Bull. Environ. Contam. Toxicol. (2006) 77:492–499
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DOI: 10.1007/s00128-006-1091-y

Bulletin of Environmental Contamination and Toxicology

Polycyclic Aromatic Hydrocarbons in Fruits and Vegetables Grown in the Czech Republic

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Received: 21 December 2005/Accepted: 14 August 2006

Polycyclic aromatic hydrocarbons (PAHs) represent an important group of environmental pollutants with carcinogenic / mutagenic potential of some representatives. The occurrence of these hazardous compounds in foodstuffs is of a health concern. The risk associated with dietary PAHs is assessed also by European Food Safety Authority, EFSA (WPPC 2004; Food law 2004).

Food crops can be contaminated by environmental PAHs occurring in atmosphere, soil and water; in addition to these sources, contamination by PAHs may results from some processing / cooking practices such as smoking, grilling, roasting etc. Considering consumers exposure, oils and fats, cereals, some vegetables and fruits were identified as major sources of PAHs in diet (Dennis et al. 1983; De Vos et al. 1990; COT 2002). The main route of food crops contamination is direct deposition of PAHs from atmosphere. Heavier PAHs preferentially associate with particulate matter, PAHs with 2 or 3 rings are predominantly present in the vapor phase and 4-ring PAHs being distributed among both phases. In any case, vegetables with large leafs are more susceptible to contamination by environmental PAHs. Depending on a surface morphology, either particles deposition and/or adsorption of low molecular mass PAHs by surface waxes occurs. The uptake of PAHs by plant roots from soil is rather limited, since these hydrophobic aromatic compounds adsorb strongly to the organic fraction of soil particles and hence their leaching into aqueous phase is very small. Moreover the translocation of potentially adsorbed PAHs from outer parts into internal parts of vegetation is also limited, thus in case of root vegetables, PAHs concentration is generally higher on plant surface than in internal tissues (Wild et al. 1992). Compared to smoked or grilled foods, levels of PAHs in fruits and vegetables are low, nevertheless the high consumption rate of these products makes them a significant dietary source (IARC 1983; Lawrence et al. 1984; Jones at al. 1989). It should be also noted, that fruits and vegetables are often grown in close proximity to urban pollution sources, hence PAHs levels might be slightly higher than in other plant crops grown in rural areas with background pollution level.

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The main objective of present study was (*i*) to determine levels of PAHs and related compounds (methylderivatives and sulfur containing heterocycles) in fruits and vegetables grown in the southern Moravia (Czech Republic) representing an important production area of these crops and (*ii*) to estimate dietary daily intake of PAHs via fruit and vegetables.

MATERIALS AND METHODS

Apple, apricot, grape, cauliflower, parsley, cabbage, cucumber and tomato samples were collected from nineteen different localities in southern Moravia (Czech Republic), in July – October 2003.

The standard mixture 1647d of 16 priority PAHs – naphthalene (Naph), acenaphthene (Ace), fluorene (Fln), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (B[*a*]A), chrysene (Chr), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), dibenz[*a,h*]anthracene (DB[*ah*]A), benzo[*g,h,i*]perylene (B[*ghi*]P) and indeno[1,2,3-*cd*]pyrene (I[*cd*]P) dissolved in acetonitrile was supplied by National Institute of Standards and Technology (NIST, USA). Standards of individual PAH derivatives – 1-methylnaphthalene (1-MeNaph), dibenzothiophene (DBT), 2-methylanthracene (2-MeAnt), 1-methylpyrene (1-MePyr), 5-methylchrysene (5-MeChr), benzo[*b*]naphtho[2,1-*d*]thiophene (B[*b*]N[*d*]T), benzo[*e*]pyrene (B[*e*]P) and 1-methylchrysene (1-MeChr) dissolved in acetonitrile (10 mg/ml) were supplied by Dr. Ehrenstorfer (Germany). Purity of individual standards was not less than 95%. Working standard solutions were prepared in acetonitrile and stored in refrigerator at 4°C.

