Milk–Arterial Plasma Transfer of PCDDs and PCDFs in Pigs

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Polychlorodibenzo-para-dioxins (PCDDs) and Polychlorodibenzofurans (PCDFs) are ubiquitous in the environment. They are mainly formed as unwanted byproducts during various chemical, industrial, and combustion processes. Thus, these pollutants can be found in the food chain. The aim of this experiment was to study the transfer of PCDD/Fs from spiked milk to arterial blood in pigs, which are considered as valid models for humans. Pigs were fed with 900 mL of milk spiked with a mixture of 17 dioxins. The levels of PCDD/Fs in the serum extracts were determined using HRGC/HRMS prior to consumption of the milk, and at 3, 5, and 7 h after milk ingestion. Concentrations of PCDD/Fs in arterial plasma increased from 3 h to 5 h and decreased at 7 h. At time point 5 h, concentrations were found between 500 and 10 000 pg g^{-1} fat. The transfer ratio ≪ plasma fat/milk fat ≫ was usually found between 0.7 and 3%. Related to the different milk concentrations, results of this study indicate a similar behavior of the studied molecules.

KEYWORDS: Dioxins; milk; blood; pigs

INTRODUCTION

Polychlorodibenzo-para-dioxins (PCDDs) and polychloro-dibenzofurans (PCDFs) are ubiquitous in the environment at normally very low concentration (*1*−*10*). They are formed as unwanted byproducts during various chemical, industrial, and combustion processes (*11*). So, even if there are some natural sources of PCDD/Fs, for example forest fires, the magnitude of these sources is small in relation to that of anthropogenic sources (*12*).

Moreover, because of the persistence and hydrophobic character of these environmental pollutants, they are found in every level of the food chain (*13*−*16*). In recent years, it has become clear that cattle represent, through meat and dairy products, the most important source of human exposure to PCDD/Fs in Europe and North America (*13*, *15*−*20*). The main portion of human exposure originates from the atmosphere via the pathway air−feed−cow−milk−man (*17*, *21*). Therefore, it is important to know the bioavailability of PCDD/Fs in humans after ingestion of polluted milk or dairy products. The amount of data available about this subject is limited (*22*−*27*). There is no standard way of measuring dioxins' bioavailability. Different methods exist ranging from intubation with a lumen tube to dioxins balance procedures. Dioxins bioavailability is linked to subsequent postprandial delivery in arterial blood. Pigs provide a valid model for conducting such studies (*28*−*29*). To our knowledge no information is available on arterial transfer of dioxins from polluted milk.

Thus, the purposes of this study were to evaluate the arterial appearance of dioxins after ingestion of milk spiked with PCDD/Fs.

MATERIALS AND METHODS

Spiked Milk. Dioxins handling and animal tests were performed in accordance with French policies. A total of 1000 mL of milk was spiked with 1 mL of a solution/mixture of native ($^{12}C_{12}$) chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) (EPA-8290 STN, Wellington Laboratories, Ontario, Canada). Physical and chemical properties of the studied PCDD/Fs are given in **Table 1**. Concentrations of PCDD/Fs in the spiked milk are presented in **Table 2**.

Animals and Diets. The animal protocol was in accordance with the general guidelines of the Council of European Communities directive 86/609/EC (*30*). Two castrated Large White pigs (body weight 40 kg) from livestock of a commercial farm were used. The pigs were fed twice a day during one week in our laboratory with a well-balanced diet (800 g/meal) based on wheat and soybean to ensure maintenance and growing needs of animals according to Henry et al. (*31*). 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 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 their normal growth rate. 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. Throughout the experimental period, the pigs were kept in individual cages allowing easy access to the cannulae for blood sampling in the portal vein and in the brachiocephalic artery.

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1696 *J. Agric. Food Chem.,* Vol. 50, No. 6, 2002

Rychen et al.

