

## Contamination of Pastures by Polycyclic Aromatic Hydrocarbons (PAHs) in the Vicinity of a Highway

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To assess PAH contamination pastures, grass and soil samples have been collected from 10 m (d1), 50 m (d2), and 150 m (d3) perpendicular to a French highway (70 000 vehicles per day) and at a control site in a rural area away from nearby contaminating sources. Total PAH concentration ranges from 767 ng/g dry weight to 3989 ng/g dry weight, according to the matrix and the distance from the highway. Distance is not a significant factor for PAH deposition on grass, while in soil it has an effect between d1 and d2 or d3. The total PAH concentration in highway samples is 8 times higher than in control site samples for grass and 7 to 4 times higher for soil. Fluoranthene, pyrene, and phenanthrene are the major PAHs in grass samples at the control site and the highway, but the concentrations are about 5 times higher near the highway. In soil samples collected near the highway, the values of concentrations between all compounds are not statistically different. PAH deposition on grass is linked to the physicochemical properties of the compounds, which lead to a specific distribution of each molecule (according to their volatility and the number of aromatic rings) while no specific behavior is revealed in soil.

**KEYWORDS:** PAHs; grass; soil; highway

### INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are potentially mutagenic compounds and are widely present in ecosystems (1). They are produced by incomplete combustion during natural events (e.g., forest fires, volcanic eruptions, etc.) and also by human activities. One of the main contaminating sources is road traffic, as PAHs are emitted by vehicle exhausts (2–4). The increase of pollution sources such as vehicle exhausts has raised concern about the possible transfer of PAHs from plants growing near highways to the lactating ruminants grazing in pastures located in the vicinity of these major roads. When emitted, PAHs are deposited next to the sources leading to a risk of contamination of different surfaces. In the case of highways, deposition occurs on either side of the road (1) (i.e., on agricultural fields such as pastures when the road runs through rural areas). PAH deposition on grasses and soils differs according to the physicochemical properties of the compounds emitted and to their ability to be transported in the gaseous or particulate phase (5).

Several authors have reported PAH profiles in the vicinity of highways or major roads in different matrixes: soil and microflora, litter, snow, specific plants, or air (6–8). Nonetheless, the potential transfer of PAHs from animal pastures to milk is not well understood, notably owing to the scarcity of investigations on pastures. Furthermore, it is known that

ruminants ingest not only grass but also soil, amounting to 0.5–18% of the total ingested dry matter (9), a fact that must be taken into account when assessing PAH transfer. Therefore, we present here a study on the contamination of pastures by PAHs in the vicinity of a highway (10).

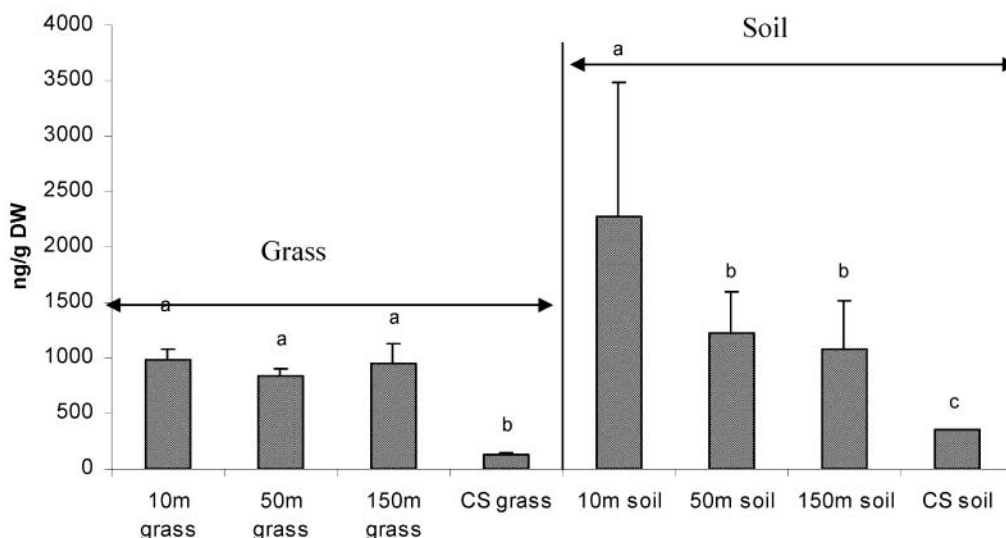
### MATERIALS AND METHODS

**Sampling Site.** To assess the PAH contamination of pastures, samples were collected near a highway located in the north east of France, orientated north–south, and which connects Luxembourg to Eastern France. Its daily traffic is 70 000 vehicles. Samples were obtained from a pasture bordering the highway where the air is characterized as being without any influence from other major sources of pollution by AERFOM (Association pour l'Exploitation du Réseau de Mesures de la qualité de l'air des vallées de la Fensch, de l'Orne, et de la Moselle, Association for assessing air quality in the valleys of the Fensch, Orne, and Moselle rivers).

