Determination of The Concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) In Water Infusion of Selected Brands of Tea Consumed in Colleges of Education in Delta State

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Abstract- This study investigated the concentrations and profiles of polycyclic aromatic hydrocarbons (PAHs) in water infusion of selected brands of tea sold in the Colleges of Education in (Warri and mosogar) Delta State Nigeria, with a view to providing information on the potential health risk associated with the consumption of these teas. A total of five different brands of teas which includes sample T1, T2, T3, T4 and T5 were collected from retail shops. The concentrations of the 16 priority PAHs in these samples was measured by gas chromatography equipped with mass selective Detector (GC-MSD) after dichloromethane/acetone extraction and clean up. The concentration varied from 128.70 to 623.40 µg kg^{-1}. The distribution pattern of PAHs in the teas were in the order of 4 Rings> 5 Rings> 6 Rings> and 3 Rings, 2 Rings was not detected. The tea infusion with water indicated concentration higher than the maximum permissible limit of (5µg/kg) which pose potential health risk to tea consumers as stated in European Commission regulation (EC) No.1881/2006.The hazard index values indicated that there was no presence of non-carcinogenic effects on exposure to PAHs for children and adult. The total cancer risk values resulting from child and adult exposure of PAHs exceeded the target value (USEPA 2009) of 1×10^{-6} suggesting that exposure to PAHs in the 5 samples carries a significant risk of cancer to humans. The dominance of high molecular weight PAHs indicated that the major sources of PAHs in these tea samples is are from combustion processes.

Keywords: Dichloromethane/Acetone Polycyclic Aromatic Hydrocarbons (Pahs), Gas Chromatography, Mass Selective Detector (GC-MSD).

I. INTRODUCTION

Tea is one of three globally recognized beverages, China is a major tea producer, having the largest tea plantation area in the world and the highest tea production, consumption and export. Research study suggest that tea contains polyphenols such as catechins, caffeine theanine, flavonoids, anthocyanins and phenolic acids [4,19].

Global tea production in 2007 was 3.60 million tonnes, of which nearly 30.6 % was produced in China. A number of studies have provided scientific evidence for the health- promoting benefits of tea consumption, including reduction of cholesterol, depression of hypertension, anti-oxidation and antimicrobial effects, and protection against cardiovascular disease and cancer [12]. However, due to both the chemical treatment of crops and post-harvest manipulation, a number of residues of chemical contaminants have been detected in tea leaves, such as heavy metals, fluoride, and pesticides [26]. These contaminants may affect tea yield and quality, and pose a health threat to tea drinkers.

Polycyclic aromatic hydrocarbons (PAHs) are a group of hundreds of organic compounds, which contain two or more fused aromatic rings made up of carbon and hydrogen. PAHs are formed by the incomplete combustion of organic material like wood, coal or oil. Studies of various matrices, such as coal combustion effluents, motor vehicle exhaust, used motor lubricating oil and tobacco smoke, have shown that PAHs are found in the outdoor air and the indoor environment. However, for non-smokers, human exposure to PAHs occurs mainly via food consumption [7]. The occurrence of PAHs in foods is due to the formation of PAHs during food processing, such as drying, smoking, and domestic food preparation such as barbecuing, roasting and frying.

PAHs comprise the largest group of chemical compounds known to be cancer causing agents. Some PAHs have been demonstrated to be carcinogenic and mutagenic. However, those PAHs that have not been found to be carcinogenic may act as synergists. The

United States Environmental Protection Agency (USEPA) has identified 16 most frequently occurring! or dangerous PAHs as priority pollutants and further classified them into carcinogenic and non carcinogen PAHs. Benzo(a) anthracene (BaA), benzo(a)pyrene (BaP), dibenzo(b)fluoranthene (DbF), benzo(k)fluoranthene (BkF), chrysene (Chry), dibenzo(a,h)anthracene (DahA) and Indeno(I ,2,3-cd)perylene (lndP) are considered as probable human carcinogens while naphthalene (Nap), acenaplithene (Ace), acenaphthylene (Acy),]anthracene (Ant), benzo(ghi)perylene (BghiP), fluoranthene (Flt), fluorene (Flu), phenanthrene (Phe) and pyrene (Pyr) are considered non-carcinogen [1]. However, these PAHs are not encountered singly in nature but as complex mixture with varying concentrations and profiles. The fact that exposure to PAHs is always due to a mixture, which is not always of constant composition, makes the assessment of health consequences difficult. Nevertheless, some studies have shown correlations between exposure to individual PAHs and occurrence of human cancer. Such substances, regardless of their carcinogenic potential, serve as markers for exposure to the entire PAH mixture [8].