Table 1. Physical and Chemical Properties of the Studied PCDD/Fs

congener	chlorine number	molecular weight	solubility mg/L (25 °C)	Log Kow	TEF
2,3,7,8 TCDD	4	321.98	1.93×10^{-5}	6.80	1
1,2,3,7,8 PeCDD	5	356.42		6.64	0.5
1,2,3,4,7,8 HxCDD	6	390.87	4.42×10^{-6}	7.80	0.1
1,2,3,7,8,9 HxCDD	6	390.87		-	0.1
1,2,3,6,7,8 HxCDD	6	390.87		-	0.1
1,2,3,4,6,7,8 HpCDD	7	425.31	2.40×10^{-5}	8.00	0.01
OCDD	8	460.76	0.74×10^{-7}	8.20	0.001
2,3,7,8 TCDF	4	305.98	4.19×10^{-4} (22,7 °C)	6.53	0.1
1,2,3,7,8 PeCDF	5	340.42		6.79	0.05
2,3,4,7,8 PeCDF	5	340.42	2.36×10^{-4} (22,7 °C)	6.92	0.5
1,2,3,4,7,8 HxCDF	6	374.87	8.25×10^{-6} (22,7 °C)	7.30	0.1
1,2,3,6,7,8 HxCDF	6	374.87	1.77×10^{-5} (22,7 °C)	-	0.1
1,2,3,7,8,9 HxCDF	6	374.87		-	0.1
2,3,4,6,7,8 HxCDF	6	374.87		-	0.1
1,2,3,4,6,7,8 HpCDF	7	409.31	1.35×10^{-6} (22,7 °C)	7.92	0.01
1,2,3,4,7,8,9 HpCDF	7	409.31		-	0.01
OCDF	8	444.76	1.16×10^{-6}	8.78	0.001

Table 2. PCDD/Fs Transfer Ratio from Milk Fat to Plasma Fat 5 Hours After Ingestion of 900 mL of Spiked Milk by Growing Pigs

congener	milk fat pg/g MG	plasma fat pg/g MG	ratio plasma/milk
2,3,7,8 TCDD	90 000	1195.05	1.33
1,2,3,7,8 PeCDD	216 000	2866.46	1.33
1,2,3,4,7,8 HxCDD	213 000	3150.81	1.48
1,2,3,6,7,8 HxCDD	209 000	4976.88	2.38
1,2,3,7,8,9 HxCDD	220 000	4692.01	2.13
1,2,3,4,6,7,8 HpCDD	189 000	5410.79	2.86
OCDD	427 000	8943.71	2.09
2,3,7,8 TCDF	74 000	514.96	0.70
1,2,3,7,8 PeCDF	155 000	2637.75	1.70
2,3,4,7,8 PeCDF	171 000	1914.08	1.12
1,2,3,4,7,8 HxCDF	242 000	3308.05	1.37
1,2,3,6,7,8 HxCDF	235 000	6500.12	2.77
2,3,4,6,7,8 HxCDF	244 000	3865.75	1.58
1,2,3,7,8,9 HxCDF	278 000	2133.12	0.77
1,2,3,4,6,7,8 HpCDF	174 000	9971.47	5.73
1,2,3,4,7,8,9 HpCDF	202 000	3460.48	1.71
OCDF	385 000	6524.94	1.69

Experimental Design. Fourteen days after surgery, 900 mL of dioxins-spiked milk was fed to each animal. Blood samples were collected as follows: (1) 60 mL of arterial blood prior to the milk distribution; (2) 60 mL of arterial blood 3 h after milk ingestion; (3) 60 mL of arterial and portal blood 5 h after milk ingestion; and (4) 60 mL of arterial blood 7 h after milk ingestion.

Blood samples were immediately centrifuged for 10 min at 3000*g* (4 °C). Plasma supernatant was then collected and stored at −20 °C before analysis of the PCDD/Fs in the blood.

Dioxins Analysis in Blood Samples. Solvents (hexane, methylene chloride) of Suprasolv quality (pesticide grade) and ethanol (absolute) were purchased from Merck Eurolab, Darmstadt, Germany. Silica (silicagel 60, 63−200 μm), basic alumina (Brockman I 90, 63−200 μm), ammonium sulfate (for analyses, ASC, ISO), and sodium sulfate (anhydrous, for analyses, ASC, ISO) were also purchased from Merck Eurolab, Darmstadt, Germany.

