DOI:10.1021/jf9003797

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Bioavailability of Polycyclic Aromatic Hydrocarbons (PAHs) from Soil and Hay Matrices in Lactating Goats

Ádrian Costera,[†] Cyril Feidt,[‡] Marie-Antoinette Dziurla,[‡] Fabrice Monteau,[§] Bruno Le Bizec,[§] and Guido Rychen*,[‡]

[†]ISTOM, 32 Boulevard du Port, 95094 Cergy-Pontoise, France, [‡]UR AFPA, Nancy-Université, INRA, 2 Avenue de la Forêt de Haye BP 172, 54505 Vandœuvre-lès-Nancy Cedex, France, and [§]LABERCA, Ecole Nationale Vétérinaire de Nantes, Atlanpôle la Chantrerie, Route de Gachet, 44307 Nantes Cedex 3, France

This experiment was aimed at determining the bioavailability of three polycyclic aromatic hydrocarbons (PAHs) in goats: phenanthrene, pyrene, and benzo[*a*]pyrene. A Latin square design procedure was carried out involving three alpine lactating goats and three PAH-contaminated matrices (soil, hay, and oil as a control). Milk and urine samples were collected to assess PAH and hydroxy-PAH excretion kinetics and to compare the carry-over rates for the different matrices. PAHs were found to be excreted mainly in urine; metabolite concentrations were about 20 times higher in urine than in milk. 1-Hydroxypyrene was the major metabolite in both body fluids (8000 ng/mL urine and 450 ng/mL milk); it may be considered as a valuable indicator of the ruminant exposure to PAHs. Apparent absorption of PAHs estimated by the metabolite excretion in urine and milk reached 34% for pyrene from soil, and the bioavailability of soil-bound PAHs was found to be similar to the bioavailability of PAHs from the other matrices.

KEYWORDS: PAHs; 1-hydroxypyrene; soil; hay; bioavailability; urine; milk; goats

INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH) transfer from various environmental matrices to food products of animal origin may be considered as a major step to assess food safety (*1*). From an environmental point of view, plants may be relevant vectors of PAHs. It has been reported that airborne pollutants in the atmosphere find their way onto leaf surfaces (*2*, *3*), and PAHs are known to be located mainly on cuticular waxes (*4*). Dairy cattle grazing near roads with important vehicle load may ingest up to 2 mg of PAHs per day (*5*). Soil samples are generally more contaminated than grass, with PAH content between 2.7 and 18 μg/g of dry matter (DM) (*6*, *7*). Soil tends to trap the hydrophobic molecules within the clay−humus complex (*3*). Binding forces between PAHs and soil vary from a simple adsorption to a covalent binding. Thus, because of these chemical interactions, the pollutants resist to hydrolysis and microbial degradations, which occur within soil (*8*). Soil ingestion by grazing ruminants is estimated between 2 and 18% of the daily ingested DM (*9*−*11*). Thus, in the case of cattle with their average intake of soil quantity around 0.5 kg per animal per day (*12*), soil ingestion could contribute up to 50 mg of PAHs per day (*13*). From the literature review, it is known that PAH metabolites may be transferred to milk from parent molecules (*14*, *15*). It is also known that urine is the principal way of excretion for the metabolized compounds (*16*−*19*).

Concerning PAH bioavailability in ruminants, few data are available on the mobility of PAHs bound to environmental matrices, such as soil or grass, and their excretion to milk or urine. The objective of this study was to determine the bioavailability of three model compounds (phenanthrene, pyrene, and benzo[*a*]pyrene) from contaminated hay and soil. Transfer of the parent compounds and their major hydroxylated (OH) metabolites (2-OH-phenanthrene, 3-OH-phenanthrene, 1-OH-pyrene, and 3-OH-benzo[*a*]pyrene) toward urine and milk was determined.

MATERIALS AND METHODS

Animals, Experimental Design, and Feeding. This study was carried out with three alpine lactating goats of approximately 50 kg of live weight and 2−3 years old. The experimental protocol was in agreement with the national and European guidelines (1986, number 86/609/CEE). Animals were given a period of 22 days to adapt to the facilities and experimental conditions. During this adaptation period, animals received feed not fortified in PAHs. The days of the PAH administration, 50 mg of each PAH (phenanthrene, pyrene, and benzo[*a*]pyrene; Sigma Aldrich and Fluka, Steinheim, Germany) was administered once, either through 500 g of hay, 25 g of soil, or 5 mL of an oil solution (matrix used as a control). These dosages were chosen to allow for the identification of PAHs and their metabolites in urine and milk. According to our experience and previous papers (*14*, *19*), such a dose was necessary to ensure a good reliability mainly in milk samples, where metabolite concentrations are rather low.

The experimental procedure was designed as a Latin square (three matrices, three goats) to study the effect of the different matrices (**Figure 1**). An interval of at least 1 week was applied between two successive administrations of contaminated matrices, to prevent the effect of a given matrix masking that of the following one.

The daily food intake of the goats comprised 500 g of hay (PAH-contaminated hay was administered once, as indicated in **Figure 1**) and a mixture of concentrate containing corn grain (500 g), soy bean (300 g),

*To whom correspondence should be addressed. E-mail: guido.rychen@ensaia.inpl-nancy.fr.

