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Concentrations, Health Risks and Sources of Polycyclic Aromatic Hydrocarbons in Nigerian Honey

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Abstract

The concentrations of the US EPA 16 priority polycyclic aromatic hydrocarbons (PAHs) were measured in honey samples collected from different regions of Nigeria with a view to providing information on the extent of contamination, regional profiles, sources and risks of PAHs in this food type. The concentrations of the PAHs were determined by using gas chromatography-mass spectrometry after extraction by ultra-sonication with *n*-hexane and dichloromethane. The concentrations of Σ16 PAHs in the honey samples ranged from 169-522 μg kg^{-1}, 97.2-1980 μg kg^{-1}, 180-641 μg kg^{-1} and 122-357 μg kg^{-1} for South-East, South-West, Niger Delta, and North Central regions respectively. The compositional patterns of PAHs in the analyzed honey samples followed the order: 5-rings > 4-rings > 3-rings > 6-rings > 2-rings. The estimated daily intake values from consumption of these honey samples ranged from not detected (nd) to 1.9 ng kg^{-1} bw day^{-1}, nd to 5.9 ng kg^{-1} bw day^{-1}, nd to 18 ng kg^{-1} bw day^{-1} and 0.6 to 33 ng kg^{-1} bw day^{-1} for BaP, PAH2, PAH4 and PAH8 respectively. The incremental life cancer risk and margin of exposure values for the majority of the samples indicate that there is no risk associated with the consumption of these honey samples. The PAH isomeric ratios and principal component analysis indicated that combustion of fossil fuels, natural gas and biomass, and automobile emissions were the main sources of PAHs in these samples from the different regions of Nigeria.

Keywords: Honey, Polycyclic aromatic hydrocarbons, Risk assessment, Nigeria

Introduction

Honey is a natural product produced by *Apis mellifera* bees from the nectar or secretions of plants, and does not usually contain any additives or preservatives[1]. It contains a number of nutritionally valuable compounds and has healing, prophylactic, anti-oxidative, anti-bacterial and immune-enhancing properties[2,3]. Honey is made up of a mixture of carbohydrates, such as fructose (25-45% m/m), glucose (25-37% m/m), maltose (2-12% m/m) and sucrose (0.5-3% m/m) with traces of other sugars, and water (14-18% m/m)[4] as well as small amounts of a wide array of vitamins, mineral substances (0.1 to 1.0% m/m)[5], amino acids and antioxidants[1]. The colour, flavour, carbohydrate composition and mineral, amino acid and antioxidant content of honey often varies with floral types[1]. The concentrations of contaminants in honey reflect the conditions of the environment and bee-keeping practices. Since the forage area of the bee hive is very large (more than 7 km^2) and the bees come in contact with the basic components of the environment (air, soil and water), the concentrations of contaminants in honey reflect their amounts in the whole region[4]. Thus, honey can serve as a useful environmental marker and bioindicator for monitoring environmental contaminants[6-9]. Despite the known nutritional and therapeutic properties of honey, the occurrence of xenobiotics, such as metals, pesticides and polycyclic aromatic hydrocarbons, in honey may constitute a serious threat to consumers.

Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of over one hundred (100) organic compounds consisting of two or more fused aromatic rings[10] and/or pentacyclic rings in linear, angular or cluster formations[11-13]. PAHs are primarily produced by the

incomplete combustion or heat-induced decomposition of matter[14-16] and natural combustion processes, such as forest fires, and volcanic eruptions. They have been classified as hazardous compounds of environmental and health concern because of the fact that a number of them have been found to exhibit carcinogenic, genotoxic and mutagenic properties[17], and long-range transportation and deposition capabilities. Consequently, the United States Environmental Protection Agency (USEPA) has listed 16 PAHs as priority environmental pollutants in order to facilitate environmental monitoring. The International Agency for Research on Cancer (IARC) has classified the 16 US EPA PAHs as follows. Benzo[*a*]pyrene (B*a*P) is carcinogenic (group 1), dibenzo[*a,h*]anthracene (D*ah*A) is probably carcinogenic (group 2A), whereas naphthalene (Nap), benzo[*a*]anthracene (B*a*A), chrysene (Chry), benzo[*b*]fluoranthene (B*b*F), benzo[*k*]fluoranthene (B*k*F) and indeno[*1,2,3-c,d*]pyrene (IndP) are classified as possible human carcinogens (group 2B) while others are not classified as carcinogenic to humans[18].

Human exposure to PAHs occurs mainly through contact with air, water and soil, and the consumption of contaminated products[19] in which PAHs may accumulate in the lipid components[20]. Dietary sources are by far the most important route of human exposure to PAHs, apart from smoking and occupational exposures. There are a number of published studies on the concentrations of PAHs in honey but most of these are restricted to European countries[9,21-25]. To the best of our knowledge, there is no published data on the PAH concentrations in honey from the different regions of Nigeria. The objective of this study was to determine the concentrations of PAHs in honey samples from different regions of Nigeria with a view to providing information on the compositional patterns, regional profiles, sources and risks associated with the consumption of these honey samples. Such information is useful for environmental and food quality management.

Results and Discussion

The concentrations of the Σ16 PAHs from different regions of Nigeria are presented in Tables 1 and 2. The concentrations of Σ16 PAHs in the analyzed honey samples ranged from 169-522 μg kg^{-1}, 97-1980 μg kg^{-1}, 180-641 μg kg^{-1} and 122-357 μg kg^{-1} for South-East, South-West, Niger Delta, and North Central regions respectively. The differences observed in the Σ16 PAH concentrations in these samples were significant ($p < 0.05$). There were also significant differences ($p < 0.05$) in the regional mean concentrations of Σ16 PAHs. However, no significant difference was observed in the mean concentrations of Σ16 PAHs observed in the South-East and Niger Delta regions. The concentration pattern of the Σ16 PAHs in the honey samples followed the order: South-West > South-East > Niger Delta > North Central. The regional concentration patterns reflect the distribution pattern of industries in Nigeria. For instance, the honey samples from the south-western part of Nigeria, that is more industrialised, showed higher concentrations of the Σ16 PAHs.

A wide concentration range for PAHs has been reported in honey and honey products in the literature. For example, Dobrinas *et al*.[9] reported PAH concentrations in the range of 56 to 2410 μg kg^{-1} in honey from Romania. In Italy, Moret *et al*.[23] reported total PAH concentrations in the range of 38 to 41300 μg kg^{-1} in raw propolis and 0.9 to 1790 μg kg^{-1} in propolis extracts. Ciemark *et al*.[26] reported Σ23 PAH concentrations up to 305 μg kg^{-1} in blossom honey. In Spain, the concentrations of 15 PAHs in 30 samples of honey collected from different regions were found to be below the limit of detection[25]. The PAH content in the honey samples in our study corresponds with that of Dobrinas *et al*.[9] from Romania and Moret *et al*.[23] from Italy but was lower than values reported for honey in Italy[21], France[22], Czech Republic[24] and Spain[25]. The contamination of these honey samples by PAHs could be due to bush burning and industrial and automobile emissions. In Nigeria, a bush fallowing farming method is commonly practiced, which involves clearing and burning of the cleared biomass at each farming season. In Nigeria, honeys are harvested mainly by the use of smoke which can also be a source of PAHs in the honey samples. Post-harvest contamination from improper packaging and processing also cannot be ruled out. Most frequently honeys are displayed for sale in open plastic containers along the major highways.

In this study, the naphthalene concentrations in the samples from the different regions were below the limit of quantification (LOQ) in all samples except for SW19 (313 μg kg^{-1}). In SW19 naphthalene constituted 28.4% of the Σ16 PAHs. The non-detectability of naphthalene in these samples may be due to its high volatility.

The 3-ringed PAH concentrations ranged from less than the LOQ to 390 μg kg^{-1}, which constituted up to 65% of the Σ16 PAHs in some of these samples. Acenaphthene (Ace) and fluorene (Flu) were the dominant 3-ringed PAHs in these samples in terms of frequency of occurrence. Ace and Flu were detected in 35% of the honey samples at concentrations in the range of 27 to 146 μg kg^{-1} and 26 to 264 μg kg^{-1} respectively. The concentrations of Flu were higher than the other 3-ringed PAHs in these honey samples. Phenanthrene (Phen) and anthracene (Ant) were detected in 28% and 22% of these honey samples at concentrations of 12 to 136

Table 1. PAH concentrations (μg kg^{-1}) from different regions in Nigeria.

