Portal absorption of 14C after ingestion of spiked milk with 14C-phenanthrene, 14C-benzo[a]pyrene or 14C-TCDD in growing pigs

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Portal absorption of ^{14}C after ingestion of spiked milk with ^{14}C-phenanthrene, ^{14}C-benzo[a]pyrene or ^{14}C-TCDD in growing pigs.

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Abstract
Polycyclic aromatic hydrocarbons (PAHs) and dioxins are lipophilic organic pollutants occurring widely in the terrestrial environment. In order to study the PAHs and 2,3,7,8 TetraChloroDibenzo-*p*-Dioxin (TCDD) transfer in the food chain, pigs have been fed with milk mixed either with ^{14}C-phenanthrene, with ^{14}C-benzo[a]pyrene or with ^{14}C-TCDD. The analysis of portal and arterial blood radioactivity showed that both PAHs and TCDD were absorbed with a maximum concentration at 4-6 h after milk ingestion. Then, the blood radioactivity decreased to reach background levels 24 h after milk ingestion. Furthermore, the portal and arterial blood radioactivities were higher for phenanthrene (even if the injected load was the lowest) than these of benzo[a]pyrene or these of TCDD, in agreement with their lipophilicity and water solubility difference. Main ^{14}C absorption occurred during the 1-3 h time period after ingestion for ^{14}C-phenanthrene and during the 3-6 h time period for ^{14}C-benzo[a]pyrene and for ^{14}C-TCDD. ^{14}C portal absorption rate was high for ^{14}C-phenanthrene (95 %), it was close to 33 % for ^{14}C-benzo[a]pyrene and very low for ^{14}C-TCDD (9 %). These results indicate that the three studied molecules have a quite different behaviour during digestion and absorption. Phenanthrene is greatly absorbed and its absorption occurs via the blood system, whereas benzo[a]pyrene and TCDD are partly and weakly absorbed respectively. However these two molecules are mainly absorbed via the portal vein.

Keywords: PAHs, TCDD, milk, portal absorption, pig

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) and dioxins are organic contaminants widely occurring at trace levels in ecosystems such as soils, sediments, atmosphere and plants (Sims and Overcash, 1983; Baek et al., 1991; McCrady et Maggard, 1993; Simonich and Hites, 1994; IARC, 1997; Lichtfouse et al., 1997, 1999; Lohmann et Jones, 1998; Kurokawa et al., 1998). Although organic micropollutants have natural sources, e.g. vegetation fires, soil records have shown that PAH and dioxins levels have increased after the start of industrial activities (Jones et al. 1989; Lohmann et Jones, 1998; Alcock et al., 1998). The occurrence of point-source pollution such as oil spills, ancient industrial sites (Henner et al., 1999), sewage sludge's (Fries, 1996) and vehicle exhausts from urban areas and highways, has raised concern on the possible transfer of organic micropollutants from plants to dairy food then to living organisms.

Nonetheless, reports on PAHs and occurrence in dairy food and animals are scarce (Madhavan and Naidu, 1995; Bosset et al., 1998 and refs therein). Moreover, food-animal transfer pathways of PAHs are so far poorly known due to the absence, to our best knowledge, of investigations involving tracers. For the dioxins, a lot of study showed that the dioxins levels in food animal are known (McLachlan, 1997; Roeder et al., 1998) and reported that the absorption mechanism for these molecules depended on the matrice and on the compounds properties (like mainly the water solubility, the lipophilicity, the chloration, the molecular weight) (Van den Berg et al., 1994; Dahl et al., 1995; Schlummer et al., 1998; Rohde et al., 1999; Morita et al., 1999). Here, we wish to report a study of portal absorption of PAHs and of dioxin using three ^{14}C-tagged compounds: ^{14}C-phenanthrene, ^{14}C-benzo[a]pyrene or ^{14}C-TCDD (TCDD: 2,3,7,8-tetracholorodibenzo-*p*-dioxin) in the growing pig. These three compounds differ either by the lipophilicity, either by the water solubility, either by fused benzene rings number.