 Published on Web 05/29/2009

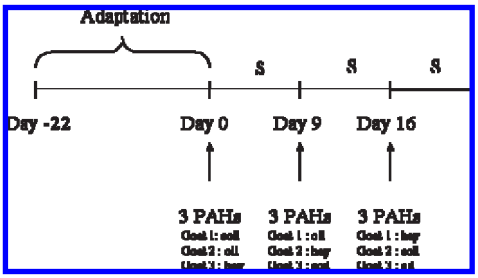

Figure 1. Experimental Latin square design. S = sampling.

pulps of beet (800 g), and minerals (20 g) to meet the needs for maintenance and dairy production of the goats (*20*). Water and salt were provided *ad libitum*. Mechanically milking was practiced twice a day (8:00 am and 5:00 pm, local time).

Preparation of PAH-Contaminated Matrices. *Oil Solution*. The oil solution used (castor oil polyether) was Cremophor EL (Fluka, Steinhem, Germany). It corresponded to a formulation that is generally used to convey hydrophobic substances in pharmacological studies (*21*). A total of 50 mg of phenanthrene, 50 mg of pyrene, and 50 mg of benzo[*a*] pyrene were dissolved in 5 mL of Cremophor EL in a glass bottle the day before the application. The oral administration was carried out using a syringe of 10 mL.

Hay. The grass used in this study was contaminated by pulverization of PAHs. A 10 m^2 grass area of a meadow (40% *Festuca arundinacea*, 30% *Dactylis glomerata*, 20% *Arrenatherum elatius*, and 10% *Lolium perenne*) was selected and pulverized with the PAH solution on a day without rain and wind. The day after the pulverization, the treated surface was harvested and the grass was dried in a climate chamber at 30 °C during 5 days to be transformed into hay.

Soil. The soil used (2.9% organic matter, 34% clay, 52% of silts, 14% sand) was taken from an experimental field far of any source of pollution on a surface layer of 10 cm. This soil resulted from a permanent meadow. To facilitate drying (at 20 °C during 15 days), homogeneity, and its treatment with PAHs, the soil was crushed and sieved at 5 mm.

Amounts of phenanthrene, pyrene, and benzo[*a*]pyrene (83 mg of each PAH) were dissolved in 50 mL of acetonitrile and added to aluminum small boats each containing 25 g of soil. Possible losses of PAHs (about 40%) during fortification and maturation were taken into account (*22*). To integrate PAHs in-depth in soil aggregates (*23*), a first humidification of the soil samples was carried out at 40% of its field capacity. Finally, the field capacity was adjusted to 80% by adding distilled water (*24*). This operation was regularly repeated during the soil maturation period at room temperature during 4 weeks (*25*). Volatilization and photodegradation were limited by an aluminum foil cover. At the end of the maturation period, preparations were divided into small pellets packed with pure cellulose wadding (Tork, Germany) and frozen at −20 °C before use to prevent the alteration of the PAHs in soil (*26*).

Sampling and Treatments. PAH concentrations were determined in control feed (concentrate and hay) and in contaminated feed matrices (oil, hay, and soil). Just before the administration of contaminated matrices (d0), milk and urine samples from each animal were also collected for analysis of PAHs and their metabolites. After administration of each contaminated matrix, sampling was carried out after 9, 24, 33, 48, and 57 h for milk and 9, 24, 33, and 48 h for urine. Urine samples were collected using urinary probes (Folatex, Porgès, Plessis Robinsons, France), which were fitted just before administration of the PAH-contaminated matrices. Collected samples (20 mL in glass bottles) of urine and milk were immediately frozen at −20 °C.

Extraction and GC−MS Analysis of PAHs and Their Major Metabolites. *PAH Determination in Oil, Hay, and Soil*. PAH extraction on oil was carried out by mixing about 100 mg of oil with 10 mL of cyclohexane. PAH extraction in hay and soil was performed on 0.5 g of hay and 1 g of soil, with 40 mL of cyclohexane/ethyl acetate (50:50, v/v). From the 2400 g of contaminated hay, three samples representing about 300 g were pooled together and grinded to 4 mm size hay samples. From this homogeneous matrix, 0.5 g was collected for analysis. A similar procedure was applied for selecting a representative 1 g of soil.

Mixtures were submitted to an ultrasonic bath for 15 min, followed with mechanical homogenization for 30 min at room temperature. After centrifugation (3400*g* at 0 °C for 30 min), the supernatant was isolated and the volume was adjusted to 100 mL with cyclohexane. Then, a volume of 100 μL of extract obtained with oil, hay, or soil was fortified with internal standards (50 ng of phenanthrene-d_{10}, pyrene-d_{10} (pyrene-d_{10}), and perylene-d_{12}) and evaporated under a gentle stream of nitrogen at 40 °C. The dry residue was dissolved in 6 mL of cyclohexane and poured onto an Envi-Chrom P solid-phase extraction (SPE) column (Supelco, St-Quentin Fallavier, France) previously conditioned with water, methanol, and cyclohexane. Washing was performed with 6 mL of cyclohexane, and PAHs were eluted with 12 mL of cyclohexane/ethyl acetate (50:50, v/v). Extracts were evaporated to almost dryness with N_2 at 40 °C and transferred in vials containing 50 ng of chrysene-d_{12} (external standard). After evaporation with N_2 at 40 °C, residues were dissolved in 100 μL of toluene before GC−MS analysis. Standard solutions of phenanthrene, pyrene, and benzo[*a*]pyrene were prepared at concentrations ranging between 0.2 and 40 μg/mL of toluene for calibration.

