95^{th} percentile) and a high priority risk (10^{-4}) for the modal incremental lifetime cancer risks, indicating a heightened public health concern. Consequentially, constant monitoring of such frequently consumed cooked rice products is needed to inform the decision for control in the long term since confounding factors could predispose some consumers to the adverse impact of PAHs.

All data relating to this manuscript can be obtained from https://data.mendeley.com/datasets/br85y85xv9/1

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Competing Interests

All authors declare that they have no known financial interests or relationships that could influence the results reported in this paper.

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