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Detection of Polycyclic Aromatic Hydrocarbon Levels in Milk Collected Near Potential Contamination Sources

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Polycyclic aromatic hydrocarbons (PAHs), mainly formed by incomplete anthropogenic organic matter combustion, are ubiquitous in the environment. To assess milk PAH contamination sources, milk samples were collected from the tank milk at farms located near potential contaminating emission sources such as cementworks, steelworks, and motorways. PAH analyses were carried out by gas chromatography coupled to mass spectrometry. Eight PAHs were identified in milk: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, fluoranthene, pyrene, and benzo[*a*]anthracene. For all potential contaminating sources, these eight PAHs were detected with similar profiles and at low concentrations except for fluorene and naphthalene, for which source−molecule interaction is pointed out.

KEYWORDS: PAHs; milk; ruminant; cementwork; motorway; steelwork

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are generated by incomplete combustion of organic material arising, in part, from natural combustion such as forest fires and volcanic eruptions. For the most part, however, human activities, such as industrial production, transportation, and waste incineration generate significant levels of PAHs (*1*−*3*). Several PAHs are known to be potential human carcinogens [benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and benzo(*g*,*h*,*i*)perylene] (*4*). These organic pollutants are ubiquitous in the environment. Due to their physical and chemical properties they migrate through the food chain into hydrophobic compartments. Thus, they accumulate in lipids at the end of the food chain (*5*−*9*). Human exposure to these compounds occurs mainly through the diet. Knowledge of transfer pathways through the food chain is a major issue in food safety. Furthermore, milk is massively used in the human diet, especially for children, who may be particularly susceptible to the presence of carcinogens.

So far, there have been few investigations of PAH contamination and transformation in milk and dairy products (*7*, *10*, *11*). The objectives of this study were to assess sources of PAHs in milk sampled at different farms located near potential contaminating sources.

MATERIALS AND METHODS

Milk Sampling. Milk samples were collected in March 2000 from 14 farms. The farms were chosen according to three parameters: type of potential contamination source(s), distance from this source (a perimeter of 4 km around the source), and cows fed with fodder produced in summer on the farm fields (winter ration composed of maize and grass silage, hay, and complements). Three kinds of potential contamination sources were selected: a cementwork (four farms sampled), a highway (four farms sampled), and combined sources (three farms simultaneously exposed to, at least, two emission sources: steelworks, cementworks, incinerators, and highways). Three control farms were chosen with fields >30 km away from any major source of contamination. For each dairy farm, one milk sample of 500 mL was collected in March, directly from the milk tank (4 °C) when stirring, then conditioned in amber bottles, and stored at −20 °C for <1 week before PAH analysis by the MicroPolluant Technology Laboratory (Centre Analytique de Spectrométrie de Masse, Thionville, France). Analysis is performed to detect the 16 compounds classified on the list established by the U.S. Environmental Protection Agency and considered as priority pollutants (*12*).

PAH Analysis. All reagents [Silicagel 60, 63−200 μm, sodium sulfate (anhydrous, for analyses, ASC, ISO), sodium oxalate (for analyses, ASC, ISO)] were purchased from Merck Eurolab, Darmstadt, Germany. Solvents (toluene, *n*-hexane, methylene chloride, methanol, diethyl ether, and petroleum ether) were of Suprasolv grade, purchased from Merck Eurolab. The internal standards mixture consisted of a cocktail of the following molecules dissolved in toluene: naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}, at a concentration of 50 ng/μL (Chemservice, West Chester, PA).

The extraction of fat was conducted following an AOAC method (Official Method 989.05, fat milk-ether extraction) with the following modifications: 2.5 g of sodium oxalate was added to 250 mL of milk in a funnel. Extraction and cleanup efficiencies for PAHs in fortified fat sample range from 40 to 125%. After mild agitation, 250 mL each of methanol, diethyl ether, and petroleum ether were subsequently added to the milk, applying agitation for 1 min after each addition. Then, the

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Table 1. Polycyclic Aromatic Hydrocarbon Mean (M) Concentrations in Tank Milk and Their Standard Deviations (SD), Expressed in Nanograms per Gram of Milk Fat

	NA[a]		ACEY[a]		ACEA[a]		FLUO[a]		ANT[a]		FLUT[a]		PYR[a]		B[a]A[a]	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
control, $n = 3$	6.4	1.33	0.8	0.6	0.3	0.4	4.6	2.3	1.5	0.3	2.4	0.4	2.4	0.5	2.0	0.2
cementwork, $n = 4$	3.8	2.9	0.2	0.4	0.5	1.0	16.2	6.8	1.4	0.3	2.3	1.3	3.7	2.9	2.2	0.3
motorway, $n = 4$	9.5	7.5	0.5	1.0	1.0	1.9	10.7	7.5	1.5	0.4	1.9	0.6	2.9	1.0	2.1	0.1
combined, $n = 3$	15.2	1.3	0.8	0.5	nd[b]		7.7	1.1	1.0	0.2	1.3	0.3	1.8	0.4	1.9	0.4

[a] NA, naphthalene; ACEY, acenaphthylene; ACEA, acenaphthene; FLUO, fluorene; ANT, anthracene; FLUT, fluoranthene; PYR, pyrene; B[a]A, benzo[a]anthracene. [b] nd, not detected.