MATERIALS AND METHODS

Spiked milk

Radioactivity handling and animal tests were performed in accordance with French policies. Thousand milliliter of milk was spiked with 50 µCi of U-^{14}C-TCDD (45.4 mCi/mmol, ChemSyn laboratories) in 1.2 mL toluene. Thousand milliliter of milk was spiked with 50 µCi of 7,10-^{14}C-benzo[a]pyrene (54 mCi/mmol, Amersham) in 1 mL toluene. Thousand milliliter of milk was spiked with 15 µCi of 9-^{14}C-phenanthrene (55 mCi/mmol, Amersham) in 1 mL ethanol. The three compounds properties were different: the difference between the TCDD and the benzo[a]pyrene is mainly the fused benzene rings number whereas the difference between the TCDD and the phenanthrene is the lipophilicity (or water solubility). The two PAHs differ in the lipophilicity or water solubility and in the fused benzene rings number (Table 1).

Table 1: Physical and chemical properties of the studied HAP and dioxin

Compounds	Fused benzene rings number	Lipophilicity Log Kow	Water solubility (mg/L)	Molecular weight
phenanthrene	3	4.5	1.29	178.20
benzo[a]pyrene	5	6.04	0.0038	252.00
TCDD	2	6.80	0.0000193	321.98

Animals and diets

The animal protocol was in accordance with the general guidelines of the Council European Communities (1986). Six castrated Large White pigs (body weight 40 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 animals according to Henry et al. (1989). Each animal was fitted with two catheters, one placed in the portal vein and another one placed in the brachiocephalic artery. Anesthesia was induced with sodium thiopentone (10 to 15 mg/kg) and maintained with fluothane inhalation (0.5 - 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 their normal growth rate (400 g/d). To prevent obstruction by blood clots the cannulaes 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-spiked experiment began once pigs had completely recovered from surgery (more than 5-6 days). Throughout the experimental period, they were kept in individual cages allowing easy access to the cannulae for blood sampling in the portal vein and in the brachiocephalic artery.

^{14}C-PAH experiment

Fourteen days after surgery, 1000 mL of either ^{14}C-benzo[a]pyrene, ^{14}C-phenanthrene either ^{14}C-TCDD spiked-milk were fed to the animals. Each studied molecule was given once to two different animals. Ten milliliter portal and arterial blood samples were then collected simultaneously (1) prior to the milk distribution and (2) 1 - 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 Bq per ml of plasma.

Portal absorption calculations

Postprandial kinetics of ^{14}C in the portal vein and the arterial blood was determined as well as postprandial kinetics of porto-arterial concentration differences. Portal absorption of ^{14}C were calculated as: "^{14}C porto-arterial differences x blood flow". ^{14}C meal absorption rate was calculated as: "portal absorption of ^{14}C / ^{14}C content in the meal". Blood flow per min and per kg body weight could be estimated thanks many references using growing pigs (Rérat et al., 1987; Simoes Nunes et al., 1989; Rérat et al., 1991; Rérat et al., 1992; Guillot et al, 1993; Guillot et al., 1994; Rérat et al., 1996; Santamaria et al., 1997; Lang et al., 1999; Le Floc'h et al., 1999; Vaugelade et al., 2000).

In fact, it is known that meal ingestion is followed by a small rise in portal blood flow during the first 1-2 postprandial hours and individual variations in pig portal blood flow have been established at between 2.8 and 5.7 % (Simoes Nunes and Malmlöf, 1992). Several authors found relatively constant blood flow values after ingestion of the meal (Guillot et al., 1993; Guillot et al., 1994; Lang et al., 1999). One can easily assume that portal blood flow variations in the present work were similar for all the animals and that they consequently interfered in the same way for all the three diets.

In this study, we have calculated portal absorption with a constant blood flow value of 41.2 ml/min/kg body weigh, which correspond with the mean value from observations of different authors (see data below) and with mean ^{14}C values of porto-arterial differences (Table 2).

