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Concentrations of polycyclic aromatic hydrocarbons in some commercial brands of candies and chocolates in Nigeria

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RESEARCH ARTICLE

Abstract

The concentrations and profiles of polycyclic aromatic hydrocarbons (PAHs) were investigated in 29 commercial brands of candies and chocolates commonly consumed in Nigeria with a view to providing information on the risks associated with the consumption of these products. The measurements were performed by means of gas chromatography-mass spectrometry after hexane/dichloromethane extraction and clean-up. The concentrations of the Σ16 PAHs in the candies and chocolates were in the range of 1.09-149.46 µg/kg and 0.95-222.62 µg/kg, respectively. The profiles of PAHs in these brands of candies and chocolates indicate dominance of low molecular weight PAHs (3- and 4-ring) over high molecular weight PAHs (5- and 6-ring). In this study, three samples of chocolate had PAH4 (benzo(a)anthracene + benzo(b) fluoranthene + benzo(a)pyrene + chrysene) concentrations above the 35 µg/kg PAH4 limit in cocoa butter stipulated by European Commission Regulation no. 835/2011.

Keywords: candies, chocolates, daily intakes, margin of exposure, polycyclic aromatic hydrocarbons, Nigeria

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of over one hundred chemical compounds containing two or more fused aromatic and/or pentacyclic rings in a linear, angular or cluster formation. A number of them have proven carcinogenic, mutagenic and genotoxic properties. Heavy PAHs (that is, those containing more than four benzene rings) are known to exhibit dioxin-like activity and to disrupt oestrogenic response (Villeneuve *et al.*, 2002). The metabolites of PAHs may bind to proteins and DNA, which causes disruption of biochemical processes, cell damage in animals and cancer in humans (Dhananjayan and Muralidharam, 2012). Those PAHs that have not been found to be carcinogenic may synergistically enhance the carcinogenicity of other PAHs (Al-Rashdan *et al.*, 2010; Maertens *et al.*, 2004; Phillips, 1999). Human exposure to PAHs does not occur singly since PAHs are typically encountered in complex mixtures (Angerer and Mueller, 2004). Out of the over 100 PAHs that occur in nature, the United States Environmental Protection Agency (EPA) has listed 16 of them as priority PAHs on the basis of their occurrence frequencies, and in order to facilitate monitoring in the environment. The International Agency for Research on Cancer (IARC) has classified the 16 PAHs as follows: benzo(a)pyrene (BaP) as group 1A (carcinogenic to humans), dibenz(a,h)anthracene (DahA) as group 2A (probably carcinogenic to humans) and naphthalene (Nap), benzo(a) anthracene (BaA), benzo(b)fluoranthene (Bbf), benzo(k) fluoranthene (Bkf), chrysene (Chy) and indeno(1,2,3-cd)pyrene (IndP) as group 2B (possibly carcinogenic to humans), while acenaphthylene (Acy), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthrancene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo(g,h,i)perylene (BghiP) are not classifiable as regards their carcinogenicity to humans (IARC, 2010). PAHs are formed primarily from incomplete combustion processes such as burning of wood, coal or oil. Owing to their mode

of formation, PAHs are ubiquitous in the environment and can enter into the food chain through air, water and soil (Ciecierska and Obiedzinski, 2007; Lage Yusty and Cortizo Daviña, 2005; Tfouni *et al.*, 2007;). PAHs are lipophilic in nature, although some of them have relatively good water solubilities (Wenzl *et al.*, 2006). PAHs are found in foodstuffs as a result of environmental conditions and thermal treatments which foods are subjected to during preparation and manufacturing. Food processing methods such as smoking, frying, roasting, drying and baking, etc., are often used to improve their taste, appearance, colour, flavour, as well as shelf life. However, these methods are known sources of food contamination by PAHs (Ciecierska and Obiedzinski 2007; Moret *et al.*, 2005; Yurchenko and Molder, 2005).

Dietary sources through the consumption of grains, vegetables and boiled fish and meat have been recognised as the major route for human exposure to PAHs, except for smokers and the occupationally exposed population (Falco *et al.*, 2005; Phillips, 1999).

Different approaches have been proposed in the literature for assessment of PAH mixtures in food, the most popular being the use of BaP as a marker and the toxic equivalency factor (TEF) (Akpambang *et al.*, 2009). The Scientific Committee on Food Additives (SCF, 2002) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) suggested the use of BaP as a marker for occurrence and the effect of PAHs in foods. The toxic equivalency approach was found not to be scientifically valid owing to lack of data from oral carcinogenicity studies on the individual PAHs, their different modes of action and the evidence of poor predictivity of the carcinogenic potency of PAH mixtures based on the currently proposed TEF value (EFSA, 2008; SCF, 2002). In 2008, the EFSA CONTAM Panel concluded that the use of BaP alone is not a suitable marker for occurrence of PAHs in food. It concluded that the eight higher molecular weight PAHs (PAH8) and a subgroup of four (PAH4) are the most suitable markers of PAHs in food and further, it suggested the use of the margin of exposure (MOE) approach for the assessment of risk exposure (EFSA, 2008).

Chocolates are produced from nonalkalised cocoa liquor by adding sucrose, cocoa butter, aroma or flavouring substances, wheat flour, buffering agents, carbohydrate, protein, fat, alkaloids, minerals and some other constituents (Belitz *et al.*, 2004). Chocolate products are more susceptible to contamination with PAHs due to the number of complex steps involved in their preparation from the cocoa bean. The steps involved include harvesting of coca, fermenting coca to cocoa beans, cleaning, drying, roasting, grinding, blending, couching and moulding. The contamination of chocolate products with PAHs can mainly occur as a result of inappropriate drying practices of the cocoa beans such as sun-drying of cocoa beans on asphalt or bitumen, or by using direct drying with fire, and the utilisation of unrefined cocoa butter for the chocolate preparation (Kumari *et al.*, 2013). Cocoa beans can also be contaminated with PAHs during storage and transportation in jute or sisal bags that have been treated with batching oil (Grob *et al.*, 1993), and by lube and hydraulic oils (Moret *et al.*, 1997). Candy is produced by dissolving sugar in water or milk to form syrup, which is boiled until it reaches the desired concentration or starts to caramelise. Candy has a wide range of textures, from soft and chewy, to hard and brittle. The textural characteristics of candy depend on the ingredients and the temperature at which the candy is produced. The production temperature of candies varies from 110 to 178 °C depending on the type of candy. Generally, high temperature and a greater sugar concentration result in hard, brittle candies while a low temperature results in soft candies.

Nigeria's confectionery sub-sector is estimated at more than 120,000 MT in volume and valued at approximately 250 million US dollars each year. Nigeria's confectioneries comprise mainly of hard candy (50%), bubble gum (30%) and toffees/others (20%). The sub-sector has grown 15 to 20% per annum over the last five years, after Nigeria lifted the import ban on chocolate in 2005 (USDA, 2013). Following from the above, the estimated per capita consumption of this confectionery is 0.71 kg per annum based on the 170,000,000 population of Nigeria. Chocolates and candies are often consumed by people of all age groups and especially by children. Children are the most vulnerable group to contamination in food items such as chocolates and candies. In Nigeria, and many other countries, chocolates and candies constitute a significant proportion of the daily food intake of children. In view of these facts, the concentrations of contaminants in human foods require constant monitoring to ensure strict compliance with regulatory control limits in order to protect public health.

A survey of the literature reveals that limited data are currently available on the PAH content of chocolates and candies. Dennis *et al.* (1991) reported the content of BaP in chocolate samples as ranging between 0.13 and 0.32 µg/kg. Lodovici *et al.* (1995) recorded the mean content of BaP in three different types of chocolate as 0.33 µg/kg. A BaP concentration of 0.18 µg/kg was detected in chocolate candy (Kazeromi *et al.*, 2001). Chocolates from the Irish market contained BaP concentrations ranging from 0.06 to 0.30 µg/kg (FSAI, 2006). Ziegenhals *et al.* (2009) reported a median concentration of 0.22 µg BaP/kg in forty samples of various types of chocolates with different cocoa contents in the German market. From the foregoing, it is apparent that most of the available data are restricted to European countries with no data currently available on the concentrations of PAHs in chocolates and candies in the Nigerian market.

The objective of the present study was to determine the concentrations of the EPA 16 priority PAHs in samples of different types of chocolates and candies in the Nigerian market with a view to providing information on the risks associated with the consumption of these products.

2. Materials and methods

Reagents

All chemicals and reagents used were of analytical grade. Dichloromethane (LC grade), alumina and silica gel were obtained from BDH (Poole, UK) while n-hexane was obtained from Aldrich (St. Louis, MO, USA). A PAH standard mixture (NIST, Baltimore, MD, USA) containing the 16 priority PAHs was used in this study. The drying agent, sodium polyacrylate, was from Aldrich (Munich, Germany).

Sample collection

A total of 29 commercial brands of candies and chocolates (consisting of 14 candy and 15 chocolate brands) were purchased from different sales outlets in Warri, Agbor, Abraka and Asaba, Nigeria. Within each brand, five samples with different batch numbers and dates of manufacture were collected. Information with respect to the origins of the examined samples is displayed in Table 1. The choice of samples was carefully made to reflect popular brands consumed by different income classes and was influenced by availability at the time of purchase. The samples were stored in a freezer at 4 °C prior to analysis.