Serum samples (30−40 g) were spiked with 16 isotopically labeled dioxins and furans (480 pg for the tetras, pentas, and hexas, and 960 pg for the heptas and octas) and then diluted with water saturated with ammonium sulfate (50 mL), ethanol (50 mL), and hexane (100 mL). The mixture was shaken in a separatory funnel for 10 min. The organic phase was collected, and the remaining aqueous phase was re-extracted once with hexane (100 mL). The organic combined extract was then evaporated to dryness, and the fat content was determined gravimetrially. The fat was then redissolved in *n*-hexane and cleaned up according to the liquid chromatographic procedures described in U.S. EPA Method 1613 (Tetra through Octa Chlorinated Dioxins and Furans

by Isotope Dilution HRGC/HRMS, October, 1994). We used two different cleanup columns from among those suggested in the method: (1) a silica column (from top to bottom, 1 g of anhydrous sodium sulfate, 1 g of activated silica, 8 g of 30% sulfuric acid impregnated silica, 1 g of activated silica, and 4 g of 23% sodium hydroxide impregnated silica). The PCDD/Fs were isolated from the lipid fraction by eluting this column with *n*-hexane. (2) a basic alumina column (from top to bottom, 1 g of anhydrous sodium sulfate, 15 g of activated basic alumina, activity I). A first fraction eluted with *n*-hexane/ methylene chloride (98:2, v:v) was discarded. A second fraction, eluted with *n*-hexane/methylene chloride (1:1, v:v) was collected and concentrated.

Just before HRGC/HRMS analysis, purified extracts were reconstituted by adding 20 μL of a standard solution containing ^{13}C 1,2,3,4 TCDD and ^{13}C 1,2,3,7,8,9 HxCDD (480 pg of each added to the extract) to monitor recoveries achieved during the HRGC/HRMS analysis.

The levels of PCDD/Fs in the serum extracts were determined using HRGC/HRMS on an Autoconcept which is a high-resolution double-focusing mass spectrometer of EB geometry produced by Mass Spectrometry International (MSI), Manchester, UK. The system was directly coupled to a high-resolution gas chromatograph (HP6890, Agilent Tecnologies) fitted with a split/splitless injector. The mass spectrometer was operated at a resolution of 10000 (10% valley definition). The system was tuned using the Autotune facility. The resolution was maintained across all the ions of interest by the use of the *y* focusing lens. The source was operated at a temperature of 250 °C with an electron voltage of 30 eV at a trap current of 300 uA. The instrument was pumped by three turbomolecular pumps to provide high vacuum conditions with no chemical background. Both the mass spectrometer and the GC were controlled through the Mach3Xe data system allowing unattended operation of the equipment. The capillary column for GC separation was a J&W DB 5 MS, 60 m length, 0.25 mm i.d., and 0.25 μm film thickness. The identification criteria specified in U.S. EPA Method 1613 with respect to the GC column performance and mass spectrometer performance were fully satisfied by the data obtained in this study (separations, resolution, and sensitivity capabilities). Laboratory blanks were analyzed with the samples, and showed no contamination (nondetectable PCDD/Fs). The recoveries of labeled compounds ranged from 60 to 118%.

RESULTS

Pigs have been fed with milk spiked with a solution/mixture of 17 PCDD/Fs. To study the arterial kinetics of the studied micropollutants, arterial blood was sampled prior to milk distribution (0 h) and at 3, 5, and 7 h after milk ingestion. Arterial plasma concentrations of the studied dioxins and furans are reported in **Figures 1** and **2**. Several peculiar features can be observed. At time point 0 h, no traces of dioxins were detected. Concentrations of PCDD/Fs increased from 3 h to 5 h after spiked milk ingestion and then decreased between 5 h and 7 h. All studied molecules presented a similar kinetic behavior and could be detected in arterial blood at 3, 5, and 7 h after milk ingestion (**Figures 1** and **2**). At time point 5 h, concentrations of the different compounds of dioxins in plasma fat were found between 500 and 10 000 pg g^{-1} fat (**Figures 1** and **2**, **Table 2**), whereas in milk fat concentrations were found between 50 000 and 450 000 pg g^{-1} fat (**Table 2**).

