Polycyclic aromatic hydrocarbons in porcine and bovine organs and tissues

M. Ciganek, J. Neca

Veterinary Research Institute, Brno, Czech Republic

ABSTRACT: Concentrations of 16 polycyclic aromatic hydrocarbons (PAH) were determined in porcine and bovine kidney, liver, lung, muscle and adipose tissue samples, and in eyeballs (lens and vitreous humour) in fattener pigs and cows. The total average PAH concentrations in individual organs were: 5.4, 6.3 (kidney); 3.8, 2.7 (liver); 4.6, 5.4 (lung); 3.6, 5.1 (muscle tissue); 0.05, 0.11 (adipose tissue); 57.9, 16.3 (lens) and 14, 6.4 (vitreous humour) for pigs and cows in ng/g of wet weight, respectively. Phenanthrene, naphthalene, pyrene and fluoranthene were predominant PAH present in samples. No significant differences ($P > 0.05$) were found among distribution of PAH in animal bodies from several localities with various PAH exposure or between their levels in porcine and bovine organs and tissues, except for eyeballs. On the contrary, significant variations of PAH concentrations ($P < 0.01$) were found between species in the same tissues from the same stable. The highest total concentrations of PAH were found in porcine and bovine lenses. Analyses of porcine and/or bovine lenses for PAH content could be used for determination of animal exposure to these compounds.

Keywords: pig; cattle; PAH; organs; tissues

Polycyclic aromatic hydrocarbons are important widespread environmental pollutants, which are formed and released into environment through natural and anthropogenic sources. They are toxic; some of them carcinogenic, persistent and bioaccumulative compounds (WHO, 1998). High PAH concentrations have been also detected in different compartments of agricultural environment, although data on PAH concentrations, distribution and fate are still only random (Von Lusky et al., 1992; Berset and Holzer, 1995; Martens et al., 1997; Ciganek et al., 1999, 2000; Raszyk et al., 1999). Sufficient information about PAH concentration in animal organs and tissues have not been obtained to the present day, despite the use of some animal organs and tissues as food or foodstuffs.

The major routes of exposure of population to PAH are from food and inhaled air. The whole body distribution of PAH has been studied extensively only in rodents. These animals were tested after oral ingestion (Vanschooten et al., 1997), intravenous dosing (Moir et al., 1998; Yuan et al., 1999) or inhalation (Jacob and Grimmer, 1996; Gerde et al., 1998; Fouchecourt et al., 1999) of pure compounds, tar pitch aerosol or contaminated soil.

The concentrations found in individual tissues depend on a number of factors, including the PAH type, the route of administration, the times after treatment at which tissues are assayed, and the presence or absence of inducers or inhibitors of hydrocarbon metabolism within the organism. The investigations have shown that (i) detectable levels of PAH occur in almost all internal organs, (ii) organs rich in adipose tissue can serve as storage depots from which the compounds are gradually released, and (iii) the gastrointestinal tract contains high levels of hydrocarbons and metabolites, even when PAH are administered by other routes.

Pharmacokinetic studies in rat (Withey et al., 1991; Jacob and Grimmer, 1996; Moir et al., 1998) and human (Jacob and Grimmer, 1996) have shown that metabolites of PAH with 2 and 3 rings are preferentially excreted in urine, while higher-molecular-weight PAH are released by faeces, and

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excretion is practically terminated three days after application. It was found that there is a relationship between concentrations of parent PAH and their metabolites in the animal body and the excreted amounts. Similar results were found in lactating goats (Grova et al., 2002) and in growing pigs (Laurent et al., 2002) after an oral administration of ^{14}C-phenanthrene, ^{14}C-pyrene and ^{14}C-benzo[a]pyrene. Detailed studies of the metabolism and excretion of PAH are limited to only to few compounds.

