Uptake and Transfer of PCDD/Fs by Cattle Fed Naturally Contaminated Feedstuffs and Feed Contaminated as a Result of Sewage Sludge Application. 2. Nonlactating Cows

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The dietary absorption and tissue distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) was investigated in 4 nonlactating Simmental cows. During Phase 1 the dietary uptake and fecal excretion of these chemicals were measured over 10 days using feed containing background levels of PCDD/Fs that were primarily of atmospheric origin. Following this, two of the animals were sacrificed and samples of different fat, muscle, and organ tissues were collected. In Phase 2 the remaining two animals were fed grass silage from a field which had a history of repeated sewage sludge applications. During the last 10 days of the 27-day feeding period, the dietary uptake and fecal excretion of PCDD/Fs were again quantified, after which these two animals were also sacrificed and sampled. The dietary absorption of the PCDD/Fs in the nonlactating cows agreed well with values reported in Part I of this series for lactating cows. In the two animals sacrificed at the end of Phase 1 that were close to a contaminant steady state, the lipid-normalized concentrations were similar in almost all tissues. The exceptions were the liver, and to a lesser extent the lungs and the spleen, which had higher levels; and the degree of elevation increased with the degree of chlorination of the PCDD/Fs. During Phase 2, the animals' body burden of several of the PCDD/F congeners increased markedly. The tissue analyses indicated that the chemicals were initially sequestered primarily in the liver, from where they were redistributed to the other tissues and organs. The rate of redistribution was related to the perfusion of the organ/tissue and decreased in the order lung>spleen>kidney>muscle>fat tissue. The rate of redistribution also decreased with increasing degree of chlorination of the PCDD/F congeners. Whereas virtually all of the 1,2,3,7,8-Cl_5DD taken up during Phase 2 had been deposited in fat tissue by the end of the 27-day feeding period, three-quarters of the Cl_8DD was still in the liver.

Keywords: *Cows; nonlactating; meat; carry over; PCDD/F; sewage sludge*

INTRODUCTION

Human exposure to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) occurs primarily through dietary ingestion of dairy products, meat, and fish (*1*−*3*). In the last years a notable amount of work has been done exploring carryover of PCDD/Fs in dairy cows (*4*−*12*). It has been shown that most of the 2,3,7,8-substituted PCDD/F congeners in cows are essentially at steady state and that the quantity of chemical excreted in the milk is equal to the quantity absorbed in the digestive tract (*11, 12*). Hence, dietary absorption is the key process governing the levels of these compounds in dairy products. It is anticipated that the degree of absorption will be influenced by the source of contaminant in the feed, because this can influence the binding of the chemical to feed components.

In many cases, much of the human exposure to PCDD/Fs via meat is attributable to beef (*1*). There is comparatively little information about the accumulation of persistent organic contaminants in nonlactating beef cattle (*13*−*15*). In addition to dietary absorption, the levels in beef products are determined by the pharmacokinetics of tissue distribution. A study with polychlorinated biphenyls (PCBs) has provided evidence that at steady state the distribution in different tissues is relatively uniform when the concentrations are normalized to the lipid content of the tissue, the one exception being the liver which shows markedly higher levels (*16*). Similar results were found for PCDD/Fs analyzed in 16 meat samples from 2 cows (*17*). However, in a recent paper it was reported that the lipid-normalized concentrations of PCDD/Fs are two to five times higher in muscle than in fat deposits (*18*).

In an earlier paper we reported on an experiment with lactating cows in which the absorption and carryover of PCDD/Fs entering the feed via atmospheric deposition (background contamination) were compared with the absorption and carryover of PCDD/Fs of sewage sludge origin (*12*). A mass balance approach was employed in which the animals were fed "naturally" contaminated feed. The dietary uptake, fecal excretion, and milk excretion of PCDD/Fs were quantified using ultra-trace analysis. It was found that the origin of the PCDD/Fs had little influence on the dietary absorption and carryover of the different congeners.