	Nap	Acy	Ace	Flu	Phen	Ant	Flt	Pyr	B*a*A	Chry	B*b*F	B*k*F	B*a*P	IndP	D*ah*A	B*ghi*P	Total	2R	3R	4R	5R	6R	PAH2	PAH4	PAH8
SE1	ND	ND	33.2	33.6	41.6	ND	ND	ND	24.7	ND	29.6	191	ND	ND	27.3	ND	381	ND	108	24.7	248	ND	ND	54.3	272
SE2	ND	ND	45.8	28.6	ND	ND	ND	ND	ND	95.7	141	113	27.3	ND	ND	ND	451	ND	74.4	95.7	281	ND	123	264	377
SE3	ND	ND	ND	ND	ND	ND	ND	153.5	ND	35.4	27.4	ND	ND	ND	ND	27.7	244	ND	ND	189	27.4	27.7	35.4	62.8	90.5
SE4	ND	ND	145.6	25.7	ND	ND	ND	ND	27.5	44.7	60.6	110	26.2	ND	ND	ND	440	ND	171	72.2	197	ND	70.9	159	269
SE5	ND	ND	26.7	30.4	ND	ND	ND	ND	37.3	ND	ND	74.3	ND	ND	ND	ND	169	ND	57.1	37.3	74.3	ND	ND	37.3	112
SE6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	86.6	147	ND	ND	59.3	ND	293	ND	ND	ND	293	ND	ND	86.6	293
SE7	ND	105	54.8	28.4	ND	ND	ND	ND	177	4.5	93.1	34.8	25.3	ND	ND	ND	522	ND	188	181	153	ND	29.8	299	334
SE8	ND	124	ND	ND	ND	ND	ND	ND	40.8	91.6	80.8	114	ND	ND	ND	ND	451	ND	124	132	195	ND	91.6	213	327
SW9	313	35.9	32.2	ND	67.2	ND	ND	61.7	77.5	ND	117	131	85.1	ND	87.1	92.9	1100	313	135	139	420	92.9	85.1	279	591
SW10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	52	ND	ND	45.1	ND	97.2	ND	ND	ND	97.1	ND	ND	ND	97.1
SW11	ND	ND	ND	43	ND	ND	ND	ND	25.9	25.7	ND	46.2	ND	ND	39.3	ND	180	ND	43	51.6	85.5	ND	25.7	51.6	137
SW12	ND	60.5	32.1	ND	ND	ND	ND	ND	98.4	ND	ND	30.5	ND	ND	25.5	ND	247	ND	92.6	98.4	56	ND	ND	98.4	154
SW13	ND	ND	ND	263.6	62.9	63.9	44.4	97.1	371	198	141	63.3	59.8	67	430	122.3	1980	ND	390	710	694	189	257	769	1450
SW14	ND	ND	ND	ND	ND	59.7	ND	210	ND	104	ND	41.9	ND	ND	ND	ND	415	ND	59.7	314	41.9	ND	104	104	149
SW15	ND	ND	ND	38.3	ND	60.8	ND	57.8	ND	31.3	ND	70.9	31.8	ND	28.6	27.4	347	ND	99.1	89.1	131	27.4	63.1	63.1	190
SW16	ND	ND	ND	81.5	136	ND	416	60.7	44.3	28.5	57.2	42.4	ND	ND	ND	ND	868	ND	218	551	99.6	ND	28.5	130	172
SW17	ND	40	26.1	65.3	ND	ND	ND	ND	ND	ND	ND	81.4	ND	ND	ND	ND	213	ND	131	ND	81.4	ND	ND	ND	81.4
SW18	ND	ND	ND	ND	ND	ND	ND	ND	24.4	ND	75.2	197	ND	ND	63.5	ND	360	ND	ND	24.4	336	ND	ND	99.6	360
SS19	ND	ND	ND	ND	ND	ND	ND	ND	ND	80.5	240	154	29	26	ND	ND	530	ND	ND	80.5	424	26	110	350	530
SS20	ND	ND	ND	ND	ND	ND	ND	142	ND	95.8	ND	74	80.3	ND	28	29.2	449	ND	ND	238	182	29.2	176	176	307
SS21	ND	62.4	48.1	ND	ND	ND	28.1	ND	111	73.1	149	92.7	21.3	ND	ND	56	641	ND	111	212	263	56	94.4	354	502
SS22	ND	ND	45.8	28.6	ND	ND	ND	ND	ND	95.7	34.9	60.5	27.3	ND	ND	ND	293	ND	74.4	95.7	123	ND	123	158	218
SS23	ND	ND	ND	ND	55.8	53.8	82.5	29.6	26.7	17.9	25.1	ND	ND	ND	ND	ND	291	ND	110	157	25.1	ND	17.9	69.7	69.7
SS24	ND	ND	ND	ND	ND	59.7	ND	104	ND	157	ND	52.4	ND	ND	ND	ND	378	ND	59.7	261	52.4	ND	157	157	209
SS25	ND	ND	ND	31.5	ND	67.1	ND	47.2	ND	ND	55.7	95.3	6	ND	ND	36.4	339	ND	98.6	47.2	157	36.4	6.0	61.7	193
SS26	ND	ND	ND	38.3	ND	60.8	ND	57.7	ND	31.3	ND	7.1	31.8	ND	2.9	0.3	230	ND	99.1	89	41.8	0.3	63.1	63.1	73.4
SS27	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	198	46	ND	ND	ND	ND	245	ND	ND	ND	244	ND	ND	198	244
SS28	ND	ND	ND	ND	ND	ND	ND	ND	28.1	ND	7.5	90	ND	ND	54.6	ND	180	ND	ND	28.1	152	ND	ND	35.6	180
SS29	ND	ND	37.8	30.4	ND	ND	ND	ND	37.3	ND	ND	127	ND	ND	ND	ND	233	ND	68.2	37.3	127	ND	ND	37.3	165
SS30	ND	ND	ND	ND	53.8	ND	106	ND	62.7	38.6	23.9	59.4	26.8	ND	ND	46.1	417	ND	53.8	207	110	46.1	65.4	152	258
NC31	ND	ND	62	ND	115.8	ND	42.1	28.4	ND	ND	ND	24.3	ND	ND	ND	ND	273	ND	178	70.5	24.3	ND	ND	ND	24.3
NC32	ND	ND	ND	ND	24.2	24.1	35	81.1	ND	ND	ND	ND	28.5	ND	ND	37.1	230	ND	48.3	116	28.5	37.1	28.5	28.5	65.6
NC33	ND	ND	ND	ND	ND	ND	ND	141.9	ND	42.9	ND	74	27.4	ND	28	29.2	343	ND	ND	185	129	29.2	70.3	70.3	202
NC34	ND	ND	ND	ND	ND	38.7	64.7	30.2	ND	ND	9.2	90.6	0.5	24.5	39.8	58.9	357	ND	38.7	94.9	140	83.4	0.5	9.7	224
NC35	ND	ND	ND	ND	62.8	ND	33.8	ND	ND	ND	ND	25.3	ND	ND	ND	ND	122	ND	62.8	33.8	25.3	ND	ND	ND	25.3
NC36	ND	ND	62	ND	11.6	ND	42.1	28.4	ND	ND	ND	24.3	ND	ND	ND	ND	168	ND	73.6	70.5	24.3	ND	ND	ND	24.3
NC37	ND	63.5	ND	ND	ND	ND	32.6	ND	ND	ND	26.4	30.3	ND	ND	ND	35.3	188	ND	63.5	32.6	56.7	35.3	ND	26.4	92
NC38	ND	ND	ND	ND	ND	ND	ND	ND	ND	28.2	29.1	36.2	10.9	ND	50.2	ND	155	ND	ND	28.2	126	ND	39.1	68.2	155
NC39	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	25.5	38.1	ND	ND	38.3	33.6	136	ND	ND	ND	102	33.6	ND	25.5	134
NC40	ND	ND	26.8	ND	49.2	ND	ND	27.8	ND	27.8	ND	41.3	25.5	ND	ND	ND	193	ND	76	55.6	66.8	ND	53.3	53.3	94.6

Napthalene (NaP), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phen), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Benzo[*a*]anthracene (B*a*A), Chrysene (Chry), Benzo[*b*]fluoranthene (B*b*F), Benzo[*k*]fluoranthene (B*k*F), Benzo[*a*]pyrene (B*a*P), Indeno[*1,2,3-c,d*]pyrene (IndP), Dibenzo[*a,h*]anthracene (D*ah*A) and Benzo[*ghi*]perylene (B*ghi*P), Rings (R), South-East (SE), South-West (SW), Niger Delta (SS), North Central (NC), Below limit of quantification (LOQ) (ND).

Table 2. Mean ± SD (Median) concentrations (μg kg^{-1}) of PAHs in honey samples.