PAHs may threaten human health when exposed to human from a wide variety of sources, such as occupation, smoking, diet, drinking water, and outdoor / indoor air particulates [11,13,17,24]. Gaseous and particle-bound PAHs can be transported over long distances before deposition, and may accumulate in vegetation. This could also indirectly cause human exposure to PAHs through food consumption and, thus, might pose a human health threat. Thus, over the years PAHs have attracted much attention.

II. MATERIALS AND METHODS

Materials

The following are some of the materials that were collected for this study: tea bags of various brands, pure ultra water samples etc. Analytical grade reagents were also employed throughout the study to ensure accuracy of results. Some of the reagents employed for this study are presented in Table 1

Table 1 Chemical reagents used for the study

Name	Grade	Manufacturer
Dichloromethane	Analytical grade	Baker Deventer. Netherlands
Anhydrous sodium sulphate	Analytical grade	
silica gel	Analytical grade	Merck. Germany

Description of Study Area:

The study areas are the Colleges of Education in Warri and Mosogar Area of Delta State.The area is in Delta State, Nigeria having many students, teachers and lecturers. Also some major activities in warri are Warri Refining and Petrochemical Company (WRPC), a subsidiary of Nigeria National Petroleum Corporation (NNPC).

Sample Collection

A total of 5 brands of teabags was collected from retail outlets in the Colleges of Education in Warri and Mosogar, Delta State. The choice of the samples was carefully made to reflect

Preparation of Tea Infusion Using Water

10g of the tea sample was submerged in 100ml boiling ultrapure water and then transferred into 500ml conical flask. Then the flask was placed in a water bath with the temperature maintained at 80^0c for 15min afterwards, the water phase was then decanted in another enclosed conical flask and cooled to room temperature. [28].

Sample Extraction and Clean-Up

Cooled tea infusion sample (50ml) will be extracted by ultrasonication at 30^0c for 30mins with 50ml dichloromethane (DCM)/acetone mixture (1:1, v/v). Thereafter, the DCM under the water was collected using a separating funnel and concentrated to 1 ml using a rotary evaporator. The extract was filtered through anhydrous Na_2SO_4 to remove traces of water. It was then cleaned up through a silica gel column and eluted with 10ml of DCM, and then concentrated to 2ml using a rotary evaporator. The concentrated extract will be transferred into a vial bottle using a pipette and labeled appropriate and stored at 4^0c before analysis. [23,28].

Sample Detection/Analysis

Samples were analyzed for ∑16 PAHs by high resolution gas chromatography (Hewlett Packard 6890) equipped with a HP5 (Cross linked PHME Siloxane) (0.025 μm film thickness x 0.25 μm x 30m) and mass selective detector (MS). A 0.5 ml aliquot of the extract was manually injected with a syringe. The injector temperature was 250^0c. The temperature program was initially set at 60^0c, held at 60^0c for 1min followed by a 20^0c/min ramping at 300^0c and then held there for 10min. Helium was used as a carrier gas at a flow rate of 1.36ml/min.

Quality Control and Quality Assurance

All solvents used (e.g. dichloromethane, acetone) was analytical grade quality. Separating funnel will be rinsed with acetone before used. All glassware's was pre-washed and rinsed with acetone before use. To evaluate the extraction efficiency for the PAH compounds, recovery studies was carried out by spiking selected already analyzed samples with known concentration of the individual PAHs compound. The recoveries for the PAH compounds ranged from 92.3 to 98.7%.

Statistical Analysis

The statistical analysis of the collected dataset was performed using Microsoft Excel.Analysis of variance (ANOVA) was carried out to determine whether the concentrations of PAHs in the teas varied significantly. A probability of 0.05 was considered a significance level.