Before homogenization non-edible parts of fruits and vegetables (stems, wrapper leaves, stones) were removed. Parsley samples were washed with fresh running water to remove surface soil.

Accredited analytical procedure (EN ISO / IEC 17025) described below was employed for examination of fruit and vegetable samples

20g of homogenized sample mixed with 80 g of anhydrous sodium sulphate was extracted in the Soxhlet extractor by 170 ml of hexane-acetone mixture (1:1, *v/v*) for 6 hours (10 cycles/hour). Extraction thimbles preextracted for 2 hours with an extraction solvent were used to obtain lower PAHs procedure blank. The extraction solvent was then carefully evaporated by rotatory vacuum evaporation at 40°C just to dryness. Residue after evaporation was quantitatively transferred into a 10-ml volumetric flask by chloroform.

The clean-up procedure was carried out by gel permeation chromatography employing Bio-Beads S-X3 (500 × 8 mm i.d. stainless steel column, mobile phase chloroform, flow rate 0.6 ml/min; injected volume 2.5 ml). The purified extracts were evaporated by rotatory vacuum evaporation at 40°C just to dryness. The

residue obtained after evaporation of chloroform was dissolved in 0.5ml of acetonitrile before HPLC/FLD determinative step.

A high performance liquid chromatographic system with fluorescence detector (Hewlett-Packard 1100 Series) was used for extracts analysis. Separation of sample components was carried out on a SUPELCOSIL^{TM} LC-PAH (250mm × 4.6mm i.d.) column with the guard column SUPELCOSIL^{TM} LC-18 (20mm × 4.0mm i.d.), (SUPELCO, USA), under the following chromatographic conditions: gradient elution (A – acetonitrile, B – water; 0 min – 55% A, 40 min – 100% A, 42 min – 100% A), mobile phase flow rate 1ml/min, injection volume 20 ml, column temperature 35°C. The external standard calibration method based on peak heights was used for quantitation of PAHs.

Procedure blank samples were handled together in the same way as real samples. The values of PAHs determined in blanks were subtracted from obtained results.

Dry matter content was determined by drying at 105°C for 24 hour (until constant weight).

RESULTS AND DISCUSSION

In the first part of study, relevant analytical procedure had to be implemented. In order to verify its accuracy and precision, recovery and repeatability experiments were carried out. Since fruit / vegetable matrices with certified concentrations of PAHs (CRM) are commercially not available, spiked samples were analysed within validation study. Apple was chosen as a representative fruit matrix for this purpose. To cover the influence of concentration, our approach included spiking at four different levels (50, 100, 150 and 200% of "natural" PAHs content in apple). In case of PAHs with nature content lower than limit of detection (LOD), the spiking levels were calculated as 50, 100, 150 and 200% of limit of quantitation (LOQ). The spiking solution was carefully prepared by mixing of individual PAH solutions to correspond as much as possible to typical PAHs pattern. To reduce a recovery information error, which can be generally caused by the different extractability of naturally incurred and intentionally added target analytes, the spike (in acetone solution) in a sample was incubated for 16 hours. Recovery was calculated as a slope of a dependence of determined PAHs concentration and theoretical concentration (calculated as a sum of nature content and spike). Recovery for most of analytes was satisfactory and ranged within 70 – 118%. The lower recoveries (10–64%) were found for the most volatile PAHs, represented by Naph, 1-MeNaph, 2-MeNaph, Ace and Fln. The repeatability of the procedure, expressed as a relative standard deviation (RSD, %) of repeated analyses (n = 6) of apple samples, ranged from 7–30%. The worse repeatability was obtained particularly for minor PAHs, levels of which in tested samples were close to a limit of quantitation. The overview of selected performance characteristics is shown in the Table 1.