Sample preparation, extraction and clean-up

The chocolate and candy samples were removed from their wrappers. At least 5 bars of chocolate of the same brand with different batch numbers were minced with a rasp and 5 g of the subsamples were mixed with the same amount of drying

Table 1. Information on the origins of the commercial brands of candies and chocolates examined.

	Brand name	Net weight (g)	Country of origin
Candies	Miss Magic	8	–
	Choco Mats	–	Singapore
	Parago orange	8	Indonesia
	Milk bomb	10	Thailand
	Black currant splash	–	Nigeria
	Milkose	–	Nigeria
	Parago grape	8	Indonesia
	Banana splash	–	Nigeria
	Watch King	–	China
	Ase Candy	–	–
	Fresh mint	–	–
	Lollipop candy	12	China
	Ase Ring candy	–	–
	Fulta fruit	7	China
Chocolates	Orient Bitter Kokolin	8	Turkey
	Track strawberry	9	Turkey
	Parago chocolate	8	Indonesia
	Choco milk chocolate	14	Indonesia
	Twix	58	Indonesia
	Nikolo	30	Uruguay
	Mini Single	14	Turkey
	Coco Crispee	15	Malaysia
	Coco Dance	14	Turkey
	Taya Gift	–	–
	Choco milo	–	Nigeria
	Tiffany	–	Dubai
	Presty	–	–
	Bon-bon	17	Argentina
	Track Hazelnut	9	Turkey

agent (sodium polyacrylate). The hard candy was ground to a fine powder and mixed with an equal amount of the drying agent. The resulting material was poured into a 33 ml cell and extracted with n-hexane and dichloromethane (1:1, v/v) in an accelerated solvent extraction system (ASE 200; Dionex Sunnyvale, CA, USA). The extraction cells were filled with solvent, pressurised to 14 MPa and heated to 120 °C for 6 min. The pressure and temperature was held constant for an extraction time of 5 min and the cells were rinsed with cold solvent (60% of cell volumes) and purged with argon for 150 s. The static extraction and purge steps were performed twice for each sample and the extracts were combined (Pies *et al.*, 2007; Wilcke *et al.*, 2005; Ziegenhals *et al.*, 2009). The extracts were evaporated to 1 ml and purified by solid phase extraction with 2 g of aluminium oxide (5% deactivated, upper part) and 2 g of silica gel (lower part). The PAHs were subsequently eluted with 15 ml of n-hexane, 15 ml n-hexane and dichloromethane (9:1) and 20 ml of hexane and dichloromethane (4:1). The eluted fractions were combined and evaporated to approximately 0.5 ml using a gentle stream of nitrogen gas.

Chemical analysis

The PAHs in the eluted fraction were measured with a gas chromatograph (GC; HP 6890) equipped with a HP5 (cross-linked PHME siloxane) column (0.25 µm film thickness, 0.25 µm × 30 m) and a HP 5973 series mass-selective detector (Agilent, Palo Alto, CA, USA). The mass spectrometer (MS) was operated in the electron impact ionisation mode (ionising energy of 70 eV) scanning from m/z 50 to 450 at 3.6 scans/s. The ion source and quadrupole temperature were 230 and 150 °C, respectively. The operating conditions were as follows: the carrier gas was helium with a linear velocity of 1 ml/min, the injection port temperature was 290 °C, the injection volume was 2 µl in pulsed splitless mode and the GC/MS interface temperature was 250 °C. The column temperature was initially held at 80 °C for 0.5 min and then increased to 230 °C at 80 °C/min and from 230 to 280 °C at 5 °C/min, and held at 280 °C for 18 min; the solvent delay was 6 min. The target and confirmation ions monitored for the analysis, and retention times for the PAH compounds are displayed in Table 2.

Quality control/quality assurance and statistical analysis

To evaluate the extraction efficiency for the target compounds, recovery studies were carried out. Some previously analysed samples were spiked with known concentrations of the individual PAH compounds and reanalysed. The spike was applied to the samples at three concentration levels. The spike recoveries for the PAH compounds ranged from 64.4 to 99.7%. The performance characteristics of the present method meet the criteria specified in European Commission Regulation 836/2011 (recovery between 50 and 120%) (EC, 2011a). The relative standard deviations for replicate analyses were less than 7.8%. The detection and quantification limits (LODs and LOQs) were evaluated on the basis of noise obtained with the analysis of a blank sample (n=4). The LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively. The percentage recoveries, r^2 values for the calibration lines, LOD and LOQ values for the PAH compounds are

Table 2. Percentage spike recoveries, r^2 values for the calibration lines, limits of detection (LOD), limits of quantification (LOQ) and identification parameters for the 16 polycyclic aromatic hydrocarbons (PAHs) analysed.

PAH compound	Percentage recovery (%)	r^2 value for calibration line	LOD (µg/kg)	LOQ (µg/kg)	Retention time (min)	m/z ion	Confirmation ion
Naphthalene	64.4	0.9991	0.006	0.02	5.25	128	127
Acenapthylene	75.4	0.9993	0.009	0.03	7.62	152	151
Acenapthene	97.2	0.9998	0.006	0.02	7.95	154	153
Fluorene	87.6	0.9998	0.006	0.02	9.02	166	165
Phenanthrene	79.7	0.9997	0.012	0.04	11.49	178	176
Anthracene	83.4	0.9992	0.006	0.02	11.64	178	176
Fluoranthene	93.6	0.9994	0.006	0.02	15.29	202	101
Pyrene	82.3	0.9997	0.006	0.02	16.05	202	101
Benzo(a)anthracene	97.6	0.9995	0.021	0.07	20.14	228	114
Chrysene	92.4	0.9996	0.003	0.01	20.23	228	114
Benzo(b)fluoranthene	90.6	0.9991	0.006	0.02	22.67	252	126
Benzo(k)fluoranthene	93.2	0.9992	0.006	0.02	22.73	252	126
Benzo(a)pyrene	99.7	0.9993	0.009	0.03	23.29	252	126
Indeno(1,2,3-cd)pyrene	87.6	0.9998	0.003	0.01	25.61	252	276
Dibenz(a,h)anthracene	93.4	0.9994	0.003	0.01	25.69	278	276
Benzo(g,h,i)perylene	98.4	0.9996	0.003	0.01	26.22	278	278

displayed in Table 2. The matrix effect was evaluated by spiking the samples of candy and chocolate with the same concentration range as was used for calibration, and then comparing the correlation coefficient and the slope of the straight line obtained with the spiked standards in the matrix with those of the original standard calibration curve. No matrix effect was observed for any of the PAHs in any type of chocolate or candy matrix. Analysis of variance and Tukey multiple-comparison tests were used to determine whether the concentrations of the PAHs varied significantly within and between groups. Differences with *P*-values less than 0.05 were considered to be statistically significant. The statistical calculations were performed with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

The results of the determination of PAHs in commercial brands of candies and chocolates are displayed in Table 3 and 4, respectively, while the mean and median concentrations and concentration ranges of each PAH in the candy and chocolate samples are summarised in Table 5. The profiles of PAHs in candy and chocolate samples are displayed in Figure 1 and 2, respectively. The concentrations of Σ16 PAHs in candies and chocolates ranged from 1.08 to 149.46 µg/kg and 0.95 to 134.93 µg/kg, respectively. The total Σ16 PAH concentrations showed significant variations within the chocolate and candy brands. Two brands of the candies showed higher total concentrations of Σ16 PAHs compared with other brands (brand CD-8 and CD-14 with concentrations of 149.46 and 135.67 µg/kg, respectively) whereas in the other samples, the concentrations of Σ16 PAHs were less than 54 µg/kg. Similarly, high concentrations of Σ16 PAHs were observed in five brands of chocolate examined (i.e. CH-1, CH-2, CH-3, CH-4 and CH-7). The chocolate samples had higher mean concentrations of Σ16 PAHs than the candy samples. The difference in the concentrations of the Σ16 PAHs in the candies and chocolates depends on the kinds of processing techniques adopted during the production of these items

Table 3. Concentrations (in µg/kg) of polycyclic aromatic hydrocarbons in commercial brands of candies purchased in Nigeria.