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Table 1. Characteristics of the Nonlactating Cows

	animal number			
	827	835	8332	842
birth date	10/16/89	02/05/91	11/17/90	06/15/91
last calf	05/12/93	09/25/93	05/13/93	12/16/93
live weight (kg)				
start (mass balance 1)	799	715	700	656
end (mass balance 1)	783	697	-	-
end (mass balance 2)	-	-	717	664

To investigate both the absorption behavior and the tissue distribution of PCDD/Fs in nonlactating animals, a parallel experiment was conducted. In a first phase, 4 nonlactating cows were fed a typical diet in which the PCDD/Fs originated primarily from background atmospheric contamination. The dietary uptake and fecal excretion of PCDD/Fs were measured during this phase, and at the end two of the animals were sacrificed. The experimental design of the second phase mirrored the first, the difference being that the remaining two animals were fed grass silage contaminated with PCDD/Fs of sewage sludge origin. Here we report on the dietary absorption and tissue distribution of PCDD/Fs measured in these 4 nonlactating cows.

MATERIALS AND METHODS

Experimental Design and Sampling. The experiment was conducted at the metabolism station of the Bavarian Centre for Animal Husbandry. Four nonlactating Simmental cows were selected from the herd at the State farm (Table 1).

Phase 1 of the study began in February 1994. The animals were placed on rations of feed that had been grown primarily on the experimental station and contained low levels of PCDD/Fs. After 10 weeks the first mass balance was conducted. During 10 days the daily ingestion of each feed type was monitored by weighing the feed offered and the feed remaining at the end of the feeding period. No feed samples were collected during this phase. Instead, we employed the feed samples collected during phase 1 of the study with lactating cows, which was conducted immediately following phase 1 of this study using feed from the same sources. A representative sample of grass silage was collected daily, whereas the other feed components were sampled once. The feces from the nonlactating cows were quantitatively collected with pails taped to the cows such that contamination with urine was prevented. They were weighed on a daily basis and a representative aliquot was taken for later analysis. At the end of phase 1, cows 827 and 835 were sacrificed, and samples of different tissues were taken for later analysis.

For phase 2 of the study, the "uncontaminated" grass silage was replaced with grass silage harvested from a meadow which had received repeated applications of sewage sludge. All other components of the feed remained the same. After 17 days on this diet the second mass balance commenced. Grass silage samples were collected daily for 7 days, and feces samples were collected daily for 10 days. Then the two remaining cows were sacrificed and tissue samples were taken from them.

The feed, feces, and tissue samples were placed in aluminum boxes, sealed with aluminum, and stored at −18 °C until analysis.

Analytical Methods. For phase 1, the feces samples for two consecutive days were pooled. All other samples were analyzed individually. The samples were freeze-dried, and the samples of grass and corn silage were pulverized in a blender. All samples were Soxhlet extracted for 16 h: the feed samples were extracted in toluene, and the tissue and feces samples were extracted in *n*-hexane/acetone (2:1 v/v). An internal standard cocktail containing 12 $^{13}C_{12}$-labeled 2,3,7,8-substituted PCDD/F congeners was added to the solvent prior to beginning the extraction.

Table 2. Feed Consumption (kilograms of dry weight per day)

	animal number			
	827	835	8332	842
corn silage				
mass balance 1	1.63	1.63	1.63	1.63
mass balance 2	-	-	1.77	1.77
minerals	0.09	0.09	0.09	0.09
grass silage				
mass balance 1	6.99	6.82	6.45	6.29
mass balance 2	-	-	7.41	7.82

The extracts were first cleaned up on a mixed column of silica gel/H_2SO_4, silica gel, and silica gel/NaOH which was eluted with *n*-hexane. The PCDD/Fs were then separated from other interfering contaminants on an alox column. $^{37}Cl_4$-labeled 2,3,7,8-Cl_4DD was added as a recovery standard, and the volume was reduced to 15 μL for analysis. The cleanup method is described in detail in Horstmann and McLachlan (*19*). For several samples it proved to be inadequate, and further cleanup steps involving florisil or gel permeation chromatography were employed.

The samples were analyzed using HRGC/HRMS on a VG-Autospec Ultima at a mass resolution of 8,000−10,000. The PCDD/F were separated on a 60 m Rtx2330 column (0.25 mm i.d., 0.1 μm film thickness) and quantified using the labeled internal standards. All 2,3,7,8-substituted congeners, with the exception of 1,2,3,7,8,9-Cl_6DF, were determined. A detailed description of the analytical parameters is found in Horstmann et al. (*20*).