The concentrations of all the target PAHs determined in examined samples are summarized in Table 2. The relative content of individual PAH groups (see Table 3) is shown in Figure 1. As documented here, in all investigated matrices, 3- and 4-ring PAHs constituted the major part of all PAHs; Phe and 1-MePhe were present at the highest concentration (units of μg/kg). 5- and 6-ring PAHs form less than 10% of the total PAHs content. Relative proportion of individual PAH groups corresponds to the different morphology and plant surface structure and thus different PAHs transfer mechanism from ambient environment into the plant. 3-ring PAHs were dominating in cucumber, tomato and apple, i.e. plants with smooth waxy surface. While trapping of atmospheric particulate matter bearing adsorbed higher molecular mass PAH is not facilitated, surface waxes may concentrate low molecular mass PAH mainly through absorption. In case of cauliflower, cabbage and grapes, large and ragged surface enables trapping of particulate matter what results in increased contribution of 4-ring PAHs. The higher content of 3-ring PAHs as compared to 4-, 5- and 6-ring PAHs in parsley (root) is probably caused by worse penetration of these PAHs occurring in soil since stronger adsorption to the organic soil fraction and/or rinsing off soil particles rich mainly in the heavier PAHs.

Table 1. Recovery, repeatability and limits of detection (LOD) of PAHs in apple samples.

PAU	Recovery (%)	Repeatability (%)	LOD (μg/kg)
Naph	35	30	0.01
1-MeNaph	10	19	0.02
2-MeNaph	12	13	0.02
Ace	47	10	0.07
Fln	64	11	0.01
DBT*	100	n/a*	0.02
Phe	81	10	0.01
Ant	95	19	0.01
Flt	115	15	0.01
1-MePhe	98	7	0.02
Pyr	95	8	0.01
2-MeAnt	88	18	0.01
1-MePyr	92	21	0.01
B[*a*]A	117	24	0.01
Chr	109	29	0.01
5-MeChr	91	14	0.01
B[*b*]N[*d*]T	73	24	0.02
B[*e*]P	86	27	0.02
B[*b*]F	82	26	0.01
1-MeChr	97	24	0.02
B[*k*]F	91	30	0.01
B[*a*]P	94	22	0.01
DB[*ah*]A	74	30	0.01
B[*ghi*]P	93	19	0.01
I[1,2,3-*cd*]P	70	17	0.01

** DBT concentration in the tested sample was less than LOD.*

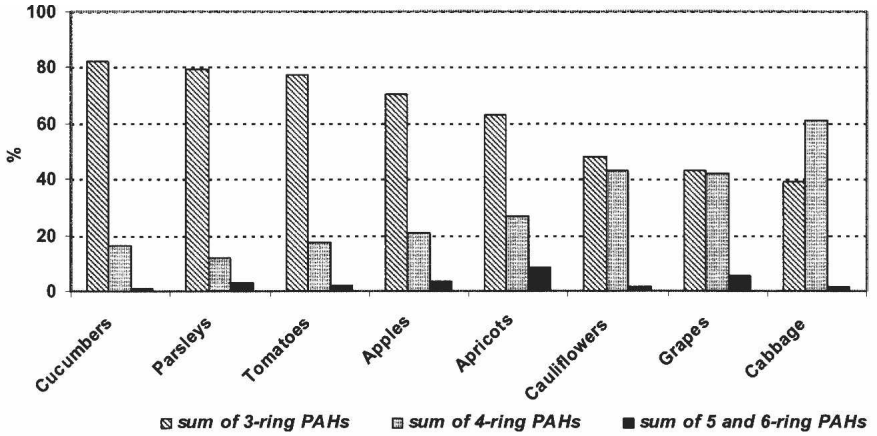

Figure 1. Relative proportion of PAH groups in fruits and vegetables.

Table 2. PAHs concentration[a] (μg/kg fresh weight) in fruits and vegetables grown in the southern Moravia in the Czech Republic.