Very little is known about the retention and turnover of PAH in other mammalian species. It can be deduced, from the few data available on hydrocarbon body burdens, that PAH are rapidly metabolised and excreted from the body, and therefore PAH themselves do not persist for long time periods. During metabolism, PAH moieties can become covalently bound to tissue constituents such as proteins and nucleic acids (Routledge et al., 2001). Protein-bound metabolites are likely to persist, therefore, for periods that do not exceed the normal lifetime of the protein itself. Long-term exposure data on PAH distribution in the pigs and cows are rare. Only Von Lusky et al. (1992) have determined benzo[*a*]pyrene levels in the spinal fat and in the brain tissues of pigs and cattle with concentrations below 0.05 ng/g.

More data have been reported on PAH distribution in fish. Pointet and Milliet (2000) determined traces of ten parents PAH in fish whole gall bladders and livers. Phenanthrene, naphthalene and fluorene (about 10 to 100 ng/g) dominated in whole gall bladder. Phenanthrene (ranged from 32 to 166 ng/g) was detected as the predominant PAH in liver. Concentrations of twelve PAH were determined in large numbers of fish liver samples from Barcelona harbour (Vives and Grimalt, 2002). The average total PAH concentrations in fish tissue in near-coastal areas of the Gulf of Mexico were 2.17 (± 3.29) ng/g wet weight (Lewis et al., 2002).

The present study focused on the determination of 16 (U.S. EPA priority PAH, see Callahan et al., 1979) in the selected porcine and bovine tissues (liver, kidney, lung, muscle and adipose tissue and eye – both eye lens and vitreous humour) in animals exposed to PAH for a fattening period in the stables. Gas chromatography with mass spectrometry (GC/MS) and high pressure liquid chromatography with fluorescence detection (HPLC/FLD) were used for identification and quantification of these compounds. The aim of the study were (a) to compare concentrations of parent PAH between selected porcine and bovine organs and tissues; (b) to establish possible differences in the occurrence and concentrations in the pig and cattle bodies; (c) to choose target organ or tissue, which could be used as a marker of PAH animal body contamination. As far as we know, this is the first comprehensive information on the real distribution of the PAH in the organs and tissues of fattened pigs and cows.

MATERIAL AND METHODS

Chemicals. Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[123-*c,d*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene were supplied by Dr. Ehrenstorfer (Augsburg, FRG). Acetonitrile and methanol, both HPLC gradient grades, were purchased from Merck (Merck, Darmstadt, Germany). Ultra pure water was obtained from a Milli-Q UF Plus water system (Millipore, Molsheim, France). The other chemicals used were of the highest purity available.

Sample preparation, extraction and clean-up. Six fattened pigs (about one year old) and four fattened cows (about four years old) were obtained from three pig and two cattle farms with different PAH exposure in the district of Hodonin in the eastern part of the Czech Republic.

Homogenised organ and tissue samples were lyophilised except for eyeballs. The eyeballs and vitreous humour were carefully removed to avoid the risk of environmental contamination. Samples were mixed with anhydrous sodium sulphate and a defined quantity was placed in a SOXTEC glass extraction thimble (Tecator, Hoeganaes, Sweden). The same amount of anhydrous sodium sulphate was used as a laboratory blank. The samples were extracted for 2 h with boiling dichloromethane and rinsed with condensed solvent vapours for 1 hour. The extracts were cleaned-up by gel permeation chromatography (GPC). The solvent was evaporated just to dryness, the residue was dissolved in 1 ml dichloromethane and the aliquot was injected into a GPC column (PLgel, 10 μm, 600 mm × 7.5 mm I.D., (Polymer Laboratories Ltd, Shropshire, UK)). Dichloromethane at the flow rate 1.0 ml/min was used as a mobile phase. Free fatty acid co-extracts were removed by column chromatography (height

40 cm, inner diameter 1 cm). Columns were filled with 10 g of deactivated Florisil (5% w/w of water). All the operations with samples were done within the shortest time possible to minimise the risk of contamination and lighting.

Aliquot of sample was redissolved in acetonitrile for HPLC/FLD analysis. For GC/MS, isooctane was used. The confirmation of analytes identification was achieved by GC/MS.