Evaluation of non-cancer health risk assessment.

The USEPA model equations USEPA (1989; 2009) were adopted in order to assess the health risks associated with human exposure to PAHs through the three exposure pathways (ingestion, inhalation and dermal contact). The hazard index (HI), which represents the non-carcinogenic risk were gotten from the calculation of the total hazard quotients resulting from the major exposure pathways as shown in Equation (1)-(2). The hazard index values significance is stated as; HI > 1 signifies adverse non-carcinogenic risk while, HI < 1 signifies there is no adverse non-carcinogenic risk.

Hazard Index (HI) = ∑ HQ

$$HQ = \frac{EDI}{RFD} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (eq1)$$

$$EDI_{ing\text{-}nc} = \frac{C \times IngR \times EF \times ED \times CF}{BW \times ATnc} \ldots\ldots\ldots\ldots (eq2)$$

Evaluation of cancer health risk assessment.

Where C represent the concentration of PAHs, AF (mg cm^{-2}) represent skin to soil adherence factor, ABS_d represent dermal absorption factor, ATnc and ATca are the respective average time for non-carcinogenic and carcinogenic effects; EF stands for exposure frequency, RFD represents oral reference dose for ingestion for specific compounds(non carcinogenic), ET represent exposure time (h d^{-1}), ED represent exposure duration; PEF represent soil to air particulate emission factor (m^3 kg^{-1}), IngR stands for ingestion rate (mg d^{-1}) and InhR represent inhalation rate (m^3d^{-1}); SA represent surface area of the skin (cm^2 $event^{-1}$), BW stands for average human body weight (kg), GIABS represent gastrointestinal absorption and CF represent conversion factor (10^{-6}). The cancer slope factor (CSF) is used to convert the ratio of the exposure dose of PAHs over a lifetime of exposure to the risk of an individual developing cancer. Potential carcinogen exposure is part of a carcinogenic risk assessment that estimates a person developing cancer over a lifetime. The incidence of cancer was estimated using Eq (3) according to USEPA guidelines [23,25,].

$$TCR = EDI \ x \ CSF \quad \ldots\ldots\ldots (3)$$

Where TCR represents total cancer risk, CSF represents the cancer slope factor for the specific compounds (carcinogenic) and EDI is extimated daily intake [23]. A risk value > 1.0×10^{-6} indicates carcinogenic effects, according to the US Environmental Protection Agency [23].

III. RESULTS AND DISCUSSION

The results of the 16 priority PAHs determined in the tea infusion samples in this study are presented in Table 2. The concentrations and profiles in these tea infusions ranged from 128.7µg/kg to 623.4µg/kg. The lowest and highest concentrations T4 and T1 respectively. The concentrations of PAHs recorded in this study were relatively high compared to those reported in literature. For example, [15] reported PAHs concentrations in the range of 11.1 to 28.2 µg/L in black tea infusions. Also, [3] reported PAHs concentrations ranging from 0.1 to 6.5 µg/L in tea infusions. The high concentration PAHs in these tea infusion may be due to tea/water ratio, infusion time, atmospheric deposition, soil contamination, drying process of leaves, storage, packaging and transportation.

Table 2 shows the concentration and profiles of PAHs in the samples. The 2 ring PAHs compound

(Naphthalene) was not detected in any of the samples investigated. Acenaphthene is the only 3 ring PAH detected in this study. It was found in only one sample (T2) at a concentration of 18.1µg/kg and constituted 7.58% of the 16 PAHs.

The concentration of 4 rings PAHs ranged from 39.1µg/kg to 576.6µg/kg with samples T1 having the highest and T2 having the lowest concentration respectively. Pyrene is the most predominant 4 ring PAH compound in this study in terms of occurrence and concentration. It was detected in all samples at concentration ranging from 24.3 µg/kg to 544.5µg/kg sample T1 and T5 had the highest and lowest concentration respectively.

BaA was detected in 60% of the samples with T1, T3 and T 5 detected concentration ranging from 12.8 to 26.0 µg/kg T3 having the highest and T1 having the lowest respectively. Chrysene was also detected in 60% of the samples at concentrations in the range of 11.90 for sample T5 to 30µg/kg for T4 respectively.