Table 2 indicates that plasma fat concentrations of PCDD/ Fs are related to milk dioxins concentrations. Thus, for most PCDD/Fs, the transfer level from milk to blood could be estimated at between 1 and 3% (**Table 2**). The transfer ratio ≪plasma fat/milk fat≫ was usually found between 0.7 and 3% (16 dioxins) and appears higher for 1,2,3,4,6,7,8 HpCDF (nearly 6%). These results indicate a quite similar behavior of the studied dioxins. All molecules are transferred from milk fat to plasma fat at a similar level (**Table 2**). In **Figure 3**, we report comparative concentration values of the different dioxins
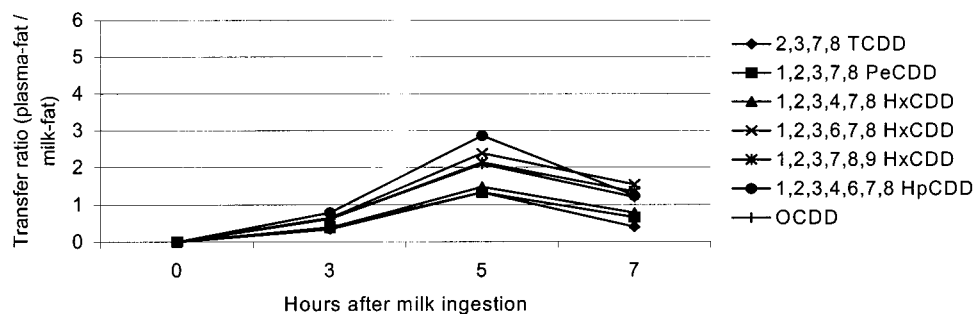
Milk–Arterial Plasma Transfer of PCDDs and PCDFs

J. Agric. Food Chem., Vol. 50, No. 6, 2002 **1697**

Figure 1. PCDDs plasma-fat/milk-fat transfer ratio following growing pigs' ingestion of 900 mL of milk spiked with 17 dioxins (mean values, $n = 2$).
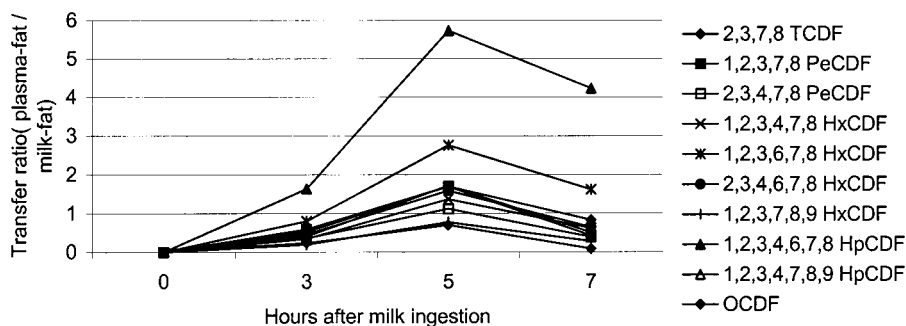

Figure 2. PCDFs plasma-fat/milk-fat transfer ratio following growing pigs' ingestion of 900 mL of milk spiked with 17 dioxins (mean values, $n = 2$).
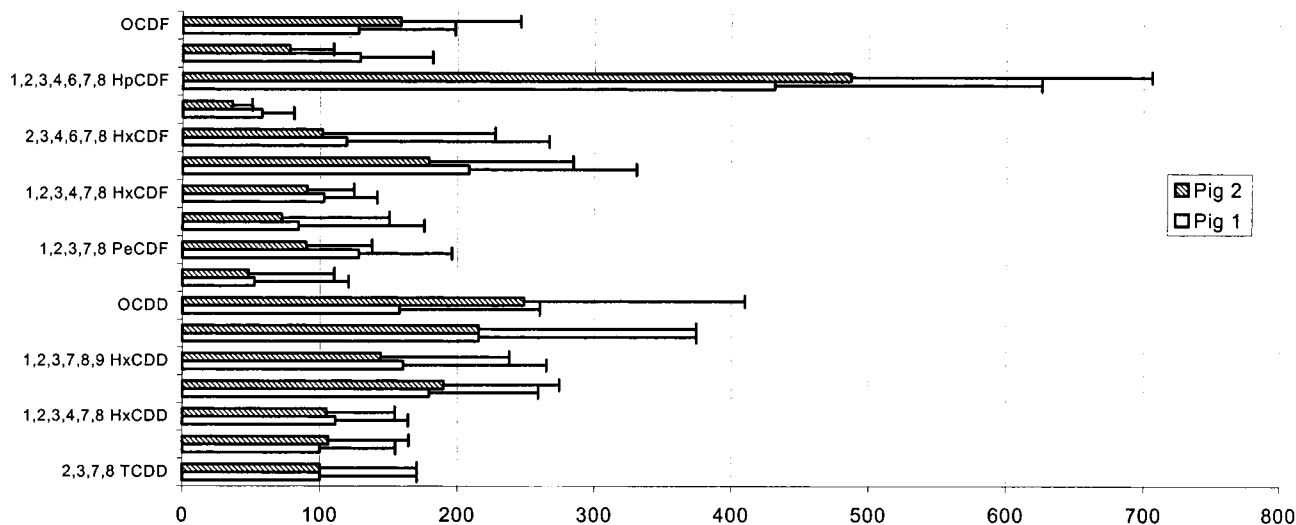