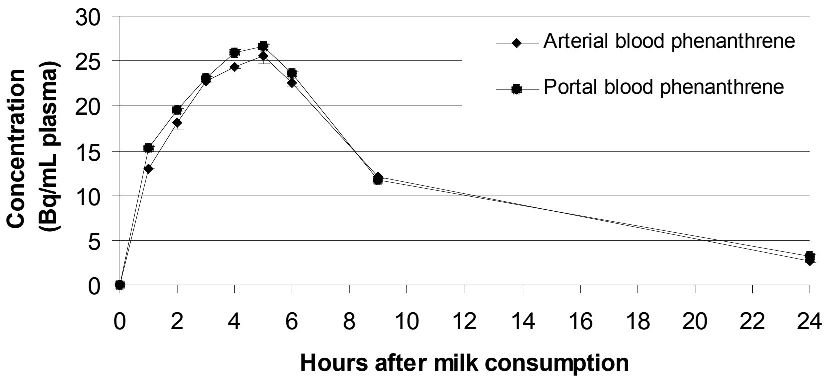
Table 2: Blood flow references in the growing pig

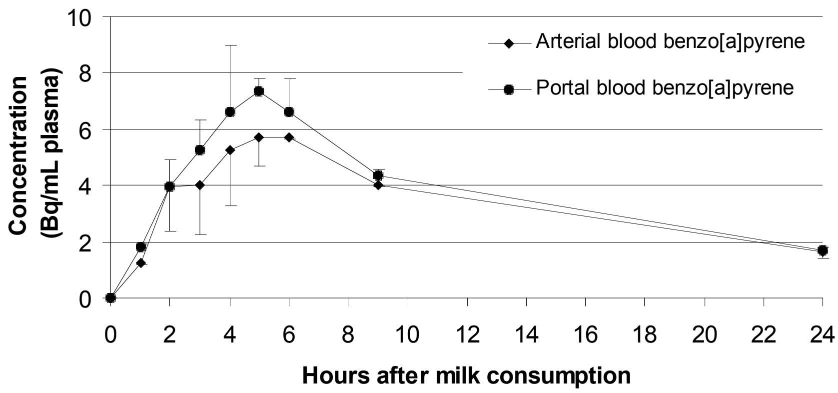
Body weight (kg)	Blood flow / min / kg (ml/min/kg)	Authors
61	39.7	Guillot et al., 1994
65	37.6	Guillot et al., 1993
8	44.7	Reeds et al., 1996
45	41	Rérat et al., 1991
45	37.9	Rérat et al, 1992
40	49.9	Rérat et al., 1996
22	45.2	Santamaria et al., 1997
64	42	Simoes Nunes et al., 1989
57	32.8	Vaugelade et al., 2000

RESULTS

Pigs have been fed with milk spiked either with ^{14}C-phenanthrene, with ^{14}C-benzo[a]pyrene either with ^{14}C-TCDD. In order to study the porto-arterial kinetics of organic micropollutants transfer, blood was simultaneously sampled in the portal vein and in the brachiocephalic artery over a 24 h period. Blood plasma radioactivity kinetics were reported in Figs. 1 and 2. Several peculiar features can be observed. The radioactivity was readily observed 1 h after milk ingestion (Figs. 1 and 2). It increased rapidly to a maximum about 4-6 h after milk ingestion, and then decreased to reach background levels after 24 h. Moreover ^{14}C plasma level from ^{14}C-phenanthrene was about 3 and 10 times more elevated than ^{14}C level from ^{14}C-benzo[a]pyrene and from ^{14}C-TCDD, respectively (Figs. 1 and 2). It's interesting to note that the milk radioactivity level from the phenanthrene was about three times lower than that of benzo[a]pyrene or that of TCDD. For each compound, the radioactivity level in blood plasma was higher for the portal vein than for the brachiocephalic artery (Figs. 1 and 2) suggestion transfer of organic micropollutants by the blood pathway.