The concentration of 5 rings PAHs ranged from 25.4 to 148.9µg/kg. Samples T4 and T2 had the highest and lowest concentration. BbF is the predominant 5 ring PAHs compound in terms of occurrence and concentration. BbF was detected in all samples at concentrations ranging from 22.30 to 101.30µg/kg with sample T2 having the lowest and T5 having the highest.

BkF was detected in 60% of the samples at a concentration ranging from 16.9 to 51.3µg/kg with T2 having the highest and T1 the lowest. BkF constituted 2.71 to 21.47% of the 16 PAHs.

BaP which is the most carcinogenic of the PAHs was detected in only T2 with a concentration of 38.5µg/kg. The concentration of BaP detected was higher than maximum recommended limit of 5µg/kg stipulated by the European Commision. DahA was detected in only T2 with a concentration of 36.8µg/kg which constituted 15.4% of the 16 PAHs.

The concentration of 6 ring PAHs was only detected in T2 with a concentration of 32.80µg/kg (BghiP) and it constituted 13.72% of the 16 PAHs.IndP was not detected in any of the samples.

The samples had more of high molecular weight PAHs Generally, Low molecular weight (LMW) PAHs are mainly from petrogenic sources and high molecular weight (HMW) PAHs are mainly contributed by pyrogenic sources [29]. The content of HMW PAHs (90%) was higher than that of LMW PAHs (10%), indicating that most PAH production might originate from the pyrogenic processes (e.g combustion processes) in these teas.

Table 2: PAHs concentration in (µg/kg) in tea infusion using water.

COMPOUNDS	T1	T2	T3	T4	T5
Naphthalene	ND	ND	ND	ND	ND
Acenaphthylene	ND	ND	ND	ND	ND
Acenaphthene	ND	18.10	ND	ND	ND
Fluorene	ND	ND	ND	ND	ND
Anthracene	ND	ND	ND	ND	ND
Phenanthene	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	26.50	15.20
Pyrene	544.50	39.10	65.30	30.30	24.30
Benz[a] anthracene	12.80	ND	26.00	16.50	ND
Chrysene	19.30	ND	ND	30.00	11.90
Benzo [b] fluoranthene	30.00	22.30	30.40	25.40	101.30
Benzo[k] fluoranthene	16.90	51.30	29.70	ND	ND
Benzo [a] pyrene	ND	38.50	ND	ND	ND
Indeno [1, 2, 3- cd]pyrene	ND	ND	ND	ND	ND
Dibenz [a, h] anthracene	ND	36.80	ND	ND	ND
Benzo [ghi] perylene	ND	32.80	ND	ND	ND

∑16PAHs	623.40	238.90	151.30	128.70	152.80
2Ring	ND	ND	ND	ND	ND
3Ring	ND	18.10	ND	ND	ND
4Ring	576.60	39.10	91.30	103.30	51.40
5Ring	46.90	148.90	60.10	25.40	101.30
6Ring	ND	32.80	ND	ND	ND
LMW-PAHs	ND	18.10	ND	ND	ND
HMW-PAHs	623.50	220.80	151.40	128.70	152.70

T- tea
ND - not detected

HEALTH RISK ASSESSMENT
The assessment of potential human health risks associated with PAHs contamination in the teas was carried out using both non-carcinogenic and carcinogenic risk indices in Table 3 and 4. The non-carcinogenic risks were evaluated using the Total Hazard Quotient (HQ) , while cancer risks were assessed based on the calculated Total Cancer Risk (TCR) values of the specific compounds.

Table 3. Hazard Index of PAHs

CHILD EXPOSURE

	Nap	Acy	Ace	Flu	Ant	Phen	Flt	Pyr	HQING
T1	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.09E-04	2.09E-04
T2	0.00E+00	0.00E+00	3.47E-05	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.50E-05	4.97E-05
T3	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.50E-05	2.50E-05
T4	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	7.62E-05	1.16E-05	8.79E-05
T5	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	4.37E-05	9.32E-06	5.30E-05

Table 4.

