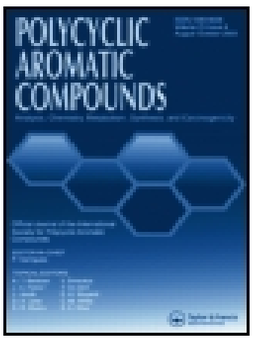

Polycyclic Aromatic Compounds

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Polycyclic Aromatic Hydrocarbons (PAHs) Occurrence and Toxicity in *Camellia sinensis* and Herbal Tea

Omowunmi H. Fred-Ahmadu and Nsikak U. Benson

Analytical and Environmental Chemistry Unit, Department of Chemistry, Covenant University, Ota, Nigeria

ABSTRACT

This study describes a survey of polycyclic aromatic hydrocarbon (PAH) concentrations in 23 green, herbal, and black tea brands widely consumed in Nigeria by determining the levels of benzo[a]pyrene, chrysene (PAH2), benzo[a]pyrene, chrysene, benz[a]anthracene, benzo[b]fluoranthene (PAH4), benzo[a]pyrene, benz[a]anthracene, benzo[k]fluoranthene, chrysene, benzo[b]fluoranthene, dibenz[ah]anthracene, benzo[ghi]per-ylene and indeno[1,2,3-cd]pyrene (PAH8). Toxic equivalence factor and mutagenic equivalence factor were applied to evaluate the toxic equivalence and mutagenic equivalence quotients relative to benzo[a]pyrene. The concentrations of PAHs indicate that Regulation 835/2011/EC was not fulfilled by benzo[a]anthracene, B[a]A, benzo[a]pyrene, B[a]P, benzo[b]fluoranthene, B[b]F, and chrysene, CHR. The PAH4 levels ranged from 1.28 to 44.57, 4.34 to 11.20, and 0.76 to 34.82 μg/kg in green, black, and herbal tea products, respectively. On the other hand, the PAH8 concentration varied between 1.63 and 65.73, 5.02 and 68.83, and 12.43 and 24.92 μg/kg in green, herbal, and black tea samples. The PAH4 and PAH8 provide more reliable indicators for determination of PAH contamination and risk characterization in food than PAH2.

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Camellia sinensis; carcinogenicity; herbal tea; mutagenicity; PAH; polycyclic aromatic hydrocarbons

Introduction

Tea is widely produced from the plant of the *Theaceae* family known as *Camellia sinensis*. Over the years, several methods have been developed for processing different types of tea. These include the non-oxidized and non-fermented process to produce the green tea, and the fully oxidized and fermented method, which results in the production of black tea. Herbal teas are widely produced from well-dried, ground (in some products), and processed roots, stem bark, seeds, or flowers of herbaceous plants, and may not necessarily contain *C. sinensis* leaves. All over the world, it is a popular dietary beverage, and is widely consumed due to its antioxidative, anticarcinogenic, and antimutagenic health benefits (1, 2). Polycyclic aromatic hydrocarbons (PAHs) are a significant class of organic compounds with known carcinogenic and mutagenic potentials, and their presence in processed tea have been documented (3–17). It has been reported that tea plant (*C. sinensis*) contains natural compounds such as flavonoids (F), theanine (T), catechin (C), epicatechin (EC), epicatechin gallate (ECG), gallocatechin (GC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), which have potent antioxidative, anticarcinogenic, and antimutagenic effects against a wide range of natural and chemical toxins (18, 1, 2, 19, 20). Other major components of tea leaf of *C. sinensis* as reported in the literature include amino acid, ash, carbohydrates, carotenoids, chlorophyll, lignin, lipids, methylxanthines, organic acids, polyphenols, protein, volatiles, and traces of other compounds and elements (21).

CONTACT Nsikak U. Benson nsikak.benson@covenantuniversity.edu.ng Analytical and Environmental Chemistry Unit, Department of Chemistry, Covenant University, Km 10 Idiroko Road, Ota, Nigeria.

Human exposure to PAHs constitute a significant threat to public health, and are usually through multi-pathways including consumption of food produce and supplements that might have been contaminated during industrial processing, and from diffuse environmental sources (22). Investigations have shown that PAHs carcinogenic and mutagenic potency are associated with biotransformation of their congeners into highly reactive chemical metabolites capable of covalently coupling with protein and nucleic acids macromolecules in human cells, resulting in alteration of DNA and growth of malignant cells (ATSDR, (23–26); IARC, (27–30)). Pro-mutagenic activity of PAHs is largely associated with their covalent bonding to DNA (EFSA, (31)).

In the last decades, PAHs monitoring in environmental matrices and food has received increasing attention, principally due to health and food safety concerns. Many international regulatory bodies such as the US Environmental Protection Agency (US-EPA) and the European Union (EU) have listed PAHs as priority contaminants, categorizing them as "16 US-EPA PAH" and "15 + 1 EU Priority PAH," respectively (US-EPA, (17, 32)). Due to the high carcinogenic potential posed by B[a]P and its pervasiveness in environmental samples, the Scientific Committee on Food recommended B[a]P as a suitable marker for PAHs occurrence and carcinogenic effects in food ((33); European Commission, (34, 35)). Consequently, legislative maximum limits were set for B[a]P in foods by the European Commission. However, in 2008, based on the review of scientific findings and data, European Food Safety Agency, EFSA CONTAM (Contaminants in Food chain) Panel reported that B[a]P alone was not suitable as marker for PAHs presence in food, but PAH4 (the sum of four PAH compounds namely benzo[a]pyrene, benzo[a]anthracene, chrysene, and benzo[b]fluoranthene), and PAH8 (the sum of benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) were proposed as more suitable markers for carcinogenic characteristics in food, with PAH8 not providing much added value compared to PAH4 (EFSA, (31)). In recent years, the rate of tea production, importation, and consumption has increased tremendously, which prompts the study on the potential occurrence, and contamination levels of PAHs in tea commercially sold in Nigeria.

Therefore, the aim of this study was to determine the occurrence of PAH2, PAH4, and PAH8 carcinogenic and mutagenic potency in different packaged tea brands marketed by retail outlets in Nigeria. The potential risks assessment of PAHs using the toxic equivalence factor (TEF), mutagenic equivalence factor (MEF), toxic equivalence (BaP-TEQ), and mutagenic equivalence (BaP-MEQ) quotients relative to benzo[a]pyrene are evaluated.