HPLC/FLD analysis. The HPLC system consisted of Waters 717plus autosampler, Waters 600E multi-solvent delivery system, Waters 474 scanning fluorescence detector (Waters, Milford, USA). A 150 × 4.6 mm Supelcosil LC-PAH column with particle diameter 5 μm (Supelco, Bellefonte, USA) was used. A gradient of water, acetonitrile and methanol was applied for separation of the analytes: 0–45 min 40–0% water, 30% acetonitrile and 30–70% methanol, 45–65 min 30–100% acetonitrile and 70–0% methanol. The flow rate of the mobile phase was 1.2 ml/min; the column temperature was set at 30°C. The fluorescence excitation and emission wavelengths were programmed as follows: 280/340 nm at 0 min, 290/370 nm at 7.5 min, 250/368 nm at 17.4 min, 270/390 nm at 19.6 min, 272/380 nm at 21.6 min, 280/400 nm at 29.8 min, 300/420 nm at 33.7 min, 300/470 nm at 44.7 min, 304/397 nm at 46.6 min.

GC/MS analysis. The confirmation using GC/MS was based on a combination of retention times and relative abundances of selected mass ions. GC separations of selected PAH was done in a DB 5 ms fused silica capillary column (15 × 0.25 mm I.D., 0.1 μm, J. & W. Scientific, Folsom, USA). Helium at a column head pressure of 70 kPa was used as the carrier gas. The separation was achieved by programming the GC oven temperature from a 1 min hold at 70°C to 150°C at 20°C/min, to 260°C at 5°C/min, and to the final temperature of 280°C at 3°C/min with a 2.5 min hold. 2 μl of sample in isooctane was injected (splitless mode) with 0.5 μl of solvent flush at the injector (temperature of 230°C). An ion trap mass spectrometer (Finnigan MAT, Austin, USA) was used for the detection and identification of the analytes. The transfer line and MS were kept at 250°C and 220°C, respectively. The mass spectrometer was operated in EI mode at electron energy of 70 eV.

Quantification and quality assurance. Washed glassware was rinsed with acetone and hexane and, in some cases, heated in a muffle furnace (450°C, 4 h) to remove any traces of chemicals. Quality assurance samples included spiked matrices, spiked controls, procedure blanks and calibration standards in isooctane and acetonitrile.

The following quantification limits were determined: 0.005 ng/g for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluorene, and pyrene; 0.01 ng/g for benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[123-*c,d*]pyrene, dibenz[*a,h*]anthracene, and benzo[*ghi*]perylene (all concentration limits are related to wet sample weight).

Concentration of PAH in the sample were expressed as the sum of 16 PAH (total PAH concentration), as sum of concentration of 7 carcinogenic PAH (benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[123-*c,d*]pyrene, dibenz[*a,h*]anthracene), and/or concentration of individual compounds. These concentrations can be calculated to the wet weight, dry weight and/or fat weight, respectively. The expression to wet weight of sample was used to compare of PAH content in selected animal organs and tissues under study.

RESULTS AND DISCUSSION

Total PAH concentrations in porcine and bovine organs and tissues

Two fattened pigs and two cows from the same stable with the same time exposure of PAH were chosen for tissue samples collection. Average concentration, standard deviation (S.D.) and range of total PAH in porcine and bovine organs and tissues are shown in Tables 1 and 2, respectively. Multiple differences were found among concentrations of PAH in selected pair of animals. As mentioned above, parent PAH are quickly metabolised and do not substantially accumulate in organisms, so no association of an intake rate with species-dependent biotransformation of PAH was found. Concentrations of this parent compounds are mostly dependent on different metabolic rates.

The highest average concentrations of total PAH were determined in porcine and bovine lenses and vitreous humour. The lowest concentrations were found in porcine and bovine adipose tissues. Average relative content of carcinogenic PAH was found to be higher in porcine adipose tissues. However these compounds are not significantly

Table 1. Total PAH concentrations (ng/g wet weight) and relative content of carcinogenic PAH in porcine tissues (n = 6)