	Cucumber, (n = 4)	Parsley, (n = 4)	Tomato, (n = 5)	Apple, (n = 3)
Dry matter content (%)	3.8	20.5	6.0	13.2
Naph	<LOD-0.70; 0.36	1.25-2.38; 1.85	<LOD-0.46; 0.31	0.58-1.01; 0.92
1-MeNaph	<LOD-0.67; 0.36	1.36-2.06; 1.61	<LOD-0.43; 0.28	0.03- 0.49; 0.35
2-MeNaph	0.21-1.40; 0.96	2.71-4.03; 3.11	0.24-1.12; 0.87	0.74- 1.57; 1.40
Ace	0.07-0.45; 0.20	0.66-3.64; 0.81	<LOD-0.16; 0.07	0.17-0.31; 0.27
Fln	0.49-1.18; 0.85	1.28-1.79; 1.31	0.79-1.58; 1.07	0.82-1.37; 1.23
DBT	<LOD; -	<LOD; -	<LOD; -	<LOD; -
Phe	1.94-4.29; 3.13	3.77-5.55; 3.97	3.08-10.64; 4.38	3.10-5.33; 4.69
Ant	0.07-0.14; 0.12	0.12-0.23; 0.15	0.11-1.29; 0.23	0.14-0.20; 0.17
Flt	0.21-0.48; 0.41	0.23-0.78; 0.39	0.29-1.12; 0.55	0.29-0.83; 0.68
1-MePhe	2.27-4.38; 3.32	4.41-5.33; 4.97	1.75-3.37; 3.20	3.85-7.04; 4.04
Pyr	0.16-0.31; 0.27	0.18-0.45; 0.28	0.26-0.89; 0.39	0.25-0.57; 0.38
2-MeAnt	0.01-0.03; 0.02	0.02-0.05; 0.03	0.03-0.08; 0.04	0.02-0.04; 0.04
1-MePyr	0.01-0.03; 0.02	<LOD; 0.01	0.02-0.04; 0.02	0.01- 0.02; 0.02
B[*a*]A	<LOD; 0.01	<LOD-0.05; 0.02	0.02-0.06; 0.03	0.01- 0.09; 0.04
Chr	0.02-0.04; 0.03	0.03-0.17; 0.05	0.05-0.12; 0.07	0.03-0.13; 0.09
5-MeChr	<LOD; -	<LOD-0.03; 0.01	< LOD; 0.01	<LOD; -
B[*b*]N[*d*]T	<LOD-0.03; 0.02	<LOD; -	0.07-0.13; 0.12	<LOD-0.10; 0.07
B[*e*]P	<LOD-0.03; 0.02	0.02-0.08; 0.03	0.05-0.07; 0.05	<LOD-0.07; 0.03
B[*b*]F	0.01-0.03; 0.02	0.05-0.16; 0.07	0.03-0.07; 0.04	0.02-0.13;0.05
1-MeChr	<LOD; -	<LOD; -	<LOD; -	<LOD; -
B[*k*]F	<LOD; 0.01	0.01-0.04; 0.02	0.01-0.03; 0.02	0.01-0.06; 0.03
B[*a*]P	0.01-0.04; 0.01	0.02-0.05; 0.03	0.02-0.05; 0.03	0.02- 0.10; 0.03
DB[ah]A	<LOD; -	<LOD; -	<LOD; -	<LOD; -
B[*ghi*]P	<LOD; 0.01	0.02-0.05; 0.03	0.02-0.06; 0.03	0.02-0.08; 0.03
I[1,2,3-*cd*]P	<LOD; -	0.01-0.04; 0.02	< LOD-0.02; < LOD	0.01-0.06; 0.04
Σ PAH[b]	5.52-13.95; 10.29	16.58-26.07; 19.00	9.86-17.98; 12.30	13.22-15.64; 15.42
Σ 15 PAH[c]	3.00-7.56; 5.48	8.00-14.58; 9.22	5.29-15.75; 7.53	5.48-9.64; 9.30
Σ carc.[d]	0.06-0.13; 0.09	0.17-0.56; 0.23	0.18-0.40; 0.20	0.14-0.66; 0.32