Materials and method

Extraction and clean-up method for PAHs

23 samples of commonly sold tea products were purchased from various supermarkets across Lagos and Ogun States of Nigeria. The general information about the investigated brands of tea is presented in Table 1.

The collection, preservation, and analysis of the considered tea brands for this research were in accordance with the European Communities Regulations No 836/2011 (European Commission, (36)). Successive coning and quartering method was applied to mix the individual tea samples, and were zip-locked and stored at −10°C until further processing. Approximately, 0.5 g of each tea sample was weighed. 15 mL of n-hexane was added and the mixture was mixed on a vortex mixer for 20 s. It was transferred to a sonicator and sonicated for 20 min at 60°C. After sonication, the mixture was centrifuged at 3,000 rpm for 10 min, and the supernatant was decanted into a 100 mL conical flask. Sonication and centrifuging were repeated twice with 10 mL of n-hexane on each occasion. The total volume of extract (approximately 35 mL) was evaporated to about 3 mL in the thermostated water bath at 55°C, filtered and collected in glass test tubes. The 100 mL flask was washed three times with 0.75 mL of n-hexane and also filtered into the test tube. 1 mL of n-hexane was then used to wash the filter paper. The total volume of approximately 6.25 mL was then taken to a thermostated water bath to reduce the volume to about 2 mL. Silica gel (60-200 mesh) was activated at 130°C overnight in a hot air oven and

Table 1. Nutritional facts and general information about tea brands.

Product name	Code	Country of origin	Flavor	Manufacturer's nutrition facts
Typhoo pure green tea	TPG	United Kingdom	—	100% green tea
Heladiv green tea	HGT	Sri Lanka	Antioxidant	Total fats 0%, Na 0%, carbohydrate 0%, Protein 0%
Gold blend green tea	GBG	Sri Lanka	Lemon and ginger	Energy 0%, Na 0%, antioxidants 100–200 mg/200 mL
Super blend green tea	SBG	Sri Lanka	Vanilla	Na 0%
Lipton green tea	LGB	USA	Blackberry pomegranate	Total fat 0 g, Na 0 mg, K 5 mg.
Lipton green tea	LGL	USA	Lemon and ginseng	Na 0 mg, K 15 mg
Lipton green tea	LGR	USA	Red Goji Raspberry	Na 0 mg, K 10 mg
Lipton green tea	LGJ	USA	Jasmine passion with fruits	Na 0 mg, K 10 mg
Loyd green sense	LGS	Poland	Aloe vera	Green tea 77%, white tea 20%
Bigelow green tea	BGT	USA	Decaffeinated, aloe vera	1–8 mg caffeine
Twinings pure green tea	TWG	United Kingdom	—	Green tea
Lipton yellow label tea	LYL	Nigeria	—	Energy 2 kJ/<1 kcal, protein 0.1 g, sugars 0 g, fat 0 g, fibre 0 g, Na 0 g.
Natural liver flush tea	NLF	China	—	—
Top tea	TTG	Nigeria	Ginger	—
Tranquilizing and brain nourishing tea	TBN	China	—	—
Moringa herbal tea	MHT	Nigeria	—	—
Sahul slim herbal tea	SSH	India	—	*Garcina indica* 0.75 g, *Cyperus rotundus* 0.5 g, Comphora mukul 0.6 g, *Garcina penduculata* 0.25 g, *Trigonella ferrum gracecum* 0.2 g, *Clerodendrum phlonidis* 0.25 g, *Tinosporn cordiofolia* 0.25 g, *Embilicia officinalis* 0.25 g, *Terminalia chebula* 0.25g, *Terminalia belerica* 0.25 g, *Zingiber officinale* 0.25 g, *Piper longum* 0.25 g, *Piper nigrum* 0.25 g, *Areca catechu* 0.25 g, *Terminalia arjuna* 0.25 g
Antihypertensive tea	AHT	China	—	—
Joint care herbal tea	JCT	China	—	—
Kidney flush tea	KFT	China	—	—
Anticancer tea	ACT	China	—	—
Top tea	TTL	Nigeria	Lime and lemon	—
Top tea (Regular)	TTR	Nigeria	—	—

cooled in a desiccator at room temperature. 3 g of the silica gel was weighed and packed into a clean column plugged with cotton wool and set up on a retort stand. 1 g of anhydrous Na_2SO_4 was added to the top of the silica gel, then 5–10 mL of n-hexane was used to condition the column. The sample was added when the solvent was 2 mL on the column and gradually eluted with n-hexane and was collected into a 100 mL conical flask. The collected eluent was concentrated to about 2 mL and taken to GC-FID for analysis.

Instrumentation

PAHs were analyzed using an Agilent 7890A with an auto-sampler Agilent 7683B, coupled to flame ionization detector (FID). The GC is equipped with an HP-5 column (19091J-413) (30 m × 0.32 mm × 0.25 µm) from Agilent (USA). The carrier gas used was helium maintained at a flow rate of 4.84 mL/min. The oven temperature program is as follows: 0.4 min at 50°C, to 195°C at 20°C/min, hold 3.0 min, to 250°C at 8°C/min, hold 5.0 min, to 290°C at 5°C/min, hold 1.0 min. Helium and nitrogen gases with 99.9999% purity were purchased from Foshan Huate Gas Coy Ltd. (China). Other equipment used in this study included hot air oven (Uniscope SM9053, Surgifriend Medicals, England), Stuart orbital shaker SSL1, Centrifuge, Langford Sonomatic 1400 Ultrasonic Bath (UK), Vortex - J.P. Selecta (Barcelona, Spain), and Thermo-Scientific MaxQ 4000 Bench-top orbital shaker (USA).