	Mean	S.D.	Range	Carcinogenic PAH (%)
Kidney	5.42	2.50	1.64–9.43	6.3
Liver	3.80	1.58	1.87–6.16	3.6
Lung	4.56	0.83	3.45–5.62	2.4
Muscle tissue	3.59	2.57	0.30–7.18	4.9
Adipose tissue	0.05	0.03	0.02–0.11	10.2
Lens	57.9	52.7	20.0–170	4.6
Vitreous humour	14.0	7.00	7.73–27.1	3.7

Table 2. Total PAH concentration (ng/g wet weight) and carcinogenic PAH relative content in bovine tissues (n = 4)

	Mean	S.D.	Range	Carcinogenic PAH (%)
Kidney	6.33	3.70	3.95–11.8	5.3
Liver	2.72	1.31	1.02–3.68	7.7
Lung	5.43	3.64	2.94–10.8	3.2
Muscle tissue	5.06	1.95	2.53–6.91	4.6
Adipose tissue	0.11	0.14	0.02–0.32	6.4
Lens	16.3	5.60	7.17–21.9	3.4
Vitreous humour	6.41	2.70	3.80–10.1	2.0

accumulated in test organs or tissues. Results also confirm that organs rich in adipose tissue do not serve as storage depots of PAH.

Significant differences ($P < 0.01$) were found only between PAH concentrations in porcine and bovine organs and tissues versus adipose tissues; no significant differences ($P > 0.05$) were found between total PAH concentration in organs and tissues of tested animals from the stable with different exposure of PAH (data not shown). No significant differences ($P > 0.05$) were found between total PAH concentration in porcine and bovine organs and tissues.

There are three main PAH exposure routes to farming animals: feeds intake, respiration and percutaneous intake. Kidney, liver, muscle and adipose tissues are exposed from circulated blood, lung is exposed by inhalation and like the other organs, by the circulating blood, but it is not easy to determine the contribution of each route. Lens and vitreous humour are exposed probably by PAH diffusion from ambient air. Because their concentration correlated (correlation coefficient ranged from 0.9218 to 0.9845) with concentrations in other organs and tissues, analyses of porcine and/or bovine lenses for PAH content could be use for determination of animal exposure to these compounds.

Individual parent PAH concentration in porcine and bovine organs and tissues

Figures 1 and 2 show average individual PAH concentration in tested porcine and bovine organs and tissues. PAH concentrations were at the range of ng/g. This finding corresponds with other published results. For example, Tokiwa et al. (1998) found the following PAH concentration in human lungs: benzo[*a*]pyrene 0.33 ± 0.11 ng/g; benzo[*b*]fluoranthene 0.16 ± 0.17 ng/g; benzo[*ghi*]perylene 0.43 ± 0.26 ng/g; pyrene 0.54 ± 0.36 ng/g; fluoranthene 0.67 ± 0.42 ng/g; chrysene 0.45 ± 0.33 ng/g dry weight. Seto et al. (1993) determined three PAH in human lungs. Benzo[*a*]pyrene, benzo[*k*]fluoranthene and benzo[*ghi*]perylene concentrations were 0.54 ± 0.35 ng/g, 0.44 ± 0.26 ng/g

