Exposure of pig fatteners and dairy cows to polycyclic aromatic hydrocarbons

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ABSTRACT: Exposure of pig fatteners and dairy cows to polycyclic aromatic hydrocarbons (PAHs) was investigated by analyses of selected indoor and outdoor samples. PAH concentrations (16 U.S. EPA priority PAHs) and data on common exposure routes were used for exposure calculations. The samples under study included water (n = 24), feed (n = 48), indoor air (n = 15), barn dust (n = 17), outdoor air (n = 6) road dust (n = 17), and soil (n = 15) collected in the summer 1999 and in the spring 2000 on 3 pig and 2 dairy cattle farms. The following mean concentrations of Σ 16 PAHs were found: 100 ng/l in water for pigs, 38 ng/l in water for cows, 82 μg/kg in feed mixtures for pigs, 128 μg/kg in feed concentrates for cows, 278 μg/kg in forages for dairy cows, 25 ng/m^3 in indoor air of pig houses, 59 ng/m^3 in indoor air of cow houses, 676 μg/kg in dust collected in pig houses, 4 475 μg/kg in dust collected in cow houses, 16 ng/m^3 in outdoor air of pig houses, 29 ng/m^3 in outdoor air of cow houses, 4 711 μg/kg in road dust collected on pig farms, 15 175 μg/kg in road dust collected on cattle farms, 826 μg/kg in soil collected around pig houses, and 1 356 μg/kg in soil collected around cow houses. The total intake of Σ 16 PAHs in the diet of cows and pigs was 14 156 μg and 164 μg PAHs per day, respectively. The exposure of cows to PAHs was 86 times higher than that of pigs. Feed was the major source of PAHs for both species (approximately 99%). Urinary 1-hydroxypyrene concentration was used as a biomarker of exposure to PAHs. The mean total amount of 1-hydroxypyrene excreted per day in porcine (2 l) and bovine (13 l) urine was 14.5 μg and 1 595 μg, respectively, which was 3.2% and 11.9% of pyrene intake.

Keywords: polycyclic aromatic hydrocarbons; air, feed; drinking water; dust; soil; exposure

Polycyclic aromatic compounds (PAHs) are widely distributed in the environment. A number of them are carcinogenic (Sjögren *et al.*, 1996) and mutagenic (Durant *et al.*, 1996). PAHs are lipophilic compounds and can be absorbed by the lung and gastrointestinal tract tissues and by the skin. Several studies have been carried out to determine the levels of exposure of humans to PAHs (Dennis *et al.*, 1983; de Vos *et al.*, 1990; Phillips 1999). The dietary sources of PAHs are cereals and vegetables, rather than meat, excepting populations with a high consumption of meat cooked over an open flame. The recently developed biomonitoring procedures allow the assessment of human exposure to PAHs, as well as identification of diet as the major exposure source. The U.S. Environment Protection Agency published "The Exposure Factors Handbook" in which available statistical data on various factors used in assessing human exposure are summarised (http://www.epa.gov/ncea/exposfac.htm). The major steps in exposure assessment include: (1) determination of exposure pathways, (2) identification of environmental media conveying the contaminant, (3) determination of the contaminant concentration, (4) determination of exposure time, frequency, and duration, and (5) identification of the exposed population. No data on the assessment of exposure of farm animals to PAHs were found in available literature.

Determination of exposure level is the first step towards the control of intake of toxic contaminants. Our study concentrated on PAHs and the subjects included pigs and cows raised on selected farms in the district of Hodonín, Czech Republic. The environment in this district is affected by extraction of soft coal (currently restricted), oil and natural gas (expanding), the operation of a large power plant combusting soft and brown coal, and by an intensive truck car traffic. The major pollution source is the power plant producing 1 390 tons of solid emissions per year (Raszyk *et al.*, 1998a).

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Polycyclic aromatic hydrocarbons have been the subject of our research since 1994. The papers published so far dealt with:

a) mutagenic factors in the environment of animal houses (Raszyk *et al*., 1998a);
b) sources of PAHs on animal farms (Raszyk *et al*., 1998b) and in feed mills (Raszyk *et al*., 1999a);
c) biomarkers of exposure to PAHs (Raszyk *et al*., 1999b) and
d) PAH concentrations in indoor air of animal houses (Ciganek *et al*., 2000).

The objectives of the present study were 1) to establish whether there exist differences in the pollution rate between pig and cattle farms; 2) to quantify the contribution of feeds and water to the overall PAH pollution burden; 3) to determine differences in exposure by PAHs between pig and cow.