Extraction of PAHs and Metabolites in Milk. PAHs (phenanthrene, pyrene, and benzo[*a*]pyrene) and their major monohydroxylated metabolites (2-OH-phenanthrene, 3-OH-phenanthrene, 1-OH-pyrene, and 3-OH-benzo[*a*]pyrene) were extracted and quantified in milk using a method adapted from Lutz et al. (*15*). Briefly, glucuronide and sulfate conjugates of PAH metabolites were hydrolyzed with purified *Helix pomatia* juice (Biosepra, Villeneuve la Garenne, France) for 15 h at 39 °C. After the addition of internal standards (phenanthrene-d_{10}, pyrene-d_{10}, perylene-d_{12}, 2-OH-fluorene, and 6-OH-chrysene), liquid−liquid extraction of PAHs and their metabolites was carried out with 40 mL of cyclohexane/ethyl acetate (50:50, v/v) under horizontal shaking for 30 min. After centrifugation (30 min at 3400*g* and 0 °C), the supernatant was evaporated under automated vacuum control. The residue was dissolved in 6 mL of cyclohexane and purified using SPE as previously described. After evaporation of the SPE eluate at 40 °C with N_2, PAHs and their metabolites were further purified by liquid−liquid extraction with 2 mL of cyclohexane and 2 mL of methanol/water (80:20; v/v). Mixtures were homogenized by vortex agitation for 10 s and centrifuged at 1400*g* at room temperature for 5 min to separate the two phases. The top cyclohexane phase containing PAHs was transferred to a new tube, and the bottom methanol/water phase containing purified PAH metabolites was submitted to a second extraction with 2 mL of cyclohexane.

To purify PAHs extracted in cyclohexane, the cyclohexane phase was submitted to saponification followed with a liquid−liquid extraction using water and cyclohexane. The cyclohexane supernatant was partially evaporated with N_2 at 40 °C and supplemented with external standard (chrysene-d_{12}). After evaporation at 40 °C with N_2, the residue was solubilized in 40 μL of toluene, before gas chromatography−mass spectrometry (GC−MS) analysis.

To purify PAH metabolites extracted in methanol/water, methanol was evaporated and the residue was extracted with water and ethyl acetate. After homogenization and centrifugation, the supernatant comprising ethyl acetate was sampled, evaporated at 40 °C with N_2, supplemented with external standard (chrysene-d_{12}), and evaporated to dryness. Derivatization of PAH metabolites was performed for 40 min at 60 °C with 20 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA, Fluke, Buchs, Switzerland) before GC−MS analysis.

Detection (LOD) and quantification limits (LOQ) of PAHs and their metabolites in milk are presented in **Table 1**. LOD is defined as a peak with a signal-to-noise ratio of 3. LOQ is defined as a peak (height or width) with a signal-to-noise of 10.

Extraction of PAHs and Metabolites in Urine. Extraction of PAHs and their metabolites in urine was performed as previously described for milk samples, up to the end of the SPE purification step but without further liquid−liquid extraction or saponification. Briefly, a volume of 5 mL of urine was acidified with 50 μL of glacial acetic acid prior to deconjugation with *Helix pomatia* juice. Extraction was carried out with cyclohexane/ethyl acetate, after sample fortification with internal standards (phenanthrene-d_{10}, pyrene-d_{10}, perylene-d_{12}, 2-OH-fluorene, and 6-OH-chrysene). After centrifugation, the supernatant was evaporated and the residue was solubilized in 6 mL of cyclohexane and purified using SPE. SPE eluates were evaporated to dryness, solubilized in 200 μL of toluene, and supplemented with external standard (chrysene-d_{12}).

Table 1. LOD and LOQ Determined for PAHs and Their Metabolites in Milk (μg/L Milk)

PAH	LOD	LOQ
phenanthrene	0.74	2.78
pyrene	0.14	0.48
benzo[*a*]pyrene	7.29	24.00
2-OH-phenanthrene	0.08	0.25
3-OH-phenanthrene	0.04	0.16
1-OH-pyrene	0.04	0.13
3-OH-benzo[*a*]pyrene	0.07	0.23

After evaporation to dryness with N_2, PAH metabolites were derivatized with 20 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA, Fluke, Buchs, Switzerland). Extracts were analyzed by GC−MS to quantify PAHs and their metabolites.

LOD and LOQ determined for PAHs and their metabolites in urine are presented in **Table 2**.

Quantification of PAHs and Metabolites by GC−MS. Analysis by GC−MS was performed with a gas chromatograph (HP-6890) coupled to a 5973 C quadrupole mass spectrometer (Agilent Technologies, Massy, France). The split/splitless injector was maintained at 280 °C, and the injection volume was 2 μL. The column used for separating PAH and their metabolites was a OV-1 (Ohio-Valley) (30 m × 0.25 mm inner diameter; film thickness, 0.25 μm). The temperature gradient was 110 °C (4.5 min), 20 °C/min until 160 °C, 15 °C/min until 300 °C (10 min hold), and then 20 °C/min until 320 °C (2 min). Analyte ionization was performed by electron ionization (70 eV), and signal acquisition was realized in the single ion monitoring (SIM) mode. Separate analyte determination was performed for parent PAHs and hydroxylated metabolites.