PAH Compound	South-East	South-West	Niger Delta	North Central
Naphthalene	0.0 ± 0.0 (0.0) ND	31.3 ± 98.9 (0.0) (ND-312.7)	0.0 ± 0.0 (0.0) ND	0.0 ± 0.0 (0.0) ND
Acenaphthylene	28.5 ± 53.1 (0.0) (ND-124)	13.6 ± 22.8 (0.0) (ND-60.5)	5.2 ± 18.0 (0.0) (ND-62.4)	6.4 ± 20.1 (0.0) (ND-63.5)
Acenaphthene	38.3 ± 48.4 (30) (ND-145.6)	9.0 ± 14.6 (0.0) (ND-32.2)	11.0 ± 20.0 (0.0) (ND-48.1)	15.2 ± 6.1 (0.0) ND-62.0
Fluorene	18.3 ± 15.3 (27.1) (ND-33.6)	49.2 ± 47.0 (0.0) (ND-264)	10.7 ± 16.0 (0.0) (ND-38.3)	0.0 ± 0.0 (0.0) ND
Phenanthrene	5.2 ± 14.7 (0.0) (ND-41.6)	26.6 ± 47.0 (0.0) (ND-136.0)	9.1 ± 21.3 (0.0) (ND-55.8)	26.4 ± 38.8 (5.8) (ND-62.8)
Anthracene	0.0 ± 0.0 (0.0) ND	18.4 ± 29.7 (0.0) (ND-63.9)	20.1 ± 29.9 (0.0) (ND-67.1)	6.3 ± 13.7 (0.0) (ND-38.7)
Fluoranthene	0.0 ± 0.0 (0.0) ND	46.2 ± 131 (0.0) (ND-417.5)	18.1 ± 36.9 (0.0) (ND-106)	25.0 ± 23.3 (33.2) (ND-64.7)
Pyrene	19.2 ± 54.3 (0.0) (ND-153.5)	48.7 ± 67.1 (28.9) (ND-210)	31.7 ± 48.0 (0.0) (ND-141.9)	33.8 ± 45.5 (28.1) (ND-142)
Benzo[*a*]anthracene	38.4 ± 58.3 (26.1) (ND-40.8)	64.1 ± 113 (25.2) (ND-371)	22.1 ± 34.7 (0.0) (ND-111)	0.0 ± 0.0 (0.0) ND
Chrysene	34.0 ± 40.7 (20.0) (ND-95.7)	38.7 ± 64.4 (12.9) (ND-198)	49.2 ± 51.0 (35.0) (ND-157)	9.9 ± 16.4 (0.0) (ND-42.9)
Benzo[*b*]fluoranthene	64.9 ± 45.0 (70.7) (ND-140.8)	39.0 ± 54.9 (0.0) (ND-141)	61.1 ± 85.1 (24.5) (ND-240.2)	9.0 ± 12.8 (0.0) (ND-29.1)
Benzo[*k*]fluoranthene	98.0 ± 60.7 (112) (ND-191)	75.7 ± 51.4 (57.7) (30.5-197)	71.6 ± 44.6 (67.3) (ND-154)	38.4 ± 26.1 (33.3) (ND-90.6)
Benzo[*a*]pyrene	9.9 ± 13.6 (0.0) (ND-27.3)	17.7 ± 31.1 (0.0) (ND-85.1)	18.5 ± 23.6 (13.7) (ND-80.3)	9.3 ± 12.8 (0.3) (ND-28.5)
Indeno[*1,2,3-c,d*]pyrene	0.0 ± 0.0 (0.0) ND	6.7 ± 21 (0.0) (ND-67.0)	2.2 ± 7.5 (0.0) (ND-26.0)	2.5 ± 7.7 (0.0) (ND-24.5)
Dibenz[*a,h*]anthracene	10.8 ± 21.8 (0.0) (ND-59.3)	72.0 ± 129 (34.0) (ND-430)	7.1 ± 17.0 (0.0) (ND-54.6)	15.6 ± 20.8 (0.0) (ND-50.2)
Benzo[*g,h,i*]perylene	3.5 ± 9.8 (0.0) (ND-27.7)	24.3 ± 45.3 (0.0) (ND-122.3)	14.0 ± 21.5 (0.0) ND-56.0	19.4 ± 21.9 (14.6) (ND-58.9)
Σ16 PAHs	368.9 ± 121.6 (411) (169-522)	581.1 ± 586.2 (353.5) (180-1980)	352 ± 137 (316) (180-641)	216.4 ± 83.1 (191) (122-357)
2 Rings	0.0 ± 0.0 (0.0) ND	31.3 ± 98.9 (0.0) (ND-313)	±0.0 (0.0) ND	0.0 ± 0.0 (0.0) ND
3 Rings	90.3 ± 70.9 (91.4) (ND-171.3)	116.9 ± 117 (95.9) (ND-390)	56.2 ± 45.3 (64.0) (ND-111)	54.1 ± 53.1 (55.6) (ND-178)
4 Rings	91.5 ± 71.0 (84.0) (ND-188.9)	197.7 ± 248 (93.8) (ND-710)	121 ± 90.0 (92.4) (ND-261)	68.7 ± 53.2 (63.1) (ND-185)
5 Rings	184 ± 95.0 (196) (27.4-293.2)	204 ± 213(98.4) (41.9-694.2)	158 ± 111 (140) (25.1-424)	72.4 ± 47.9 (61.8) (24.3-140)
6 Rings	3.5 ± 9.8 (0.0) (ND-27.7)	31.0 ± 62.9 (0.0) (ND-189.3)	16.2 ± 21.3 (0.2) (ND-56.0)	21.9 ± 27.4 (14.6) (ND-83.4)
PAH2	43.8 ± 46.8 (32.6) (ND-123)	56.4 ± 80.2 (27.1) (ND-257.3)	67.7 ± 64.2 (64.3) (ND-176)	19.2 ± 26.8 (0.3) (ND-70.3)
PAH4	147.1 ± 102.0 (123) (37.3-299)	160 ± 228(99.0) (ND-769)	151 ± 110 (155) (35.6-354)	28.2 ± 27.4 (26.0) (ND-70.3)
PAH8	259 ± 104 (283) (90.5-377)	338 ± 420(163) (81.4-1450)	246 ± 144(214) (69.0-530)	104 ± 73.0 (93.3) (24.3-224)

μg kg^{-1} and 24 to 67 μg kg^{-1} respectively. Ant was below the LOQ in the honey samples from the South-East region of Nigeria. Acenaphthylene (Acy) was detected in 15% of the samples at concentrations in the range of 36 to 124 μg kg^{-1}.

The 4-ringed PAHs were found in 88% of the samples at concentrations varying from 24 to 710 μg kg^{-1}. Chry was the dominant 4-ringed PAH in the honey samples in terms of concentration and frequency of occurrence. In these samples, Chry was detected at concentrations in the range of 4.5 to 198 μg kg^{-1} which constituted 0.9 to 42.1% of the Σ16 PAHs. Pyrene (Pyr)

and B*a*A were detected in 43% and 35% of the samples at concentrations in the range of 30 to 210 μg kg^{-1} and 25 to 371 μg kg^{-1} respectively. B*a*A and fluoranthene (Flt) were not detected in the honey samples from the North Central and South-East parts of Nigeria respectively. However, Flt was detected in 28% of the total samples examined at concentrations between 28 and 418 μg kg^{-1} which constituted 2.2 to 48% of the Σ16 PAHs in these samples.

The 5-ringed PAH concentrations ranged from 25.1 to 694 μg kg^{-1} which constituted 8.9 to 100% of the Σ16 PAHs in these samples. The 5-ring PAHs were the dominant PAHs in terms of concentrations and frequency of occurrence compared with 2-, 3- and 6-ringed PAHs. The dominant 5-ringed PAH was B*k*F which had 95% occurrence in the honey samples. B*k*F was detected at concentrations that varied from 7.1 to 197 μg kg^{-1}. B*b*F was detected in 58% of the honey samples at concentrations of 7.5 to 240 μg kg^{-1} which constituted up to 45.3% of the Σ16 PAHs in some of these samples. B*a*P was detected in 45% of the honey samples at concentrations ranging from 0.5 to 85 μg kg^{-1}. There is no legislation regulating the concentration of B*a*P in honey and honey products. Consequently, these results were compared with the limit for B*a*P specified for infant food. The concentrations of B*a*P in 45% of the samples examined were above the 1 μg kg^{-1} limit specified for B*a*P in infant food[27]. The concentrations of B*a*P in this study are comparable to those found in honey from Romania (0.5 to 141 μg kg^{-1})[23] and in propolis and propolis-based dietary supplements in Italy (0.8 to 42 μg kg^{-1})[19] but were higher than B*a*P concentrations reported in honey from France[22], Czech Republic[24], Spain[25] and Poland[26].