ADULT EXPOSURE

	Nap	Acy	Ace	Flu	Ant	Phen	Flt	Pyr	HQING
T1	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	6.38E-05	6.38E-05
T2	0.00E+00	0.00E+00	1.06E-05	0.00E+00	0.00E+00	0.00E+00	0.00E+00	4.58E-06	1.52E-05
T3	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	7.65E-06	7.65E-06
T4	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.33E-05	3.55E-06	2.68E-05
T5	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.34E-05	2.85E-06	1.62E-05

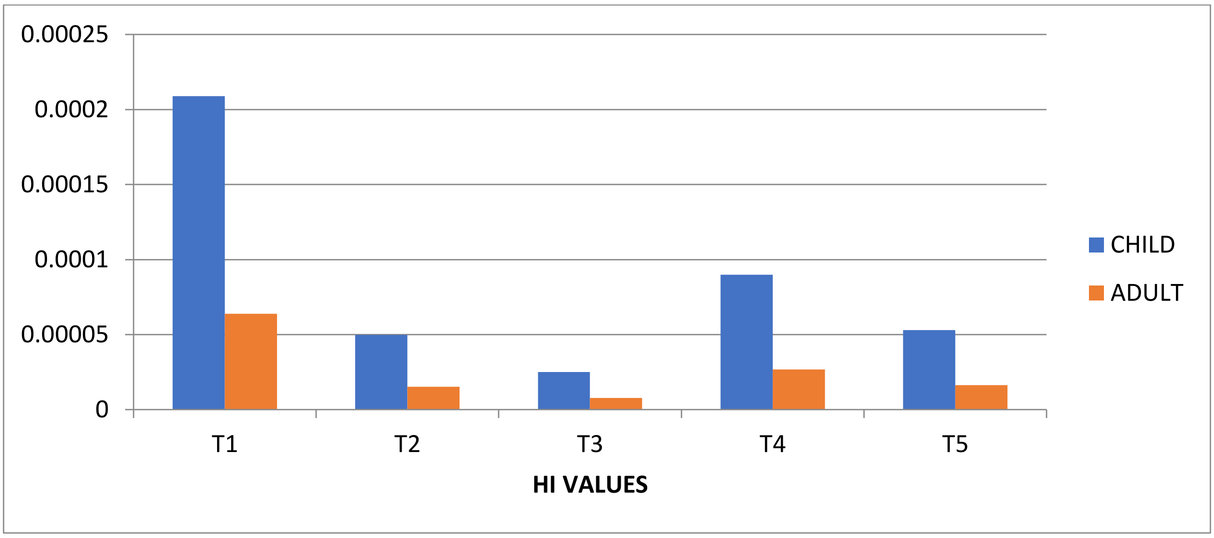

Figure 1: Non-carcinogenic risk for children and adult exposure to poly aromatic hydrocarbons PAHs

The non-cancer risk arising from the exposure of adults and children to the concentration of PAHs via oral ingestion (HQing) is shown in Table 3, 4 and fig 1. The Hazard Index values for the individual exposure pathways were below 1. The HI values suggested that there was no (non-carcinogenic) health effects for child and adult exposure to PAHs with child having the highest concentration. The carcinogenic risk for children and adult exposure are presented in Table 5 and 6 respectively.

Table 5 Carcinogenic risk for children exposure

	BaA	Chry	BbF	BkF	BaP	IndP	DahA	TCRing
T1	1.08E-03	1.62E-05	2.52E-03	1.42E-04	1.42E-02	0.00E+00	0.00E+00	1.79E-02
T2	0.00E+00	0.00E+00	1.87E-03	4.31E-04	4.31E-02	0.00E+00	3.09E-02	7.63E-02
T3	2.18E-03	0.00E+00	2.55E-03	2.49E-04	2.49E-02	0.00E+00	0.00E+00	2.99E-02
T4	1.39E-03	2.52E-05	2.13E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	3.54E-03
T5	0.00E+00	1.00E-05	8.51E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	8.52E-03