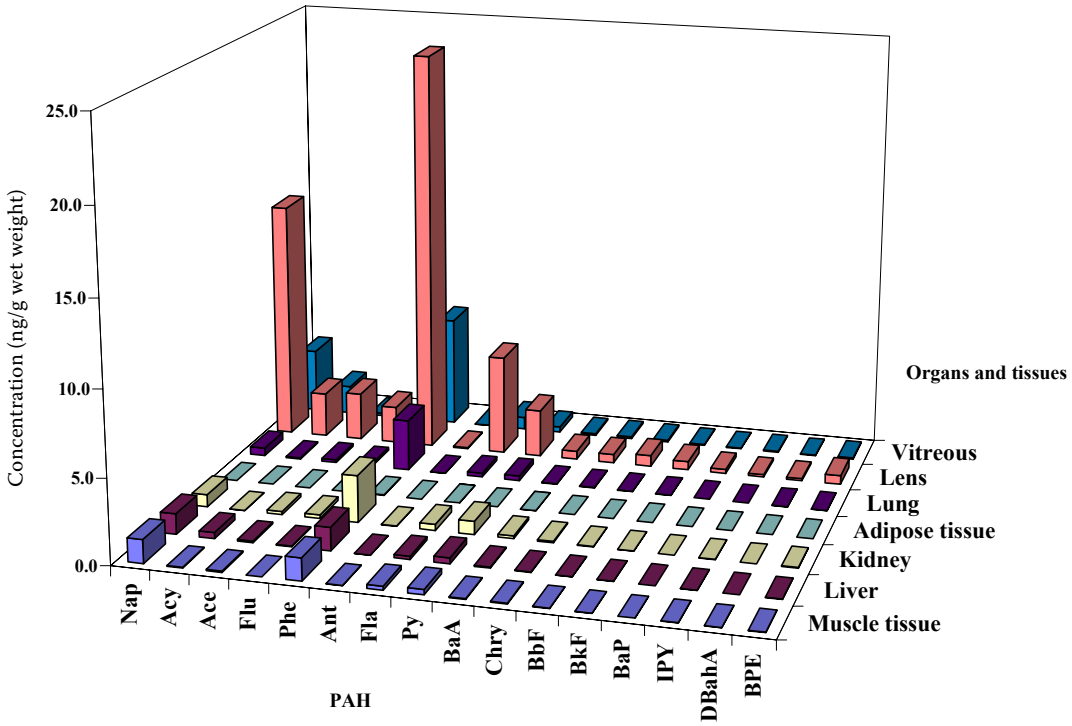

Figure 1. PAH distribution in porcine organs and tissues

Nap = naphthalene, Acy = acenaphthylene, Ace = acenaphthene, Flu = fluorene, Phe = phenanthrene, Ant = anthracene, Fla = fluoranthene, Py = pyrene, BaA = benzo[*a*]anthracene, Chry = chrysene, BbF = benzo[*b*]fluoranthene, BkF = benzo[*k*]fluoranthene, BaP = benzo[*a*]pyrene, IPY = indeno[1,2,3-*cd*]pyrene, DBahA = dibenz[*a,h*]anthracene, BPE = benzo[*ghi*]perylene

and 0.87 ± 056 ng/g dry lung, respectively. Lodovici et al. (1998) detected six PAH in human lung tissue and reported concentration of benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene and dibenzo[*a,h*]anthracene ranged from 0.1 to 0.9 ng/g dry weight. Concentrations of individual PAH ranging from <0.01 ng/g dibenz[*a,h*]anthracene) to 6.9 ng/g fluoranthene in lard and dripping (Dennis et al., 1991). De Vos et al., (1990) found that meat and meat products contain from 0.5 (benz[*a*]anthracene) to 3.0 ng/g wet weight of phenanthrene. Phenanthrene was found a more abundant compound followed naphthalene, fluorene, pyrene, acenaphthylene, acenaphthene and fluorene. In this study the highest PAH concentrations were detected in lens and vitreous humour. These results are in good agreement with published data. Gallenga et al. (1997) determined five PAH in lenticular tissue with total concentration 27.1 ng/g wet weight [pyrene 3.2 ng/g, fluoranthene 9.7 ng/g, triphenylene 2.7 ng/g, benz[*a*]anthracene 4.6 ng/g and chrysene 6.9 ng/g]. The lens appeared to be a suitable tissue for chemical monitoring analysis.

Average content of PAH in porcine and bovine organs and tissues

PAH are composed of two or more aromatic rings. In this study concentrations of five PAH isomeric groups were determined; the low molecular weight PAH with two (naphthalene) and three rings (acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene); and the high molecular PAH with four (fluoranthene, pyrene, benz[*a*]anthracene and chrysen), five (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and dibenz[*a,h*]anthracene) and six rings (indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene). Relative average content of the isomeric groups and individual compounds is summarized in Table 3. These results indicated that only PAH with lower molecular weight (2 and 3 rings) were notably present in all samples. High