	CD-1	CD-2	CD-3	CD-4	CD-5	CD-6	CD-7	CD-8	CD-9	CD-10	CD-11	CD-12	CD-13	CD-14
NaP	0.02	0.25	<0.02	<0.02	51.7	1.3	0.77	2.51	1.52	0.87	0.07	0.06	3.1	<0.02
Acy	0.6	0.03	0.07	0.10	0.07	1.51	0.57	1.5	0.71	<0.03	0.15	0.13	<0.03	0.42
Ace	0.13	0.06	0.02	0.33	<0.02	0.53	0.45	45.89	0.34	<0.02	0.13	0.09	0.11	0.11
Flu	2.01	0.38	0.5	1.37	<0.02	0.94	0.37	<0.02	<0.02	<0.02	2.01	1.38	2.55	0.2
Phe	0.15	0.16	0.21	0.14	<0.04	0.61	0.84	10.14	<0.04	0.07	0.15	0.14	<0.04	0.2
Ant	<0.02	0.04	0.41	3.56	<0.02	0.47	1.15	8.57	<0.02	0.08	<0.02	<0.02	<0.02	0.02
Flt	0.02	0.41	0.08	0.36	<0.02	0.26	0.21	24.77	<0.02	0.02	0.02	0.01	<0.02	0.02
Pyr	2.12	0.16	0.78	1.97	<0.02	0.96	2.37	7.87	<0.02	0.05	2.12	0.03	<0.02	0.62
BaA	0.21	0.13	0.07	0.65	<0.07	0.75	1.02	6.37	<0.07	<0.07	0.21	0.53	<0.07	4.5
Chy	0.01	0.05	0.07	0.84	1.43	1.14	0.27	1.52	<0.01	<0.01	0.01	0.01	<0.01	2.29
Bbf	0.02	0.05	0.03	0.36	<0.02	1.58	1.25	3.76	<0.02	<0.02	0.02	0.06	<0.02	3.15
Bkf	<0.02	0.08	0.07	<0.02	<0.02	0.49	1.29	5.06	<0.02	<0.02	<0.02	0.02	<0.02	1.04
BaP	0.1	0.03	0.55	<0.03	<0.03	3.67	2.12	1.52	<0.03	<0.03	0.1	0.08	<0.03	2.18
IndP	0.31	0.33	1.12	<0.01	<0.01	7.43	10.42	7.59	<0.01	<0.01	0.31	0.52	<0.01	7.19
DahA	0.02	0.05	0.24	<0.01	<0.01	1.46	16.53	12.55	<0.01	<0.01	0.02	0.01	<0.01	33.59
BghiP	0.01	0.03	0.02	<0.01	<0.01	1.87	6.28	9.84	<0.01	<0.01	0.01	0.25	<0.01	80.14
∑16 PAHs	5.73	2.24	4.24	9.68	53.2	24.97	45.91	149.46	2.57	1.08	5.33	3.32	5.76	135.67
2-ring	0.02	0.25	0	0	51.7	1.3	0.77	2.51	1.52	0.87	0.07	0.06	3.1	0
3-ring	2.89	0.67	1.21	5.5	0.07	4.06	3.38	66.1	1.05	0.15	2.44	1.74	2.66	0.95
4-ring	2.36	0.75	1	3.82	1.43	3.11	3.87	40.53	0	0.06	2.36	0.58	0	7.43
5-ring	0.14	0.21	0.89	0.36	0	7.2	21.19	22.89	0	0	0.14	0.17	0	39.96
6-ring	0.32	0.36	1.14	0	0	9.3	16.7	17.43	0	0	0.32	0.77	0	87.33
PAH2	0.11	0.08	0.62	0.84	1.43	4.81	2.39	3.04	0	0	0.11	0.09	0	4.47
PAH4	0.34	0.26	0.72	1.85	1.43	7.14	4.66	13.17	0	0	0.34	0.68	0	12.12
PAH8	0.68	0.75	2.17	1.85	1.43	18.39	39.18	48.21	0	0	0.68	1.48	0	134.08

CD = candies; NaP = naphthalene; Acy = acenapthylene; Ace = acenapthene; Flu = fluorene; Phe = phenanthrene; Ant = anthracene; Flt = fluoranthene; Pyr = pyrene; BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; BaP = benzo(a)pyrene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene; BghiP = benzo(g,h,i)perylene; PAH2 = Chy + BaP; PAH4 = BaA + Chy + Bbf + BaP; PAH8 = BaA + Chy + Bbf + Bkf + BaP + IndP + DahA + BghiP.

Table 4. Concentrations (in µg/kg) of polycyclic aromatic hydrocarbons in commercial brands of chocolates purchased in Nigeria.

	CH-1	CH-2	CH-3	CH-4	CH-5	CH-6	CH-7	CH-8	CH-9	CH-10	CH-11	CH-12	CH-13	CH-14	CH-15
NaP	<0.02	0.21	<0.02	<0.02	1.71	0.95	20.47	0.56	0.86	1.25	<0.02	0.02	1.06	1.34	0.88
Acy	0.05	1.22	0.19	4.86	2.67	<0.03	7.25	0.23	0.55	<0.03	0.1	0.08	0.83	<0.03	<0.03
Ace	0.23	0.06	0.24	2.81	1.85	<0.02	6.67	0.05	0.22	<0.02	0.04	0.02	0.41	<0.02	<0.02
Flu	0.05	<0.02	22.25	4.81	1.74	<0.02	5.74	0.02	<0.02	<0.02	0.72	<0.02	0.65	<0.02	<0.02
Phe	<0.04	<0.04	2.51	1.94	2.8	<0.04	3.89	0.04	<0.04	0.65	0.34	0.13	0.13	2.98	0.96
Ant	5.59	<0.02	0.28	4.2	0.94	<0.02	4.29	0.11	<0.02	0.75	0.78	1.04	0.37	0.2	0.04
Flt	0.75	0.12	4.32	1.0	2.01	<0.02	7.0	0.02	<0.02	0.05	0.1	1.63	0.16	1.19	0.12
Pyr	7.35	4.77	4.07	2.91	1.7	<0.02	0.85	0.14	<0.02	0.43	1.19	0.86	0.21	1.48	0.21
BaA	4.68	64.48	2.62	1.7	0.93	<0.07	9.57	0.13	<0.07	<0.07	0.08	1.53	0.54	<0.07	<0.07
Chy	105.27	6.61	6.02	3.12	1.35	<0.01	13.81	0.86	<0.01	<0.01	0.09	0.75	0.97	<0.01	<0.01
Bbf	10.96	1.57	8.54	5.76	1.19	<0.02	0.39	0.67	<0.02	<0.02	0.04	0.1	0.16	<0.02	<0.02
Bkf	<0.03	0.02	38.13	2.37	0.65	<0.03	0.32	0.02	<0.03	<0.03	0.1	0.83	0.29	<0.03	<0.03
BaP	<0.02	0.49	3.76	1.46	1.41	<0.02	39.16	0.37	<0.02	<0.02	0.76	1.12	2.59	<0.02	<0.02
IndP	<0.01	6.39	10.6	26.64	3.44	<0.01	<0.01	0.55	<0.01	<0.01	5.35	0.01	1.35	<0.01	<0.01
DahA	<0.01	11.62	15.54	9.74	17.68	<0.01	<0.01	0.03	<0.01	<0.01	0.45	0.89	0.75	<0.01	<0.01
BghiP	<0.01	<0.01	3.55	16.84	11.44	<0.01	<0.01	<0.01	<0.01	<0.01	0.09	0.35	0.93	<0.01	<0.01
∑16 PAHs	134.93	97.56	122.62	90.16	53.51	0.95	119.4	3.8	1.63	3.13	10.23	9.36	11.4	7.19	2.21
2-ring	0	0.21	0	0	1.71	0.95	20.47	0.56	0.86	1.25	0	0.02	1.06	1.34	0.88
3-ring	5.92	1.28	25.47	18.62	10	0	27.84	0.45	0.77	1.4	1.98	1.27	2.39	3.18	1
4-ring	118.05	75.98	17.03	8.73	5.99	0	31.23	1.15	0	0.48	1.46	4.77	1.88	2.67	0.33
5-ring	10.96	13.7	65.97	19.33	20.93	0	39.87	1.09	0	0	1.35	2.94	3.79	0	0
6-ring	0	6.39	14.15	43.48	14.88	0	0	0.55	0	0	5.44	0.36	2.28	0	0
PAH2	105.27	7.1	9.78	4.58	2.76	0	52.97	1.23	0	0	0.85	1.87	3.56	0	0
PAH4	120.91	73.15	20.94	12.04	4.88	0	62.93	2.03	0	0	0.97	3.5	4.26	0	0
PAH8	120.91	91.18	88.76	67.63	38.09	0	63.25	2.63	0	0	6.96	5.58	7.58	0	0

CH = chocolates; NaP = naphthalene; Acy = acenapthylene; Ace = acenapthene; Flu = fluorene; Phe = phenanthrene; Ant = anthracene; Flt = fluoranthene; Pyr = pyrene; BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; BaP = benzo(a)pyrene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene; BghiP = benzo(g,h,i)perylene; PAH2 = Chy + BaP; PAH4 = BaA + Chy + Bbf + BaP; PAH8 = BaA + Chy + Bbf + Bkf + BaP + IndP + DahA + BghiP.

and the background concentration of PAHs in the cocoa butter, which is the major ingredient for production of chocolates. The concentrations of Σ16 PAHs in the samples examined were comparable with the concentrations of Σ16 PAHs reported in the literature for chocolates and candies. For example, Ziegenhals *et al.* (2009) reported total PAH concentrations in chocolates on the German market as 1.33 to 6.85 µg/kg, while Kumari *et al.* (2012) recorded a Σ16 PAH content in the range of 2.70 to 235.91 µg/kg in chocolate candies.