Calculations and Data Processing. The concentrations of studied PAHs and their metabolites in milk and urine were used to present the more representative kinetics of excretion in these biological matrices. The carry-over rates (CORs) from the contaminated matrices (oil, hay, and soil) to the two biological matrices (urine and milk) were also established and compared. They were established by the cumulated excreted quantity of each pollutant between 0 and 24, 0 and 48, and 0 and 57 after ingestion for milk and between 0 and 9, 0 and 24, and 0 and 48 h for urine. The formula of COR calculation used was as follows:

$$\mathrm{COR} = \frac{Qq}{Mm} \times 100 \quad (1)$$

where COR is the carry-over rate (%), Q is the milk or urine quantity produced (g), q is the pollutant concentration in milk or urine (μg/L), M is the matrix quantity ingested (g), and m is the pollutant concentration in the studied matrix (μg/L).

With regard to the metabolites, the CORs were established in the same way from the corresponding parent compounds. Thus, the CORs of 2-OH-phenanthrene and 3-OH-phenanthrene were calculated from phenanthrene; the COR of 1-OH-pyrene was calculated from pyrene; and the COR of 3-OH-benzo[*a*]pyrene was calculated from benzo[*a*]pyrene. An analysis of variance (ANOVA) was carried out on the COR of each compound. The MIXED procedure of SAS software (version 9.1, 2004) and the option of repeated measurements with three controlled factors were used. The studied factors were the ingested matrix, time, and the interaction matrix−time. The treatments were compared on the basis of adjusted average by the Tukey−Kramer multiple test. A 5% threshold of significance was retained.

RESULTS

PAH Ingestion. Fortification of oil, hay, and soil was carried out to compare the transfer of around 50 mg of PAHs via these matrices. The final levels of fortification of the studied matrices are given in **Table 3**. They indicate that the mode of fortification implemented was satisfactory. The average quantities of PAHs ingested by the animals are presented in **Table 4**.

Despite 500 g of hay, which were placed at the disposal of the animals, goats 1, 2, and 3 ingested 102, 319, and 192 g, respectively.

Table 2. LOD and LOQ Determined for PAHs and Their Metabolites in Urine (μg/L Urine)

PAH	LOD	LOQ
phenanthrene	0.23	0.77
pyrene	0.22	0.75
benzo[*a*]pyrene	0.12	0.41
2-OH-phenanthrene	1.56	5.20
3-OH-phenanthrene	1.54	5.15
1-OH-pyrene	0.72	2.38
3-OH-benzo[*a*]pyrene	0.48	1.60

Table 3. PAH Concentrations in the Studied Matrices (n = 1) Following Their Fortification (mg/g)

PAH	matrix	expected concentration	real concentration
phenanthrene	oil	11.0	11.0
	hay	0.1	0.1
	soil	2.0	1.9
pyrene	oil	11.0	11.0
	hay	0.1	0.1
	soil	2.0	1.7
benzo[*a*]pyrene	oil	11.0	11.0
	hay	0.1	0.08
	soil	2.0	1.9

Table 4. Average Quantities (mg) of Ingested PAHs (n = 3)

matrix	phenanthrene	pyrene	benzo[*a*]pyrene
oil	50	50	50
hay	24	28	17
soil	49	43	50

This situation explains the reduced quantity of PAHs introduced via this matrix (**Table 4**). Thus, the ingested average quantities of PAHs from hay were approximately half the quantities ingested from oil or soil (rate of ingestion hay/oil: 0.48, 0.56, and 0.34 and rate of ingestion soil/oil: 0.98, 0.86, and 1 for phenanthrene, pyrene, and benzo[*a*]pyrene, respectively).

Excretion Kinetics of PAHs and Their Metabolites into Urine and Milk. 1-OH-Pyrene was the major metabolite in urine for all three matrices (**Figure 2**). Generally, excretion of 1-OH-pyrene was highest between 9 and 24 h and was found to decline after 24 h. The concentration of 1-OH-pyrene reached 8000 ng/mL at 9 h after the ingestion of contaminated soil. The kinetics of the 3-OH-phenanthrene (**Figure 3**) were similar to kinetics of 1-OH-pyrene but with much lower concentrations (highest concentrations of 2000 ng/mL obtained at 9 h after ingestion of contaminated soil). The concentrations found for 2-OH-phenanthrene were even weaker than those of 3-OH-phenanthrene, while having similar kinetics (600 ng/mL at 9 h from soil; data not presented). Finally, from the parent compounds, only pyrene ($<$ 16 ng/mL) could be determined. In fact, the levels of phenanthrene and benzo[*a*]pyrene in urine were close to the detection limits (0.23 and 0.12 μg/L, respectively).