Quality control

A meticulous regime of analytical quality control was observed at all times during the entire course of this research. Analyses of procedural blanks were periodically carried out to ascertain quality of analytical results and ensure that there was no laboratory contamination and operational errors. The average blank concentration was subtracted from each sample to correct for methodological and equipment errors. The limit of detection and limit of quantitation were calculated as 3 and 10 times the standard deviation of the blank, respectively. The recoveries of each individual PAH varied from 90.24 to 108.92% for PHE and DahA, respectively. The calibration curves were obtained using a series of stock standard prepared through serial dilution with n-hexane to give 6 calibration standard PAH solutions containing 5, 10, 20, 30, 40, and 50 µg/L of stock solution. Calibration curves for all analyzed PAH standards ($n = 6$) had values of residual standard deviations that ranged between 77.02% and 100.60%, demonstrating good repeatability for the analytical method. Triplicate determinations were made on all extracted tea samples.

Carcinogenic and mutagenic risk assessments

The assessment of the carcinogenicity and mutagenicity of PAHs due to human exposure from tea consumption was performed by calculating the carcinogenic equivalents (BaP-TEQ) and the mutagenic equivalents (BaP-MEQ) relative to a reference standard benzo[a]pyrene (B[a]P), respectively. B(a)P-equivalent carcinogenicity and mutagenicity assessments were calculated by multiplying the mean concentrations of the non-volatile Σ8PAHs by their respective toxic equivalent factors (TEF) as indicated below (37–41):

$$BaP - TEQ = \sum_i C_i \, TEF_i \tag{1}$$

$$BaP - MEQ = \sum_i C_i \, MEF_i \tag{2}$$

where C_i, TEF_i, and MEF_i are the individual PAH concentration, TEF, and MEF, respectively. The contributing Σ8PAHs are benzo[a]pyrene (B[a]P), benzo[a]anthracene (B[a]A), chrysene (CHR), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), indeno[1,2,3-c,d]pyrene (I[cd]P), dibenzo[a,h]anthracene (D[ah]A), and benzo[ghi]perylene (B[ghi]P).

Results and discussion

Levels of PAHs

Tables 2 and 3 present the concentrations of PAHs determined in 23 green, herbal, and black tea samples commonly marketed in Nigeria. The mean concentrations of Σ_{16}PAHs in green tea samples varied between 1.63 ± 0.33 and 75.53 ± 6.07 µg/kg in Typhoo Pure Green Tea (TPG) and Loyd Green Sense Tea samples, respectively. However, the mean levels of Σ_{16}PAHs in the herbal and black tea samples varied from 4.71 ± 0.23 to 79.61 ± 7.02 µg/kg and 12.52 ± 0.15 to 26.89 ± 0.68 µg/kg, respectively. The result however indicated that the lowest mean Σ_{16}PAHs was obtained for the Typhoo Pure Green Tea samples. Out of the 23 branded tea samples analyzed, 22 samples tested positive for PAH2, PAH4, and PAH8. The TPG tested negative for PAH2 and PAH4, but indicated the occurrence of PAH8 owing to the presence of D[ah]A. The PAH4 levels ranged from 1.28–44.57, 4.34–11.20, and 0.76–34.82 µg/kg in green, black and herbal tea products, respectively. On the other hand, the PAH8 concentration varied between 1.63 and 65.73, 5.02 and 68.83, and 12.43 and 24.92 µg/kg in green, herbal, and black tea samples. Of the individual PAHs, B[ghi]P was not detected in any of the investigated tea samples. The B[a]P was most commonly detected in samples that were negative for B[k]F, B[b]F, B[ghi]P, and I[cd]P, although some brands indicated relatively low concentrations of these PAHs. In the green tea samples, the highest and lowest PAH8 to B[a]P ratios were found in HGT and LGJ, with approximately 77% and 21% contribution to the total PAHs concentrations in respective tea samples. The highest maximum PAH8 to B[a]P

Table 2. Mean levels (μg/kg) of carcinogenic PAHs in selected green tea samples.

Compound	TPG	HGT	GBG	SBG	LBG	LGL	LGR	LGJ	LGS	BGT	TWG
B[a]P	BDL	1.22	1.12	10.05	2.36	1.28	2.83	4.80	8.82	3.73	15.9
CHR	BDL	6.10	3.24	8.43	12.92	BDL	BDL	BDL	26.02	6.96	0.83
PAH2	—	7.32	4.36	18.48	15.28	1.28	2.83	4.80	34.84	10.69	16.73
Ratio to B[a]P		6.0	3.90	1.84	6.47	1.0	1.0	1.0	3.95	2.90	1.05
B[a]A	BDL	3.97	2.04	5.25	4.29	BDL	BDL	BDL	9.31	4.47	BDL
B[b]F	BDL	10.26	5.15	14.33	6.35	BDL	BDL	BDL	0.42	BDL	BDL
PAH4	—	21.55	11.55	38.06	25.92	1.28	2.83	4.80	44.57	15.16	16.73
Ratio to B[a]P		17.66	10.31	3.79	10.98	1.0	1.0	1.0	5.05	4.06	1.05
B[k]F	BDL	BDL	BDL	0.59	BDL	BDL	BDL	BDL	3.30	BDL	BDL
D[ah]A	1.63	13.17	8.37	12.15	6.88	2.59	2.69	1.86	13.29	9.03	6.71
B[ghi]P	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
I[cd]P	BDL	5.11	1.77	6.51	3.15	BDL	BDL	BDL	4.57	4.20	BDL
PAH8	1.63	39.83	21.69	57.31	35.95	3.87	5.52	6.66	65.73	28.39	23.44
Ratio to B[a]P	—	32.65	19.37	5.70	15.23	3.02	1.95	1.39	7.45	7.61	1.47
Σ_{16}PAHs	1.63	42.43	23.72	58.74	43.24	3.87	5.59	6.76	73.53	28.61	23.45

ratio in the herbal tea samples was found in SSH with 45% contribution to total PAHs levels, while the lowest ratio was recorded for KFT with 24% contribution to Σ_{16}PAHs. Among the black tea samples, the TTL indicated the highest PAH8 to B[a]P ratio, while the TTR had the lowest ratio. The percentage contribution of both black tea samples to the Σ_{16}PAHs concentration was 36% and 19%, respectively.