MATERIAL AND METHODS

Chemicals

PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeo[123-*c,d*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]-perylene) were supplied by Dr. Ehrenstorfer (Augsburg, FRG). The organic solvents used were for organic trace analysis. All the other chemicals used were of the highest purity available.

Site description

The contents of PAHs in selected samples and exposure rates were determined on three pig (I–III) and two cattle farms (IV–V).

The pig farm I housed 17 000 fatteners in 15 separate premises. All the pigs were fed a liquid diet prepared with drinking water from a public source. Finger drinkers were also at disposal. The premises were heated with soft and brown coal.

The pig farm II housed 10 000 fatteners fed a liquid diet. Water from a private source was used for liquid diet preparation. No drinkers were installed. The premises were heated with natural gas.

The pig farm III housed 1 650 fatteners fed a liquid diet prepared with drinking water from a public source. Finger drinkers were also at disposal. The premises were heated with brown coal.

The cattle farm IV housed 338 dairy cows fed forages (hay, haylage, silage) and concentrates including also rapeseed meal, wheat bran, and malt flower. One half of drinking water was supplied from a public source and the other from a well on the farm. The animals are watered from drinking bowls containing water mixed from the two sources. The premises were heated with bituminous coal.

The cattle farm V housed 265 dairy cows fed the same diet as those on the farm IV. The animals were watered from drinking bowls supplied with water from a public source. The premises were equipped with electric heaters.

Sampling

Feed samples (n = 48) were collected in feed rooms. The set included 22 samples of feed mixtures for pigs, 14 samples of concentrate mixtures for dairy cows, 12 samples of hay. Minimum amount of each sample was 500 g.

Water samples (n = 24) were collected from drinkers or in the feed rooms. The set included 14 and 10 samples collected on pig and cattle farms, respectively. Minimum sample volume was 2 000 ml.

Barn dust samples (n = 17) were collected with an electric vacuum cleaner with an output of 1.62 m^3 of air per 1 min. The samples were collected into disposable paper bags. The mean sampling area and sampling time were 100 m^2 and 15 min, respectively, and mean sample sizes were 500 g for the pig houses and 400 g for the cattle houses. Larger particles, amounting to approximately 5% w/w, had been separated by sieving through 7 mm mesh before the samples were analysed.

Outdoor dust samples (n = 17) were collected from asphalted service roads using the technique described above. The sample size ranged from 500 to 1 000 g. Larger particles, amounting to approximately 10% w/w, had been separated by sieving through 7 mm mesh before the samples were analysed.

Soil samples were collected at a distance of not more than 10 m from the animal houses from the depth of 10 cm. The minimum sample size was 1 000 g.

Air samples were collected with a large-volume pump with an output of approx. 20 m^3/h (PS-1 Graseby-Andersen, USA) and the following sampling materials: glass microfibre filters Z-5 (Filpap, Štětí, Czech Republic) with a diameter of 102 mm and thickness of 350 μm; polyurethane foam filters of the polyether type N 2227 (Gumotex, Břeclav, Czech Republic) with a density of 0.022 g/cm^3 put into glass thimbles with a supporting noncorrosive grid (Graseby-Andersen, USA).

Analytical methods

Analytical methods used for the detection and determination of the 16 PAHs in feeds, drinking water, dust, soil, and air were described in detail in our earlier papers (Raszyk *et al*., 1998a; Ciganek *et al.,* 2000). Briefly, PAHs were extracted with dichloromethane, co-extracts were separated by gel permeation chromatography and the analytes were determined by GC-MS. Total urine 1-hydroxypyrene was determined by reverse HPLC with fluorimetric detection preceded by enzymatic hydrolysis of 1-hydroxypyrene conjugates with glucuronic acid and sulphate (Raszyk *et al*., 1999b).

RESULTS AND DISCUSSION

Concentrations of PAHs in the indoor and outdoor environment

Mean concentrations of PAHs in the indoor and outdoor environment of the farms are shown in Tables 1 and 2. The following data have been tabulated: sample material incl. concentration units; (*n*) number of analysed samples; Σ PAHs) mean concentration of sum of 16 PAHs; (Σcarc. PAHs) mean concentration of sum of the 7 carcinogenic PAHs; (Py) mean concentration of pyrene; (BaP) mean concentration of benzo(a)pyrene; (% carc. PAHs) percentage of carcinogenic PAHs from the sum of the 16 PAHs.

The concentration of sum of 16 PAHs was higher on cattle farms than on pig farms. The highest mean concentrations of 15 175 μg/kg and 4 711 μg/kg were found in road dust samples collected on cattle and pig farms, respectively. The highest proportion of 7 carcinogenic PAHs (34%) was detected in soil samples collected on pig farms.