1-OH-Pyrene was also the PAH metabolite with the highest concentration in milk after soil ingestion (450 ng/mL; **Figure 4**). The excretion of 1-OH-pyrene appeared later in milk compared to urine, with highest levels observed between 24 and 48 h. After 48 h, the concentrations of 1-OH-pyrene were found to decline for all studied matrices. Kinetics of 3-OH-phenanthrene and 2-OH-phenanthrene were similar to that of 1-OH-pyrene for all studied matrices but with much lower concentrations. Highest concentrations were found at 24 h for 3-OH-phenanthrene (30 ng/mL) and at 33 h for 2-OH-phenanthrene after ingestion of contaminated soil (7 ng/mL). Milk concentrations

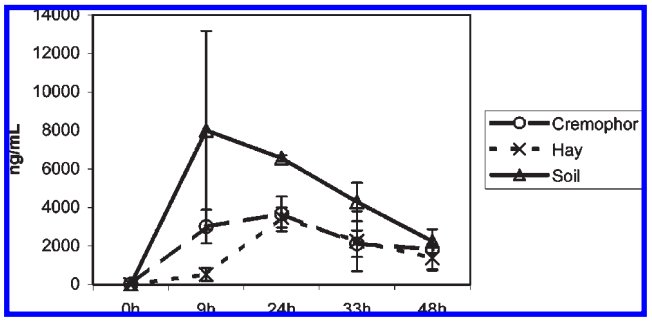

Figure 2. Kinetics (over 48 h) of 1-OH-pyrene excretion in urine (ng/mL) following the ingestion of the studied matrices.

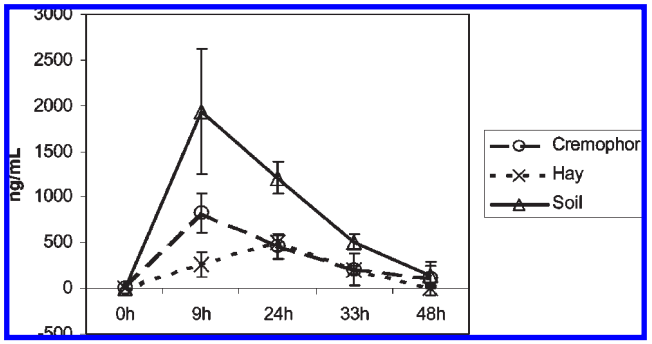

Figure 3. Kinetics (over 48 h) of 3-OH-phenanthrene excretion into urines (ng/mL) following the ingestion of the studied matrices.

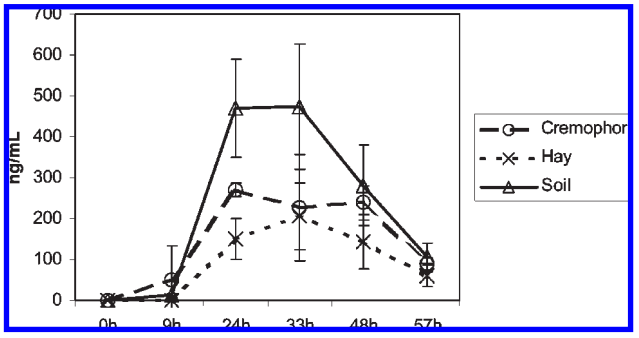

Figure 4. Kinetics (over 57 h) of 1-OH-pyrene excretion into milk (ng/mL) following the ingestion of the studied matrices.

of 3-OH-benzo[*a*]pyrene were always lower than the limit of quantification (0.23 μg/L).

Concerning the parent compounds in milk, concentrations of phenanthrene and benzo[*a*]pyrene were below the limits of detection (0.74 and 7.29 ng/mL, respectively). Kinetics of pyrene are presented in **Figure 5**. Excretion of pyrene occurs mainly during the first 24 h after the administration of the contaminated matrices, with a peak at 9 h (4.5 ng/mL).

Transfer of the Studied Compounds to Urine and Milk. Over the 0−48 h period and whatever the compounds, significant differences were found between COR from soil and COR from hay or chremophor to urine (**Table 5**). Soil COR was at least 2 times higher than hay or chremophor COR. The 3-OH-benzo[*a*]pyrene CORs were very weak (< 0.06%) for all three studied matrices.

Table 5 indicates that 1-OH-pyrene was the most transferred molecule into urine, with a COR of 32.8%. Generally, COR of 1-OH-pyrene were at least 6 times higher than 3-OH-phenanthrene COR and at least 27 times higher than 2-OH-phenanthrene COR. **Table 5** also shows significant differences ($p < 0.05$) for the three studied periods of transfer (0−9, 0−24, and 0−48 h),

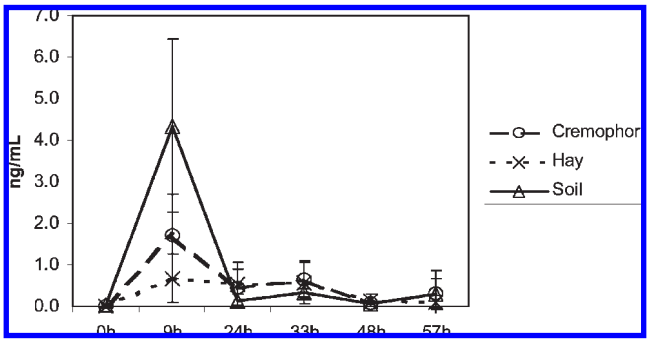

Figure 5. Kinetics (over 57 h) of pyrene excretion in milk (ng/mL) following the ingestion of the studied matrices.