A comparison of PAHs contamination results of imported and locally produced tea samples in Nigeria with similar reports conducted in other countries, indicates that PAH2, PAH4, and PAH8 concentrations in green tea were fairly low with mean levels recorded as 11.66, 18.25, and 26.36 μg/kg, respectively. Similar data was obtained for PAH4 in green tea samples as reported by Kamangar et al. (42). According to Londoño et al. (11), green tea samples market in Argentina recorded 40.5, 63.2, and 84.9 μg/kg average contamination contents for PAH2, PAH4, and PAH8, respectively. Schlemitz and Pfannhauser (43) indicated a similarly enhanced concentration of 73.7 μg/kg for PAH4 in green tea samples commercially available in Austria. Evaluating the results for black tea samples, the mean concentration for PAH2 (6.30 μg/kg), PAH4 (7.54 μg/kg), and PAH8 (17.44 μg/kg) indicated contamination levels that are comparatively low against studies reported by other researchers in different countries (44, 45, 46, 11). However, the average concentrations of PAH2 (13.07 μg/kg), PAH4 (15.41 μg/kg), and PAH8 (24.84 μg/kg)

Table 3. Mean levels (μg/kg) of carcinogenic PAHs in selected herbal and black tea samples.

	Herbal tea								Black tea			
Compound	NLF	TBN	MHT	SSH	AHT	JCT	KFT	ACTT	LYL	TTG	TTL	TTR
B[a]P	6.56	11.07	0.76	1.91	8.48	28.35	4.03	8.40	2.04	6.22	2.75	5.21
CHR	BDL	6.89	BDL	20.21	2.53	5.37	BDL	BDL	1.48	2.94	3.65	0.89
PAH2	6.56	17.96	0.76	22.12	11.01	33.72	4.03	8.40	3.52	9.16	6.40	6.10
Ratio to B[a]P	1.0	1.62	1.0	11.58	1.30	1.19	1.0	1.0	1.73	1.47	2.33	1.17
B[a]A	BDL	4.24	BDL	10.22	1.20	1.10	BDL	BDL	0.82	2.03	2.11	BDL
B[b]F	BDL	BDL	BDL	1.97	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
PAH4	6.56	22.20	0.76	34.31	12.21	34.82	4.03	8.40	4.34	11.19	8.51	6.10
Ratio to B[a]P	1.0	2.01	1.0	17.96	1.44	1.23	1.0	1.0	2.13	1.80	3.09	1.17
B[k]F	BDL	0.55	BDL	1.40	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
D[ah]A	1.22	10.19	3.44	28.24	10.75	1.75	0.53	6.41	8.99	11.91	8.57	6.33
B[ghi]P	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
I[cd]P	BDL	3.79	0.82	4.88	1.48	BDL	BDL	BDL	BDL	1.82	1.99	BDL
PAH8	7.78	36.73	5.02	68.83	24.44	36.57	4.56	14.81	13.33	24.92	19.07	12.43
Ratio to B[a]P	1.19	3.32	6.61	36.04	2.88	1.29	1.13	1.76	6.53	4.01	6.93	2.39
Σ_{16}PAHs	7.92	37.03	5.03	79.61	25.9	50.21	4.71	14.81	14.28	26.89	19.16	12.52

B[a]A = benzo[a]anthracene, B[a]P = benzo[a]pyrene, B[b]F = benzo[b]fluoranthene, B[ghi]P = benzo(g,h,i)perylene, B[k]F = benzo[k]fluoranthene, CHR = chrysene, D[ah]A = dibenzo[a,h]anthracene, I[cd]P = indeno[1,2,3cd]pyrene, BDL = Below limit of detection.

obtained for herbal tea samples in the present study could not be compared with results of similar studies due to sparse information.

Carcinogenic and mutagenic assessments

The calculated BaP-equivalent carcinogenic and mutagenic PAHs potency representing potential cancer and mutation effects evaluation are shown in Table 4.

The presence of PAHs in processed and raw foods may be attributed to a number of factors including the environmental quality of the soil, and the consequences of the preparation, production, and manufacturing processes. Exposure of humans to PAHs through oral, dermal, and inhalation routes, particularly the group known as PAH8, is generally considered as a major threat to health, and they are classified as the most potent carcinogens ((37, 47); IARC, (28)). Therefore, their occurrence in combination or individually in dietary products is considered as likely indicators of PAHs carcinogenicity (EFSA, (31)). In the present study, benzo[a]pyrene was observed in all investigated tea samples except Typhoo Pure Green Tea, while dibenz[g,h,i]perylene was not detected in any of the herbal, black, or green tea samples. Therefore, it can be ascertained that benzo[a]pyrene's contribution to the overall carcinogenicity and mutagenicity of the studied samples was quite significant. The observed range of BaP-TEQ was 3.04–7.82 µg/kg, 0.16–16.58 µg/kg, and 1.19–28.69 µg/kg for black, green, and herbal teas, respectively, while BaP-MEQ ranged between 4.47 and 10.45 µg/kg for black tea, 0.47 and 19.82 µg/kg for green tea, and 1.19 and 28.69 µg/kg for herbal tea. The aggregate measure of carcinogenicity or toxicity (BaP-TEQ), with respect to the listed contributing congeners shows that Joint Care Herbal Tea indicated the highest value of 28.69 µg/kg, which is indicative of its high carcinogenic risk, while Typhoo Pure Green Tea had the lowest value of 0.16 µg/kg.

The mutagenic equivalency quotient (BaP-MEQ) values on the other hand show that Joint Care Herbal Tea and Typhoo Pure Green Tea had the highest and lowest mutagenic risk quotients with 29.04 and 0.47 µg/kg, respectively (Table 4). The results of this study reveal herbal teas to be more potentially carcinogenic and mutagenic than green and black teas. This may be attributed to the number

Table 4. Derived carcinogenic and mutagenic potency factors associated with B[a]P.

Sample code	BaP-TEQ (µg/kg)	BaP-MEQ (µg/kg)
TPG	0.16	0.43
HGT	4.52	9.62
GBG	2.88	5.60
SBG	14.02	19.82
LBG	4.56	7.50
LGL	1.54	2.03
LGR	3.10	3.62
LGJ	4.99	5.34
LGS	12.17	15.77
BGT	5.57	8.14
TWG	16.58	17.87
LYL	3.04	4.74
NLF	6.68	6.91
TTG	7.82	10.45
TBN	13.02	15.73
MHT	1.19	2.02
SSH	6.78	13.44
AHT	9.85	12.20
JCT	28.69	29.04
KFT	4.08	4.18
ACT	9.05	10.26
TTL	4.06	6.09
TTR	5.86	7.06

TEF: toxic equivalency factors for cancer potency relative to B[a]P (48).
MEF: mutagenic potency factor relative to B[a]P (26).