The 6-ringed PAHs were detected in 38% of the honey samples in the range of 0.3 to 189 μg kg^{-1}, which constituted up to 25% of the Σ16 PAHs in some of the samples. Benzo[*ghi*]perylene (B*ghi*P) was the most abundant 6-ringed PAH compound in the honey samples. Indeno[*1,2,3-c,d*]pyrene (IndP) was detected in three samples at concentrations of 25 to 67 μg kg^{-1}. IndP was not detected in any of the samples from South-East Nigeria.

Although, B*a*P is considered as a suitable indicator for occurrence and effect of PAHs in food, this relation is not fully convincing. Therefore, the European Food Safety Authority (EFSA) has suggested the use of PAH2 (Chry + B*a*P), PAH4 (PAH2 + B*a*A + B*b*F) and PAH8 (PAH4 + B*k*F + D*ah*A + IndP + B*ghi*P) as indicators for occurrence and effects of PAHs in food[28]. The indicators: PAH2, PAH4 and PAH8 were detected in 60, 88 and 100% of the samples at concentrations in the range of 0.5 to 257 μg kg^{-1}, 9.7 to 769 μg kg^{-1}, and 24 to 1450 μg kg^{-1} respectively. The average concentrations of PAH8 in the four regions were 259 μg kg^{-1} (South-East), 338 μg kg^{-1} (South-West), 246 μg kg^{-1} (Niger Delta) and 104.1 μg kg^{-1} (North Central). The results indicated that honey samples from south-western Nigeria contained higher concentrations of PAH8 than the other regions.

Dietary Intake and Risk Assessment

The estimated dietary intake of PAHs based on B*a*P and the EFSA suggested indicators for occurrence and effect of PAHs in food are displayed in Table 3. The maximum estimated daily intake values for B*a*P, PAH2, PAH4 and PAH8 were 1.9 ng kg^{-1} bw day^{-1}, 5.9 ng kg^{-1} bw day^{-1}, 18 ng kg^{-1} bw day^{-1} and 33 ng kg^{-1} bw day^{-1} respectively. The results of the present study revealed that the intake values of these indicators of PAHs were higher in the South-West region than the Niger Delta, South-East and North Central regions of Nigeria. The dietary intakes of PAHs in this study are lower than intake values reported from consumed canned fish[13], smoked/grilled fish[29], fresh and smoked fish[30,31], milk[32], biscuits[33], chocolates and candies[34] and ready to eat foods in Nigeria[35] but were comparable to intake values obtained from consumption of tea in Nigeria[36]. Moret *et al*.[23] reported intake values for B*a*P and PAH8 of 0.0 to 1.3 ng kg^{-1} bw day^{-1} and 0.01 to 47 ng kg^{-1} bw day^{-1} respectively through consumption of raw propolis and propolis extracts used as dietary supplements. The estimated margin of exposure (MOE) based on B*a*P and the EFSA suggested indicators for effects and occurrence of PAHs in foods are displayed in Table 3. The B*a*P-MOE, PAH2-MOE, PAH4-MOE and PAH8-MOE values were greater than 10,000 which indicate no health concern associated with the consumption of these honeys at the current ingestion rate.

The computed BaP$_{TEQ}$ and BaP$_{MEQ}$ concentrations from ingestion of these honey samples are displayed in Table 4. BaP$_{TEQ}$ is directly associated with carcinogenicity, whereas BaP$_{MEQ}$ (mutagenic activity) may not be directly associated with cancer[37,38] and may have to do with other non-cancerous adverse health effects such as pulmonary disease, birth defects, impotency, low IQ, etc.[39-41]. The BaP$_{TEQ}$ for the honey samples ranged from 0.2 to 549 μg kg^{-1}. The main contributors to Σ BaP$_{TEQ}$ values of these honey samples were B*a*A, B*a*P, B*b*F, and D*ah*A, while the contribution of IndP, Chry and B*k*F to the Σ BaP$_{TEQ}$ was minimal. BaP$_{MEQ}$ values obtained in this study ranged from 2.67 to 281 μg kg^{-1} with significant contributions from B*b*F, B*k*F and B*a*P. The honey samples from the South-West region had higher mean BaP$_{TEQ}$ and BaP$_{MEQ}$ values than the other regions.

The estimated incremental life cancer risk associated with the consumption of these honey samples ranged

Table 3. Estimated daily intakes (ng kg^{-1} bw day^{-1}) and margin of exposure for honey samples.

	Daily intake				Margin of exposure			
	B*a*P	PAH2	PAH4	PAH8	B*a*P	PAH2	PAH4	PAH8
SE1	0.0	0.0	1.2	6.2	–	–	274000	78800
SE2	0.6	2.8	6.0	8.6	112000	60500	56400	56900
SE3	0.0	0.8	1.4	2.1	0	210000	237000	237000
SE4	0.6	1.6	3.6	6.1	117000	105000	93700	79800
SE5	0.0	0.0	0.9	2.5	–	–	399000	192000
SE6	0.0	0.0	2.0	6.7	–	–	172000	73200
SE7	0.6	0.7	6.8	7.6	121000	245000	49700	64200
SE8	0.0	2.1	4.9	7.5	–	81300	69800	65600
SW9	1.9	1.9	6.4	13.5	36000	87500	53300	36300
SW10	0.0	0.0	0.0	2.2	–	–	–	221000
SW11	0.0	0.6	1.2	3.1	–	290000	289000	157000
SW12	0.0	0.0	2.2	3.5	–	–	151000	139000
SW13	1.4	5.9	18	33	51300	28900	19400	14800
SW14	0.0	2.4	2.4	3.3	–	71700	143000	147000
SW15	0.7	1.4	1.4	4.3	96400	119000	236000	113000
SW16	0.0	0.7	3.0	3.9	–	261000	115000	125000
SW17	0.0	0.0	0.0	1.9	–	–	–	264000
SW18	0.0	0.0	2.3	8.2	–	–	150000	59600
SS19	0.7	2.5	8.0	12	106000	68000	42600	40500
SS20	1.8	4.0	4.0	7.0	38200	42300	84600	69800
SS21	0.5	2.2	8.1	12	144000	78900	42100	42700
SS22	0.6	2.8	3.6	5.0	112000	60500	94300	98300
SS23	0.0	0.4	1.6	1.6	–	416000	214000	308000
SS24	0.0	3.6	3.6	4.8	–	47500	94900	103000
SS25	0.1	0.1	1.4	4.4	51100	1240000	241000	111000
SS26	0.7	1.4	1.4	1.7	96400	118000	236000	292000
SS27	0.0	0.0	4.5	5.6	–	–	75300	88000
SS28	0.0	0.0	0.8	4.1	–	–	418000	119000
SS29	0.0	0.0	0.9	3.8	–	–	399000	130000
SS30	0.6	1.5	3.5	5.9	114000	114000	98000	83300
NC31	0.0	0.0	0.0	0.6	–	–	–	883000
NC32	0.7	0.7	0.7	1.5	108000	261000	523000	327000
NC33	0.6	1.6	1.6	4.6	112000	106000	212000	107000
NC34	0.0	0.0	0.2	5.1	6130000	14900000	1540000	96000
NC35	0.0	0.0	0.0	0.6	–	–	–	848000
NC36	0.0	0.0	0.0	0.6	–	–	–	883000
NC37	0.0	0.0	0.6	2.1	–	–	564000	233000
NC38	0.2	0.9	1.6	3.5	281000	19000	218000	139000
NC39	0.0	0.0	0.6	3.1	–	–	584000	158000
NC40	0.6	1.2	1.2	2.2	120000	140000	279000	227000

between 8.0×10^{-10} to 1.8×10^{-6} (Table 5). The results indicated that these samples gave incremental life cancer risk (ILCR) values lower than the acceptable risk level of one in a million chance of additional cancer over a 52 year lifetime ($1/10^6$). This indicates that there is no additional risk arising from the consumption of these honey samples. The ILCR values are in strong agreement with the MOE values.

Source Analysis from Isomeric Ratios

Different isomeric ratios of PAHs have been used to identify the proportion of pyrogenic and petrogenic PAHs in environmental matrices[42]. These diagnostic ratios include Ant/(Ant + Phen), Flt/(Flt + Pyr), B*a*A/(B*a*A + Chry), IndP/(IndP + B*ghi*P), Phen/Ant, Flt/Pyr and LMW/HMW. The values obtained for the various diagnostic ratios in this work are shown in Table 6. In this study, the ratio of low molecular weight (LMW) to high molecular weight (HMW) PAHs ranged from 0.00 to 1.88. LMW/HMW > 1 indicates petrogenic while LMW/HMW < 1 indicates pyrogenic sources. Three samples showed LMW/HMW ratios greater than 1 which indicates that the sources of PAHs in these samples were pyrogenic in nature. A ratio of Ant/(Ant + Phen) < 0.1 and Flt/(Flt + Pyr) < 0.4 is indicative of petroleum sources, while Ant/(Flt + Pyr) > 0.1 implies

Table 4. B*a*P_{TEQ} and B*a*P_{MEQ} (μg kg^{-1}) in honey samples.