PAH concentrations in barn dust, road dust and soil

Results of analyses indicate that, among the indoor samples, barn dust was the most significant source of contamination. Mean concentrations of Σ 16 PAHs and Σ 7 carcinogenic PAHs (chrysene, benz[*a*]-anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-cd]pyrene and dibenz-[*a,h*]anthracene) were higher on the cattle farms than on the pig farms (4 475 vs. 676 μg/kg and 162 vs. 38 μg/kg, respectively).

Analyses of road dust samples provide an information on topical contamination of the immediate surroundings of animal houses. Mean concentrations of Σ 16 PAHs and Σ 7 carcinogenic PAHs were higher on the cattle farms than on the pig farms (15 175 vs. 4 711 μg/kg and 899 vs. 364 μg/kg, respectively). Windy weather and opening of windows and doors can increase the concentration of PAH-conveying dust particles in the indoor environment of animal houses.

Results of soil analyses are indicative of chronic contamination of immediate surroundings of animal houses. Mean concentrations of Σ 16 PAHs and Σ 7 carcinogenic PAHs in the soil samples were higher on the cattle farms than on the pig farms (1 356 vs. 826 μg/kg and 394 vs. 269 μg/kg, respectively).

Exposure of farm animals to PAHs

PAH-exposed animal species undergo inhalation exposure, dermal exposure and oral exposure to a certain extent. PAHs do not usually enter the body through the skin under normal condition, therefore dermal exposure was neglected in our study. Average PAH concentrations in the selected samples were used to calculate the level of exposure.

Oral exposure

Data on concentrations of PAHs in feed mixtures were used to calculate the exposure of feeder pigs. At an intake of 2 kg of a feed mixture, a finisher with a live weight of 100 kg received 164 μg of Σ 16 PAHs (14.4 μg of pyrene) per day.

Concentration of PAHs in roughages are relevant in ruminants. Calculations of a average intake were based on data on concentrations of PAHs in hay. At an intake of 50 kg of roughages, a cow received 13 900 μg of Σ 16 PAHs (1 505 μg of pyrene) per day. At a daily intake of 2 kg of concentrate mixtures, one cow received additional 256 μg of Σ 16 PAHs (90 μg of pyrene) per day. Total intakes of Σ 16 PAHs and pyrene in the diet were 14 156 μg and 1 595 μg per day, respectively, which was a 86fold (Σ 16 PAHs) and 111fold (pyrene) of the amount received by finishing pigs. Expressed in terms of relative intake, a pig with a bodyweight of 100 kg received 1.64 μg of Σ 16 PAHs per 1 kg per day, while that of a cow with a bodyweight of 400 kg 35.4 μg of Σ 16 PAHs per 1 kg per day, or a 22fold of the amount received by the pig.

Analyses of drinking water can complete the information on oral exposure to PAHs. At a consumption of 8 l of water, a finishing pig with a bodyweight of 100 kg received 0.8 μg of Σ 16 PAHs (0.068 μg of pyrene) per day. Similarly a cow with a bodyweight of 400 kg consuming 40 l of water received 1.52 μg of Σ 16 PAHs (0.092 μg of pyrene) per day, which was twice as much as in the pig. Expressed in terms of relative intake, a pig received with drinking water additional 0.008 μg and a cow 0.0038 μg of Σ 16 PAHs per 1 kg of bodyweight per day.

Inhalation exposure

Our earlier investigations, done in the same premises, demonstrated that the mean indoor air concentrations of the 16 PAHs on the pig and the cattle farms were 25 and 56 ng/m^3, respectively (Ciganek *et al.*, 2000). At a mean inspired air volume of 10 m^3 per 24 h, a pig with a bodyweight of 100 kg received 0.25 μg of Σ 16 PAHs (0.03 μg of pyrene); the corresponding values for a cow with a bodyweight of 400 kg were 40 m^3 and 2.24 μg of Σ 16 PAHs (0.28 μg of pyrene). Expressed in terms of relative intake, a pig received by breathing additional 0.0025 μg and a cow 0.0056 μg of Σ 16 PAHs per kg bodyweight per day.

Total PAHs exposure

Overall results of pigs and cows exposure by PAHs from different exposure sources are shown in Table 3.

Considering the summary (feed + water + air) relative intake, the exposure was approximately 20 times higher in the cows than in the pigs (35.4 vs. 2.57 ng per 1 kg body weight per day).