The two-ring PAH, NaP, was detected in 79 and 21% of the candies and chocolate brands respectively at concentrations ranging from 0.02 to 51.70 µg/kg. Higher NaP concentrations were observed in CD-5 and CH-7 for candies and chocolates respectively. The percentage contribution of NaP to the Σ16 PAH concentrations of these products ranged from 0.6 to 100%.

The 3-ring PAHs (Acy, Ace, Flu, Phe and Ant) constituted 0.1 to 57.1% and 1.2 to 47.9% of the Σ16 PAHs in the candies and chocolates, respectively. The most dominant PAH compounds in these samples were Acy and Ace. Acy and Ace were detected at concentrations ranging from 0.03 to 7.25 µg/kg and 0.02 to 45.89 µg/kg, respectively. All the samples had Acy and Ace concentrations less than 50 µg/kg. Kumari *et al.* (2012) reported the prominence of Acy in chocolate candies at concentrations in the range of 3.99 to 183.47 µg/kg. Flu was detected in 71% of the candy samples at concentrations in the range of 0.20 to 2.55 µg/kg and in 53% of the chocolate samples at concentrations in the range of 0.02 to 22.25 µg/kg. The highest sample concentration was observed in CH-3 while other brands had Flu concentrations less than 6.0 µg/kg. Phe was detected in 86% of the candies and 73% of the chocolates at concentrations in the range of 0.07 to 10.14 µg/kg and 0.04 to 3.89 µg/kg respectively. Phe constituted 0.1 to 7.2% and 1.0 to 43.2% of the Σ16 PAH content in

Table 5. Means ± standard deviations (SD) (median) and range (in µg/kg) of polycyclic aromatic hydrocarbons (PAHs) in candies and chocolate samples.[1]

PAH compound	Candies		Chocolates	
	Mean ± SD (median)	Range (µg/kg)	Mean ± SD (median)	Range (µg/kg)
naphthalene	4.44±13.64 (0.51)	(<0.02-51.7)	1.95±5.15 (0.86)	(<0.02-20.47)
acenapthylene	0.42±0.52 (0.14)	(<0.03-1.51)	1.20±2.14 (0.19)	(<0.03-7.25)
acenapthene	3.44±12.22 (0.12)	(<0.02-45.89)	0.84±1.80 (0.06)	(<0.02-6.67)
fluorene	0.84±0.88 (0.44)	(<0.02-2.55)	2.40±5.79 (0.02)	(<0.01-22.25)
phenanthrene	0.92±2.67 (0.15)	(<0.01-10.14)	1.09±1.35 (0.34)	(<0.02-3.89)
anthracene	1.02±2.37 (0.03)	(<0.02-8.57)	1.24±1.84 (0.37)	(<0.02-5.59)
fluoranthene	1.87±6.59 (0.02)	(<0.02-24.77)	1.23±1.98 (0.16)	(<0.02-7.0)
pyrene	1.36±2.08 (0.70)	(<0.02-7.87)	1.75±2.14 (0.86)	(<0.02-7.35)
benzo(a)anthracene	1.03±1.93 (0.21)	(<0.07-6.37)	5.75±16.45 (0.54)	(<0.07-64.48)
chrysene	0.55±0.76 (0.06)	(<0.01-2.29)	9.26±26.83 (0.86)	(<0.01-105.27)
benzo(b)fluoranthene	0.73±1.26 (0.04)	(<0.02-3.76)	1.96±3.52 (0.16)	(<0.02-10.96)
benzo(k)fluoranthene	0.58±1.36 (0.02)	(<0.02-5.06)	2.85±9.78 (0.02)	(<0.02-38.13)
benzo(a)pyrene	0.74±1.16 (0.09)	(<0.03-3.67)	3.41±9.95 (0.49)	(<0.03-39.16)
indeno(1,2,3-cd)pyrene	2.52±3.78 (0.32)	(<0.01-10.42)	3.62±7.11 (0.01)	(<0.01-26.64)
dibenz(a,h)anthracene	4.61±9.86 (0.02)	(<0.01-33.59)	3.78±6.39 (0.03)	(<0.01-17.68)
benzo(g,h,i)perylene	7.03±21.25 (0.02)	(<0.01-80.14)	2.22±5.03 (0.01)	(<0.01-16.84)
∑16 PAHs	31.77±47.15 (5.77)	(2.23-149.46)	52.76±67.06 (10.22)	(0.95-222.62)

[1] For PAH compounds whose concentrations were below limits of quantification (LOQ), the half of the LOQ values were used in computation of the mean and median values.

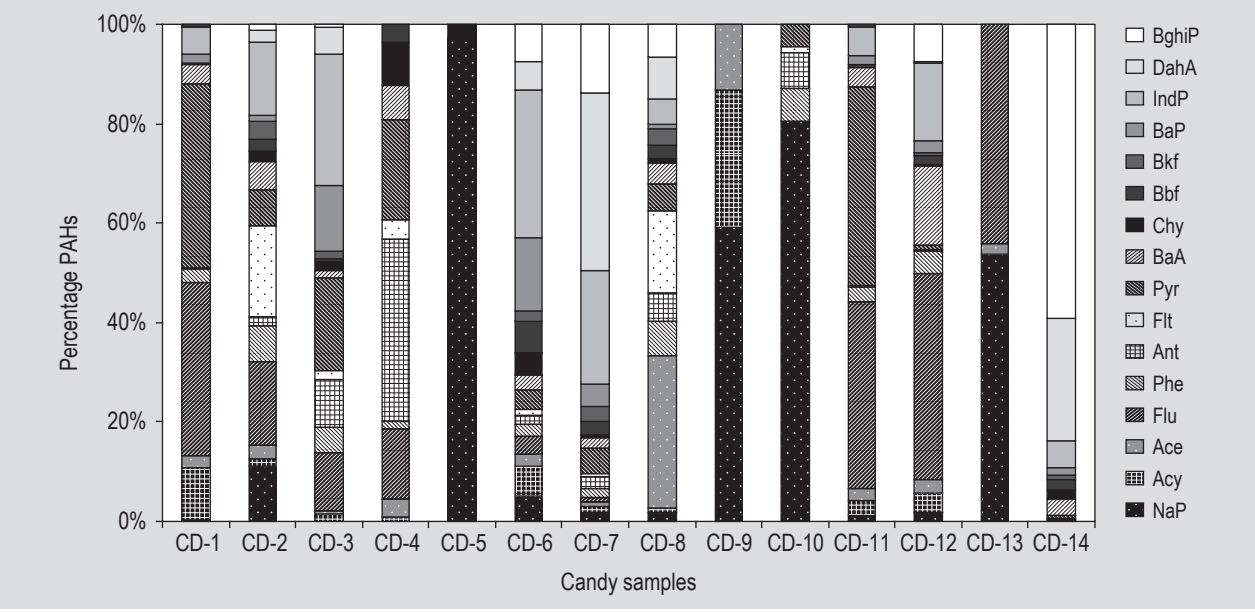

Figure 1. Polycyclic aromatic hydrocarbon profile in candy samples.

candies and chocolates, respectively. Ant was found in 57% of candies and 47% of chocolate samples. Chocolates had higher concentrations of Ant than the candy samples. Ant was detected at concentrations of 0.02-8.57 µg/kg in candies and 0.04-5.59 µg/kg in chocolates and constituted 1.8 to 37% and 0.1 to 24.9% of the Σ16 PAHs in candies and chocolates, respectively.

The four-ring PAHs (Flt + Pyr + BaA + Chy) constituted 5.3 to 79.8% and 0.7 to 77.1% of the total PAH content

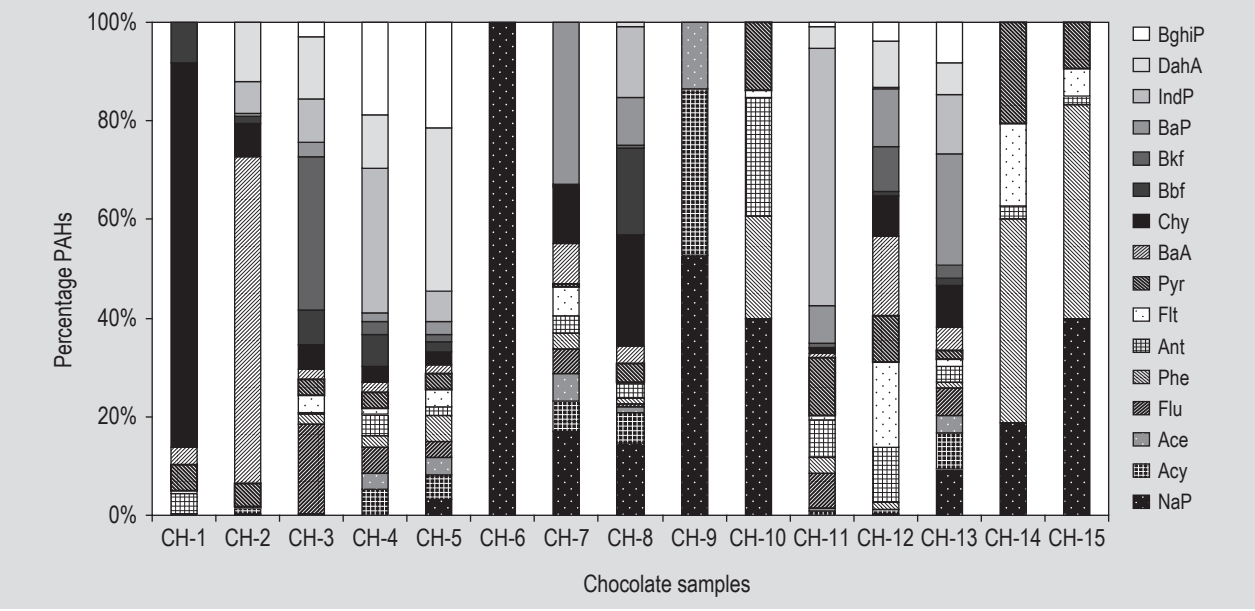

Figure 2. Polycyclic aromatic hydrocarbon profile in chocolate samples.