RESULTS AND DISCUSSION

Quality of the Analytical Data. The recovery of 2,3,7,8-Cl_4DD was 80−90%. With every batch of 9 samples a method blank was analyzed. The quantities in the blanks were in almost all cases at least an order of magnitude lower than the quantities in the samples, with the exception of 2,3,7,8-Cl_4DF, 1,2,3,7,8-Cl_5DF, 1,2,3,4,7,8,9-Cl_7DF, and Cl_8DF in many of the tissue samples. If the quantity in the sample was less than three times the quantity in the blank, the value was not included in the data set. No consistent evidence of interferences was found in the chromatograms with the exception of 2,3,7,8-Cl_4DD in grass silage. This interference was estimated to account for 20% of the signal, and the data were corrected accordingly.

Uptake of PCDD/Fs. The average consumption of the different feed types is given in Table 2. The dry weight feed uptake was well balanced, ranging between 5.8 and 6.2 kg d. wt./100 kg l. wt.$^{0.75}$ during phase 1. During phase 2 more corn silage and grass silage were fed, resulting in an uptake of 6.7 kg d. wt./100 kg l. wt.$^{0.75}$ by animal 8332 and 7.4 kg d. wt./100 kg l. wt.$^{0.75}$ by animal 842. This was done to ensure a positive body-fat balance, and this is reflected in the slightly higher live weights at the end of phase 2.

The PCDD/F concentrations in the feed were reported earlier with the results for the lactating cows (*12*). Although no feed samples were taken during phase 1 for the nonlactating cows, the same feed was used as for phase 1 for the lactating cows. Because phase 1 for the nonlactating cows was conducted immediately before phase 1 for the lactating cows, the concentrations measured during the latter were assumed to be representative for the former. The barley, sugar beet pulp, and concentrate fed to the lactating cows contained relatively low levels of PCDD/Fs, so the differences between the diets of the lactating and nonlactating animals had little influence on the overall contaminant