	B*a*A	Chry	B*b*F	B*k*F	B*a*P	IndP	D*ah*A	B*a*P_{TEQ}	B*a*A	Chry	B*b*F	B*k*F	B*a*P	IndP	D*ah*A	B*a*P_{MEQ}
SE1	2.5	ND	3.0	1.9	ND	ND	27	34.6	2.0	ND	7.4	21	ND	ND	7.92	38.3
SE2	ND	0.1	14.1	1.1	27.3	ND	ND	42.6	ND	1.6	35.2	12.5	27.3	ND	ND	76.6
SE3	ND	0.04	2.7	ND	ND	ND	ND	2.78	ND	0.6	6.9	ND	ND	ND	ND	7.5
SE4	2.8	0.04	6.1	1.1	26.2	ND	ND	36.2	2.3	0.8	15.2	12.1	26.2	ND	ND	56.4
SE5	3.7	ND	ND	0.7	ND	ND	ND	4.47	3.1	ND	ND	8.2	ND	ND	ND	11.2
SE6	ND	ND	8.7	1.5	ND	ND	59	69.4	ND	ND	21.7	16.2	ND	ND	17.2	55.1
SE7	17.7	0.00	9.3	0.6	25.3	ND	ND	52.6	14.5	0.08	23.3	3.8	25.3	ND	ND	67
SE8	4.1	0.1	8.1	1.1	ND	ND	ND	13.4	3.4	1.6	20.2	12.5	ND	ND	ND	37.6
SW9	7.8	ND	11.7	1.3	85.1	ND	87	193	6.4	ND	29.2	14.5	85.1	ND	25.3	160
SW10	ND	ND	ND	0.5	ND	ND	45	45.6	ND	ND	ND	5.7	ND	ND	13.1	18.8
SW11	2.6	0.03	ND	0.5	ND	ND	39	42.4	2.1	0.4	ND	5.1	ND	ND	11.4	19.0
SW12	9.8	ND	ND	0.3	ND	ND	25	35.7	8.1	ND	ND	3.4	ND	ND	7.4	18.8
SW13	37.1	0.2	14.1	0.6	59.8	6.7	430	549	30.4	3.4	35.2	7.0	59.8	20.8	125	281
SW14	ND	0.1	ND	0.4	ND	ND	ND	0.52	ND	1.8	ND	4.6	ND	ND	ND	6.4
SW15	ND	0.03	ND	0.7	31.8	ND	29	61.1	ND	0.5	ND	7.8	31.8	ND	8.3	48.4
SW16	4.4	0.03	5.7	0.4	ND	ND	ND	10.6	3.6	0.5	14.3	4.7	ND	ND	ND	23.1
SW17	ND	ND	ND	0.8	ND	ND	ND	0.81	ND	ND	ND	9.0	ND	ND	ND	9.0
SW18	2.4	ND	7.5	2.0	ND	ND	64	75.4	2.0	ND	18.8	21.7	ND	ND	18.4	60.9
SS19	ND	0.1	24.0	1.5	29	2.6	ND	57.2	ND	1.4	60.1	17	29	8.1	ND	116
SS20	ND	0.1	ND	0.7	80.3	ND	28	109	ND	1.6	ND	8.1	80.3	ND	8.1	98.2
SS21	11.1	0.1	14.9	0.9	21.3	ND	ND	48.2	9.1	1.2	37.1	10.2	21.3	ND	ND	79
SS22	ND	0.1	3.5	0.6	27.3	ND	ND	31.5	ND	1.6	8.7	6.7	27.3	ND	ND	44.3
SS23	2.7	0.02	2.5	ND	ND	ND	ND	5.20	2.2	0.3	6.3	ND	ND	ND	ND	8.8
SS24	ND	0.2	ND	0.5	ND	ND	ND	0.68	ND	2.7	ND	5.8	ND	ND	ND	8.4
SS25	ND	ND	5.6	1.0	6	ND	ND	12.5	ND	ND	13.9	10.5	6	ND	ND	30.4
SS26	ND	0.03	ND	0.1	31.8	ND	2.9	34.8	ND	0.5	ND	0.8	31.8	ND	0.8	34
SS27	ND	ND	19.9	0.5	ND	ND	ND	20.2	ND	ND	49.5	5.1	ND	ND	ND	54.5
SS28	2.8	ND	0.8	0.9	ND	ND	55	59.1	2.3	ND	1.9	9.9	ND	ND	15.8	29.9
SS29	3.7	ND	ND	1.3	ND	ND	ND	5.0	3.1	ND	ND	14	ND	ND	ND	17.1
SS30	6.3	0.04	2.4	0.6	26.8	ND	ND	36.1	5.1	0.7	6.0	6.5	26.8	ND	ND	45.1
NC31	ND	ND	ND	0.2	ND	ND	ND	0.24	ND	ND	ND	2.7	ND	ND	ND	2.7
NC32	ND	ND	ND	ND	28.5	ND	ND	28.5	ND	ND	ND	ND	28.5	ND	ND	28.5
NC33	ND	0.04	ND	0.7	27.4	ND	28	56.2	ND	0.7	ND	8.1	27.4	ND	8.1	44.4
NC34	ND	ND	0.9	0.9	0.5	2.45	40	44.6	ND	ND	2.3	10	0.5	7.6	11.6	31.9
NC35	ND	ND	ND	0.3	ND	ND	ND	0.3	ND	ND	ND	2.8	ND	ND	ND	2.8
NC36	ND	ND	ND	0.2	ND	ND	ND	0.2	ND	ND	ND	2.7	ND	ND	ND	2.7
NC37	ND	ND	2.6	0.3	ND	ND	ND	2.9	ND	ND	6.6	3.3	ND	ND	ND	9.9
NC38	ND	0.03	2.9	0.4	10.9	ND	50	64.4	ND	0.5	7.3	4.0	10.9	ND	14.6	37.2
NC39	ND	ND	2.6	0.3	ND	ND	38.3	41.2	ND	ND	6.4	4.2	ND	ND	11.1	21.7
NC40	ND	0.03	ND	0.4	25.5	ND	ND	25.9	ND	0.5	ND	4.5	25.5	ND	ND	30.5

Benzo[*a*]anthracene (B*a*A), Chrysene (Chry), Benzo[*b*]fluoranthene (B*b*F), Benzo[*k*]fluoranthene (B*k*F), Benzo[*a*]pyrene (B*a*P), Indeno[*1,2,3-cd*]pyrene (IndP), Dibenz[*a,h*]anthracene (D*ah*A) and Benzo[*ghi*]perylene (B*ghi*P), South-East (SE), South-West (SW), Niger Delta (SS), North Central (NC), Below limit of quantification (ND).

biomass and coal combustion. A Flt/(Flt + Pyr) ratio between 0.4 and 0.5 implies liquid fossil fuel combustion and Flt/(Flt + Pyr) > 0.5 suggests biomass and coal combustion. Ratios of B*a*A/(B*a*A + Chry) < 0.2 and IndP/(IndP + B*ghi*P) > 0.5 and B*a*A/(B*a*A + Chry) > 0.35 indicate that the source of PAHs is biomass and coal combustion[42]. In this study, the ratio of Flt/(Flt + Pyr) ranged from 0.30 to 1.00, Ant/(Ant + Phen) ranged from 0.37 to 1.00; B*a*A/(B*a*A + Chry) ranged from 0.31 to 1.00 and IndP/(IndP + B*ghi*P) > 0.5 indicates that sources of PAHs in these honey samples were mainly due to biomass and fossil fuel combustion.

The ratio of ∑COMB/∑PAHs provides useful information on the degree to which the origins of PAHs are related to combustion of typical organics[43]. ∑COMB is the sum of Flt, Pyr, Chry, B*k*F, B*a*P, B*ghi*P and IndP, while ∑PAH is the sum of the concentrations of the 16 PAHs measured in this study. The ratio of ∑COMB/∑PAH values in this study ranged from 0.19 to 0.94 indicating high fractions of combustion origin in these sites. In addition, the total index[15] was also estimated as the sum of single indices (discussed earlier) normalized for the limit value (low temperature sources-high temperature sources) reported in the literature[42].

Table 5. Excess cancer risk of PAHs in honey samples.