Grass and soil samples were collected in autumn 2001. At this time of the year, the permanent pasture was covered with grass mainly composed of *poacea*. To assess the specific risk of exposure due to the highway, samples were also collected at the same time from another permanent pasture, also covered with *poacea*, located on a control farm in a rural area, away from any contaminating sources. The chosen field is surrounded by woods, and the nearest road is 700 m away.

**Sampling Method.** Samples of both grass and soil were collected downwind along a transect perpendicular to the verge of the highway. Nine sub-samples were collected at each point, 10 m (d1), 50 m (d2), and 150 m (d3) from the road. On the two sampling sites, grass was cut with prewashed scissors (using distilled water, acetone, and hexane)

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**Figure 1.** Total PAH in grass and soil samples from 0 to 150 m (CS = control site) (a,b,c) for a same matrix, mean values with a different letter differ significantly ( $p < 0.05$ ).

2 cm from the plant base to avoid collecting roots and soil. Each subsample was wrapped into tin foil then pooled together at the laboratory to obtain approximately 500 g of wet matter to represent each of the three distances. Grass samples were immediately stored in a climate chamber regulated at 22 °C and 60–80% relative humidity for 5 days. Dried samples were then ground to 1.5-mm pieces to perform PAH analyses. The surficial part of soil (0–3 cm) was collected at the same places, by use of a prewashed garden dibble (using distilled water, acetone, hexane). Roots and pieces of grass were removed, and subsamples were pooled together (to give approximately 500 g of wet matter) in an aluminum tub and placed in the same climate chamber as the grass. Soil samples were then ground and sieved through a 2-mm sieve. Grass and soil samples were stored in solvent-washed glass pots to perform the PAH analysis.

**PAH Analysis.** Detailed extraction procedures are described in the NF ISO 15302 method (...dissolution of a measured quantity of fat matter in an organic solvent which is introduced through an alumine filled chromatography column to separate the lipidic fraction from the analytes. After elution, the extract is concentrated...) and were conducted by Micropolluant Technology Ltd (Thionville, France). PAHs were quantified by gas chromatography coupled to mass spectrometry in the single ion monitoring mode, using internal deuterated standards: naphthalene- $d_8$  ( $m/z$  136) for naphthalene, acenaphthene- $d_{10}$  ( $m/z$  164) for acenaphthene, acenaphthylene and fluorene, phenanthrene- $d_{10}$  ( $m/z$  188) for phenanthrene, anthracene, fluoranthene, and pyrene, chrysene- $d_{12}$  ( $m/z$  240) for chrysene and benzo[*a*]anthracene and perylene- $d_{12}$  ( $m/z$  264) for perylene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene. Analytical detection levels is 0.5 ng PAH  $g^{-1}$  grass and 0.5 ng PAH  $g^{-1}$  soil. Losses of analytes were quantified by scientists from Micropolluants Technologies as follows: 40–70% for acenaphthylene to fluorene and 0–30% for the heaviest compounds.

**Statistical Analysis.** Effects on origin (soil and grass) and effects of distance (d1, d2, d3) on molecular concentrations were determined by analysis of variance using the SAS statistical software General Linear Model (GLM) procedure (11). The student's *t*-test was used for comparison of the means at a significant level of 0.05. Data are presented as mean  $\pm$  standard deviation.