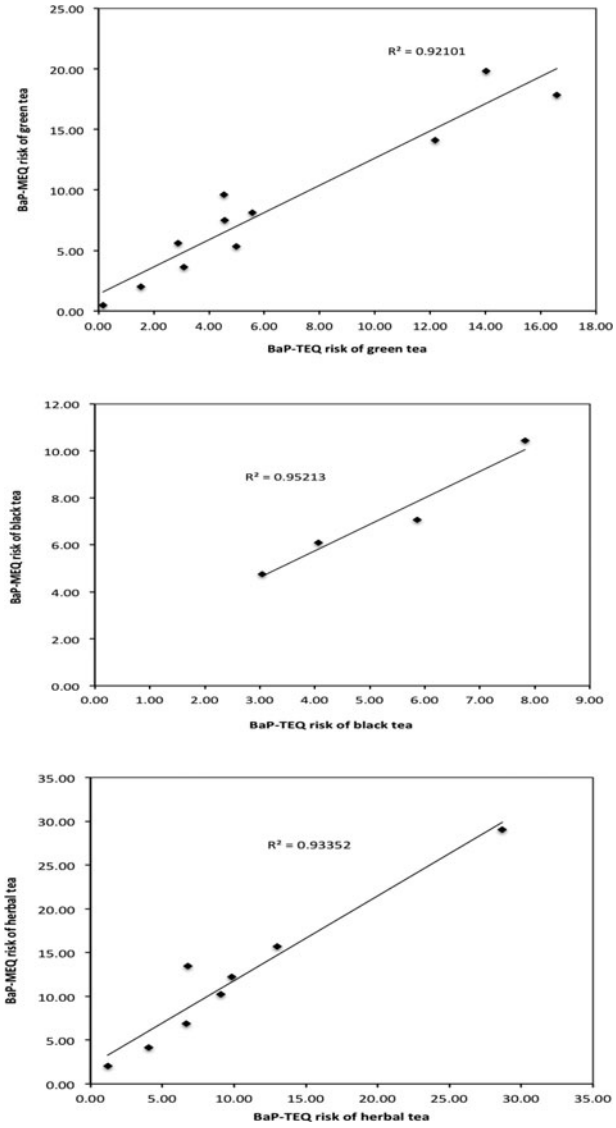

Figure 1. Linear relationships between calculated BaP-carcinogenic and -mutagenic equivalents associated with green, black, and herbal tea samples.

of contributing ingredients blended to form the herbal teas as observed on the product labels, which may have been contaminated with PAHs during cultivation or the processing stages of the tea products. This is also indicative of higher retention rates of high molecular weight PAHs in herbal teas than in the black and green teas. Generally, the BaP-MEQ values are higher than the BaP-TEQ values for all the classes of tea in this study. The mean BaP-TEQ and BaP-MEQ followed the same sequence of herbal > green > black, suggesting similar carcinogenic and mutagenic potentials of the tea samples. A dominant contribution from B[a]P, followed by D[ah]A to BaP-TEQ and BaP-MEQ was observed for all samples with contributions from other PAH congeners being less than 1%. There was no contribution from B[ghi]P to the toxic or mutagenic potency of any of the samples. An evaluation of the linear relationships between the calculated BaP-carcinogenic and –mutagenic potency associated with green, black, and herbal tea samples indicated positive and significant correlations $r^2 = 0.921$ (green tea), $r^2 = 0.952$ (black tea samples), and $r^2 = 0.934$ (herbal tea products) (Figure 1).