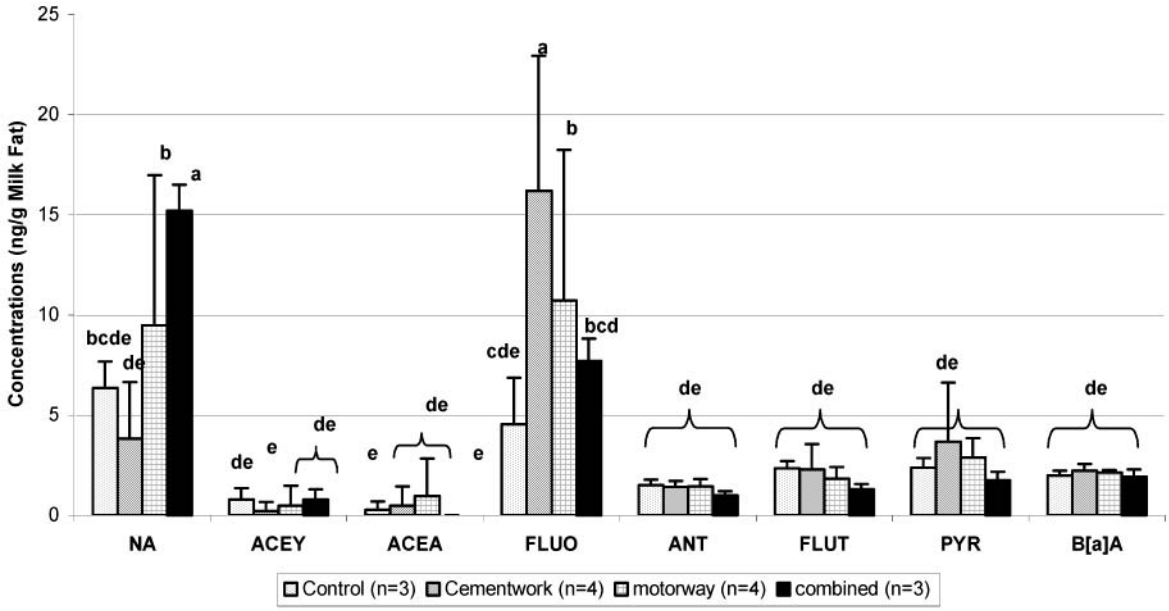

Figure 1. Concentration of polycyclic aromatic hydrocarbons (PAHs) in milk sampled near various potential contamination sources. For each compound, mean values with a different letter differ significantly ($p < 0.05$). NA, naphthalene; ACEY, acenaphthylene; ACEA, acenaphthene; FLUO, fluorene; ANT, anthracene; FLUT, fluoranthene; PYR, pyrene; B[a]A, benzo[*a*]anthracene.

mixture was left for decantation. The organic layer was collected, filtered through anhydrous sodium sulfate, and evaporated. The fat content was then determined gravimetrically.

The isolation of PAHs from the lipid matrix was conducted as follows: A glass chromatographic column (i.d. = 20 mm, height = 400 mm) was filled with activated silica and eluted with methylene chloride and *n*-hexane. Two grams of fat (precision = 0.001 g) was dissolved in 5 mL of *n*-hexane. This extract was applied to the column, eluted with a mixture of *n*-hexane/methylene chloride (v/v 3:2). The final extract was then concentrated using a rotary evaporator and under a gentle stream of pure nitrogen. Solvent exchange to toluene occurred, until the final volume of 1 mL was reached. Ten microliters of internal standards was added, and gas chromatography coupled with mass spectrometry (GC-MS) analysis was performed.

The determination of PAHs in final extract was conducted on a 6890^+ gas chromatograph coupled to a 5973 mass spectrometer from Agilent Technologies. A DB-XLB (from J&W, 60 m length, 0.25 mm i.d., 0.25 μm film thickness) capillary column was connected to the gas chromatograph coupled to a mass spectrometer apparatus for the chromatographic separation. The analysis was performed in selected ion monitoring (SIM) mode in order to attain sensitivities in accordance with food sample contaminant concentrations. The determined analytes are the U.S. Environmental Protection Agency 16 (see U.S. EPA 610 method).