Figure 3. Comparative concentrations of the 17 PCDD/Fs in arterial blood 5 hours after ingestion of spiked milk (values are related to milk concentrations, TCDD = 100, mean values + standard deviation).

compared to that of 2,3,7,8 TCDD and related to the different milk concentrations. The PCDD/Fs arterial profile is quite analogous for each animal and indicates a good repeatability of the measurements. Considering that analytical uncertainties were close to 30−40%, taking into account every analytical step, values found between 50 and 150 can be considered as equivalent, so most of the molecules present a behavior similar to that of 2,3,7,8 TCDD. There is only one furan (1,2,3,4,6,7,8 HpCDF) which appears in blood fat in a concentration higher than that of 2,3,7,8 TCDD.

At 5 h after milk feeding, blood was simultaneously sampled in the portal vein and in the brachiocephalic artery in order to get some information on a possible absorption of PCDD/Fs by the blood pathway (delivery of the dioxins to the organism by the portal vein). **Table 3** indicates dioxins and furans concentrations in both blood vessels. For each studied molecule, portal and arterial concentrations were quite similar, suggesting a same absorption pathway for each molecule.

DISCUSSION

The aim of this investigation was to study PCDD/Fs transfer from polluted milk to arterial blood. The measure of these events is of great physiological importance because it allows precise information on the bioavailability of dioxins and the transfer level from food to the organism. For all studied molecules, the highest concentrations were found 5 h after ingestion of the spiked milk. This result suggests that PCDD/Fs absorption is connected with milk fat absorption (*32*), which differs notably from peak absorption of glucose (45 min) and protein (30 min) (*33*). Thus, PCDD/Fs absorption appears concomitant with fat absorption. Moser and McLachlan (*34*), Rohde et al. (*35*), and Shlummer et al. (*36*) gave the same suggestion. Considering portal and arterial concentrations of dioxins were similar at 5 h (**Table 3**), we could expect that dioxins are not absorbed via the blood pathway, but via the lymphatic pathway (*37*). Indeed, there are two main pathways of nutrient absorption through the

1698 *J. Agric. Food Chem.,* Vol. 50, No. 6, 2002

Rychen et al.

Table 3. PCDD/Fs Transfer Ratio from Milk Fat to Arterial or Portal Plasma Fat 5 Hours after Ingestion of 900 mL of Spiked Milk by Growing Pigs (mean values + standard deviation)

	transfer ratio (arterial plasma-fat/ milk-fat)	transfer ratio (portal plasma-fat/ milk-fat)
2,3,7,8 TCDD	1.33 ± 0.94	1.06 ± 0.75
1,2,3,7,8 PeCDD	1.33 ± 0.73	1.37 ± 0.75
1,2,3,4,7,8 HxCDD	1.48 ± 0.70	1.37 ± 0.65
1,2,3,6,7,8 HxCDD	2.38 ± 1.06	2.34 ± 1.04
1,2,3,7,8,9 HxCDD	2.13 ± 1.39	2.30 ± 1.50
1,2,3,4,6,7,8 HpCDD	2.86 ± 2.11	2.42 ± 1.78
OCDD	2.10 ± 1.36	1.82 ± 1.18
2,3,7,8 TCDF	0.70 ± 0.91	0.92 ± 1.19
1,2,3,7,8 PeCDF	1.70 ± 0.90	1.80 ± 0.95
2,3,4,7,8 PeCDF	1.12 ± 1.22	1.24 ± 1.34
1,2,3,4,7,8 HxCDF	1.37 ± 0.51	1.32 ± 0.49
1,2,3,6,7,8 HxCDF	2.77 ± 1.63	2.50 ± 1.47
2,3,4,6,7,8 HxCDF	1.58 ± 1.96	1.38 ± 1.71
1,2,3,7,8,9 HxCDF	0.77 ± 0.31	0.77 ± 0.31
1,2,3,4,6,7,8 HpCDF	5.73 ± 2.58	4.90 ± 2.21
1,2,3,4,7,8,9 HpCDF	1.71 ± 0.70	1.37 ± 0.56
OCDF	1.69 ± 0.93	1.62 ± 0.89

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. Lakshmanan et al. (*38*) showed that the transfer of 2,3,7,8 TCDD was primarily via the lymphatic route and was predominantly associated with chylomicrons. This study is in agreement with our hypothesis.

