Milk-blood transfer of ^{14}C -tagged polycyclic aromatic hydrocarbons (PAHs) in pigs

Claire Laurent, Cyril Feidt, Eric Lichtfouse, Nathalie Grova, François Laurent, Guido Rychen

► To cite this version:

Claire Laurent, Cyril Feidt, Eric Lichtfouse, Nathalie Grova, François Laurent, et al.. Milk-blood transfer of ^{14}C -tagged polycyclic aromatic hydrocarbons (PAHs) in pigs. *Journal of Agricultural and Food Chemistry*, 2001, 49 (5), pp.2493 -2496. 10.1021/jf0014011 . hal-00263867

HAL Id: hal-00263867

<https://hal.science/hal-00263867>

Submitted on 27 Mar 2008

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Milk-blood transfer of ^{14}C -tagged polycyclic aromatic hydrocarbons (PAHs) in pigs

Claire LAURENT, Cyril FEIDT, Eric LICHTFOUSE, Nathalie GROVA,
François LAURENT, Guido RYCHEN*.

Ecole Nationale d'Agronomie et des Industries Alimentaires, INRA-INPL, BP 172, F-54505 Vandoeuvre-lès-Nancy, France. *Corresponding author : Guido RYCHEN

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are lipophilic organic pollutants occurring widely in the terrestrial environment. To study the transfer of PAHs in the food chain, pigs have been fed with milk spiked either with [^{14}C]phenanthrene or with [^{14}C]benzo[a]pyrene. The analysis of blood radioactivity showed that both PAHs were absorbed with a maximum concentration at 5-6 h after milk ingestion, similarly to fat metabolism. The blood radioactivity then decreased to reach background levels 24 h after milk ingestion. Furthermore, the blood radioactivity was higher for phenanthrene (even if the injected load was the lowest) than for benzo[a]pyrene, in agreement with their solubility difference. These findings suggest that milk fat and PAHs were absorbed during the same time period.

Keywords : PAHs, milk, blood, animal, pig, transfer, toxicity, bioavailability

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are organic contaminants widely occurring at trace levels in ecosystems such as soils, sediments, the atmosphere and plants (1-5). Although PAHs have natural sources, for example, vegetation fires, soil records have shown that PAH levels have increased after the start of industrial activities (6). The occurrence of point-source pollution such as oil spills, ancient industrial sites (7), sewage sludges (8) and vehicle exhausts from urban areas and highways, has raised concern on the possible transfer of PAHs from plants to dairy food and then to living organisms. Nonetheless, reports on PAHs occurrence in dairy food and animals are scarce (9, 10). Moreover, food-animal transfer pathways of PAHs are so far poorly known due to the absence of investigations involving tracers. Here, we wish to report a study of milk-blood transfer of PAHs using two ^{14}C -tagged compounds, the three-ring phenanthrene of moderate water-solubility (1.2 mg/L) and the five-ring benzo[a]pyrene of low water-solubility (3.8 $\mu\text{g/L}$).

MATERIALS AND METHODS

Spiked milk

Radioactivity handling and animal tests were performed in accordance with French policies. One liter of milk was spiked with 50 μCi of $[7,10\text{-}^{14}\text{C}]\text{benzo[a]pyrene}$ (54m Ci/mmol, Amersham) in 1 mL toluene. One liter of milk was spiked with 15 μCi of $[9\text{-}^{14}\text{C}]\text{phenanthrene}$ (55 mCi/mmol, Amersham) in 1 mL ethanol.