Table 2. *Continued.*

	Apricot, (n = 5)	Cauliflower, (n = 3)	Grape, (n = 3)	Cabbage, (n = 3)
Dry matter content (%)	12.5	8.1	18.1	8.5
Naph	<LOD-0.16; 0.13	0.57-1.01; 0.63	<LOD-0.37; <LOD	0.12-1.40; 1.06
1-MeNaph	<LOD-0.43; 0.02	0.23-0.62; 0.31	<LOD-0.28; <LOD	0.33-44.36; 0.78
2-MeNaph	<LOD-0.82; 0.53	1.65-2.81; 1.72	<LOD-0.88; 0.11	1.28-2.34; 1.47
Ace	<LOD-0.29; 0.20	0.07-0.37; 0.29	<LOD-0.28;<LOD	0.27-19.10; 0.87
Fln	0.23-1.45; 0.59	0.85-1.28; 1.12	0.12-0.67; 0.33	0.94-1.52; 1.27
DBT	<LOD; -	<LOD; -	<LOD; -	<LOD; -
Phe	2.16-6.09; 2.37	2.92-3.65; 3.47	1.59-2.78; 2.76	1.44-3.71; 2.56
Ant	0.09-0.55; 0.11	0.10-0.19; 0.14	0.08-0.18; 0.14	0.15-0.31; 0.24
Flt	0.23-3.20; 0.58	0.29-2.67; 1.33	0.92-2.16; 1.18	1.26-4.04; 3.84
1-MePhe	1.00-3.08; 2.59	3.34-3.85; 3.58	1.02-3.02; 1.74	1.37-6.67; 2.62
Pyr	0.13-2.15; 0.36	0.22-2.16; 1.36	0.47-1.43; 0.79	0.24-1.04; 0.28
2-MeAnt	<LOD-0.03; 0.01	0.02-0.02; 0.02	0.03-0.07; 0.04	0.04-0.43; 0.29
1-MePyr	<LOD-0.05; 0.01	<LOD-0.05; 0.01	0.02-0.09; 0.04	<LOD-0.09; 0.04
B[*a*]A	<LOD-0.56; 0.04	<LOD-0.33; 0.03	0.06-0.25; 0.08	0.04-0.34; 0.17
Chr	0.03-0.92; 0.09	0.02-0.34; 0.03	0.17-0.49; 0.27	0.03-0.26; 0.04
5-MeChr	< LOD; -	<LOD-0.02; 0.01	<LOD; -	<LOD-0.13; 0.02
B[*b*]N[*d*]T	<LOD -0.31; <LOD	<LOD-0.10; 0.09	0.06-0.27; 0.11	0.05-0.44; 0.05
B[*e*]P	0.05-0.51; 0.08	<LOD-0.22; 0.03	0.04-0.11; 0.07	0.04-0.28; 0.06
B[*b*]F	0.04-0.80; 0.09	0.02-0.38; 0.02	0.10-0.17; 0.10	0.02-0.35; 0.04
1-MeChr	<LOD; -	<LOD; -	<LOD; -	<LOD; -
B[*k*]F	0.01-0.39; 0.03	0.01-0.17; 0.01	0.05-0.09; 0.05	0.01-0.02; 0.01
B[*a*]P	0.03-0.72; 0.09	0.01-0.33; 0.05	0.05-0.13; 0.09	0.02-0.09; 0.03
DB[ah]A	<LOD-0.07; <LOD	<LOD-0.03; <LOD	<LOD; -	<LOD-0.06; <LOD
B[*ghi*]P	0.03-0.46; 0.08	0.01-0.21; 0.02	0.04-0.09; 0.04	0.02-0.02; 0.02
I[1,2,3-*cd*]P	0.01-0.45; 0.04	0.01-0.20; 0.01	0.04-0.07; 0.04	0.01-0.05; 0.02
Σ PAH[b]	6.99-14.43; 7.82	10.52-19.07; 16.06	4.92-11.27; 10.61	12.34-78.09; 20.09
Σ 15 PAH[c]	3.91-12.39; 4.57	5.19-12.87; 8.85	3.75-8.18; 6.82	9.03-25.14; 13.14
Σ carc.[d]	0.17-4.36; 0.45	0.12-1.99; 0.13	0.55-1.30; 0.63	0.28-1.09; 0.29