To our knowledge, this study presents for the first time the appearance profile of ingested milk dioxins in arterial blood. In particular, we observed that the level and profile of dioxins in arterial plasma seems to be unrelated to the physical or chemical properties of the molecules (**Table 1**). The small variations in responses can be attributed to the analytical uncertainties (up to 30−40%, considering every analytical steps), more than real differences in the absorption of dioxins and furans in pig's blood. Indeed, feeding of the pigs with the fortified milk, sampling of the blood, and the whole analytical process from the fat extraction to the analysis by HRGC/HRMS introduces small deviations at each step. Thus, our results demonstrate that the pig absorbed all studied dioxins which were found in similar concentrations in arterial blood. When compared to milk, PCDD/Fs concentrations in plasma are found between 0.7 and 3%. The absence of concentration differences in arterial plasma for chemical compounds with different physical and chemical properties could be explained by the fact that any molecules could be metabolized or quickly distributed in tissues, especially fat tissue. These results have also to be discussed with previous results, and more particularly, those related to fecal excretion rates of PCDD/Fs in breast fed infants (*22*−*24*, *27*, *44*, *45*). In these studies absorption was estimated by the differences between PCDD/Fs ingestion and PCDD/Fs fecal excretion. In some cases (*22*, *23*, *44*), PCDD/Fs concentrations in feces were on a fat basis in the range of the corresponding concentrations in mother's milk. In other studies (*21*, *27*, *45*) the lower chlorinated PCDD/Fs were highly absorbed compared to heptachlorinated or octachlorinated dioxins which were highly excreted in the feces of the infants. Thus, dioxins absorption is a very complex mechanism which has to be determined precisely. However, in several studies it has been suggested that dioxins absorption is mainly dependent on their physical and chemical properties, and that the dioxins absorption efficiency decreases as the degree of chlorination or the lipophilicity increases (*15*, *27*, *39*, *40*). In general, these authors suggest that smaller compounds are more efficiently absorbed than larger

molecules. McLachlan et al. (*21*), Olling et al. (*41*), and Van den Berg et al. (*42*) showed in lactating cows that the degree of chlorination influences the molecules metabolism: lower chlorinated molecules are probably more metabolized than higher chlorinated compounds. Regarding these observations, we have to remember that feces (*22*, *23*, *27*, *44*, *45*) or milk (*21*, *39*, *41*, *42*) PCDD/Fs concentrations cannot be easily compared to arterial blood concentrations. Indeed, feces include PCDD/Fs which have been absorbed and recycled by biliary salts, and milk includes PCDD/Fs which have been carried by blood and filtered by the mammary gland. Therefore, it may be possible that PCDD/Fs arterial profiles are not similar to PCDD/Fs milk or feces profiles. Thus, further research work has to be carried out to precisely determine PCDD/Fs metabolism in the monogastric or ruminant animal. It would be of great interest to study the dioxins distribution in the different organs and tissues of the animals; particularly in the liver, the kidney, and the body fat. We should also analyze the digestive content and feces to get information on PCDD/Fs which have not been absorbed. This study has also shown the usefulness of the pig model to precisely determine the bioavailability of dioxins from contaminated milk.

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LITERATURE CITED

(1) Sims, R. C.; Overcash, M. R. Fate of polynuclear aromatic compounds (PNAs) in soil−plant systems. *Residue Rev.* **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) McCrady, J. K.; Maggard, S. P. Uptake and photodegradation of 2,3,4,7,8-tetrachlorodibenzo-*p*-dioxin sorbed to grass foliage. *Environ. Sci. Technol.* **1993**, *27*, 343−350.

(4) Simonich, S. L.; Hites, R. A. Vegetation−atmosphere partitioning of polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* **1994**, *28*, 939−943.