in candies and chocolates respectively. Four-ringed PAHs were not detected in CD-9 and CD-13 in candies and CH-6 and CH-9 in the chocolate samples. The Flt concentration ranged from 0.01 to 24.77 µg/kg and 0.02 to 7.00 µg/kg in candies and chocolates, respectively. The highest sample concentration of Flt was observed in candies (CD-8). The percentage contribution of Flt to the Σ16 PAH concentrations in the candies and chocolates never exceeded 18.4%. Pyr was found in 78 and 87% of the candies and chocolates examined. Higher Pyr concentrations were found in chocolates than candies. Pyr was detected at concentrations in the range of 0.03-7.87 µg/kg and 0.14 to 7.35 µg/kg in candies and chocolates, respectively. The highest Pyr concentrations were observed in CD-8 and CH-1. An exceptionally high concentration (64.48 µg/kg) of BaA was observed in the chocolate brand CH-2 whereas other brands had BaA contents less than 10.0 µg/kg. BaA was detected in 71% and 67% of the candies and chocolates studied. Chy was detected in 72% of the brands at concentrations ranging between 0.01 and 105.27 µg/kg. Chocolates had higher concentrations of Chy than candies. An exceptionally high content of Chy was found in sample CH-1 compared with the other brands studied. In other samples, the Chy concentration was below 14.0 µg/kg. Chy constituted 0.2 to 8.7% of the Σ16 PAHs in candies and 0.9 to 68.7% in the chocolates.

The 5-ring PAHs (Bbf + Bkf + BaP + DahA) constituted 2.0-33.7% of the Σ16 PAHs in candies and chocolates. Five-ring PAHs were not detected in CD-5, CD-9, CD-10, CD-13 and CH-6, CH-9, CH-10, CH-14, CH-15 for candies and chocolates, respectively. Bbf was detected at concentrations of 0.02 to 3.76 µg/kg and 0.04 to 10.96 µg/kg in candies and chocolates, respectively. Bkf was detected in 50% of the candies and 60% of the chocolates at concentrations in the range of 0.02 to 5.06 µg/kg and 0.02 to 38.13 µg/kg, respectively. CH-3 had an elevated level of Bkf compared with other brands of candies and chocolates studied. In other brands, the concentrations of Bkf never exceeded 5.1 µg/kg. BaP was detected in 52% of the samples at concentrations in the range of 0.03 and 3.67 µg/kg in candies and 0.37 to 39.16 µg/kg in chocolates. The European Commission (EC) Regulation no. 835/2011 stipulates that the permissible limit of BaP in cocoa butter, a major ingredient of chocolate, should be 5.0 µg/kg, while that of PAH4 (BaP + BaA + Chy + Bbf) should be 35.0 µg/kg as from 1st April 2013 and 30 µg/kg as from 1st April 2015 (EC, 2011b). In this study, CH-1, CH-2 and CH-7 had PAH4 concentrations above the permissible limit while the concentration of BaP in CH-7 was above the permissible limit for BaP. A wide concentration range of BaP in chocolate and cocoa butter samples has been reported in the literature. For instance, BaP concentrations of 0.33 µg/kg (Lodovici *et al.*, 1995), 0.13 to 0.32 µg/kg (Dennis *et al.*, 1991), 0.07 to 0.63 µg/kg (Ziegenhals *et al.*, 2009), and not detected to 12.7 6 µg/kg (Kumari *et al.*, 2012) have been reported. The concentrations of BaP found in our samples were similar to the BaP concentrations in candies and chocolates in the literature. However, higher concentrations of BaP in the range of 7.698 to 148.04 µg/kg have been reported in cocoa bean subjected to different drying methods (Wandan *et al.*, 2011). Lowor *et al.* (2012) reported BaP contents of 0.08 to 0.76 µg/kg and 0.16 to 35.51 µg/kg in cocoa beans dried by the roadside and cocoa beans stored in diesel-contaminated jute sacks respectively.

The six-ring PAHs (IndP + BghiP) constituted 13.3 to 60% and 5.9 to 86.6% of the Σ16 PAHs in chocolates and

candies, respectively. Six-ringed PAHs were not detected in 41% of the samples examined. The 6-ringed PAHs were detected at concentrations of 0.32 to 87.33 µg/kg. Some of the studied candies and chocolates had exceptionally high contents of IndP, DahA and BghiP. These samples were CD-7, CD-8, CD-14, CH-4 and CH-5. Generally, the PAH8 concentration in the candies and chocolates was in the range of 0.68-134.08 µg/kg and 2.63-102.91 µg/kg respectively. Chocolates had higher PAH8 concentrations than candies. The PAH8 constituted up to 90.8% of the Σ16 PAH content in some samples.

Estimation of dietary intake and risk assessment

In this study an ingestion rate of 1.9 g per day was used for estimation of intakes of PAHs from consumption of candies and chocolates, based on the per capital consumption rate of 0.71 kg per annum. In the present study, the ingestion rate was applied in the case of BaP, PAH2, PAH4 and PAH8. Table 6 and 7 report the estimated daily intakes of BaP, PAH2, PAH4 and PAH8 in ng/kg body weight (bw)/day from the consumption of chocolate and candies in Nigeria for the child and adult scenarios. The estimated daily intake of BaP, PAH2, PAH4 and PAH8 for adults from chocolate ranged from not detected (nd)-1.24, nd-3.33, nd-3.83 and

Table 6. Estimated dietary intakes (ng/kg bw/day) and the margin of exposure (MOE) from consumption of 1.9 g of candy per day.[1]

	Daily intake				MOE			
	BaP	PAH2	PAH4	PAH8	BaP	PAH2	PAH4	PAH8
Adult								
CD-1	0.00	0.00	0.01	0.02	22,105,263	48,803,828	31,578,947	22,755,418
CD-2	0.00	0.00	0.01	0.02	73,684,211	67,105,263	41,295,547	20,631,579
CD-3	0.02	0.02	0.02	0.07	4,019,139	8,658,744	14,912,281	7,130,730
CD-4	0.00	0.03	0.06	0.06	–	6,390,977	5,803,698	8,364,154
CD-5	0.00	0.05	0.05	0.05	–	3,754,141	7,508,281	10,820,758
CD-6	0.12	0.15	0.23	0.58	602,323	1,116,096	1,503,759	841,418
CD-7	0.07	0.08	0.15	1.24	1,042,701	2,246,201	2,304,043	394,938
CD-8	0.05	0.10	0.42	1.53	1,454,294	1,765,928	815,250	320,964
CD-9	0.00	0.00	0.00	0.00	–	–	–	–
CD-10	0.00	0.00	0.00	0.00	–	–	–	–
CD-11	0.00	0.00	0.01	0.02	22,105,263	48,803,828	31,578,947	22,755,418
CD-12	0.00	0.00	0.02	0.05	27,631,579	59,649,123	15,789,474	10,455,192
CD-13	0.00	0.00	0.00	0.00	–	–	–	–
CD-14	0.07	0.14	0.38	4.25	1,014,003	1,200,989	885,878	115,406
Child								
CD-1	0.01	0.01	0.04	0.09	5,526,316	12,200,957	7,894,737	5,688,854
CD-2	0.00	0.01	0.03	0.10	18,421,053	16,776,316	10,323,887	5,157,895
CD-3	0.07	0.08	0.09	0.27	1,004,785	2,164,686	3,728,070	1,782,683
CD-4	0.00	0.11	0.23	0.23	–	1,597,744	1,450,925	2,091,038
CD-5	0.00	0.18	0.18	0.18	–	938,535	1,877,070	2,705,190
CD-6	0.46	0.61	0.90	2.33	150,581	279,024	375,940	210,355
CD-7	0.27	0.30	0.59	4.96	260,675	561,550	576,011	98,735
CD-8	0.19	0.39	1.67	6.11	363,573	441,482	203,812	80,241
CD-9	0.00	0.00	0.00	0.00	–	–	–	–
CD-10	0.00	0.00	0.00	0.00	–	–	–	–
CD-11	0.01	0.01	0.04	0.09	5,526,316	12,200,957	7,894,737	5,688,854
CD-12	0.01	0.01	0.09	0.19	6,907,895	14,912,281	3,947,368	2,613,798
CD-13	0.00	0.00	0.00	0.00	–	–	–	–
CD-14	0.28	0.57	1.54	16.98	253,501	300,247	221,470	28,852

[1] CD = candies; BaP = benzo(a)pyrene; PAH2 = Chy + BaP; PAH4 = BaA + Chy + Bbf + BaP; PAH8 = BaA + Chy + Bbf + Bkf + BaP + IndP + DahA + BghiP (BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene; BghiP = benzo(g,h,i)perylene).