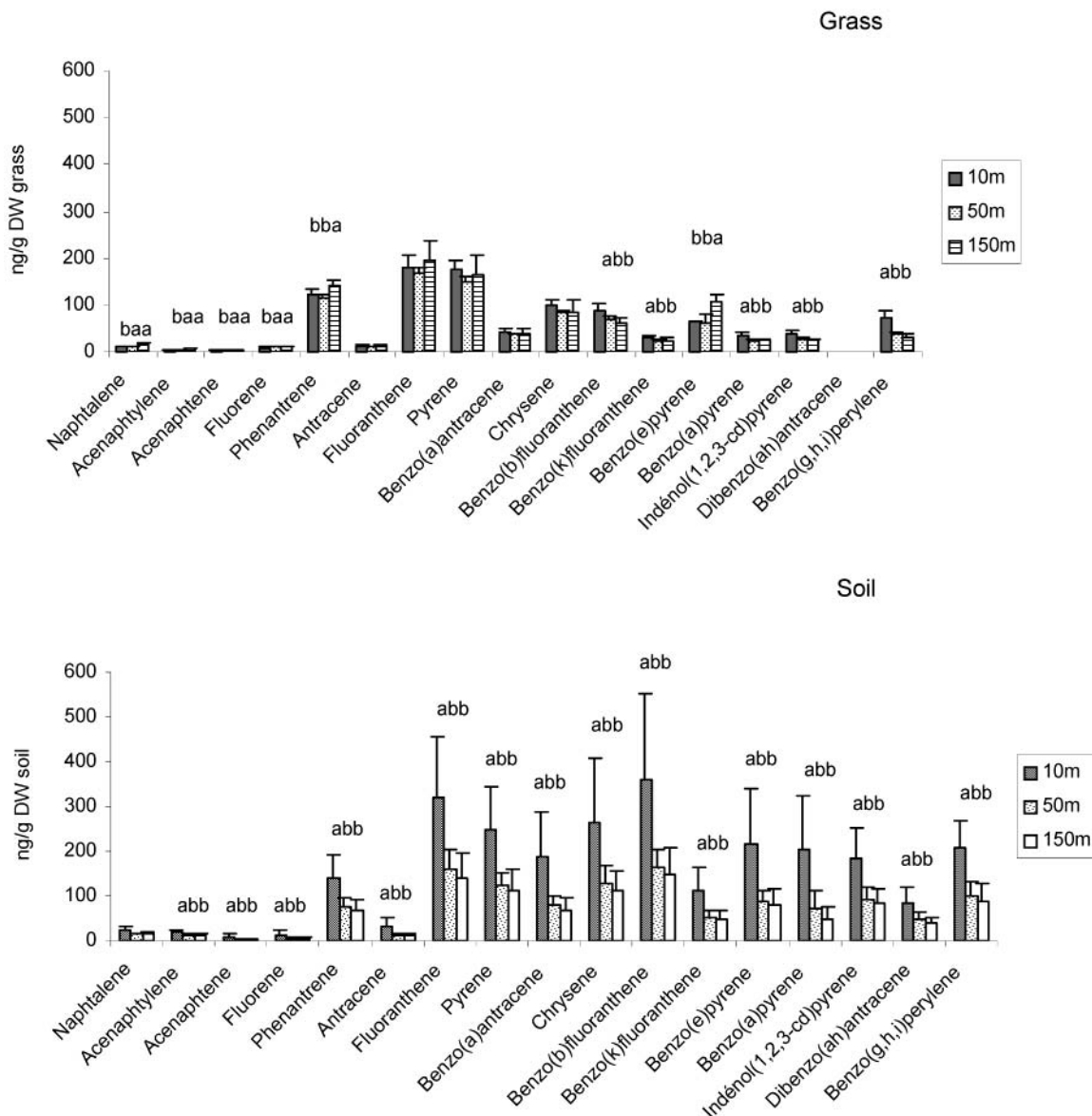
## RESULTS AND DISCUSSION

**Total PAH Concentrations in Grass and Soil from the Pasture Near the Highway.** The total PAH concentration of both matrixes collected on the pasture ranged from 767 ng/g DW to 3989 ng/g DW, depending on the matrix and the distance from the highway. Statistical analyses demonstrated that the distance from the road is not a significant factor for PAH

distribution in grass; the total PAH concentration remained similar for the studied distances (d1, d2, d3), 981 ng/g DW, 836 ng/g DW, and 950 ng/g DW, respectively. However, PAH distribution in soil was partly influenced by distance; total concentration decreased from the distance d1 (2627 ng/g DW) to the distance d2 (1228 ng/g DW), then remained similar for d2 and d3 (1078 ng/g DW) (Figure 1). The total PAH concentration in both grass and soil samples was also compared with total PAHs concentration of the control site. Our results revealed a significant difference for grass (a factor of 8) and soil (a factor of 7 for d1, a factor of 4 for d2 and d3). Furthermore, in grass from the control site, several high molecular weight compounds were not detected (benzo[*e*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, benzo[*g,h,i*]perylene).