(5) Duarte-Davidson, R.; Sewart, A.; Alcock, R. E.; Cousins, I. T.; Jones, K. C. Exploring the balance between sources, deposition, and environmental burden of PCDD/Fs in the U.K. terrestrial environment: An aid to identifying uncertainties and research needs. *Environ. Sci. Technol.* **1997**, *31*, 1−11.

(6) IARC (International Agency for Reasearch on Cancer). Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Volume 69; IARC: Lyon, France, 1997; p 666.

(7) 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.

(8) Lichtfouse, E.; Apitz, S.; Nanny, M. The biogeochemistry of polycyclic aromatic hydrocarbons. *Org. Geochem.* **1999**, *30*, 873−969.

(9) Kurokawa, Y.; Matsueda, T.; Nakamura, M.; Takada, S.; Fukamachi, K. Distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in various sizes of airborne particles. *Chemosphere* **1998**, *37*, 2161−2171.

(10) Lohmann, R.; Jones, K. C. Dioxins and furans in air and deposition: a review of levels, behaviour and process. *Sci. Total Environ.* **1998**, *219*, 53−81.

Milk–Arterial Plasma Transfer of PCDDs and PCDFs

J. Agric. Food Chem., Vol. 50, No. 6, 2002 **1699**

(11) Quaβ, U.; Fermann, M. W.; Broker, G. Identification of relevant industrial sources of dioxins and furans in Europe. Landesumweltamt NRW (ed.) Report number LUA Materialien Nr. 43, 1997.

(12) Eduljee, G. H.; Dyke, P. An updated inventory of potential PCDD and PCDF emission sources in the U.K. *Sci. Total Environ.* **1996**, *177*, 303–321.

(13) Fries, G. F. A review of the significance of animal food products as potential pathways of human exposures to dioxins. *J. Anim. Sci.* **1995**, *73*, 1639–1650.

(14) McLachlan, M. S. Accumulation of PCDD/F in agricultural food chain. *Organohalogen Compd.* **1995**, *26*, 105–108.

(15) McLachlan, M. S. A simple model to predict accumulation of PCDD/Fs in an agricultural food chain. *Chemosphere* **1997**, *34*, 1263–1276.

(16) Roeder, R. A.; Garber, M. J.; Schelling, G. T. Assessment of dioxins in food from animal origins. *J. Anim. Sci.* **1998**, *76*, 142–151.

(17) Fürst, P.; Fürst, C.; Groebel, W. Levels of PCDDs and PCDFs in food-stuffs from the federal republic of Germany. *Chemosphere* **1990**, *20*, 787–792.

(18) Theelen, R. M. C.; Liem, A. K. D.; Slob, W.; van Wijnen, J. H. Intake of 2,3,7,8 chlorine substituted dioxins, furans, and planar PCBs from food in The Netherlands: Media and Distribution. *Chemosphere* **1993**, *27*, 1625–1635.

(19) Eduljee, G. H.; Gair, A. J. Validation of a methodology for modelling PCDD and PCDF intake via the foodchain. *Sci. Total Environ.* **1996**, *187*, 211–229.

(20) Domingo, J. L.; Schuhmacher, M.; Granero, S.; Llober, J. M. PCDDs and PCDFs in food samples from Catalonia, Spain. An assessment of dietary intake. *Chemosphere* **1999**, *38*, 3517–3528.

(21) McLachlan, M. S.; Thoma, H.; Reissinger, M.; Hutzinger, O. PCDD/F in an agricultural food chain. Part 1: PCDD/F mass balance of a lactating cow. *Chemosphere* **1990**, *20*, 1013–1020.

(22) Jödicke, B.; Ende, M.; Helge, H.; Neubert, D. Fecal excretion of PCDDs/PCDFs in a 3-month-old breast-fed infant. *Chemosphere* **1992**, *25*, 1061–1065.

(23) Körner, W.; Dawidowsky, N.; Hagenmaier, H. Fecal excretion of PCDDs and PCDFs in two breast-fed infants. *Chemosphere* **1993**, *27*, 157–162.

(24) McLachlan, M. S. Digestive tract absorption of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in a nursing infant. *Toxicol. Appl. Pharmacol.* **1993**, *123*, 68–72.

(25) Pluim, H. J.; Wever, J.; Koppe, J. G.; Sikke vd, J. W.; Olie, K. Intake and fecal excretion of PCDD/F in breast-fed infants at different ages. *Chemosphere* **1993**, *26*, 1947–1952.