Animals and diets

The animal protocol was in accordance with the general guidelines of the Council European Communities (11). Two castrated Large White pigs (body weight = 48 kg) from the herd of a commercial farm were used. The pigs were fed twice daily during one week in our laboratory with a well balanced diet (800 g/meal) based on wheat and soybean to meet maintenance and growing needs of the animals as prescribed by Henry et al. (12). Each animal was fitted with two catheters, one in the portal vein and one in the brachiocephalic artery. Anesthesia was induced with sodium thiopentone (10 to 15 mg/kg) and maintained with fluothane inhalation (0.5 to 1.5 % as required). The animals were fitted with a cuffed endotracheal tube; and the lungs were mechanically ventilated at a minute volume of 150 ml/kg. Surgery was performed under strictly aseptic conditions. The animals began to eat the day after the operation and rapidly recovered to assume their normal growth rate (400 g/day). To prevent obstruction by blood clots, the cannulas were rinsed daily with a heparinized (100 IU/ml) NaCl solution (9 g/l). This was achieved under aseptic conditions to avoid any risk of infection. The ^{14}C spike experiment began once pigs had completely recovered from surgery (> 5-6 days). Throughout the experimental period, they were kept in individual cages allowing easy access to the cannulas for blood sampling in the portal vein and in the brachiocephalic artery.

^{14}C -PAH experiment

Fourteen days after surgery, 1 L of either $[^{14}\text{C}]\text{benzo[a]pyrene}$ or $[^{14}\text{C}]\text{phenanthrene}$ spiked-milk were fed to the animals. Ten-milliliter portal and arterial blood samples were then collected simultaneously (1) prior to the milk distribution and (2) 1, 2, 3, 4, 5, 6, 9 and 24 h after milk ingestion. Blood samples were immediately centrifuged 10 min at 3000 g (4°C). Plasma supernatant was then collected and stored at -20°C. ^{14}C in plasma was measured by direct counting (10 min) of duplicate 1 mL samples in 10 mL Ultimagold scintillation fluid (Beckman) using a Tricarb 460 CD liquid scintillation counter (Packard). Radioactivity is expressed in becquerels per milliliter of plasma.

RESULTS AND DISCUSSION

Pigs have been fed with milk spiked either with $[^{14}\text{C}]\text{phenanthrene}$ or with $[^{14}\text{C}]\text{benzo[a]pyrene}$. To study the kinetics of PAH transfer, blood was sampled in the portal vein and in the brachiocephalic artery over a 24-h period. Values of blood plasma radioactivity are reported in Table 1. Several noteworthy observations were made. First, the radioactivity was readily observed 1 h after milk ingestion (Figures 1,2). It increased rapidly to a maximum at about 5-6 h, then decreased to reach background levels after 24 h. Second, the blood plasma radioactivity was higher at peak maximum for phenanthrene than for benzo[a]pyrene. It's interesting to note that the radioactivity level from the phenanthrene was about 3 times lower than that of benzo[a]pyrene. Third, although radioactivity levels were slightly higher in the portal vein compared to the brachiocephalic artery, the absorption kinetics were similar (no time shift). These findings have several implications.

Table 1. ^{14}C radioactivity level in portal and arterial blood after ingestion of [^{14}C]benzo[a]pyrene or [^{14}C]phenanthrene by the growing pig

Benzo[a]pyrene h after ingestion	Arterial blood Bq/mL	Portal blood Bq/mL
0	0 $\pm$ 0	0 $\pm$ 0
1	1.13 $\pm$ 0.02	1.78 $\pm$ 0.01
2	2.82 $\pm$ 0.11	3.22 $\pm$ 0.15
3	2.61 $\pm$ 0.01	4.45 $\pm$ 0.07
4	3.81 $\pm$ 0.10	5.12 $\pm$ 0.24
5	4.96 $\pm$ 0.16	6.98 $\pm$ 0.11
6	5.73 $\pm$ 0.01	7.47 $\pm$ 0.28
9	4.04 $\pm$ 0.06	4.50 $\pm$ 0.01
24	1.49 $\pm$ 0.01	1.65 $\pm$ 0.00