Our results are in accordance with previous findings in soil studies. For example, a rapid decrease in the first 10 meters and then a slow decrease were observed in soil samples along a major highway in the Czech Republic (6). Concerning plant matrixes, in a previous study, concentrations in leaf litter showed a rapid decrease from 30 to 100 m away from an Australian highway (3). In our study, no differences could be found for grass whatever the distance from the highway is. It is therefore necessary to carry out further experiments to test several hypotheses (daily traffic, season of sampling, age of grass, etc.). The total PAH concentration value near the highway is higher, for both grass and soil, than the total PAH concentration at the control site but has the same levels as in the vicinity of other highways (8,6).

**PAH Distribution Along the Distance d1, d2, and d3 for Grass and Soil Samples.** In grass samples, three groups of molecules were distinguished according to the evolution of their concentration (Figure 2). A first group is composed of PAHs whose concentrations remained similar for d1 and d2 and increased after d2 (naphthalene, acenaphthylene, acenaphthene, and fluorene). A second group is composed of PAHs whose concentrations remained the same from d1 to d3 (anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, and chrysene). A third group is composed of PAHs whose concentrations decreased between d1 and d2 and remained stable between d2 and d3 (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*g,h,i*]perylene).



**Figure 2.** Role of the distance for each PAHs in grass and soil samples. (a,b) for the same compound, mean values with a different letter differ significantly ( $p < 0.05$ ).

In soil samples, all PAHs composed of more than two rings behaved the same from d1 to d3; their concentrations decreased significantly between d1 and d2 or d3 (**Figure 2**).

PAH distribution in grass from a pasture in the first 150 m perpendicular to a highway seems to be linked to the physico-chemical properties of the compounds. The first group of molecules statistically identified for the evolution of their concentrations along the distance is composed of PAHs of 2 to 3 aromatic rings, which are the most volatile and the lowest molecular weight PAHs (naphthalene, acenaphthylene, acenaphthene, and fluorene). These compounds are also mainly found in gaseous phase in ambient air, mainly adsorbed on vegetal matrix, and can be transported by wind. The risk of exposure is higher for d3 than for d1 and d2. The second group identified in grass samples, whose concentrations remained stable from 0 to 150 m, is composed (with the exception of anthracene) of PAHs of 4 aromatic rings (fluoranthene, pyrene, benzo[a]-anthracene, and chrysene). They have a higher molecular weight than the previous compounds, and are mainly found in ambient air both in gaseous and particulate phase. For these compounds, the distance to the emission source does not influence their

distribution, suggesting a risk of exposure all along the 150 m and a more stable deposition on grass. The third group, whose concentrations decreased after d1, is composed of the heaviest PAHs of 5 or 6 aromatic rings and mainly coupled with particles in ambient air (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno(1,2,3-cd)pyrene, and benzo(g,h,i)perylene). These compounds are rapidly deposited, suggesting a lack of transport. The risk of exposure is the highest in d1.

In soil samples from the pasture bordering the highway, the PAH distribution did not seem to be linked to the physico-chemical properties of the compounds (**Figure 2**). The difference between the compounds was lower in soil than in grass. From d1 to d2, the compounds remained in the same proportions with a possible distinction between one group whose concentrations remained low and another group whose concentrations remained high. The first group is mainly composed of low molecular weight, less than four aromatic rings, and high volatility compounds (which are also the least persistent compounds in soil, acenaphthylene, acenaphthene, fluorene, and anthracene). The second group consists mainly of high molecular weight compounds, low volatility, and four and more than four aromatic

**Table 1.** Relative Proportion of the Different PAHs in Grass for Distance 50 m (d2) and Control Site (Molecules Are Classified According to Their Contribution)