Table 5. Cumulated COR (%) of Metabolites in Urine from the Ingested Matrices for Studied Periods[a]

metabolite	matrix	0−9 h	0−24 h	0−48 h
1-OH-pyrene	oil	2.8 b	12.5 a AB	19.2 a B
	hay	1.2 b	6.9 ab B	16.0 a B
	soil	8.1 c	22.4 b A	32.8 a A
3-OH-phenanthrene	oil	0.8 b	2.0 a B	2.5 a B
	hay	0.7	1.7 B	2.0 B
	soil	1.7 b	4.1 ab A	5.0 a A
2-OH-phenanthrene	oil	0.2 B	0.3 B	0.5 B
	hay	0.1 B	0.3 B	0.4 B
	soil	0.5 c A	0.9 b A	1.2 a A

[a] A and B in the same column and for a given compound: average values with different letters indicate a significant difference ($p \leq 0.05$) between matrices. a, b, and c in the same line: average values with different letters indicate a significant difference ($p \leq 0.05$) between time periods.

demonstrating that transfer of the OH metabolites increased until 48 h.

COR of parent compounds in urine was extremely weak (values lower than 0.06%, data not shown). In milk, transfer of metabolites (except 3-OH-benzo[*a*]pyrene) were significantly higher ($p < 0.0001$) than the transfer of all of the parent compounds, which was very weak (COR ≤ 0.009%). The highest COR was found for 1-OH-pyrene, which was almost 10 times higher than the COR of the other metabolites. It is also interesting to note that no significant matrix effect ($p < 0.05$) was observed on the transfer of 1-OH-pyrene to milk (the COR ranged from 0.93 to 1.74% for the 0−57 h period).

DISCUSSION

In general, the transfer values obtained in this study reveal a substantial apparent absorption of PAHs in the organism of lactating goats. The rate of absorption is at least equivalent to the sum of the excreted metabolites in urine and milk. Thus, apparent absorption of pyrene reaches 34% (**Table 5**). This study did not demonstrate absorption of benzo[*a*]pyrene in lactating goats. These findings are in agreement with that of van Schooten et al. (*27*), who showed that PAHs were largely absorbed by the gastrointestinal tract in rats exposed to a contaminated industrial soil (35 mg of pyrene/kg of dry weight). These authors found that the cumulative urinary 1-OH-pyrene excretion during the first 3 days after fortified soil dosing was 0.2% of the original dose. In the present study, the cumulative COR found for this metabolite into urine was 32.8% (**Table 5**). This difference can be due to the level of soil fortification, which was 1000 times higher in this study, and also due to the specificity of the ruminant digestive tract. In fact, bioaccessibility of the soil contaminants depends upon the contaminant chemistry, the soil properties, and the chemical conditions in the gastrointestinal system. Bioavailability

of soil contaminants primarily depends upon the ability of the stomach and the small intestine to dissolve the contaminant (bioaccessibility) and the ability of the intestinal membranes to absorb the contaminant (*28*). Tang et al. (*29*) found that oral bioaccessibility of PAHs in the intestinal lumen ranged from 9.2 to 60.5%. Furthermore, in gastric content, bioaccessibility was found to range from 3.9 to 54.9%. Jurjanz and Rychen (*30*) found that the *in vitro* bioaccessibility of soil-bound PAHs in successive digestive compartments in cows range from 2.3 to 24%.

The higher COR found for the used soil compared to hay and oil (**Table 5**) is an original result. This finding is interesting given the fact that soil tends to trap the hydrophobic molecules within the clay−humus complex (*10*) and that the pollutants resist hydrolysis and microbial degradations, which occur in soil (*8*). This suggests that the hydrolytic potential of the ruminal microorganisms is such that the release of the PAHs is almost complete whatever the contaminated matrices ingested. The question arising was to know whether our soil was representative of a natural contaminated soil. As described in the Materials and Methods (*24*−*26*), the soil used in this study was fortified according to validated procedures with adequate storage and maturation periods. However, Vessigaud (*31*) highlighted the fact that PAH desorption from aged contaminated soils may be slower and more difficult than that from artificial contaminated soils. In parallel, results of Jurjanz and Rychen (*30*) showed that aging generally reduced the bioaccessibility of soil-bound PAHs. Van Schooten et al. (*27*) suggest that the soil matrix is capable of reducing the absorption of at least pyrene in rats compared to animals that ingested a pure PAH mixture. This is in contrast to our results; in fact, higher CORs were systematically found from soil when compared to hay and oil (**Table 5**).

The form of the excretion curves, similar for all molecules and all matrices, reveals the capacity of the ruminant to absorb, transform, and eliminate quickly the PAHs and their metabolites (**Figures 2**−**4**). It appeared that 1-OH-pyrene is by far the predominant metabolite in urine and milk. Lutz et al. (*15*) observed a similar profile of metabolite excretion in ruminant milk. Supporting Chahin et al. (*19*) observations, 1-OH-pyrene seems to be a good indicator of the PAH exposure of lactating goats as well as for humans (*16*, *18*). Moreover, in the present study, we showed that 1-OH-pyrene was a good indicator of this exposure whatever the ingested matrix. As described by Foth et al. (*32*), the incomplete absorption of benzo[*a*]pyrene and the "first pass effect" before the systemic circulation could explain why benzo[*a*]pyrene and 3-OH-benzo[*a*]pyrene were excreted in urine and milk in negligible quantities (contrary to pyrene, phenanthrene, and their metabolites). In addition, Tang et al. (*29*) showed that bioaccessibility of individual PAHs in soils generally decreased with increasing ring number in both gastric and small intestinal conditions. According to these authors, PAH bioaccessibility decreases with an increase of K_{ow}, directly dependent upon the ring number.