	B*a*A	Chry	B*b*F	B*k*F	B*a*P	IndP	D*ah*A	Excess cancer risk
SE1	8.1×10^{-9}	0.0	9.7×10^{-9}	6.2×10^{-9}	0.0	0.0	8.9×10^{-8}	1.1×10^{-7}
SE2	0.0	3.1×10^{-10}	4.6×10^{-8}	3.7×10^{-9}	8.9×10^{-8}	0.0	0.0	1.4×10^{-7}
SE3	0.0	1.2×10^{-10}	2.0×10^{-9}	0.0	0.0	0.0	0.0	9.1×10^{-9}
SE4	9.0×10^{-9}	1.5×10^{-10}	2.0×10^{-8}	3.6×10^{-9}	8.6×10^{-8}	0.0	0.0	1.2×10^{-7}
SE5	1.2×10^{-8}	0.0	0.0	2.4×10^{-9}	0.0	0.0	0.0	1.5×10^{-8}
SE6	0.0	0.0	2.8×10^{-8}	4.8×10^{-9}	0.0	0.0	1.9×10^{-7}	2.3×10^{-7}
SE7	5.8×10^{-8}	1.5×10^{-11}	3.1×10^{-8}	1.1×10^{-9}	8.3×10^{-8}	0.0	0.0	1.7×10^{-7}
SE8	1.3×10^{-8}	3.0×10^{-10}	2.7×10^{-8}	3.7×10^{-9}	0.0	0.0	0.0	4.4×10^{-8}
SW9	2.5×10^{-8}	0.0	3.8×10^{-8}	4.3×10^{-9}	2.8×10^{-7}	0.0	2.9×10^{-7}	6.3×10^{-7}
SW10	0.0	0.0	0.0	1.7×10^{-9}	0.0	0.0	1.5×10^{-7}	1.5×10^{-7}
SW11	8.5×10^{-9}	8.4×10^{-11}	0.0	1.5×10^{-9}	0.0	0.0	1.3×10^{-7}	1.4×10^{-7}
SW12	3.2×10^{-8}	0.0	0.0	1.0×10^{-9}	0.0	0.0	8.4×10^{-8}	1.2×10^{-7}
SW13	1.2×10^{-7}	6.5×10^{-10}	4.6×10^{-8}	2.1×10^{-9}	2.0×10^{-7}	2.2×10^{-8}	1.4×10^{-6}	1.8×10^{-6}
SW14	0.0	3.4×10^{-10}	0.0	1.4×10^{-9}	0.0	0.0	0.0	1.7×10^{-9}
SW15	0.0	1.0×10^{-10}	0.0	2.3×10^{-9}	1.0×10^{-7}	0.0	9.4×10^{-8}	2.0×10^{-7}
SW16	1.5×10^{-8}	9.3×10^{-11}	1.9×10^{-8}	1.4×10^{-9}	0.0	0.0	0.0	3.5×10^{-8}
SW17	0.0	0.0	0.0	2.7×10^{-9}	0.0	0.0	0.0	2.7×10^{-9}
SW18	8.0×10^{-9}	0.0	2.5×10^{-8}	6.5×10^{-9}	0.0	0.0	2.1×10^{-7}	2.5×10^{-7}
SS19	0.0	2.6×10^{-10}	7.9×10^{-8}	5.1×10^{-9}	9.5×10^{-8}	8.5×10^{-9}	0.0	1.9×10^{-7}
SS20	0.0	3.1×10^{-10}	0.0	2.4×10^{-9}	2.6×10^{-7}	0.0	9.2×10^{-8}	3.6×10^{-7}
SS21	3.6×10^{-8}	2.4×10^{-10}	4.9×10^{-8}	3.0×10^{-9}	7.0×10^{-8}	0.0	0.0	1.6×10^{-7}
SS22	0.0	3.1×10^{-10}	1.1×10^{-8}	2.0×10^{-9}	8.9×10^{-8}	0.0	0.0	1.0×10^{-7}
SS23	8.8×10^{-9}	5.9×10^{-11}	8.2×10^{-9}	0.0	0.0	0.0	0.0	1.7×10^{-8}
SS24	0.0	5.14×10^{-10}	0.0	1.7×10^{-9}	0.0	0.0	0.0	2.2×10^{-9}
SS25	0.0	0.0	1.8×10^{-8}	3.1×10^{-9}	2.0×10^{-8}	0.0	0.0	4.1×10^{-8}
SS26	0.0	1.0×10^{-10}	0.0	2.3×10^{-10}	1.0×10^{-7}	0.0	9.5×10^{-9}	1.1×10^{-7}
SS27	0.0	0.0	6.5×10^{-8}	1.5×10^{-9}	0.0	0.0	0.0	6.6×10^{-8}
SS28	9.2×10^{-9}	0.0	2.5×10^{-9}	3.0×10^{-9}	0.0	0.0	1.8×10^{-7}	1.9×10^{-7}
SS29	1.2×10^{-8}	0.0	0.0	4.2×10^{-9}	0.0	0.0	0.0	1.6×10^{-8}
SS30	2.1×10^{-8}	1.3×10^{-10}	7.8×10^{-9}	2.0×10^{-9}	8.8×10^{-8}	0.0	0.0	1.2×10^{-7}
NC31	0.0	0.0	0.0	8.0×10^{-10}	0.0	0.0	0.0	8.0×10^{-10}
NC32	0.0	0.0	0.0	0.0	9.3×10^{-8}	0.0	0.0	9.3×10^{-8}
NC33	0.0	1.4×10^{-10}	0.0	2.4×10^{-9}	9.0×10^{-8}	0.0	9.2×10^{-8}	1.8×10^{-7}
NC34	0.0	0.0	3.1×10^{-9}	3.0×10^{-9}	1.6×10^{-9}	8.0×10^{-9}	1.3×10^{-7}	1.5×10^{-7}
NC35	0.0	0.0	0.0	8.3×10^{-10}	0.0	0.0	0.0	8.3×10^{-10}
NC36	0.0	0.0	0.0	8.0×10^{-10}	0.0	0.0	0.0	8.0×10^{-10}
NC37	0.0	0.0	8.7×10^{-9}	9.9×10^{-10}	0.0	0.0	0.0	9.6×10^{-9}
NC38	0.0	9.2×10^{-11}	9.5×10^{-9}	1.2×10^{-9}	3.6×10^{-8}	0.0	1.6×10^{-7}	2.1×10^{-7}
NC39	0.0	0.0	8.4×10^{-9}	1.3×10^{-9}	0.0	0.0	1.3×10^{-7}	1.4×10^{-7}
NC40	0.0	9.1×10^{-11}	0.0	1.4×10^{-9}	8.4×10^{-8}	0.0	0.0	8.5×10^{-8}

Benzo[*a*]anthracene (B*a*A), Chrysene (Chry), Benzo[*b*]fluoranthene (B*b*F), Benzo[*k*]fluoranthene (B*k*F), Benzo[*a*]pyrene (B*a*P), Indeno[*1,2,3-cd*]pyrene (IndP), Dibenz[*a,h*]anthracene (D*ah*A); South-East (SE), South-West (SW), Niger Delta (SS), North Central (NC).

$$\text{Total index} = \frac{\text{Ant}/(\text{Ant}+\text{Phen})}{0.1} + \frac{\text{Flt}/(\text{Flt}+\text{Pyr})}{0.4} + \frac{\text{B}a\text{A}/(\text{B}a\text{A}+\text{Chry})}{0.2} + \frac{\text{IndP}/(\text{IndP}+\text{B}ghi\text{P})}{0.5}$$

It should be noted that PAHs associated with high temperature processes (combustion) have a total index that is greater than 4, while PAHs originating from low temperature processes (petroleum products) have a total index that is less than 4. The total index values in our samples ranged from 1.49 to 10.7. Eight samples had total index values less than 4 (low temperature processes) while 20 samples had total index values greater than 4 which confirms that most of the PAHs in the honey samples originated predominantly from combustion processes.

Multivariate Analysis

The results of the principal component analysis of PAHs in honey from the four geographical zones of Nigeria are displayed in Table 7. In the Niger Delta (South-South), three components were extracted. Factor 1 accounts for 23.38% of the variance and is dominated by high loadings in Acy, B*a*A and B*ghi*P, and weak loadings in Flt and Ace. Acy and B*a*A are mark-

Table 6. Diagnostic ratios of PAHs in honey samples.