Table 7. Estimated dietary intakes (ng/kg bw/day) and the margin of exposure (MOE) from consumption of 1.9 g of chocolate per day.[1]

	Daily intake				MOE			
	BaP	PAH2	PAH4	PAH8	BaP	PAH2	PAH4	PAH8
Adult								
CH-1	0.00	3.33	3.83	3.83	–	50,997	88,800	127,977
CH-2	0.02	0.22	2.32	2.89	4,511,278	756,116	146,778	169,705
CH-3	0.12	0.31	0.66	2.81	587,906	548,918	512,743	174,332
CH-4	0.05	0.15	0.38	2.14	1,514,059	1,172,144	891,764	228,799
CH-5	0.04	0.09	0.15	1.21	1,567,749	1,945,080	2,200,173	406,240
CH-6	0.00	0.00	0.00	0.00	–	–	–	–
CH-7	1.24	1.68	1.99	2.00	56,449	101,348	170,616	244,643
CH-8	0.01	0.04	0.06	0.08	5,974,395	4,364,570	5,289,085	5,883,530
CH-9	0.00	0.00	0.00	0.00	–	–	–	–
CH-10	0.00	0.00	0.00	0.00	–	–	–	–
CH-11	0.02	0.03	0.03	0.22	2,908,587	6,315,789	11,068,909	2,223,230
CH-12	0.04	0.06	0.11	0.18	1,973,684	2,870,813	3,067,669	2,773,062
CH-13	0.08	0.11	0.13	0.24	853,485	1,507,983	2,520,385	2,041,383
CH-14	0.00	0.00	0.00	0.00	–	–	–	–
CH-15	0.00	0.00	0.00	0.00	–	–	–	–
Child								
CH-1	0.00	13.33	15.32	15.32	–	12,749	22,200	31,994
CH-2	0.06	0.90	9.27	11.55	1,127,820	189,029	36,695	42,426
CH-3	0.48	1.24	2.65	11.24	146,976	137,230	128,186	43,583
CH-4	0.18	0.58	1.53	8.57	378,515	293,036	222,941	57,200
CH-5	0.18	0.35	0.62	4.82	391,937	486,270	550,043	101,560
CH-6	0.00	0.00	0.00	0.00	–	–	–	–
CH-7	4.96	6.71	7.97	8.01	14,112	25,337	42654	61,161
CH-8	0.05	0.16	0.26	0.33	1,493,599	1,091,142	1,322,271	1,470,883
CH-9	0.00	0.00	0.00	0.00	–	–	–	–
CH-10	0.00	0.00	0.00	0.00	–	–	–	–
CH-11	0.10	0.11	0.12	0.88	727,147	1,578,947	2,767,227	555,808
CH-12	0.14	0.24	0.44	0.71	493,421	717,703	766,917	693,265
CH-13	0.33	0.45	0.54	0.96	213,371	376,996	630,096	510,346
CH-14	0.00	0.00	0.00	0.00	–	–	–	–
CH-15	0.00	0.00	0.00	0.00	–	–	–	–

[1] CH = chocolates; BaP = benzo(a)pyrene; PAH2 = Chy + BaP; PAH4 = BaA + Chy + Bbf + BaP; PAH8 = BaA + Chy + Bbf + Bkf + BaP + IndP + DahA + BghiP (BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene; BghiP = benzo(g,h,i)perylene).

nd-3.83 ng/kg bw/day, respectively. For a child, consuming the same quantity of chocolate per day, the dietary intake of BaP, PAH2, PAH4 and PAH8 were in the range of nd-4.96, nd-13.33, nd-15.32 and nd-15.32 ng/kg bw/day, respectively. The highest intake values of PAH2, PAH4 and PAH8 were observed in both the child and adult cases who consumed brand CH1 in preference to other brands, while the highest intake of BaP was obtained from consumption of the brand CH-7. In general, the dietary intakes of BaP, PAH2, PAH4 and PAH8 from consumption of candies were much lower than the intake values for chocolates in this study. The upper intake values for consumption of 1.9 g of candy per day for the adult scenario were 0.12, 0.15, 0.42 and 4.25 ng/kg bw/day for BaP, PAH2, PAH4 and PAH8, respectively. On the other hand, the upper intake values for the child scenario were 0.46, 0.61, 1.67 and 16.98 ng/kg bw/day for BaP, PAH2, PAH4 and PAH8, respectively. Higher intake values of PAH8 were obtained from consumption of the brands CD-6, CD-7, CD-8 and CD-14 both adult and child scenarios. However, for a child or adult who consumes up to 20 g of chocolate per day (worst case scenario) they would

Table 8. Estimated BaP carcinogenic equivalent (BaP_{TEQ}) and BaP mutagenic equivalent (BaP_{MEQ}) (µg/kg) from the consumption of 1.9 g of candy per day.[1]

	BaA	Chy	Bbf	Bkf	BaP	IndP	DahA	BaP_{TEQ}
CD-1	0.021	0.000	0.002	ND	0.100	0.031	0.020	0.174
CD-2	0.013	0.000	0.005	0.001	0.030	0.033	0.050	0.132
CD-3	0.007	0.000	0.003	0.001	0.550	0.112	0.240	0.913
CD-4	0.065	0.001	0.036	ND	ND	ND	ND	0.102
CD-5	ND	0.001	ND	ND	ND	ND	ND	0.001
CD-6	0.075	0.001	0.158	0.005	3.670	0.743	1.460	6.112
CD-7	0.102	0.000	0.125	0.013	2.120	1.042	16.530	19.932
CD-8	0.637	0.002	0.376	0.051	1.520	0.759	12.550	15.894
CD-9	ND	ND	ND	ND	ND	ND	ND	0.000
CD-10	ND	ND	ND	ND	ND	ND	ND	0.000
CD-11	0.021	0.000	0.002	ND	0.100	0.031	0.020	0.174
CD-12	0.053	0.000	0.006	0.000	0.080	0.052	0.010	0.201
CD-13	ND	ND	ND	ND	ND	ND	ND	0.000
CD-14	0.450	0.002	0.315	0.010	2.180	0.719	33.590	37.267
	BaA	**Chy**	**Bbf**	**Bkf**	**BaP**	**IndP**	**DahA**	**BaP_{MEQ}**
CD-1	0.017	0.000	0.005	ND	0.100	0.096	0.006	0.224
CD-2	0.011	0.001	0.013	0.009	0.030	0.102	0.015	0.180
CD-3	0.006	0.001	0.008	0.008	0.550	0.347	0.070	0.989
CD-4	0.053	0.014	0.090	ND	ND	ND	ND	0.158
CD-5	ND	0.024	ND	ND	ND	ND	ND	0.024
CD-6	0.062	0.019	0.395	0.054	3.670	2.303	0.423	6.926
CD-7	0.084	0.005	0.313	0.142	2.120	3.230	4.794	10.687
CD-8	0.522	0.026	0.940	0.557	1.520	2.353	3.640	9.557
CD-9	ND	ND	ND	ND	ND	ND	ND	0.000
CD-10	ND	ND	ND	ND	ND	ND	ND	0.000
CD-11	0.017	0.000	0.005	ND	0.100	0.096	0.006	ND
CD-12	0.043	0.000	0.015	0.002	0.080	0.161	0.003	0.305
CD-13	ND	ND	ND	ND	ND	ND	ND	0.000
CD-14	0.369	0.039	0.788	0.114	2.180	2.229	9.741	15.460

[1] CD = candies; BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; BaP = benzo(a)pyrene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene; ND = not detected.

ingest as much as 52, 140.4, 161.2 and 161.2 ng/kg bw/day for BaP, PAH2, PAH4 and PAH8, respectively.