Portal absorption of ^{14}C estimated as "Porto-arterial differences x blood flow" as well as ^{14}C absorption rates evaluated as "portal absorption of ^{14}C / ^{14}C content in the milk" are presented in Table 3. According to our calculations, major part of absorption occurs during the 0-6 h time period (90 % for ^{14}C-phenanthrene, 93 % for ^{14}C-benzo[a]pyrene and 96 % for ^{14}C-TCDD). However, absorption of these three molecules differed in the time: main ^{14}C absorption occurred during the 1-3 h time period after ingestion for ^{14}C-phenanthrene (51 %) and during the 3-6 h time period for ^{14}C-benzo[a]pyrene (83 %) and for ^{14}C-TCDD (90 %) (Table 3). Moreover, over the whole studied period, we found a global portal absorption of about 14 μCi for phenanthrene, 16 μCi for benzo[a]pyrene and only 4 μCi for TCDD. Ingestion of ^{14}C-phenanthrene, ^{14}C- benzo[a]pyrene and ^{14}C-TCDD was of 15 μCi, 50 μCi and 50 μCi respectively. Thus, absorption rates were quite different (Table 3) for the three molecules: it was high for phenanthrene (95 %), low for TCDD (9 %) and intermediate for benzo[a]pyrene (about 33 %).





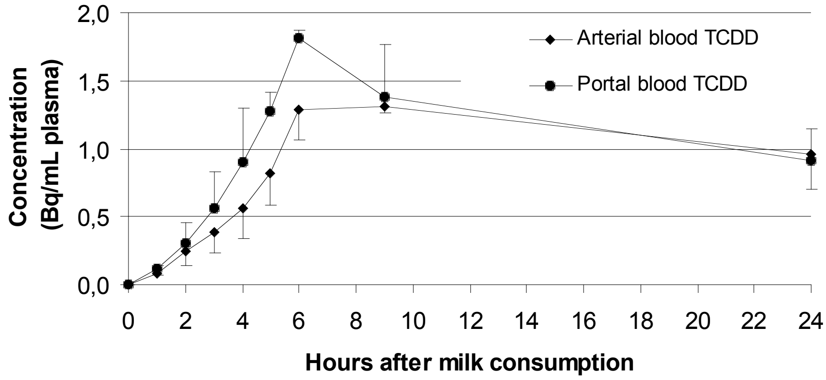


Figure 1 : Portal and arterial kinetics of ^{14}C after ingestion by the growing pig of 1000 mL milk spiked with ^{14}C phenanthrene, ^{14}C benzo[a]pyrene or ^{14}C TCDD (mean value, n=2).

DISCUSSION

The radioactivity peaks observed after 4-6h of PAHs and TCDD consumption are in the same range of those observed for milk fat absorption (Dubois et al., 1996), and differ notably from peak absorption of glucose (45 min) and protein (30 min) (Mahe et al., 1994). Thus, this finding suggests that the organic micropollutant absorption is linked with the fat absorption. However, blood absorption of ^{14}C after ingestion of 1000 mL milk spiked with ^{14}C phenanthrene, ^{14}C benzo[a]pyrene or ^{14}C TCDD is different within time (Table 3). So this result suggests that the benzo[a]pyrene or TCDD absorption mechanism is different from that of phenanthrene. Benzo[a]pyrene or TCDD are probably transferred in the lipid phase during their gut absorption: these two molecules are very lipophilic compounds (log Kow 6.04 and 6.80 respectively) and their main blood absorption occurs in the 3-6 h time period of their consumption (83 % for the benz[a]pyrene and 90% for the TCDD, Table 3).