Phenanthrene h after ingestion	Arterial blood Bq/mL	Portal blood Bq/mL
0	0 $\pm$ 0	0 $\pm$ 0
1	12.89 $\pm$ 0.01	15.32 $\pm$ 0.08
2	18.06 $\pm$ 0.67	19.58 $\pm$ 0.09
3	22.70 $\pm$ 0.22	23.08 $\pm$ 0.26
4	24.35 $\pm$ 0.25	26.00 $\pm$ 0.25
5	25.63 $\pm$ 0.95	26.69 $\pm$ 0.03
6	22.57 $\pm$ 0.39	23.55 $\pm$ 0.16
9	12.03 $\pm$ 0.38	11.72 $\pm$ 0.23
24	2.60 $\pm$ 0.06	3.25 $\pm$ 0.05

The radioactivity peaks observed after 4-6 h of PAH ingestion were in the same range of those observed for milk fat absorption (13), and differed notably from peak absorption of glucose (45 min) and protein (30 min) (14). This result thus suggested that PAH absorption was most likely related to fat absorption. This behavior was supported by the fact that phenanthrene and benzo[a]pyrene kinetics of absorption were similar, notably with the absence of time shift (Figures 1 and 2). Indeed, one would expect a much faster transfer of phenanthrene in blood because the water-solubility of phenanthrene (1.2 mg/L) was much higher than that of benzo[a]pyrene (3.8 $\mu\text{g/L}$). On the contrary, because both absorption curves were similar, it can be concluded that both compounds were not transferred in the aqueous phase, thus favoring the lipid phase as transport means. Finally, PAH absorption, related to fat absorption, may be in agreement with the high lipophilicity of phenanthrene (Log Kow = 4.5) and benzo[a]pyrene (Log Kow = 6.3), and with the behavior of other lipophilic contaminants such as dioxins (15-19). On these grounds, it can be suggested that PAHs and fat milk were absorbed during the same time period. Nonetheless, other transfer mechanisms cannot be excluded, notably because this study has not taken into account the radioactivity transferred to animal compartments other than blood plasma.

For each compound, the radioactivity level in blood plasma was higher for the portal vein than for the brachiocephalic artery (Figures 1 and 2). This finding suggests a preferential transfer of PAHs by the blood pathway. Indeed, there are two main pathways of nutrient absorption through guts: (1) the blood pathway which involves direct transfer of blood into

the portal vein and (2) the slower blood transfer by the lymphatic pathway, which can be seen partly at the brachiocephalic artery. Alternatively, the lower radioactivity level observed in the brachiocephalic artery can be explained by rapid absorption of PAHs in the liver and other tissues. Whatever pathway, our study has clearly shown that PAHs and milk fat were absorbed during the same time period.

Figure 1. Portal and arterial kinetics of ^{14}C after ingestion by the growing pig of 1 L spiked milk with [^{14}C]benzo[a]pyrene or [^{14}C]phenanthrene.

Figure 2. Porto-arterial differences of ^{14}C after ingestion by the growing pig of 1 L spiked milk with [^{14}C]benzo[a]pyrene or [^{14}C]phenanthrene.

LITERATURE CITED

1. Sims, R. C.; Overcash, M. R. Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. *Residue Reviews* **1983**, 88, 1-68.
2. Baek, S. O.; Field, R. A.; Goldstone, M. E.; Kirk, P. W.; Lester, J. N.; Perry, R. A review of atmospheric polycyclic aromatic hydrocarbons: source, fate and behavior. *Water Air Soil Pollut.* **1991**, 60, 279-300.
3. Simonich, S. L.; Hites, R. A. Vegetation-atmosphere partitioning of polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* **1994**, 28, 939-943.
4. Lichtfouse, E.; Budzinski, H.; Garrigues, Ph.; Eglinton, T. I. Ancient polycyclic aromatic hydrocarbons in modern soils : ^{13}C , ^{14}C and biomarker evidence. *Org. Geochem.* **1997**, 26, 353-359.
5. Lichtfouse, E.; Apitz, S.; Nanny, M. (Eds.). The Biogeochemistry of Polycyclic Aromatic Hydrocarbons. *Org. Geochem.* **1999**, 30, 873-969.
6. Jones, K. C.; Stratford, J. A.; Waterhouse, K. S.; Furlong, E. T.; Giger, W.; Hites, R.; Schaffner, C.; Johnston, A. E. Increases in the polynuclear aromatic hydrocarbon content of an agricultural soil over the last century. *Environ. Sci. Technol.* **1989**, 23, 95-101.
7. Henner, P.; Schiavon, M.; Druelle, V.; Lichtfouse, E. Phytotoxicity of ancient gaswork soils. Effect of polycyclic aromatic hydrocarbons (PAHs) on plant germination. *Org. Geochem.* **1999**, 30, 963-969.