Margin of exposure

The MOE approach was adopted to assess the risk of various indicators of occurrence and effects of PAHs in foods (EFSA, 2008). The MOE is the ratio of a defined point on the dose response curve (reference point) for the adverse effect of the compound in the animal carcinogenicity study of the estimated human intake of the compound. The reference point is the benchmark dose lower limit ($BMDL_{10}$) which is derived from mathematical modelling of experimental tumour data with the observed range. The $BMDL_{10}$ values for BaP, PAH2, PAH4 and PAH8 are 0.07, 0.17, 0.34 and 0.49 mg/kg bw/day, respectively (EFSA, 2008). The computed MOE values based on an ingestion rate of 1.9 g of chocolate per day for the adult and child scenarios are displayed in Table 6 and 7. MOE values less than 10,000 indicate a potential concern for the consumer's health and a possible need for management action (EFSA, 2008). The estimated MOE values based on BaP, PAH2 and PAH4 indicated that all the samples examined had MOE values greater than 10,000 and therefore there are no serious health concerns for the consumers (both for adult and child scenarios). However, for a worst case scenario of consumption of a 20 g bar of chocolate per day (based on the average weight of the studied samples) nine samples of these studied brands

Table 9. Estimated BaP carcinogenic equivalent (BaP_{TEQ}) and BaP mutagenic equivalent (BaP_{MEQ}) (µg/kg) from the consumption of 1.9 g of chocolate per day.[1]

	BaA	Chy	Bbf	Bkf	BaP	IndP	DahA	BaP_{TEQ}
CH-1	0.468	0.105	1.096	ND	ND	ND	ND	1.669
CH-2	6.448	0.007	0.157	0.000	0.490	0.639	11.620	19.361
CH-3	0.262	0.006	0.854	0.381	3.760	1.060	15.540	21.863
CH-4	0.170	0.003	0.576	0.024	1.460	2.664	9.740	14.637
CH-5	0.093	0.001	0.119	0.007	1.410	0.344	17.680	19.654
CH-6	ND	ND	ND	ND	ND	ND	ND	0.000
CH-7	0.957	0.014	0.039	0.003	39.160	ND	ND	40.173
CH-8	0.013	0.001	0.067	0.000	0.370	0.055	0.030	0.536
CH-9	ND	ND	ND	ND	ND	ND	ND	0.000
CH-10	ND	ND	ND	ND	ND	ND	ND	0.000
CH-11	0.008	0.000	0.004	0.001	0.760	0.535	0.450	1.758
CH-12	0.153	0.001	0.010	0.008	1.120	0.001	0.890	2.183
CH-13	0.054	0.001	0.016	0.003	2.590	0.135	0.750	3.549
CH-14	ND	ND	ND	ND	ND	ND	ND	0.000
CH-15	ND	ND	ND	ND	ND	ND	ND	0.000
	BaA	**Chy**	**Bbf**	**Bkf**	**BaP**	**IndP**	**DahA**	**BaP_{MEQ}**
CH-1	0.384	1.790	2.740	ND	ND	ND	ND	4.913
CH-2	5.287	0.112	0.393	0.002	0.490	1.981	3.370	11.635
CH-3	0.215	0.102	2.135	4.194	3.760	3.286	4.507	18.199
CH-4	0.139	0.053	1.440	0.261	1.460	8.258	2.825	14.436
CH-5	0.076	0.023	0.298	0.072	1.410	1.066	5.127	8.072
CH-6	ND	ND	ND	ND	ND	ND	ND	0.000
CH-7	0.785	0.235	0.098	0.035	39.160	ND	ND	40.312
CH-8	0.011	0.015	0.168	0.002	0.370	0.171	0.009	0.744
CH-9	ND	ND	ND	ND	ND	ND	ND	0.000
CH-10	ND	ND	ND	ND	ND	ND	ND	0.000
CH-11	0.007	0.002	0.010	0.011	0.760	1.659	0.131	2.578
CH-12	0.125	0.013	0.025	0.091	1.120	0.003	0.258	1.636
CH-13	0.044	0.016	0.040	0.032	2.590	0.419	0.218	3.359
CH-14	ND	ND	ND	ND	ND	ND	ND	0.000
CH-15	ND	ND	ND	ND	ND	ND	ND	0.000

[1] CH = chocolates; BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; BaP = benzo(a)pyrene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene; ND = not detected.

of confectionary (three candies and six chocolates) had PAH8-MOE values less than 10,000 for the child scenario, which implies that children who consume these brands of candies and chocolates, in preference to others, will be at a greater risk of exposure to carcinogenic PAHs.

Carcinogenic and mutagenic potency

PAHs occur as mixtures and a valuable appraisal of the risk to human health from various PAH exposures is established by the toxicity or carcinogenic potency of the individual PAH compound relative to BaP. The BaP toxic equivalent factor (BaP_{TEQ}) and the BaP mutagenic equivalent factor (BaP_{MEQ}) have been used for risk assessment of PAHs in soil, dust and foods (Durant, 1996, 1999; EPA, 1993; Larsen and Larsen, 1998; Nisbet and LaGoy, 1992; Thompson *et al.*, 1990).

The BaP carcinogenic equivalent (BaP_{TEQ}) for the individual PAHs was estimated by using the formula:

$$BaP_{TEQ} = \Sigma C_i \times BaP_{TEF} \quad (1)$$

Table 10. Estimated excess cancer risk from the consumption of 1.9 g of candy per day.[1]

	BaA	Chy	Bbf	Bkf	BaP	IndP	DahA	Excess cancer risk
Adult								
CD-1	8.17×10^{-12}	3.89×10^{-15}	7.78×10^{-13}	0.00	3.89×10^{-11}	1.21×10^{-11}	7.78×10^{-12}	6.77×10^{-11}
CD-2	5.06×10^{-12}	1.95×10^{-14}	1.95×10^{-12}	3.11×10^{-13}	1.17×10^{-11}	1.28×10^{-11}	1.95×10^{-11}	5.13×10^{-11}
CD-3	2.72×10^{-12}	2.72×10^{-14}	1.17×10^{-12}	2.72×10^{-13}	2.14×10^{-10}	4.36×10^{-11}	9.34×10^{-11}	3.55×10^{-10}
CD-4	2.53×10^{-11}	3.27×10^{-13}	1.40×10^{-11}	0.00	0.00	0.00	0.00	3.96×10^{-11}
CD-5	0.00	5.56×10^{-13}	0.00	0.00	0.00	0.00	0.00	5.56×10^{-13}
CD-6	2.92×10^{-11}	4.43×10^{-13}	6.15×10^{-11}	1.91×10^{-12}	1.43×10^{-9}	2.89×10^{-10}	5.68×10^{-10}	2.38×10^{-9}
CD-7	3.97×10^{-11}	1.05×10^{-13}	4.86×10^{-11}	5.02×10^{-12}	8.25×10^{-10}	4.05×10^{-10}	6.43×10^{-9}	7.75×10^{-9}
CD-8	2.48×10^{-10}	5.91×10^{-13}	1.46×10^{-10}	1.97×10^{-11}	5.91×10^{-10}	2.95×10^{-10}	4.88×10^{-9}	6.18×10^{-9}
CD-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD-10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD-11	8.17×10^{-12}	3.89×10^{-15}	7.78×10^{-13}	0.00	3.89×10^{-11}	1.21×10^{-11}	7.78×10^{-12}	0.00
CD-12	2.06×10^{-11}	3.89×10^{-15}	2.33×10^{-12}	7.78×10^{-14}	3.11×10^{-11}	2.02×10^{-11}	3.89×10^{-12}	7.83×10^{-11}
CD-13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD-14	1.75×10^{-10}	8.91×10^{-13}	1.23×10^{-10}	4.05×10^{-12}	8.48×10^{-10}	2.80×10^{-10}	1.31×10^{-8}	1.45×10^{-8}
Child								
CD-1	6.53×10^{-12}	3.11×10^{-15}	6.22×10^{-13}	0.00	3.11×10^{-11}	9.64×10^{-12}	6.22×10^{-12}	5.41×10^{-11}
CD-2	4.04×10^{-12}	1.56×10^{-14}	1.56×10^{-12}	2.49×10^{-13}	9.33×10^{-12}	1.03×10^{-11}	1.56×10^{-11}	4.10×10^{-11}
CD-3	2.18×10^{-12}	2.18×10^{-14}	9.33×10^{-13}	2.18×10^{-13}	1.71×10^{-10}	3.48×10^{-11}	7.46×10^{-11}	2.84×10^{-10}
CD-4	2.02×10^{-11}	2.61×10^{-13}	1.12×10^{-11}	0.00	0.00	0.00	0.00	3.17×10^{-11}
CD-5	0.00	4.45×10^{-13}	0.00	0.00	0.00	0.00	0.00	4.45×10^{-13}
CD-6	2.33×10^{-11}	3.55×10^{-13}	4.91×10^{-11}	1.52×10^{-12}	1.14×10^{-9}	2.31×10^{-10}	4.54×10^{-10}	1.90×10^{-9}
CD-7	3.17×10^{-11}	8.40×10^{-14}	3.89×10^{-11}	4.01×10^{-12}	6.59×10^{-10}	3.24×10^{-10}	5.14×10^{-9}	6.20×10^{-9}
CD-8	1.98×10^{-10}	4.73×10^{-13}	1.17×10^{-10}	1.57×10^{-11}	4.73×10^{-10}	2.36×10^{-10}	3.90×10^{-9}	4.94×10^{-9}
CD-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD-10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD-11	6.53×10^{-12}	3.11×10^{-15}	6.22×10^{-13}	0.00	3.11×10^{-11}	9.64×10^{-12}	6.22×10^{-12}	5.41×10^{-11}
CD-12	1.65×10^{-11}	3.11×10^{-15}	1.87×10^{-12}	6.22×10^{-14}	2.49×10^{-11}	1.62×10^{-11}	3.11×10^{-12}	6.26×10^{-11}
CD-13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD-14	1.4×10^{-10}	7.12×10^{-13}	9.80×10^{-11}	3.23×10^{-12}	6.78×10^{-10}	2.24×10^{-10}	1.05×10^{-8}	1.16×10^{-8}

[1] CD = candies; BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; BaP = benzo(a)pyrene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene.