Data Analysis. Statistical analysis involved calculating the means and standard deviations. Variance analysis was performed using SAS statistical software (*13*) (ANOVA, SAS Institute, Cary, NC). Student's *t* test was used for comparison of the means at a significance level of 0.05. Data are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

PAHs Detected in Milk from Various Sources. The concentrations of PAHs in milk sampled at various locations are reported in **Table 1**. Only 8 of the 16 compounds researched were detected: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, fluoranthene, pyrene, and benzo[*a*]anthracene, with concentrations ranging between 0.1 and 16.2 $ng \cdot g^{-1}$ of milk fat. PAHs with more than four aromatic cycles were not detected. Benzo[*a*]pyrene, considered to be the most toxic compound, was not detected in this matrix. Except for the benzo[*a*]anthracene, only nonmutagenic PAHs were detected (*4*). A previous study (*11*) observed different summer milk PAHs profiles: only five compounds were detected (naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene). In the atmosphere, PAHs are mainly combined with particles, and the transport of these airborne particles depends on their size, their volatilization properties, and meteorological conditions (*14*). Detected PAHs present low molecular weight from 128.2 $g \cdot mol^{-1}$ (naphthalene) to 228.35 $g \cdot mol^{-1}$ (benzo[*a*]anthracene) and high volatility measured by vapor pressure from 1.04 to 2.71 10^{-5} Pa, compared to the less volatile compound, which is 2.6×10^{-9} Pa (indenol[1,2,3-*c*,*d*]pyrene) (*15*). These properties may explain PAHs' capacity to be transported over long distances and to be deposited on fields far from any contaminating sources (i.e., control farms) and consequently on those located at <4 km from the source. This hypothesis is confirmed by Bryselbout et al. (*16*), who demonstrated that light com-

Table 2. Variance Analysis Results ($n = 14$)

	source effect	molecule effect	molecule–source interaction	SEM
P	0.1890	0.000	0.0002	2.71

pounds with four or fewer aromatic cycles could be transported over a longer distance than heavy compounds with more than four aromatic cycles and that they deposited rapidly after emissions; the soil concentration massively decreases 6 m away from the motorway.

Interaction Source—PAHs in Milk Samples. Two compounds, naphthalene and fluorene, show statistically higher concentrations ($p < 0.05$) than the other six PAHs (**Figure 1**). Statistical analysis did not show a source effect, but a highly significant interaction between source and molecule is found ($p < 0.001$) (**Table 2**). Indeed, naphthalene is detected with a statistically higher level (15.2 ± 1.3 ng·g^{-1} of milk fat) in milk sampled from farms located near combined sources. Fluorene is detected with a statistically higher level (16.2 ± 6.8 ng·g^{-1} milk fat) in milk sampled from farms located near cementworks. Statistical analysis performed on the six other PAHs showed no molecule−source interaction and statistically different from zero concentrations (**Figure 1**). These results suggest that milk residual contamination is independent of the type of source. Naphthalene and fluorene present higher concentrations in winter milk for all kinds of sources. Indicatory PAHs from various emissions sources (cementworks, mobile sources, incinerators, and steelworks) were identified in ambient air (*3*). PAH milk profiles can be related with indicatory PAHs of mobile sources (acenaphthylene, fluorene, and fluoranthene) and combined sources (acenaphthylene, fluorene, and fluoranthene) except for compounds with more than four aromatic cycles (chrysene, benzo[*a*]pyrene, and indeno[1,2,3-*cd*]pyrene). Our results are partly in agreement with previous findings in summer milk (*11*). Three major PAHs were phenanthrene (10.9 ± 4.5 ng·g^{-1} of milk fat), naphthalene (8.0 ± 3.0 ng·g^{-1} of milk fat), and pyrene (7.0 ± 4.8 ng·g^{-1} of milk fat). Several hypotheses exist with regard to the absence of phenanthrene in winter milk and the absence of fluorene in summer milk: (i) seasonal effect of PAH concentration [with 2 or 4 times higher concentrations in winter (*1*)]; (ii) different source emission profiles during the year [quantities and characteristics of PAHs emitted from industrial stacks depend on several factors: the type of input, the manufactoring process, air pollution control devices, etc. (*3*)]; (iii) other contamination pathways such as the air breathed, depending on the type of source, or soil ingestion with soil PAH profiles different from the fodder profiles. Similar milk PAH profiles emphasize basic contamination levels independent of the studied site.

Conclusion. This study gives new insight into PAH contamination in milk produced near different potential PAH emissions sources. It indicates for the first time that PAHs in milk show similar profiles whatever the sources of emissions, except for naphthalene and fluorene, for which a source−molecule interaction has been shown. To specify PAH milk contamination through ruminant, we need further information concerning emission source profiles and ruminant feed contamination levels in winter.

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