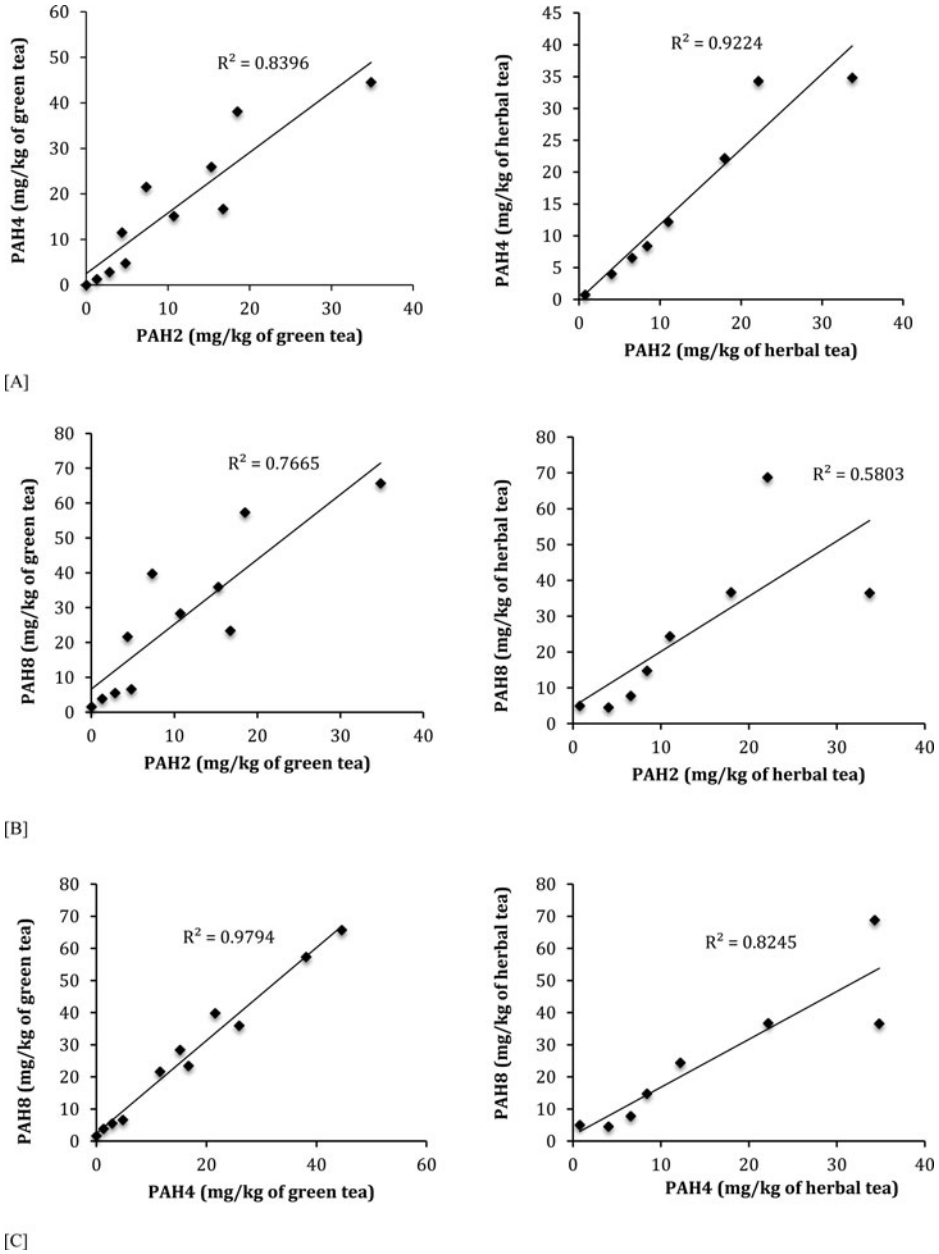

Figure 2. Linear regression between (A) PAH2 & PAH4, (B) PAH2 & PAH8, and (C) PAH4 & PAH8.

Correlation between *PAH2, PAH4, and PAH8*

In the present study, the interrelationships between PAH2, PAH4, and PAH8 were evaluated using simple linear regression model. The correlations between PAH2 and PAH4, PAH2 and PAH8, and PAH4 and PAH8 in green and herbal tea samples considered in this study are presented in Figure 2. It is generally observed that a positive relationship exists between these three categories of PAHs. However, the most significant correlation was obtained for PAH4 against PAH8 in green tea ($r^2 = 0.979$), and PAH2 against PAH4 in herbal tea ($r^2 = 0.922$). According to European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM Panel), the interrelation between PAH2 and PAH4 or PAH8 was 0.92, and between PAH4 and PAH8 was 0.99, based on analysis of 111 tea and coffee samples (EFSA, (31)). The

result obtained in this study for correlations between PAH2 and PAH4 for herbal tea samples yielded values that were similar to EFSA's report. The interrelationship obtained for PAH4 against PAH8 (green tea) was approximately equated with result indicated by EFSA (31). Other relationships were moderately lower than values observed by EFSA.

In addition to evaluating the occurrence of carcinogenic PAHs in the green, black, and herbal tea samples, the correlations between PAH2, PAH4, PAH8, and Σ_{16}PAHs were evaluated. The correlation analyses between PAH8 and Σ_{16}PAHs, PAH4 and Σ_{16}PAHs, PAH2 and Σ_{16}PAHs indicated corresponding coefficients that ranged between 0.98 and 0.99, 0.86 and 0.98, 0.67 and 0.78, respectively. Our data reveal positive and significant interrelationships between these groups of PAHs. However, the considerable differences in the strength of correlations corroborate the CONTAM Panel's report that in food categories and subcategories, PAH4 and PAH8 are better indicators of the occurrence of PAHs than PAH2, and could therefore be applied in calculating the health risks associated with PAHs exposures (EFSA, (31)).

Conclusions

PAHs occurrence and risk assessment in 11 green, 8 herbal, and 4 black tea samples widely consumed in Nigeria were investigated. The study compared the PAH2, PAH4, and PAH8 categories profiles in imported and local processed tea brand samples. All of the selected PAH categories were present in all the tea samples except in TPG that was characterized by the absence of PAH2 and PAH4, but showed relatively low occurrence of PAH8 associated with the detection of dibenz[a,h]anthracene. Benzo[a]pyrene was present in all herbal, black, and green tea samples analyzed except in TPG samples, while dibenz[g,h,i]perylene was not detected in any of the samples. Results reveal significant concentrations of B[a]A, B[a]P, B[b]F, and CHR in most green, black, and herbal tea brands, and largely exceed the limits stipulated in the Regulation 835/2011/EC for foodstuffs. This indicates a potential health concerns for consumers of these beverages. The computed aggregate measure of carcinogenicity (BaP-TEQ) shows that JCT may likely pose high carcinogenic risk to the consumers of the investigated tea sample, while TPG chances of carcinogenic threat to human health would be considered as being insignificant. PAH4 and most especially PAH8 are found to be suitable indicators of the occurrence of PAHs in food products compared to PAH2.

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ORCID

Nsikak U. Benson http://orcid.org/0000-0002-1285-579X

References

1. da Silva Pinto, M. "Tea: A New Perspective on Health Benefits." *Food Res. Int.* 53, no. 2 (2013): 558–67.
2. Jain, A., C. Manghani, S. Kohli, D. Nigam, and V. Rani. "Tea and Human Health: The Dark Shadow." *Toxicol. Lett.* 220 (2013): 82–7.
3. Bishnoi, N., U. Mehta, U. Sain, and G. G. Pandit. "Quantification of Polycyclic Aromatic Hydrocarbons in Tea and Coffee Samples of Mumbai (India) by High Performance Liquid Chromatography." *Environ. Monit. Assess.* 107 (2005): 399–406.
4. Ciemniak, A., and K. Mocek. "Polycyclic Aromatic Hydrocarbons in Tea and Tea Infusions." *Rocz. Panstw. Zakl. Hig.* 61, no. 3 (2010): 243–248.
5. Duedahl-Olesen, L., M. A. Navaratnam, J. Jewula, and A. H. Jensen. "PAH in Some Brands of Tea and Coffee." *Polycycl. Aromat. Compd.* 35, no. 1 (2015): 74–90, doi: 10.1080/10406638.2014.918554