The excretion kinetics of PAHs and their metabolites in milk showed that the metabolite concentrations are much more important than those of the parent compounds (up to 500 times for 1-OH-pyrene). This fact may be explained by the higher solubility of hydroxy metabolites compared to parent compounds and a larger affinity of these compounds to cross the mammary epithelial barrier (*33*). Parent compounds are very slightly transferred toward milk whatever the administered matrices. This is in agreement with the results obtained by West and Horton (*34*) in ewes (transfer of 0.012% for 3-methylcholanthrene and benzo[*a*]pyrene). These authors indicated that, after 24 h, the hydrocarbons had been metabolized between 33 and 55%. In this study, metabolites were transferred toward milk with COR lower than 2%. Lutz et al. (*15*) also obtained values of transfer to milk lower than 1.5%, following a chronic administration of contaminated soil to milking cows. To our knowledge, no data are available concerning the potential toxicity of 1-OH-pyrene, which is the most transferred molecule.

From this study, it can be concluded that apparent absorption of PAHs from soil and hay is far from being negligible. Indeed, the absorption rates, which correspond at least to the urine and milk excretion rates, have reached values up to 34%. This study also reveals that bioavailability of soil-bound PAHs is at least similar to the bioavailability of PAHs from hay or oil. Because soil is generally more contaminated than fodder with PAHs, the results presented here suggest that greater attention should be focused on potential soil ingestion by grazing ruminant animals. Thus, soil ingestion should be considered for the risk assessment in the food chain. Further studies on PAH transfer from naturally contaminated matrices should now be carried out.

ACKNOWLEDGMENT

The authors thank Dr. Arsène Randrianariveloseheno and Hervé Toussaint for their assistance.

LITERATURE CITED

(1) Llobet, J. M.; Falco, G.; Bocio, A.; Domingo, J. L. Exposure to polycyclic aromatic hydrocarbons through consumption of edible marine species in Catalonia, Spain. *J. Food Prot*. **2006**, *69*, 2493–2499.

(2) Wild, S. R.; Jones, K. C.; Johnston, A. E. The polynuclear aromatic hydrocarbon (PAH) content of herbage from a long-term grassland experiment. *Atmos. Environ*. **1992**, *26A*, 1299–1307.

(3) Phillips, D. H. Polycyclic aromatic hydrocarbons in the diet. *Mut. Res*. **1999**, *443*, 139–147.

(4) Wang, Y.-Q.; Zuo, Q.; Jiao, X.-C.; Wu, S.-P.; Tao, S. Polycyclic aromatic hydrocarbons in plant leaves from Peking University campus and nearby in summer season. *Huanjing Kexue* **2004**, *25*, 23–27.

(5) Crépineau, C.; Rychen, G.; Feidt, C.; Le Roux, Y.; Lichtfouse, E.; Laurent, F. Contamination of pastures by polycyclic aromatic hydrocarbons (PAHs) in the vicinity of a highway. *J. Agric. Food Chem*. **2003**, *51*, 4841–4845.

(6) Wild, S. R.; Jones, K. C. Polynuclear aromatic hydrocarbons in the United Kingdom environment: A preliminary source inventory and budget. *Environ. Pollut*. **1995**, *88*, 91–108.

(7) Meharg, A. A.; Wrigth, J.; Dyke, H.; Osborn, D. Polycyclic aromatic hydrocarbon (PAH) dispersion and deposition to vegetation and soil following a large scale chemical fire. *Environ. Pollut*. **1998**, *99*, 29–36.

(8) Verstraete, W.; Devliegher, W. Formation of non-bioavailable organic residues in soil: Perspectives for site remediation. *Biodegradation* **1996**, *7*, 471–485.

(9) Fries, G. F.; Paustenbach, D. J. Evaluation of potential transmission of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-contaminated incinerator emissions to humans via foods. *J. Toxicol. Environ. Health* **1990**, *29*, 1–43.

(10) Wild, S. R.; Jones, K. C. Organic chemicals entering agricultural soils in sewage sludges: Screening for their potential to transfer to crop plants and livestock. *Sci. Total Environ*. **1992**, *119*, 85–119.

(11) Fries, G. F. Transport of organic environmental contaminants to animal products. *Rev. Environ. Contam. Toxicol*. **1995**, *141*, 71–109.

(12) Fries, G. F. Ingestion of sludge applied organic chemicals by animals. *Sci. Total Environ*. **1996**, *185*, 93–108.

(13) Bryselbout, C.; Henner, P.; Carsignol, J.; Lichtfouse, E. Polycyclic aromatic hydrocarbons in highway plants and soils. Evidence for a local distillation effect. *Analysis* **2000**, *28*, 32–35.

(14) Grova, N.; Monteau, F.; Le Bizec, B.; Feidt, C.; André, F.; Rychen, G. Determination of phenanthrene and hydroxyphenanthrenes in various biological matrices at trace levels using gas chromatography−mass spectrometry. *J. Anal. Toxicol*. **2005**, *29*, 175–181.