Table 6 Carcinogenic risk for adult exposure

	BaA	Chry	BbF	BkF	BaP	IndP	DahA	TCRing
T1	3.29E-04	4.95E-06	7.70E-04	4.34E-05	0.00E+00	0.00E+00	0.00E+00	1.15E-03
T2	0.00E+00	0.00E+00	5.72E-04	1.32E-04	9.88E-03	0.00E+00	9.45E-03	2.00E-02
T3	6.67E-04	0.00E+00	7.80E-04	7.62E-05	0.00E+00	0.00E+00	0.00E+00	1.52E-03
T4	4.24E-04	7.70E-06	6.52E-04	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.08E-03
T5	0.00E+00	3.05E-06	2.60E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.60E-03

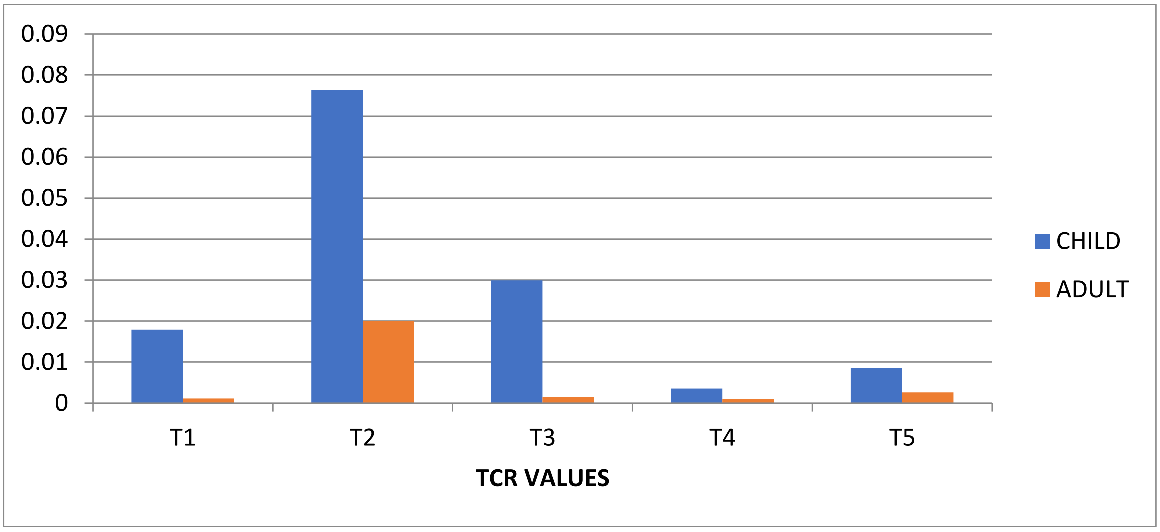

Figure 2: Carcinogenic risk for children and adult exposure to poly aromatic hydrocarbons PAHs

The cancer risk arising from the exposure of adults and children to the concentration of PAHs via oral ingestion (TCRing) is shown in Table 5, 6 and fig 2. The values for the individual exposure pathways were above $1x10^{-6}$. The cancer risk values suggested that there was potential carcinogenic health risk for child and adult exposure to PAHs with child having the highest concentration.

IV. SUMMARY AND FINDINGS.

In this study, 5 samples of tea were obtained from stores in the College of Education in Warri and Mosogar Delta State to determine the concentration of PAHs in these teas. The results indicated the presence of PAHs in the tea infusion in water may result in potential health risk to consumers with children been more exposed to this risk. The concentration of benzo(a) pyrene observed in this study exceeded the maximum tolerable limits of (5µg/kg) stated in Commission Regulation (EC) No. 1881/2006 by the European Commission.

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VI. CONCLUSION

Based on the findings, the samples collected surpassed internationally accepted contamination limits for PAHs. The hazard index values indicated no presence of non-carcinogenic risk associated PAHs while the total cancer risk value for children was found to be higher than that for adults. The data obtained indicated more of high molecular weight PAHs. These high molecular weight (HMW) PAHs are mainly contributed by pyrogenic sources Therefore, this suggests that these teas were contaminated with PAHs originating from combustion-related activities. This calls for caution in consumption of these teas and the regulation of PAHs and other contaminants in food by the appropriate authorities in Nigeria to safeguard the health of the nation

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