6. Fiedler, H., C. K. Cheung, and M. H. Wong. "PCDD/PCDF, Chlorinated Pesticides and PAHs in Chinese Teas." *Chemosphere* 46 (2002): 1429–1433.
7. Iwegbue, C. M. A., H. Agadaga, F. I. Bassey, L. C. Overah, G. O. Tesi, and G. E. Nwajei. "Concentrations and Profiles of Polycyclic Aromatic Hydrocarbons in Some Commercial Brands of Tea-, Coffee-, and Cocoa-based Food Drinks in Nigeria." *Int. J. Food Prop.* 18, no. 10 (2015): 2124–33.
8. Kayali-Sayadi, M. N., S. Rubio-Barroso, M. P. Cuesta-Jimenez, and L. M. Polo-Díez. "Rapid Determination of Polycyclic Aromatic Hydrocarbons in Tea Infusion Samples by High-Performance Liquid Chromatography and Fluorimetric Detection based on Solid-phase Extraction." *Analyst* 123 (1998): 2145–8.
9. Khiadani, M., M. M. Amin, F. M. Beik, A. Ebrahimi, M. Farhadkhani, and F. Mohammadi-Moghadam. "Determination of Polycyclic Aromatic Hydrocarbons Concentration in Eight Brands of Black Tea which are used more in Iran." *Int. J. Environ. Health Eng.* 2 (2013): 1–5.
10. Li, X. Y., N. Li, H. D. Luo, L. R. Lin, Z. X. Zou, Y. Z. Jia, et al. "A Novel Synchronous Fluorescence Spectroscopic Approach for the Rapid Determination of Three Polycyclic Aromatic Hydrocarbons in Tea with Simple Microwave-Assisted Pretreatment of Sample." *J. Agric. Food Chem.* 59, no. 11 (2011): 5899–905.
11. Londoño, V. A. G., C. M. Reynoso, and S. L. Resnik. "Polycyclic Aromatic Hydrocarbons (PAHs) Survey on Tea (*Camellia sinensis*) Commercialized in Argentina." *Food Control* 50 (2015): 31–7.
12. López-Jiménez, F. J., A. Ballesteros-Gómez, and S. Rubio. "Determination of Polycyclic Aromatic Hydrocarbons (PAH4) in Food by Vesicular Supramolecular Solvent-based Microextraction and LC-Fluorescence Detection." *Food Chem.* 143 (2014): 341–7.
13. Orecchio, S., V. Ciotti, and L. Cullotta. "Polycyclic Aromatic Hydrocarbons (PAHs) in Coffee Brew Samples: Analytical Method by GC–MS, Profile, Levels and Sources." *Food Chem. Toxicol.* 47 (2009): 819–26.
14. Pincemaille, J., C. Schummer, E. Heinen, and G. Moris. "Determination of Polycyclic Aromatic Hydrocarbons in Smoked and Non-Smoked Black Teas and Tea Infusions." *Food Chem.* 145 (2014): 807–13.
15. Schulz, C., H. Fritz, and A. Ruthenschrör. "Occurrence of 15 + 1 EU Priority Polycyclic Aromatic Hydrocarbons (PAH) in Various Types of Tea (*Camellia sinensis*) and Herbal Infusions." *Food Addit. Contam, Part A* 31, no. 10 (2014): 1723–35.
16. Shi, Y., H. Wu, C. Wang, X. Guo, J. Du, and L. Du. "Determination of Polycyclic Aromatic Hydrocarbons in Coffee and Tea Samples by Magnetic Solid-Phase Extraction Coupled with HPLC–FLD." *Food Chem.* 199 (2016): 75–80.
17. Thea, A. E., D. Ferreira, L. A. Brumovsky, and M. E. Schmalko. "Polycyclic Aromatic Hydrocarbons (PAHs) in Yerba Mate (*Ilex paraguariensis* St. Hil) Traditional Infusions (*mate* and *tereré*)." *Food Control* 60 (2016): 215–20.
18. Cooper, R., D. J. Morre, and D. M. Morre. "Medicinal Benefits of Green Tea: Part I. Review of Noncancer Health Benefits." *J. Altern. Complement. Med.* 11, no. 3 (2005): 521–8.
19. Lambert, J. D., S. Sang, and C. S. Yang. "Biotransformation of green tea polyphenols and the biological activities of those metabolites." *Mol. Pharm.* 4, no. 6 (2007): 819–25.
20. Rameshrad, M., B. M. Razavi, and H. Hosseinzadeh. "Protective Effects of Green Tea and its Main Constituents Against Natural and Chemical Toxins: A Comprehensive Review." *Food Chem. Toxicol.* 100 (2017): 115–37.
21. Graham, H. N. "Green Tea Composition, Consumption, and Polyphenol Chemistry." *Prev. Med.* 21, no. 3 (1992): 334–50.
22. Benson, N. U., J. P. Essien, F. E. Asuquo, and A. L. Eritobor. "Occurrence and Distribution of Polycyclic Aromatic Hydrocarbons in Surface Microlayer and Subsurface Seawater of Lagos Lagoon, Nigeria." *Environ. Monit. Assess.* 186, no. 9 (2014): 5519–29, doi: 10.1007/s10661-014-3800-z
23. ATSDR, Agency for Toxic Substances and Disease Registry. Toxicological profile for polycyclic aromatic hydrocarbons. Retrieved March 4, 2016 from http://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=122&tid=25 (1995).
24. Dipple, A., R. C. Moschel, and C. A. H. Bigger. "Polynulear Aromatic Carcinogens. In: Chemical Carcinogens." in *ACS Monograph*, vol. 182, ed. C. E. Searle (Washington, DC: American Chemical Society Press, 1984), 41–163.
25. Dipple, A., P. A. Pigott, S. K. Agarwal, H. Yagi, J. M. Sayer, and D. M. Jerina. "Optically Active Benzo[c]phenanthrene Diol-epoxides Bind Extensively to Adenine in DNA." *Nature* 327 (1987): 535–6.
26. Durant, J. L., A. L. Lafleur, W. F. Busby Jr, L. L. Donhoffner, B. W. Penman, and C. L. Crespi. "Mutagenicity of $C_{24}H_{14}$ PAH in Human Cells Expressing CYP1A1." *Mutat. Res.* 446, no. 1 (1999): 1–14.
27. Gelboin, H. V. "Benzo[alpha]pyrene Metabolism, Activation and Carcinogenesis: Role and Regulation of Mixed-Function Oxidases and Related Enzymes." *Physiol. Rev.* 60 (1980): 1107–66.
28. IARC - International Agency for Research on Cancer. "Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures." *Monogr. Eval. Carcinog. Risks Hum.* 92 (2010): 1–853.
29. Nordquist, M., D. R. Thakker, K. P. Vyas, H. Yagi, M. Levin, D. E. Ryan, D. E. Thomas, A. H. Conney, and D. M. Jerina. "Metabolism of Chrysene and Phenanthrene to Bayregion Diol-epoxide by Rat Liver Enzymes." *Mol. Pharmacol.* 19 (1981): 168–78.
30. Teranishi, K., H. Kokichi, and H. Watanabe. "Quantitative Relationship between Carcinogenicity and Mutagenicity of Polyaromatic Hydrocarbons in *Salmonella typhimurium* mutant." *Mutat. Res.* 31 (1975): 97.
31. EFSA. "Scientific Opinion of the Panel on Contaminants in the Food Chain on a Request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food." *EFSA J.* 724 (2008): 1–114.
32. US-EPA (United States Environmental Protection Agency). Polycyclic Aromatic Hydrocarbons, PAHs. Retrieved October 31, 2016 from https://archive.epa.gov/epawaste/hazard/wastemin/web/pdf/pahs.pdf (2008).