8. Fries, G. F. Ingestion of sludge applied organic chemicals by animals. *Sci. Total Environ.* **1996**, *185*, 93-108.
 9. Madhavan, N. D.; Naidu, K. A. Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. *Hum. Exp. Toxicol.* **1995**, *14*, 503-506.
 10. Bosset, J. O.; Bütikofer, U.; Dafflon, O.; Koch, H.; Scheurer-Simonet, L.; Sieber, R. Occurrence of polycyclic aromatic hydrocarbons in cheese with and without a smoked flavour. *Sci. Alim.* **1998**, *18*, 347-359.
 11. Council of European Communities. Directives of the Council concerning the animal protection for the use of living animals in scientific investigations. *Official Journal of European Communities* 86/609 **1986**, L 358, 1-28.
 12. Henry, Y.; Perez, J. M.; Seve, B. Alimentation des porcs en croissance. In *Alimentations des animaux monogastriques : porc, lapin, volailles* **1989**, Deuxième édition, INRA, 147 rue de l'université, 75431 Paris cedex 07, 49-76.
 13. Dubois, C.; Arnaud, M.; Férézou, J.; Beaumier, G.; Porugal, H.; Pauli, A.M.; Bernard, P.M.; Bécue, T.; Lafont, H.; Lairon, D. Postprandial appearance of dietary deuterated cholesterol in the chylomicron fraction and whole plasma in healthy subjects. *Amer. J. Clin. Nutr.* **1996**, *64*, 47-52.
 14. Mahe, S.; Roos, N.; Benamouzig, R.; Sick, H.; Baglieri, A.; Huneau, J. F.; Tome D. True exogenous and endogenous nitrogen fractions in the human jejunum after ingestion of small amounts of ¹⁵N-labeled casein. *J. Nutr.* **1994**, *124*, 548-555.
 15. Tuinstra, L.G.M.Th.; Roos, A.H.; Berende, P.L.M.; van Rhijn, J.A.; Taag, W.A.; Mengelers, M.J.B. Excretion of polychlorinated dibenzo-p-dioxins and -furans in milk of cows fed on dioxin in the dry period. *J. Agric. Food Chem.* **1992**, *40*, 1772-1776.
 16. Schlummer, M.; Moser, G. A.; McLachlan, M. S. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans : Mass balances and mechanistic considerations. *Toxicol. Appl. Pharmacol.* **1998**, *152*, 128-137.
 17. Rohde, S.; Moser, G. A.; Pöpke, O.; McLachlan, M. S. Clearance of PCDD/Fs via the gastrointestinal tract in occupationally exposed persons. *Chemosphere* **1999**, *38*, 3397-3410.
 18. Morita, K.; Matsueda, T.; Iida, T.; Hagesagawa, T. Chlorella accelerates dioxin excretion in rats. *J. Nutr.* **1999**, *129*, 1731-1736.
 19. Moser, G. A.; McLachlan, M. S. A non-absorbable dietary fat substitute enhances elimination of persistent lipophilic contaminants in humans. *Chemosphere*, **1999**, *39*, 1513-1521.
-