Where BaP_{TEF} is the cancer potency relative to BaP and C_i is the individual PAH concentration.

The BaP mutagenic equivalent (BaP_{MEQ}) for the individual PAHs was evaluated by using the equation:

$$BaP_{MEQ} = \Sigma C_i \times BaP_{MEF} \quad (2)$$

Where BaP_{MEQ} is the mutagenic potency relative to BaP and C_i is the individual PAH concentration.

The BaP carcinogenic equivalency factors (BaP_{TEF}) of the seven carcinogenic PAHs used were BaP=1, BaA=0.1, Bbf=0.1, Bkf=0.01, Chr=0.001, DahA=1 and IndP=0.1 (EPA, 1993). The BaP mutagenic potency factors (BaP_{MEF}) were BaP=1, BaA=0.082, Bbf=0.25, Bkf=0.11, Chr=0.017, DahA=0.29 and IndP=0.31 (Durant, 1996).

The BaP TEQ is directly associated with carcinogenicity, but the BaP MEQ (mutagenic activity) may not be directly associated with cancer (Zeiger, 1998, 2001) and may have implied other non-cancerous adverse effects such as pulmonary diseases, birth defects, impotency, low IQ, etc. (DeMarini *et al.*, 2004; Seagrave 2002). The BaP_{TEQ} and BaP_{MEQ} for the candies and chocolates are displayed on Table 8 and 9, respectively. The ΣBaP_{TEQ} values ranged from nd to 37.27 and 40.17 µg/kg for candies and chocolates, respectively. The major contributors to the ΣBaP_{TEQ} value are BaP, IndP and DahA. The chocolate sample CH-7 had a higher carcinogenic potency factor

Table 11. Estimated excess cancer risk from the consumption of 1.9 g of chocolate per day.[1]

	BaA	Chy	Bbf	Bkf	BaP	IndP	DahA	Excess cancer risk
Adult								
CH-1	1.82×10^{-10}	4.10×10^{-11}	4.26×10^{-10}	0.00	0.00	0.00	0.00	6.49×10^{-10}
CH-2	2.51×10^{-9}	2.57×10^{-12}	6.11×10^{-11}	7.78×10^{-14}	1.91×10^{-10}	2.49×10^{-10}	4.52×10^{-9}	7.53×10^{-9}
CH-3	1.02×10^{-10}	2.34×10^{-12}	3.32×10^{-10}	1.48×10^{-10}	1.46×10^{-9}	4.12×10^{-10}	6.05×10^{-9}	8.50×10^{-9}
CH-4	6.61×10^{-11}	1.21×10^{-12}	2.24×10^{-10}	9.22×10^{-12}	5.68×10^{-10}	1.04E-09	3.79×10^{-9}	5.69×10^{-9}
CH-5	3.62×10^{-11}	5.25×10^{-13}	4.63×10^{-11}	2.53×10^{-12}	5.48×10^{-10}	1.34×10^{-10}	6.88×10^{-9}	7.65×10^{-9}
CH-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-7	3.72×10^{-10}	5.37×10^{-12}	1.52×10^{-11}	1.24×10^{-12}	1.52×10^{-8}	0.00	0.00	1.56×10^{-8}
CH-8	5.06×10^{-12}	3.35×10^{-13}	2.61×10^{-11}	7.78×10^{-14}	1.44×10^{-10}	2.14×10^{-11}	1.17×10^{-11}	2.09×10^{-10}
CH-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-11	3.11×10^{-12}	3.50×10^{-14}	1.56×10^{-12}	3.89×10^{-13}	2.96×10^{-10}	2.08×10^{-10}	1.75×10^{-10}	6.84×10^{-10}
CH-12	5.95×10^{-11}	2.92×10^{-13}	3.89×10^{-12}	3.23×10^{-12}	4.36×10^{-10}	3.89E-13	3.46×10^{-10}	8.49×10^{-10}
CH-13	2.10×10^{-11}	3.77×10^{-13}	6.22×10^{-12}	1.13×10^{-12}	1.01×10^{-9}	5.25×10^{-11}	2.92×10^{-10}	1.38×10^{-9}
CH-14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Child								
CH-1	1.46×10^{-10}	3.27×10^{-11}	3.41×10^{-10}	0.00	0.00	0.00	0.00	5.19×10^{-10}
CH-2	2.01×10^{-9}	2.06×10^{-12}	4.88×10^{-11}	6.22×10^{-14}	1.52×10^{-10}	1.99×10^{-10}	3.61×10^{-9}	6.02×10^{-9}
CH-3	8.15×10^{-11}	1.87×10^{-12}	2.66×10^{-10}	1.19×10^{-10}	1.17×10^{-9}	3.30×10^{-10}	4.83×10^{-9}	6.80×10^{-9}
CH-4	5.29×10^{-11}	9.70×10^{-13}	1.79×10^{-10}	7.37×10^{-12}	4.54×10^{-10}	8.29×10^{-10}	3.03×10^{-9}	4.55×10^{-9}
CH-5	2.89×10^{-11}	4.20×10^{-13}	3.70×10^{-11}	2.02×10^{-12}	4.39×10^{-10}	1.07×10^{-10}	5.50×10^{-9}	6.11×10^{-9}
CH-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-7	2.98×10^{-10}	4.29×10^{-12}	1.21×10^{-11}	9.95×10^{-13}	1.22×10^{-8}	0.00	0.00	1.25×10^{-8}
CH-8	4.04×10^{-12}	2.67×10^{-13}	2.08×10^{-11}	6.22×10^{-14}	1.15×10^{-10}	1.71×10^{-11}	9.33×10^{-12}	1.67×10^{-10}
CH-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-11	2.49×10^{-12}	2.80×10^{-14}	1.24×10^{-12}	3.11×10^{-13}	2.36×10^{-10}	1.66×10^{-10}	1.40×10^{-10}	5.47×10^{-10}
CH-12	4.76×10^{-11}	2.33×10^{-13}	3.11×10^{-12}	2.58×10^{-12}	3.48×10^{-10}	3.11×10^{-13}	2.77×10^{-10}	6.79×10^{-10}
CH-13	1.68×10^{-11}	3.02×10^{-13}	4.98×10^{-12}	9.02×10^{-13}	8.05×10^{-10}	4.20×10^{-11}	2.33×10^{-10}	1.10×10^{-9}
CH-14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH-15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[1] CH = chocolates; BaA = benzo(a)anthracene; Chy = chrysene; Bbf = benzo(b)fluoranthene; Bkf = benzo(k)fluoranthene; BaP = benzo(a)pyrene; IndP = indeno(1,2,3-cd)pyrene; DahA = dibenz(a,h)anthracene.

than the other samples investigated. The total mutagenic potency factor for these products ranged from nd to 15.46 μg/kg and nd to 40.31 μg/kg for candies and chocolates, respectively with significant contributions from Bkf, BaP, IndP and DahA. Samples CH-2, CH-3, CH-4, CH-5 and CH-7 had higher carcinogenic and mutagenic potency factors than other samples examined.

Estimation of excess cancer risk

The excess cancer risk was estimated by using the general equation:

$$\text{Excess cancer risk} = \frac{\text{EI} \times \text{ED} \times \text{CSF}}{\text{BW} \times \text{AT}} \times 10^{-6} \quad (3)$$

Where EI is the estimated intake, ED is the exposure duration in years (adults = 30 years), CSF is the oral cancer slope factor (mg/kg/d), BW is human body weight (assuming 60 kg weight), AT is the averaging time for carcinogens in years (assuming 48.9 years for the average Nigerian) and 10^{-6} is the conversion factor. The CSF data for individual PAHs, obtained from the integrated risk information system reported by the Kansas Department of Health and Environment (KDHE, 2010), are BaA=0.73, Chy=0.0073, Bbf=0.73, Bkf=0.073, BaP=7.3, IndP=0.73 and DahA=7.3.

The excess cancer risk values estimated from the consumption of these brands of candies and chocolates for the adult and child scenarios are presented in Table 10 and 11. The maximum excess cancer risk values for the adult and child scenarios were below the EPA excess cancer risk guideline value of 1.0×10^{-6} chance of developing cancer. However, for the child/adult who consumes as much as a chocolate bar of 20 g per day, the excess cancer risk values in 46% of these brands of candies and chocolates exceeded the cancer risk guideline value of 1.0×10^{-6} (EPA, 1989).

4. Conclusions

The concentrations of 16 PAHs in the 29 commercial brands of chocolates and candies were generally elevated with three samples having PAH4 concentrations above the permissible limit. The lower molecular weight 2- to 4-ringed PAHs were dominant over 5-and 6-ringed PAHs in the majority of these samples. The estimated MOE values were greater than 10,000 in all samples of candies and chocolates based on PAH8 concentrations for the adult and child scenarios, which indicates no health concern at the current ingestion rate. However, excessive consumption of these products could lead to exposure to high concentrations of carcinogenic PAHs, therefore parental guidance is required to control the consumption of these food items by children. The levels of PAHs found in these brands of chocolates and candies demonstrated the urgent need for limits for PAHs in foods in Nigeria and a systematic monitoring of PAHs in foods for possible risk management action.

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