Table 1. Mean concentrations of polycyclic aromatic hydrocarbons (PAHs) in the indoor and outdoor environment of pig houses

Sampled material	*n*	Σ PAHs	Σ carcinogenic PAHs	Py	BaP	% carcinogenic PAHs
Water (ng/l)	14	100	51	8.5	6.7	9.2
Feed mixtures (μg/kg)	22	82	2.3	7.2	0.3	4.0
Indoor air (ng/m^3)	9	25	1.3	3.0	0.17	5.1
Barn dust (μg/kg)	11	676	38	26	1.1	9.0
Outdoor air (ng/m^3)	3	16	1.0	1.4	0.09	7.8
Road dust (μg/kg)	11	4 711	364	140	8.3	13
Soil (μg/kg)	9	826	269	78	12	34

Table 2. Mean concentrations of polycyclic aromatic hydrocarbons (PAHs) in the indoor and outdoor environment of cow houses

Sampled material	*n*	Σ PAHs	Σ carcinogenic PAHs	Py	BaP	% carcinogenic PAHs
Water (ng/l)	10	38	11	2.3	0.1	8.7
Feed concentrates (μg/kg)	14	128	34	1.8	0.6	12
Forages (μg/kg)	12	278	24	30.1	3.0	16
Indoor air (ng/m^3)	6	56	4.3	7.0	0.5	7.0
Barn dust (μg/kg)	4	4 475	162	33.6	1.2	17
Outdoor air (ng/m^3)	4	29	2.4	2.5	0.3	7.0
Road dust (μg/kg)	4	15 175	899	206	10.0	13
Soil (μg/kg)	4	1 356	394	90	15.0	30

Table 3. Total PAHs exposure

	Pigs	Cows
Feeds (μg/day)	256	14 156
Water (μg/day)	0.80	1.5
Inspired air (μg/day)	0.25	2.2
Sum of intake (μg/day)	257	14 160
Exposure (μg/kg/day)	2.6	35

Feed was the major source of PAHs on the pig and the cattle farms (99.5% in pigs and 99.9% in cows). The contributions of drinking water and indoor air contaminants were negligible.

Most authors who studied human exposure identified the diet as the major source of PAHs. Based on a total daily consumption of 1.46 kg food and beverages, the total daily dietary load of PAHs in the UK was calculated to be 3.70 μg (Dennis *et al.*, 1983). A similar study of the Dutch diet estimated an average daily intake of PAHs at between 5 and 17 μg/day (de Vos *et al.*, 1990). The Dutch study also concluded that cereal products were the major source of PAHs, and that significant contributors were also vegetable oils and their products. In our study the total daily intakes of PAHs in pigs and cows were approximately 20 and 150 times higher, respectively.

Urinary pyrene excretion

Urinary concentration of 1-hydroxypyrene can be used as a biological indicator of recent intake of PAHs (Jongeneelen, 1994, 1997, 2001; Strickland and Daehee, 1999) (see Table 4). Metabolites of PAHs with 2 and 3 rings are preferentially excreted in the urine, while higher molecular ones are excreted in faeces (Jacob and Grimmer, 1996).

Table 4. Urinary pyrene excretion

	Pigs (*n* = 49)	Cows (*n* = 40)
1-Hydroxypyrene in urine (μg/l)	0.23	14.5
1-Hydroxypyrene excreted (μg/day)	0.46	189
Pyrene intake (μg/day)	14.5	1 595
Pyrene excretion (% of intake)	3.2	11.9

Urine samples collected in the same premises were analysed for 1-hydroxypyrene concentrations in an earlier study (Raszyk *et al.*, 1999a). Mean concentrations of total 1-hydroxypyrene in porcine and bovine urine were 0.23 and 14.5 μg/l. Mean total amounts of 1-hydroxypyrene excreted per day in porcine (2 l) and bovine (13 l) urine were 14.5 μg and 1 595 μg, respectively, which amounted to 3.2% a 11.9% of the calculated intake pyrene exposure (Table 4).

CONCLUSIONS

As far as we known, this is the first comprehensive information on the exposure of cattle and pigs to PAHs quantifying also the role of possible contamination sources.

The exposure of cows to PAHs was found to be 86 times higher than the exposure of pigs.

The major source of PAHs for both the species was the feed. The contribution of drinking water and inhaled air to the total PAH burden was negligible.

Mean total amounts of 1-hydroxypyrene excreted per day in porcine (2 l) and bovine (13 l) urine were 14.5 μg and 1 595 μμg, respectively, which amounted to 3.2% a 11.9% of pyrene intake.

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