	LMW/ HMW	Ant/ (Ant + Phe)	B*a*A/ (B*a*A + Chry)	Flt/ (Flt + Pyr)	IndP/ (IndP + B*ghi*P)	B*a*P/ B*ghi*P	CombPAH/ Sum PAH	Total index
SE1	0.40	0.00	1.00	0.00	0.00	0.00	0.57	5.00
SE2	0.20	0.00	0.00	0.00	0.00	0.00	0.52	0.00
SE3	0.00	0.00	0.00	0.00	0.00	0.00	0.89	0.00
SE4	0.64	0.00	0.38	0.00	0.00	0.00	0.47	1.90
SE5	0.51	0.00	1.00	0.00	0.00	0.00	0.66	5.00
SE6	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00
SE7	0.56	0.00	0.98	0.00	0.00	0.00	0.46	4.88
SE8	0.38	0.00	0.31	0.00	0.00	0.00	0.55	1.54
SW9	0.69	0.00	1.00	0.00	0.00	0.92	0.41	5.00
SW10	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.00
SW11	0.31	0.00	0.50	0.00	0.00	0.00	0.54	2.51
SW12	0.60	0.00	1.00	0.00	0.00	0.00	0.52	5.00
SW13	0.25	0.59	0.65	0.31	0.35	0.49	0.52	10.7
SW14	0.17	1.00	0.00	0.00	0.00	0.00	0.86	10.0
SW15	0.40	1.00	0.00	0.00	0.00	1.16	0.63	10.0
SW16	0.33	0.00	0.61	0.87	0.00	0.00	0.68	5.23
SW17	1.61	0.00	0.00	0.00	0.00	0.00	0.38	0.00
SW18	0.00	0.00	1.00	0.00	0.00	0.00	0.61	5.00
SS19	0.00	0.00	0.00	0.00	1.00	0.00	0.55	2.00
SS20	0.00	0.00	0.00	0.00	0.00	2.75	0.94	0.00
SS21	0.21	0.00	0.60	1.00	0.00	0.38	0.60	5.51
SS22	0.34	0.00	0.00	0.00	0.00	0.00	0.63	0.00
SS23	0.60	0.39	0.60	0.74	0.00	0.00	0.54	8.78
SS24	0.19	1.00	0.00	0.00	0.00	0.00	0.84	10.0
SS25	0.41	1.00	0.00	0.00	0.00	0.16	0.55	10.0
SS26	0.76	1.00	0.00	0.00	0.00	106	0.56	10.0
SS27	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00
SS28	0.00	0.00	1.00	0.00	0.00	0.00	0.66	5.00
SS29	0.41	0.00	1.00	0.00	0.00	0.00	0.71	5.00
SS30	0.15	0.00	0.62	1.00	0.00	0.58	0.81	5.59
NC31	1.88	0.00	0.00	0.60	0.00	0.00	0.35	1.49
NC32	0.27	0.41	0.00	0.30	0.00	0.77	0.79	4.83
NC33	0.00	0.00	0.00	0.00	0.00	0.94	0.92	0.00
NC34	0.12	0.37	0.00	0.68	0.29	0.01	0.75	6.03
NC35	1.06	0.00	0.00	1.00	0.00	0.00	0.48	2.50
NC36	0.78	0.00	0.00	0.60	0.00	0.00	0.56	1.49
NC37	0.51	0.00	0.00	1.00	0.00	0.00	0.52	2.50
NC38	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.00
NC39	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.00
NC40	0.62	0.00	0.00	0.00	0.00	0.00	0.63	0.00

Phenanthrene (Phen), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Benzo[*a*]anthracene (B*a*A), Chrysene (Chry), Benzo[*a*]pyrene (B*a*P), Indeno[*1,2,3-cd*]pyrene (IndP), Benzo[*ghi*]perylene (B*ghi*P), South-East (SE), South-West (SW), Niger Delta (SS), North Central (NC).

ers for gasoline vehicle related sources[44-46]. Factor 2, accounting for 19.89% of the total variance, was dominated by high positive loadings in B*b*F, B*k*F and IndP, with high negative bipolar loadings in Phen and Flt. Because B*b*F and IndP are known marker compounds of oil combustion[43,47], this factor may represent oil combustion related sources emanating from the activities of the oil and gas industries in the Niger Delta. Factor 3, accounting for 14.48% of the variance, is dominated by Pyr, Chry and B*a*P. Because Pyr, Flt and B*a*P are typical markers of coal combustion[44,48] they could represent coal combustion related sources.

In the South-East region, three components were extracted accounting for 70.9% of the total variance. Factor 1 accounted for 29.59% and was dominated by B*k*F and D*ah*A with negative loadings in Pyr and B*ghi*P. B*k*F and D*ah*A are representative compounds for diesel emissions[43,49,50]. Factor 2 accounted for 25.25% of the total variance with high positive loadings for Ace, Flu, B*a*A, and B*a*P. Acy and Flu are tracers for wood combustion sources[44].

For the South-West region, three components were extracted accounting for 76.27% of the total variance. Factor 1 constituted 42.80% of the total variance, and

Table 7. PCA analysis of PAHs in honey samples.

	South East			South West			Niger Delta			North Central			
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 4
Nap					.836								
Acy			.605		.695		.816				.585		− .561
Ace		.775			.828		.476	.369			− .800		
Flu	.332	.780	− .383	.824			− .422				− .799		
Phen	.420		− .646			.932		− .692	− .442				
Ant				.637	− .486	− .307	− .454	− .536		.902			
Flt						.935	.490	− .628	− .424	.843		− .300	
Pyr	− .876	− .337		.420	− .355			− .382	.845			.914	
B*a*A		.639		.910			.900						
Chry			.769	.864	− .445				.674	− .518			.468
B*b*F	.355		.795	.745	.382	.426		.671			.740	.618	
B*k*F	.914				.479			.804		.334		− .546	.754
B*a*P		− .784	.410	.707	.569				.743			.926	
IndP				.940				.637		.829			.490
D*ah*A	.533	− .596		.952					.310		.446		.844
B*ghi*P	− .876	− .337		.904	.373		.799			.701	.626		
Variance %	29.59	25.25	16.04	42.80	21.44	12.03	23.28	19.89	14.48	30.45	24.51	19.66	10.80

Napthalene (NaP), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phen), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Benzo[*a*]anthracene (B*a*A), Chrysene (Chry), Benzo[*b*]fluoranthene (B*b*F), Benzo[*k*]fluoranthene (B*k*F), Benzo[*a*]pyrene (B*a*P), Indeno[*1,2,3-cd*]pyrene (IndP), Dibenzo[*a,h*]anthracene (D*ah*A) and Benzo[*ghi*]perylene (B*ghi*P).

was dominated by Flu, Ant, B*a*A, Chry, B*b*F, B*a*P, IndP, D*ah*A and B*ghi*P. Diesel emissions are characterized by B*a*A, Chry, B*b*F, IndP, and D*ah*A[43,44,49-51]. B*ghi*P and B*a*P have been identified as tracers for automobile emissions because these compounds were found to be enriched in traffic tunnels[47,52-54]. Factor 2 accounts for 21.44% of the total variance, with high loadings in Nap, Acy, Ace and B*a*P. Acy, Ace and Phen are characteristic of fossil fuel/biomass combustion[45,49,55,56]. Factor 3 has high loadings in Phen and Flt.

The PCA factor loadings for the North Central region indicate that four components were extracted accounting for 85.42% of the total variance. Factor 1 has high loadings in Ant, Flt, IndP and B*ghi*P. Flt, IndP, and B*ghi*P are typical markers for coke production[52,53], while Ant is a product of wood combustion. This factor probably represents sources from coal, straw and wood combustion. Factor 2 has high positive loadings in Acy, B*b*F, and B*ghi*P with high negative bipolar loadings in Ace and Flu. B*b*F and B*ghi*P are markers for gasoline combustion, while Acy is a product of wood combustion. This factor represents a mixture derived from gasoline and wood combustion. Factor 3 accounts for 19.66% of the total variance with high loadings in Pyr, B*b*F and B*a*P. Factor 3 is related to exhaust emissions from stationary sources[44,57,58]. Factor 4 has high positive loadings in B*k*F and D*ah*A, and weak positive loadings in Chry and IndP. B*k*F and D*ah*A are tracers of diesel emissions[43,49,50].

Conclusions

The regional distribution patterns of ∑16 PAHs in these honeys followed the order: South-West > South-East > Niger Delta > North Central. The 5-ringed PAHs were the dominant PAH compounds in these honey samples. The incremental life cancer risk values for the majority of the samples were above the acceptable risk level of one in a million chance of additional cancer over a 52 year lifetime ($1/10^6$), while the margin of exposure values, based on the EFSA suggested indicators for occurrence and effects of PAHs in food, were greater than 10000. This indicated there is no additional risk associated with the consumption of these honeys based on the current ingestion rate, however, a considerable risk may arise with excessive consumption of these products. The PAH isomeric ratios and principal component analysis indicated that combustion of fossil fuels, natural gas and biomass, and automobile emissions were the main sources of PAHs in these honey samples from the different regions of Nigeria. This study provided useful information for environmental and food quality management in Nigeria.