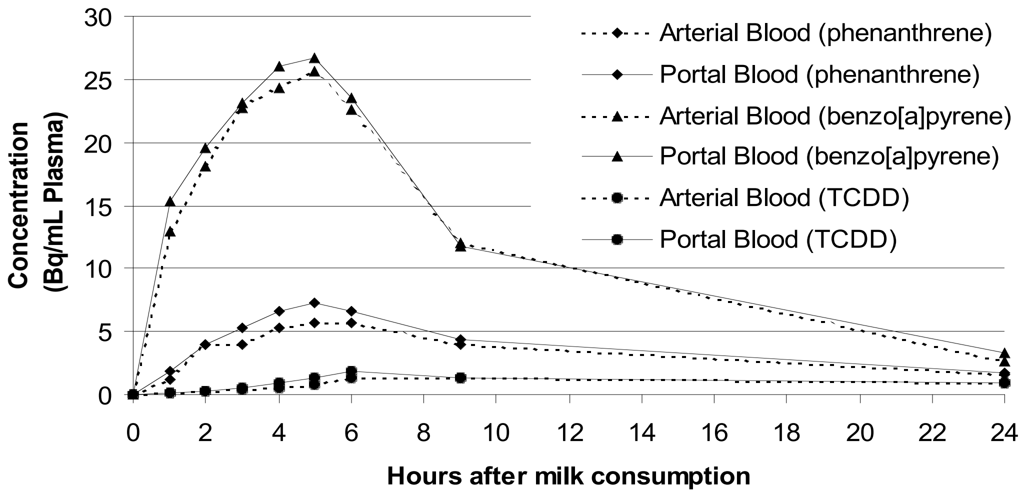


Figure 2 : Portal and arterial kinetics of ^{14}C after ingestion by the growing pig of 1000 mL milk spiked with ^{14}C phenanthrene, ^{14}C benzo[a]pyrene or ^{14}C TCDD (mean value, n=2).

The phenanthrene could be transferred either in the aqueous phase or in the lipid phase. Indeed, the water solubility of this molecule (1.2 mg/L) is much higher than that of benzo[a]pyrene or that of TCDD (3.8 µg/L and 0.02 µg/L respectively) and its portal absorption occurs for a long time (0-6 hours after its consumption, Table 3). ^{14}C portal and arterial concentrations after ^{14}C-TCDD ingestion are rather low and portal absorption rate of TCDD is weak (9 %) when compared to phenanthrene (95 %) or benzo[a]pyrene (33 %). This result suggests that the fused benzene rings number (respectively 2, 3 and 5 rings) does not influence the absorption mechanism of organic micropollutants. So the absorption mechanism of PAHs and TCDD seems mainly dependent to lipophilicity and water solubility.

For each compound and for the whole studied period, the radioactivity level in blood plasma is higher for the portal vein than for the brachiocephalic artery (Figs. 1 and 2). This finding suggests a possible transfer of PAHs and TCDD via the blood pathway. Indeed, there are two main pathways of nutrient absorption through the gut: (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.

Table 3: Portal Absorption of ^{14}C after ingestion of 1000 mL milk spiked with ^{14}C phenanthrene, ^{14}C benzo[a]pyrene, or ^{14}C TCDD in the growing pig (mean value, n=2)

	^{14}C (μCi)		
	phenanthrene	benzo[a]pyrene	TCDD
1 – 3 h	7.34	1.63	0.26
3 – 6 h	5.58	13.63	3.89
6 – 24 h	1.40	1.19	0.18
0 – 24 h (a)	14.32	16.45	4.33
meal content (b)	15	50	50
% absorption rate (a/b)	95	33	9

It is interesting to notice the particular behaviour of ^{14}C from ^{14}C-TCDD. Moreover the low absorption of TCDD appears original and surprising since several authors (Tuinstra et al., 1992; Schlummer et al., 1998; Rohde et al., 1999; Morita et al., 1999; Moser and McLachlan, 1999) suggested a high digestion and absorption rate of TCDD in experiments when they used the balance method. Moreover, Henderson and Patterson (1988) suggested that TCDD is mainly absorbed via the lymphatic pathway (via in vitro methods). However, these author have used methods which seem rather less precise (balance or in vitro methods) than the portal absorption method used in this study. Whatever pathway, our study has clearly shown that the PAHs and TCDD absorption mechanism via contaminated milk ingestion is undoubtedly limited by their lipophilicity and their water solubility.

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