highway	ng/g DW	stat sig <sup>a</sup>	%	cum %	control site	ng/g DW	%	cum %
fluoranthene	168.25	a	20.11	20.11	fluoranthene	28.64	23.57	23.57
pyrene	148.72	b	17.77	37.88	pyrene	24.26	19.97	43.54
phenanthrene	114.27	c	13.66	51.54	phenanthrene	22.99	18.92	62.49
chrysene	82.81	d	9.9	61.44	benzo[ <i>b</i> ]fluoranthene	16.94	13.94	76.43
benzo[ <i>b</i> ]fluoranthene	68.81	de	8.22	69.66	chrysene	10.94	9.00	85.43
benzo[ <i>e</i> ]pyrene	61.52	e	7.35	77.01	benzo[ <i>k</i> ]fluoranthene	5.84	4.80	90.23
benzo[ <i>a</i> ]anthracene	38.87	f	4.65	81.66	anthracene	4.89	4.02	94.25
benzo[ <i>g,h,i</i> ]perylene	37.86	f	4.53	86.19	naphthalene	2.39	1.97	96.22
indeno[1,2,3- <i>cd</i> ]pyrene	27.68	fg	3.31	89.50	benzo[ <i>a</i> ]anthracene	2.33	1.92	98.14
benzo[ <i>a</i> ]pyrene	24.09	fg	2.88	92.38	fluorene	2.31	1.86	100
benzo[ <i>k</i> ]fluoranthene	23.24	fg	2.78	95.16				
anthracene	11.57	g	1.38	96.54				
naphthalene	11.32	g	1.35	97.89				
fluorene	10.06	g	1.2	99.09				
acenaphthylene	4.62	g	0.55	99.64				
acenaphthene	2.87	g	0.36	100				

<sup>a</sup> Statistical significance, mean values affected with a different letter differ significantly ( $P < 0.05$ ).

**Table 2.** Relative Proportion of the Different PAHs in Soil for Distance 50 m (d2) (Molecules Are Classified According to Their Contribution)

highway	ng/g DW	stat sig <sup>a</sup>	%	cum %	control site	ng/g DW	%	cum %
benzo[ <i>b</i> ]fluoranthene	162.55	a	13.22	13.22	fluoranthene	58.73	16.61	16.61
fluoranthene	161.52	a	13.14	26.36	benzo[ <i>b</i> ]fluoranthene	48.18	13.63	30.24
chrysene	126.24	ab	10.27	36.63	pyrene	45.20	12.79	43.03
pyrene	122.65	ab	9.98	46.61	chrysene	40.72	11.52	54.55
benzo[ <i>ghi</i> ]perylene	101.63	ab	8.27	54.88	phenanthrene	28.21	7.98	62.53
indeno[1,2,3- <i>cd</i> ]pyrene	92.68	ab	7.54	62.42	indeno[1,2,3- <i>cd</i> ]pyrene	25.36	7.17	69.7
benzo[ <i>e</i> ]pyrene	88.75	ab	7.22	69.64	benzo[ <i>a</i> ]anthracene	23.06	6.52	76.22
benzo[ <i>a</i> ]anthracene	78.62	bc	6.39	76.03	benzo[ <i>ghi</i> ]perylene	20.98	5.94	82.16
phenanthrene	75.59	bc	6.15	82.18	naphthalene	19.99	5.65	87.81
benzo[ <i>a</i> ]pyrene	70.01	bc	5.69	87.87	benzo[ <i>k</i> ]fluoranthene	15.81	4.47	92.28
benzo[ <i>k</i> ]fluoranthene	53.63	bc	4.36	92.23	dibenzo[ <i>ah</i> ]anthracene	10.63	3.01	95.29
dibenzo[ <i>ah</i> ]anthracene	47.68	bc	3.88	96.11	acenaphthylene	4.98	1.41	96.7
naphthalene	14.54	c	1.18	97.29	anthracene	3.46	0.98	97.68
anthracene	12.94	c	1.05	98.34	fluorene	3.27	0.92	98.6
acenaphthylene	11.53	c	0.94	99.28	benzo[ <i>a</i> ]pyrene	1.93	0.55	99.15
fluorene	5.22	c	0.43	99.71	benzo[ <i>e</i> ]pyrene	1.61	0.46	99.61
acenaphthene	3.64	c	0.29	100	acenaphthene	1.40	0.39	100

<sup>a</sup> Statistical significance, mean values affected with a different letter differ significantly ( $P < 0.05$ ).

rings. These are the most persistent compounds in soil, because their persistence in this matrix increases with the number of aromatic rings (12) (fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*ah*]anthracene, and benzo[*g,h,i*]perylene). These results suggest an accumulation of more than three ring compounds in soil. This is in accordance with previous studies; these PAHs are strongly adsorbed to the soil (13), while the low molecular weight compounds are known to be degraded by the indigenous microbial population (14).