Materials and Methods

Samples and Sample Collection

The sampling procedures have been previously de-

scribed by Iwegbue *et al*.[59] Briefly, a total of 40 samples of honey, consisting of raw samples from beehives and bottled samples from vendors, were collected from different locations across Nigeria. The honey samples were categorized into four geographical regions: (i) south-west region (latitude 6° N to 9° N; longitude 2° E to 5° E), (ii) Niger Delta (latitude 2° N to 8° N; longitude 5° E to 9° E), (iii) south-east region (latitude 5° N to 7° N; longitude 6° E to 8° E), and (iv) north central region (9° N to 14° N; longitude 2° E to 12° E). The major activities in these regions are agriculture, urbanization and industrial development. The south-western and south-eastern regions have higher concentrations of manufacturing industries than the northern region where there is predominantly mining and large-scale farming. The Niger Delta region, and some parts of the eastern region, house the crude oil production facilities with their associated multiple gas flaring units. Within a given location in a region at least 2 to 4 samples were collected and mixed together. Most vendors claimed that the honey samples collected were sourced from the wild except for 5 samples collected from bee farms in different locations. The bottled samples were labelled "undiluted pure natural honey" although the practice of enhancing honey with sugar syrup cannot be completely ruled out[59].

Reagents

All chemicals and reagents used were of analytical grade. Acetone and *n*-hexane were purchased from Rieldel-de Haën (Seelze, Germany, with a purity of 99.8 %) while dichloromethane (LC grade), anhydrous sodium sulfate (purity 99%), alumina and silica gel were purchased from BDH Chemicals (Poole, UK). A PAH standard mixture containing the US EPA 16 priority PAHs was purchased from Supelco (Bellefonte, PA, USA). Working mixed standard solutions containing all the PAHs were prepared by dilution of the stock solution with acetone and stored at −20°C in the dark to avoid volatilization and photodegradation.

Sample Preparation, Extraction and Clean-up

A mass of 5.0 g of the honey sample was mixed with the same amount of anhydrous Na_2SO_4, until the mixture became free-flowing. A 30 mL aliquot of hexane/dichloromethane (1 : 1 v/v) was added to the resulting material and placed in an ultra-sonic bath, and sonicated at 30°C for 30 minutes. The organic extract was filtered and the process was repeated three times by sonication of the residue with a fresh mixture of hexane/dichloromethane each time as described above. The extracts for each sample were combined and reduced to 1 mL by using a rotary evaporator, and subsequently cleaned-up by solid phase extraction with 2 g of aluminum oxide. The PAHs were eluted with 15 mL of hexane, 15 mL hexane and dichloromethane (9 : 1 v/v) and 20 mL of hexane and dichloromethane (4 : 1 v/v). The eluted fractions were combined and evaporated to approximately 0.5 mL with a gentle stream of nitrogen.

Chemical Analysis

The PAHs in the eluted fractions were measured with a gas chromatograph (HP 6890 Palo Alto, CA) equipped with a J&W Durabond 5 (cross-linked phenyl methyl siloxane) column (0.25 μm film thickness, 0.25 μm × 30 m) and a HP 5973 series mass-selective detector. The mass spectrometer was operated in the electron impact ionization mode (ionizing energy of 70 eV) scanning from m/z 50 to 450 at 3.6 scans/s. The ion source and quadrupole temperatures were 230°C and 150°C respectively. The operating conditions were as follows: the injection port and the GC/MS interface temperatures were 290°C and 250°C respectively. 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°C to 280°C at 5°C/min, and held at 280°C for 18 min; the solvent delay was 6 min. The injection volume was 2 μL in pulsed splitless mode and the carrier gas was helium with a linear velocity of 1 mL/min.

Quality Control/Quality Assurance and Statistical Analysis

The quantification was carried out by the use of external calibrations which were obtained with PAH solutions at five concentration levels. To evaluate the extraction efficiency for the target compounds, known concentrations of standard PAH mixtures were added to fresh portions of already analyzed samples at three concentration levels and all analysis steps from extraction to chromatographic analysis were repeated. The recoveries for the PAH compounds were in the range of 66 to 103%. The relative standard deviations for replicate analyses (n = 3) were less than 6%. The r^2 values for the calibration lines for the PAH compounds ranged from 0.9994 to 0.9999 while the limits of detection and quantification for the PAH compounds ranged from 0.03 to 0.2 μg kg^{-1} and from 0.1 to 0.7 μg kg^{-1} respectively. The average inter-day and intra-day precision ranged between 1.8 and 6.9%. The performance characteristics of the present method meet the criteria specified in European Commission Regulation 836/2011 (recovery between 50 and 120%)[60]. Analysis of variance (ANOVA) and Tukey multiple-comparison tests were used to determine whether the concentrations of the PAHs varied significantly within and between the regions respectively. Differences with p values less than 0.05 ($p < 0.05$) were considered to be statistically significant. The statistical calculations were performed

with SPSS version 20.5.

Estimation of Dietary Intakes and Risk Assessment

The estimated daily intake (EDI) of PAHs from the consumption of these honey samples was evaluated by using the formula:

$$\text{EDI}\ (\mu g\ kg^{-1}\ bw\ day^{-1}) = \frac{MI \times C_{PAH}}{BW} \quad (1)$$

where MI is the mass of product ingested per day. In this study, an ingestion rate of 1.4 g/day was used based on the per capita consumption of 0.5 kg per annum per person. C_{PAH} is the concentration of the various indicators for the occurrence and effects of PAHs in foods such as B*a*P, PAH2, PAH4 and PAH8[28].

Margin of Exposure

The margin of exposure (MOE) approach was adopted to assess the risk of PAHs in these honey samples by using the various indicators of occurrence and effects of PAHs in foods (B*a*P, PAH2, PAH4 and PAH8)[28]. 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 to the estimated average daily intake of the compound by humans[61].

$$MOE = \frac{BMDL_{10} \times 10^6}{EDI} \quad (2)$$

The Benchmark Dose Lower Limit ($BMDL_{10}$) is the reference point that was derived from mathematical modelling of experimental tumour data within the observed range. The $BMDL_{10}$ values for B*a*P, PAH2, PAH4 and PAH8 are 0.07, 0.17, 0.34 and 0.49 mg kg^{-1} bw day^{-1}, respectively[28].

Toxic Equivalency Factor

PAHs occur as mixtures and the risk to human health from various PAH exposures can be established by the toxicity or carcinogenic potency of the individual PAH compound relative to B*a*P. The risks of PAHs in soil, dust and foods have been assessed using the B*a*P toxic equivalent factor (BaP_{TEQ}) and the B*a*P mutagenic equivalent factor (BaP_{MEQ})[62-67].

The B*a*P carcinogenic equivalent (BaP_{TEQ}) for the individual PAHs is given by the formula:

$$BaP_{TEQ} = \sum C_i \times BaP_{TEF} \quad (3)$$

where BaP_{TEF} is the cancer potency relative to B*a*P and C_i is the individual PAH concentration.

The B*a*P mutagenic equivalent (BaP_{MEQ}) for the individual PAHs is given by the equation:

$$BaP_{MEQ} = \sum C_i \times BaP_{MEF} \quad (4)$$

where BaP_{MEF} is the mutagenic potency relative to B*a*P and C_i is the individual PAH concentration. The B*a*P carcinogenic equivalency factors (BaP_{TEFs}) of the seven carcinogenic PAHs used were: B*a*P (1), B*a*A (0.1), B*b*F (0.1), B*k*F (0.01), Chry (0.001), D*ah*A (1) and IndP (0.1)[64]. The B*a*P mutagenic potency factors (BaP_{MEFs}) were B*a*P (1), B*a*A (0.082), B*b*F (0.25), B*k*F (0.11), Chry (0.017), D*ah*A (0.29) and IndP (0.31)[66].

Estimation of Excess Cancer Risk

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

$$\text{Excess cancer risk} = \frac{EI \times ED \times CSF}{BW \times AT} \times 10^{-6} \quad (5)$$

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^{-1} d^{-1}), BW is the human body weight (assuming 60 kg weight), AT is the averaging time for carcinogens in years (assuming 52 years for the average Nigerian) and 10^{-6} is the conversion factor. The CSF data for individual PAHs are B*a*A = 0.73, Chry = 0.0073, B*b*F = 0.73, B*k*F = 0.073, B*a*P = 7.3, IndP = 0.73 and D*ah*A = 7.3[64].

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