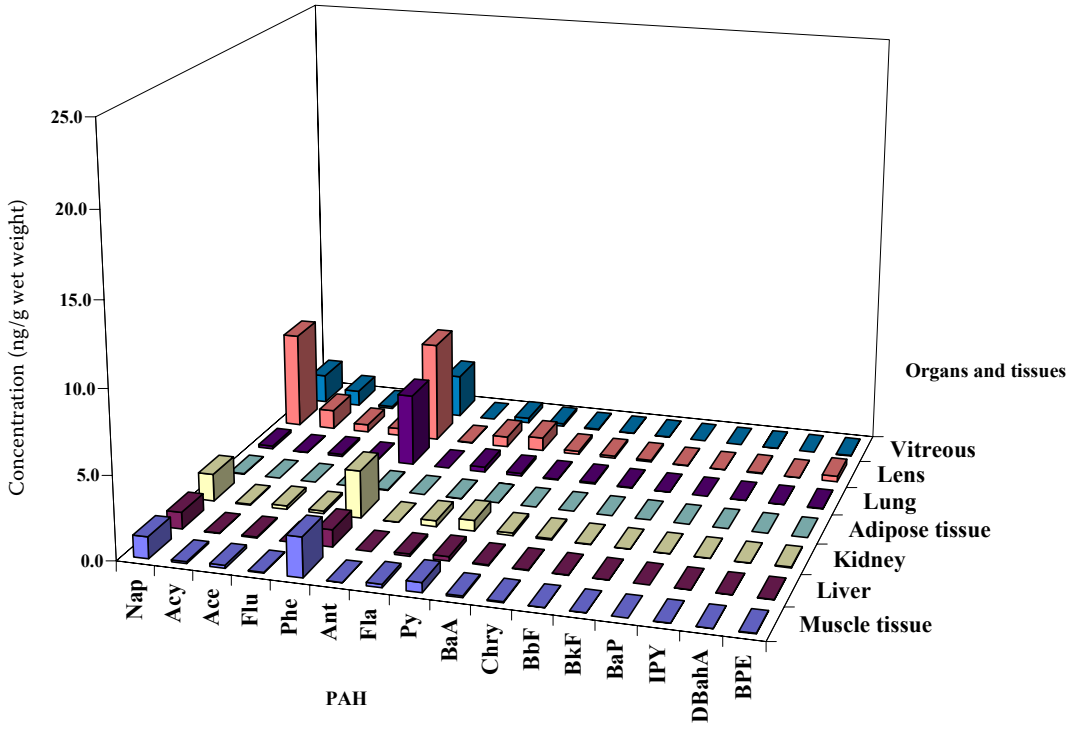

Figure 2. PAH distribution in bovine organs and tissues

Nap = naphthalene, Acy = acenaphthylene, Ace = acenaphthene, Flu = fluorene, Phe = phenanthrene, Ant = anthracene, Fla = fluoranthene, Py = pyrene, BaA = benzo[*a*]anthracene, Chry = chrysene, BbF = benzo[*b*]fluoranthene, BkF = benzo[*k*]fluoranthene, BaP = benzo[*a*]pyrene, IPY = indeno[1,2,3-*cd*]pyrene, DBahA = dibenz[*a,h*]anthracene, BPE = benzo[*ghi*]perylene

molecular PAH, compounds with significant carcinogenic potencies, were present only at low concentrations.

Positive correlations were found between the PAH concentrations in indoor air (data not shown) and those in porcine (correlation coefficient 0.89926) and bovine lungs (0.90179) respectively, higher than in all other tissues. Among the PAH, phenanthrene was the most abundant compound in lung samples. Average relative contributions of phenanthrene in porcine and bovine lungs were 66.6% and 79.8%, respectively. This was the highest content of this compound from the all other samples. Naphthalene was the second most abundant compound. Relatively low concentration of naphthalene in lung samples, in comparison to other samples, could be explained by its high volatility leading to its low retention. Similar distribution of prevalent PAH was found for low molecular three-ring PAH. These compounds were predominant in the porcine and bovine lenses and vitreous humour (mainly naphthalene and phenanthrene).

Low quantities of anthracene were detected in all samples studied. Its relative content in environmental samples is mostly 10% of phenanthrene concentration. In all samples, this content was lower than 1%. This finding is probably connected with its higher chemical reactivity (lower stability) in comparison with phenanthrene.