33. Semanová, J., B. Skláršová, P. Šimon, and P. Šimko. "Elimination of Polycyclic Aromatic Hydrocarbons from Smoked Sausages by Migration into Polyethylene Packaging." *J. Food Chem.* 201 (2016): 1–6.
34. European Commission. "Regulation No 1881/2006 of 19 December 2006 Setting Maximum Levels of Certain Contaminants in Foodstuffs." *Official J. Eur. Union* L364/5 (2006).
35. European Commission. "Decision No 2002/658/EC of 12 August 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results." *Official J. Eur. Union* L221/8 (2002).
36. European Commission. "Commission Regulation (EU) No 836/2011 of 19 August 2011 Amending Regulation (EC) No 333/2007 Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Lead, Cadmium, Mercury, Inorganic Tin, 3-MCPD and benzo(a)pyrene in Foodstuffs." *Official J. Eur. Union* L 215 (2011): 9–16.
37. Benson, N. U., W. U. Anake, A. E. Adedapo, O. H. Fred-Ahmadu, and K. P. Eke. "Polycyclic Aromatic Hydrocarbons in Imported *Sardinops sagax*: Levels and Health Risk Assessments Through Dietary Exposure in Nigeria." *J. Food Compos. Anal.* 57 (2017): 109–16.
38. Koh, C.-H., J. S. Khim, K. Kannan, D. L. Villeneuve, K. Senthilkumar, and J. P. Giesy. "Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and Polycyclic Aromatic Hydrocarbons (PAHs) and 2,3,7,8-TCDD Equivalents (TEQs) in Sediment from the Hyeongsan River, Korea." *Environ. Pollut.* 132 (2004): 489–501.
39. Rogula-Kozłowska, W., B. Kozielska, and K. Klejnowski. "Concentration, Origin and Health Hazard from Fine Particle-Bound PAH at Three Characteristic Sites in Southern Poland." *Bull. Environ. Contam. Toxicol.* 91 (2013): 349–55, doi: 10.1007/s00128-013-1060-1
40. Zhang, L., L. Dong, L. Ren, S. Shi, L. Zhou, T. Zhang, et al. "Concentration and Source Identification of Polycyclic Aromatic Hydrocarbons and Phthalic Acid Esters in the Surface Water of the Yangtze River Delta." *China J. Environ. Sci.* 24, no. 2 (2012): 335–42.
41. Zhang, J., C. Sun, Q. Ma, and Y. Chen. "Human Health And Ecological Risk Assessment Of 16 Polycyclic Aromatic Hydrocarbons In Drinking Source Water From A Large Mixed-Use Reservoir." *Int. J. Environ. Res. Public Health* 12 (2015): 13956–69; doi:10.3390/ijerph121113956
42. Kamangar, F., M. M. Schantz, C. C. Abnet, R. B. Fagundes, and S. M. Dawsey. "High Levels of Carcinogenic Polycyclic Aromatic Hydrocarbons in Mate Drinks." *Cancer Epidemiol. Biomarkers Prev.* 17, no. 5 (2008): 1262–8.
43. Schlemitz, S., and W. Pfannhauser. "Supercritical Fluid Extraction of Mononitrated Polycyclic Aromatic Hydrocarbons from Tea-Correlation with the PAH Concentration." *Z. Lebensm. -Forsch. A* 205, no. 4 (1997): 305–10.
44. Drabova, L., J. Pulkrabova, K. Kalachova, M. Tomaniova, V. Kocourek, and J. Hajslova. "Rapid Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tea Using Two-Dimensional Gas Chromatography Coupled with Time of Flight Mass Spectrometry." *Talanta* 100 (2012): 207–16.
45. Grover, L. S., S. A. Singh, and B. Pal. "Priority PAHs in Orthodox Black Tea During Manufacturing Process." *Environ. Monit. Assess.* 185 (2013): 6291–4.
46. Ishizaki, A., K. Saito, N. Hanioka, S. Narimatsu, and H. Kataoka. "Determination of Polycyclic Aromatic Hydrocarbons in Food Samples by Automated on-line in-Tube Solid-phase Microextraction Coupled with High-Performance Liquid Chromatography-Fluorescence Detection." *J. Chromatogr. A* 1217, no. 35 (2010): 5555–63.
47. Conte, F., C. Copat, S. Longo, G. Oliveri Conti, A. Grasso, G. Arena, A. Dimartino, M. V. Brundo, and M. Ferrante. "Polycyclic Aromatic Hydrocarbons in *Haliotis tuberculata* (Linnaeus, 1758) (Mollusca, Gastropoda): Considerations on Food Safety and Source Investigation." *Food Chem. Toxicol.* 94 (2016): 57–63.
48. Nisbet, I., and P. LaGoy. "Toxic Equivalency Factors (TEFs) for Polycyclic Aromatic Hydrocarbons (PAHs)." *Regul. Toxicol. Pharmacol. RTP.* 16 (1992): 290–300.