(15) Lutz, S.; Feidt, C.; Monteau, F.; Rychen, G.; Le Bizec, B.; Jurjanz, S. Effect of exposure to soil-bound polycyclic aromatic hydrocarbons on milk contaminations of parent compounds and their monohydroxylated metabolites. *J. Agric. Food Chem.* **2006**, *54*, 263–268.
(16) Bouchard, M.; Viau, C. Urinary excretion kinetics of pyrene and benzo[*a*]pyrene metabolites following intravenous administration of the parent compounds or the metabolites. *Toxicol. Appl. Pharmacol.* **1996**, *139*, 301–309.
(17) Grova, N.; Feidt, C.; Laurent, C.; Rychen, G. [^{14}C]Milk, urine and faeces excretion kinetics in lactating goats after an oral administration of [^{14}C]polycyclic aromatic hydrocarbons. *Int. Dairy J.* **2002**, *12*, 1025–1031.
(18) Pigini, D.; Cialdella, A. M.; Faranda, P.; Tranfo, G. Comparison between external and internal standard calibration in the validation of an analytical method for 1-hydroxypyrene in human urine by high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1013–1018.
(19) Chahin, A.; Guiavarc'h, Y. P.; Dziurla, M. A.; Toussaint, H.; Feidt, C.; Rychen, G. 1-Hydroxypyrene in milk and urine as a bioindicator of polycyclic aromatic hydrocarbon exposure of ruminants. *J. Agric. Food Chem.* **2008**, *56*, 1780–1786.
(20) Jarrige, R. *Alimentation des Bovins, Ovins et Caprins*; INRA: Paris, France, 1988; p 471.
(21) Gelderblom, H.; Verweij, J.; Nooter, K.; Sparreboom, A. Cremophor EL: The drawbacks and advantages of vehicle selection for drug formulation. *Eur. J. Cancer* **2001**, *37*, 1590–1598.
(22) Lutz, S. Evaluation du risque de transfert des hydrocarbures aromatiques polycycliques du sol vers le lait chez le ruminant laitier. Ph.D. in Agronomical Sciences. INPL, Nancy, France, July **2006**.
(23) Le Masson, P. Mise au point d'une approche *in vitro* de la disponibilité des HAP du sol pour les ruminants. Master Degree, INPL, Nancy, France, **2003**.
(24) Liste, H. H.; Alexander, M. Butanol extraction to predict bioavailability of PAHs in soil. *Chemosphere* **2002**, *46*, 1011–1017.
(25) Kottler, B. D.; Alexander, M. Relationship of properties of polycyclic aromatic hydrocarbons to sequestration in soil. *Environ. Pollut.* **2001**, *113*, 293–298.
(26) Rost, H.; Loibner, A. P.; Hasinger, M.; Braun, R.; Szolar, O. H. J. Behavior of PAHs during cold storage of historically contaminated soil samples. *Chemosphere* **2002**, *49*, 1239–1246.
(27) Van Schooten, F. J.; Moonen, E. J. C.; van der Wal, L.; Levels, P.; Kleinjans, J. C. S. Determination of the polycyclic aromatic hydrocarbons (PAH) and their metabolites in blood, feces and urine of rats orally exposed to PAH contaminated soils. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 317–322.
(28) Grøn, C.; Anderser, L. Human bioaccessibility of heavy metals and PAH from soil. Technology programme for soil and groundwater contamination. Danish Environmental Protection Agency. Enviromental Project, **2003**, Vol. *840*, p 113.
(29) Tang, X.-Y.; Tang, L.; Zhu, Y.-G.; Xing, B.-S.; Duan, J.; Zheng, M.-H. Assessement of the bioaccessibility of polycyclic aromatic hydrocarbons in soils from Beijing using an in vitro test. *Environ. Pollut.* **2006**, *140*, 279–285.
(30) Jurjanz, S.; Rychen, G. In vitro bioaccessibility of soil-bound polycyclic aromatic hydrocarbons in successive digestive compartments in cows. *J. Agric. Food Chem.* **2007**, *55*, 8800–8805.
(31) Vessigaud, S. Re-mobilisation des hydrocarbures aromatiques polycycliques de terres industrielles de cokerie et mécanismes impliqués dans le "vieillissement" du fluoranthène. Ph.D. in Agronomical Sciences. INPL, Nancy, France, February 2007.
(32) Foth, H.; Khal, R.; Khal, G. F. Pharmacokinetics of low doses of benzo(*a*)pyrene in the rat. *Food Chem. Toxicol.* **1988**, *26*, 45–51.
(33) Cavret, S.; Feidt, C.; Le Roux, Y.; Laurent, F. Short communication: Study of mammary epithelial role in polycyclic aromatic hydrocarbons transfer to milk. *J. Dairy Sci.* **2005**, *88*, 67–70.
(34) West, C. E.; Horton, B. J. Transfert of polycyclic aromatic hydrocarbons from diet to milking rats, rabbits and sheep. *Life Sci.* **1976**, *19*, 543–551.

Received February 3, 2009. Revised manuscript received May 7, 2009. This work was supported by grants from the Agence de l'Environnement et de la Maîtrise de l'Energie (ADEME) and the Institut National de la Recherche Agronomique (INRA).