**Major PAHs in Grass and Soil in the Vicinity of the Highway and at the Control Site.** As the distance from the verge of the road did not have a statistically detectable effect for the grass, the PAH profile in this matrix was studied for the distance d2 (50 m). Statistical analysis was performed to determine the relative proportion of each compound in the profile (Table 1). Major compounds are fluoranthene (concentration > 160 ng/g DW), pyrene (concentration > 140 ng/g DW), and phenanthrene (concentration > 110 ng/g DW). These three molecules represent more than 50% of the total PAHs. The same analysis was performed on grass control site samples, and the results were similar; fluoranthene, pyrene, and phenanthrene are also the main compounds of the mixture. Their concentrations represent more than 60% of the total concentra-

tions. Nevertheless, the values of the concentrations were much lower in the control site samples, less than 30 ng/g DW.

The PAH profile in soil samples was also examined for the distance d2 (50 m); the distance has a statistically detectable effect on soil concentrations after the distance d1 (10 m) (Table 2). Major compounds are benzo[*b*]fluoranthene and fluoranthene (concentration > 160 ng/g DW), whose concentrations represent more than 25% of the total, whereas the values of the concentrations of the other compounds were not significantly different (Table 2), meaning that no major compounds could actually be detected for the soil samples. Major compounds at the control site were similar, with fluoranthene and benzo[*b*]fluoranthene representing 30% of the total concentrations, but their concentration values were much lower (less than 60 ng/g DW).

The PAH profile in grass samples near the highway was mainly composed of four and five aromatic ring molecules (except phenanthrene); 81% of the total concentration is composed of this kind of compound (fluoranthene, pyrene, chrysene, benzo[*b*]fluoranthene, benzo[*e*]pyrene, and benzo[*a*]anthracene). The deposition of these compounds on grass can be in gaseous or in particulate phase, according to the climatic conditions. They all present a medium volatility. In soil, the PAH profile is more due to high number of aromatic ring compounds; the six aromatic ring molecules (benzo[*g,h,i*]-



perylene and indeno(1,2,3,cd)pyrene) were already present in the first 60% of the total concentrations. This result suggests an accumulation in soil; PAH persistence in soil increases with the number of aromatic rings (12), while when deposited on grass, they can be photodegraded, windblown, or washed-off (15).

The influence of the highway is measured when comparing concentration values with those of the control site. Vehicle exhausts lead to an increasing of concentrations of all PAHs. In grass samples from the control site, several high molecular weight compounds were not detected (benzo[e]pyrene, benzo[a]pyrene, indeno(1,2,3)pyrene, and benzo(g,h,i)perylene). In soil samples, the same compounds were detected in both control site samples and highway samples, but in the latter, they were in much higher concentrations (a factor of 3 to 5). Furthermore, major PAHs detected in the pasture by the highway (fluoranthene, pyrene, phenanthrene) are characteristic of vehicular emission (16), suggesting an actual risk of contamination for the pastures bordering major roads.

## CONCLUSION

Three points in this study give new insight into PAH pasture contamination. From the verge of a highway to a distance of 150 m, the grass and soil are actually contaminated by these compounds at a significantly higher level than in a rural control site. PAH deposition on grass is linked to the physicochemical properties of the compounds, leading to a specific distribution of each molecule (according to their volatility and the number of aromatic rings). The lighter PAHs found on the grass are more readily transported to 50 and 150 m. The heavier PAHs cannot undergo the same kind of airborne or waterborne transport, so they remain close to the road. The PAHs detected in the soil are not distributed from 0 to 150 m, according to their physicochemical properties, because the soil interacts immediately with the compounds (microbial degradation, adsorption, half-lives of the molecules) and there is accumulation in this matrix (no photodegradation, no wind-blown...). The difference of the evolution of concentrations between grass and soil is a result that needs to be confirmed by further experiments. For example, we will carry out a study using test plants (lollium perenne) to assess the kinetics of PAHs deposition and accumulation in a vegetal matrix. Major PAHs detected near the highway are quite similar to those detected at the control site but in higher concentrations. The highway is actually a PAH contamination source whose effect is still detected 150 m away on agricultural fields. Nevertheless, PAH deposition and consequently the risk of toxicity, may differ from one site to another, according to the intensity of the vehicular traffic or the season of sampling. It would now be of great interest to produce a risk assessment model incorporating the deposition features of the pollutants and their physicochemical properties and to link these to soil and grass consumption by lactating animals to provide an estimate of risk.

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