(26) Abraham, K.; Hille, A.; Ende, M.; Helge, H. Intake and fecal excretion of PCDDs, PCDFs, HCB and PCBs (138, 153, 180) in a breast-fed and a formula-fed infant. *Chemosphere* **1994**, *29*, 2279–2286.

(27) Dahl, P.; Lindström, G.; Wiberg, K.; Rappe, C. Absorption of polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans by breast-fed infants. *Chemosphere* **1995**, *30*, 2297–2306.

(28) Pointillart, A.; Cayron, B.; Gueguen, L. Utilisation du calcium et du phosphore et minéralisation osseuse chez le porc consommant du yaourt. *Sci. Aliments* **1986**, *6*, 15–30.

(29) Rowan, A. M.; Moughan, P. J.; Wilson, M. N.; Maher, K.; Tasman-Jones, C. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model for digestion studies in man. *Br. J. Nutr.* **1994**, *71*, 29–42.

(30) Council of European Communities. Directives of the council concerning the animal protection for the use of living animals in scientific investigations. *Off. J. Eur. Communities: Legis.* **1986**, 86/609, L358, 1–28.

(31) Henry, Y.; Perez, J. M; Sève, B. (Feeding of growing pigs) Alimentation des porcs en croissance. In *L'alimentation des Animaux Monogastriques*: *Porc, Lapin, Volailles*, 2 éme édition; INRA: 147 rue de l'Université, 75431 PARIS Cedex 07, 1989; pp 49–76.

(32) Thomson, A. B. R.; Schoeller, C.; Keelan, M.; Smith, L.; Clandinin, M. T. Lipid absorption: passing through the unstirred layers, brush-border membrane, and beyond. *Can. J. Physiol. Pharmacol.* **1992**, *71*, 531–555.

(33) 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.

(34) 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.

(35) Rohde, S.; Moser, G. A.; Päpke, O.; McLachlan, M. S. Clearance of PCDD/Fs via the gastrointestinal tract in occupationally exposed persons. *Chemosphere* **1999**, *38*, 3397–3410.

(36) 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. Pharmamacol.* **1998**, *152*, 128–137.

(37) 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. *Am. J. Clin. Nutr.* **1996**, *64*, 47–52.

(38) Lakshmanan, M. R.; Campbell, B. S.; Chirtel, S. J.; Ekarohita, N.; Ezekiel, M. Studies on the mechanism of absorption and distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *J. Pharmacol. Exp. Ther.* **1986**, *239*, 673–677.

(39) McLachlan, M. S. Model of the fate of hydrophobic contaminants in cows. *Environ. Sci. Technol.* **1994**, *28*, 2407–2414.

(40) Diliberto, J. J.; Jackson, J. A.; Birnbaum, L. S. Comparison of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) disposition following pulmonary, oral, dermal, and parenteral exposures to rats. *Toxicol. Appl. Pharmacol.* **1996**, *138*, 158–168.

(41) Olling, M.; Derks, H. J. G. M.; Berende, P. L. M.; Liem, A. K. D.; Jong, A. P. J. M. Toxicokinetics of eight ^{13}C-labeled polychlorinated dibenzo-*p*-dioxins and -furans in lactating cows. *Chemosphere* **1991**, *23*, 1377–1385.

(42) Van den Berg, M.; de Jongh, J.; Poiger, H.; Olson, R. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *CRC Rev. Toxicol.* **1994**, *24*, 1–74.

(43) Morita, K.; Matsueda, T.; Iida, T.; Hagesagawa, T. Chlorella accelerates dioxin excretion in rats. *J. Nutr.* **1999**, *129*, 1731–1736.

(44) Pluim, H. J.; Wever, J.; Koppe, J. G.; Slikke vd, J. W.; Olie, K. Intake and fecal excretion of chlorinated dioxins and dibenzofurans in breast-fed infants at different ages. *Chemosphere* **1993**, *26*, 1947–1952.

(45) Abraham, K.; Hille, A.; Ende, M.; Helge, H. Intake and fecal excretion of PCDDs, PCDFs, HCB, and PCBs (138, 153, 180) in a breast-fed and a formula-fed infant. *Chemosphere* **1994**, *29*, 2279–2286.