CONCLUSIONS

Distribution of 16 U.S. EPA priority PAH was determined in porcine and bovine tissues collected in differently polluted areas.

Similar average levels of PAH contamination were determined in porcine and bovine tissues.

The highest concentrations of total PAH were determined in lenses and vitreous humour, the lowest PAH concentrations were found in adipose tissue.

Phenanthrene was the compound with the highest relative content from all PAH under study, with

Table 3. Average content of parent PAH in porcine and bovine organs and tissues

Content (%)	Porcine organs and tissues							Bovine organs and tissues						
	muscle tissue	liver	kidney	adipose tissue	lung	lens	vitreous	muscle tissue	liver	kidney	adipose tissue	lung	lens	vitreous
Naphthalene	38.9	32.0	13.3	13.3	10.4	24.5	27.3	25.8	37.2	25.9	32.0	3.3	35.7	27.2
Acenaphthylene	1.3	9.2	0.3	0.5	1.7	4.6	12.2	2.2	0.9	0.9	3.9	0.3	7.1	14.5
Acenaphthene	2.2	2.4	3.3	1.5	3.7	4.9	1.1	3.5	2.3	3.5	0.6	2.4	2.8	2.1
Fluorene	n.d.	1.2	3.9	n.d.	3.4	3.8	0.8	0.9	n.d.	2.5	n.d.	0.4	2.7	6.0
Phenanthrene	37.0	36.4	51.3	51.5	66.6	41.4	46.8	47.1	38.0	45.3	39.2	79.8	37.3	40.5
Anthracene	0.3	0.3	0.4	0.3	0.4	0.1	0.1	0.3	0.4	0.3	0.4	0.1	0.2	0.1
Fluoranthene	6.4	4.9	6.7	10.6	5.0	10.2	5.2	3.8	4.9	5.7	10.8	5.7	3.9	4.6
Pyrene	8.4	9.1	14.3	12.6	6.1	4.9	2.3	11.5	9.5	10.2	7.1	3.0	4.9	2.6
Benz[*a*]anthracene*	1.2	1.5	3.0	2.0	0.6	0.9	0.7	2.0	2.3	2.3	1.2	1.0	1.1	0.8
Chrysene*	1.4	1.0	1.4	2.6	0.7	0.9	0.8	1.1	1.5	1.3	1.8	1.5	0.8	0.4
Benzo[*b*]fluoranthene*	0.8	0.5	0.5	1.5	0.5	1.1	0.7	0.4	0.7	0.5	1.1	0.7	0.7	0.4
Benzo[*k*]fluoranthene*	0.5	0.2	0.2	1.6	0.3	0.9	0.4	0.2	0.4	0.2	0.4	0.3	0.2	0.2
Benzo[*a*]pyrene*	0.1	0.1	0.1	0.1	0.1	0.4	0.3	0.1	0.2	0.2	0.1	0.2	0.1	n.d.
Indeno[1,2,3-*cd*]pyrene*	0.5	0.3	0.3	0.6	0.2	0.2	0.5	0.2	0.4	0.3	0.5	0.3	0.4	0.1
Dibenz[*a,h*]anthracene*	0.3	0.2	0.1	0.5	0.2	0.2	0.3	0.1	0.3	0.1	0.3	0.2	0.1	0.1
Benzo[*ghi*]perylene	0.7	0.6	0.8	0.9	0.3	0.9	0.6	0.6	1.0	0.8	0.6	0.6	2.1	0.6

*compound with carcinogenic potencies (WHO, 1998)

maximal relative content in bovine and porcine lung. No significant differences were found in distribution of PAH in porcine and bovine bodies from the different sites with various PAH exposure.

Nevertheless, positive correlations of PAH concentrations in the indoor air and porcine and bovine lung concentration were found.

Chemical analyses of lenses and lung might be used for routine determination of PAH content.

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Corresponding Author:

RNDr. Miroslav Ciganek, PhD., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 533 331 811, fax +420 541 211 229, e-mail: ciganek@vri.cz