[a] min-max; median; [b] sum of all target compounds; [c] sum of 16 EPA priority pollutant PAHs (except acenaphthylene); [d] Sum of B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P

- all values < LOD, median not calculated

Table 3. PAH groups according to ring number

Number of rings	PAH
2,3	Naph,1-MeNaph, 2-MeNaph, Ace, Fln, DBT, Phe, Ant, 1-MePhe, 2-MeAnt
4	Flt, Pyr, B[*a*]A, Chr, 1-MePyr, 5-MeChr, B[*b*]N[*d*]T, 1-MeChr
5,6	B[*e*]P, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P, I[1,2,3-*cd*]P

Very limited information on typical PAH levels in fruits and vegetables has been reported in literature. Nevertheless, the PAHs concentrations (average) found in the Italian study (Lodovici et al. 1995) in tomato (0.64 μg/kg) and cauliflower

(2.79 μg/kg) samples were comparable to our data (Table 2). Similar results were also reported in study, published by Camargo (2003); the PAHs content (mean) in tomato, apple and grape were 14.62, 4.05 and 3.87 μg/kg, respectively. The only available relevant information available in the Czech Republic are results of long-term monitoring study conducted in 22 localities during the years 1995 – 2000. (Volka et al. 2002). PAH levels in apples were in the range 2.17–14.55 μg/kg (10% and 90% percentile) with median value 4.66 μg/kg. The highest levels of PAHs, when comparing with other plant matrices involved in the above mentioned monitoring study, occurred in moss followed by needles (order of magnitude 10 – 1000 μg/kg dry weight and 10 – 100 μg/kg dry weight, respectively). In another study (Ledererová, 1998) focused on PAHs contamination levels in apples in the region Teplice (Czech Republic), similar levels (3.07 – 5.56 μg/kg) were found.
Based on the consumption data for fruits and vegetables available in the Czech Republic (report of Czech Statistical Office, 2003) the dietary intake of PAHs was estimated. Calculated values for B[*a*]P employed in most monitoring studies as the marker compound representing carcinogenic PAHs is shown in Table 4 together with complementary data. In spite of relatively low PAH levels, the highest contribution to the dietary intake was found for apples, since relatively high consumption (23.8 kg); the exposure due to consumption of some vegetable as parsley and cabbage is negligible.

The study by Vousta et al. (1998) considered rather different set of matrices (cabbage, carrot, leek, lettuce and endive), nevertheless the value of estimated daily intake for B[*a*]P were of the same order of magnitude, i.e. tenth - units ng/day.

Table 4. Estimated dietary daily intake of PAHs via fruits and vegetables in the Czech Republic (ng/day).

PAH	Apple	Apricot	Cauliflower	Cabbage	Cucumber	Grape	Parsley	Tomato
Consumption [a]	23.8	2.1	3.2	0.7	5.5	3.3	1.0	12.6
B[*a*]P	2.3	0.5	0.4	0.1	0.5[d]	0.8	0.1	1.0
∑carc.[b]	20.6	2.6	1.2	0.6	1.3	5.7	0.6	7.0
∑12 PAH[c]	448.6	22.8	56.4	13.6	60.6	49.7	14.6	205.8

[a] Consumption – kg, annual per capita averages
[b] Sum of B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P
[c] Sum of Phe, Ant, Flt, Pyr, B[*a*]A, Chr, B[*b*]F, B[*k*]F, B[*a*]P, DB[*ah*]A, B[*ghi*]P and I[1,2,3-*cd*]P
[d] For calculation the limit of quantitation (LOQ) was used.

Acknowledgments. This work was supported by Ministry of Education, Youth and Sports of The Czech Republic within projects MSM 6046137305 and Inco Copernicus IC 15-CT98-0339 (OK 377).

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