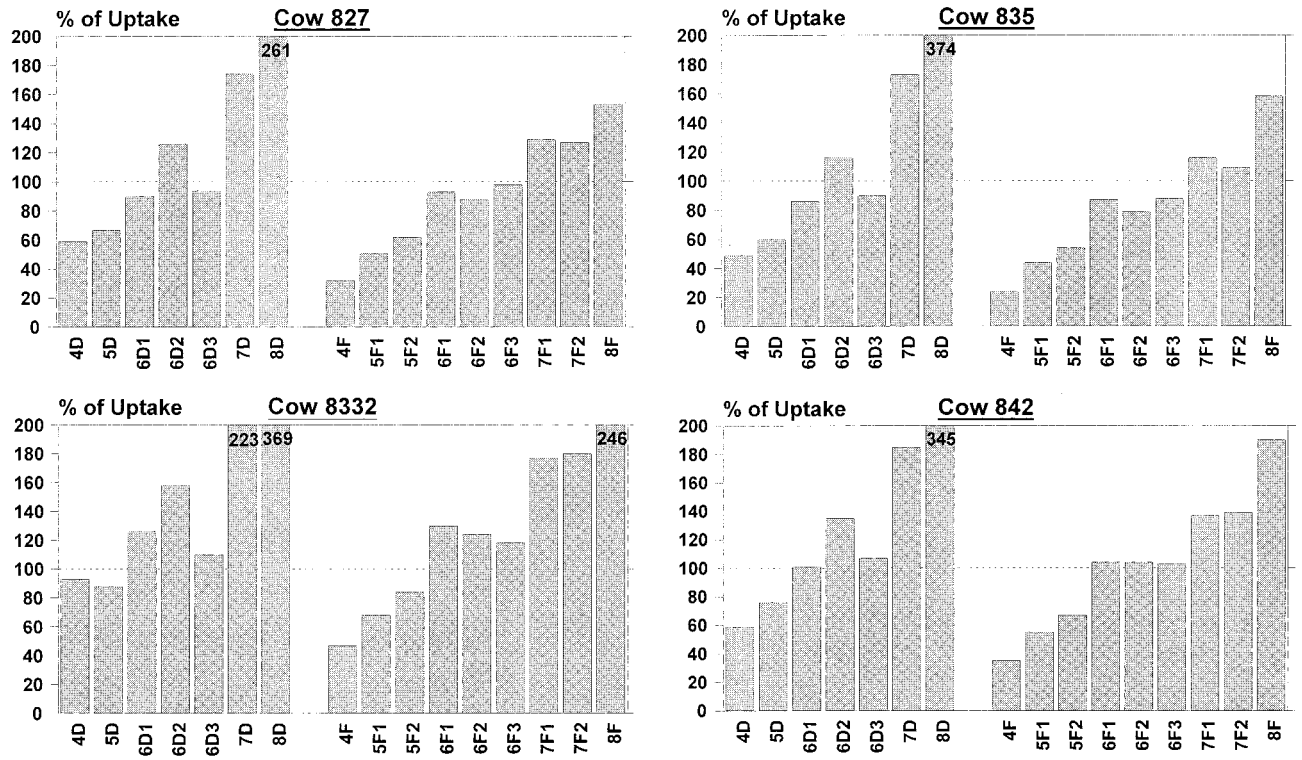

Figure 1. Average fecal excretion of PCDD/F congeners for each of the cows during phase 1 expressed as a percentage of the dietary uptake. 4D, 2,3,7,8-Cl_4DD; 5D, 1,2,3,7,8-Cl_5DD; 6D1, 1,2,3,4,7,8-Cl_6DD; 6D2, 1,2,3,6,7,8-Cl_6DD; 6D3, 1,2,3,7,8,9-Cl_6DD; 7D, 1,2,3,4,6,7,8-Cl_7DD; 8D, Cl_8DD; 4F, 2,3,7,8-Cl_4DF; 5F1, 1,2,3,4/7,8-Cl_5DF; 5F2, 2,3,4,7,8-Cl_5DF; 6F1, 1,2,3,4,7,8/9-Cl_6DF; 6F2, 1,2,3,6,7,8-Cl_6DF; 6F3, 2,3,4,6,7,8-Cl_6DF; 7F1, 1,2,3,4,6,7,8-Cl_7DF; 7F2, 1,2,3,4,7,8,9-Cl_7DF; 8F, Cl_8DF.

uptake. Grass silage was the major source of PCDD/Fs in the feed during phase 1, and the quantity of grass silage consumed was similar for the lactating and nonlactating animals. The ration of the second significant source of PCDD/Fs in the feed, corn silage, was the same. Hence, the PCDD/F uptake during phase 1 was similar for the lactating and nonlactating animals.

Comparing phase 1 and phase 2, the uptake of 2,3,7,8-Cl_4DD and the Cl_{4-6}DFs by the nonlactating cows was very similar. For the remaining persistent PCDD/Fs, the uptake during phase 2 was considerably higher: about a factor of 4, 8, 15, and 50 for 1,2,3,7,8-Cl_5DD, 1,2,3,4,7,8-Cl_6DD, the Cl_7DFs, and the remaining compounds (1,2,3,6,7,8-Cl_6DD, 1,2,3,7,8,9-Cl_6DD, Cl_7DD, Cl_8DD, and Cl_8DF), respectively. Soil particles in the grass silage containing PCDD/Fs from past sewage sludge application were the probable source of the contamination. As described in detail in the earlier paper, the feed concentrations during phase 1 were quite homogeneous, whereas during phase 2 the levels varied widely, reflecting the heterogeneous distribution of the contaminated soil in the grass silage (*12*).

Dietary Absorption During Phase 1. Figure 1 shows the average fecal excretion of the PCDD/F congeners during phase 1 in each of the 4 nonlactating cows expressed as % of the dietary uptake. The difference between fecal excretion and the dietary uptake is the dietary absorption (in %). For cows 827, 835, and 842 the dietary absorption is ~70% for 2,3,7,8-Cl_4DF; 40−50% for 2,3,7,8-Cl_4DD, 1,2,3,7,8-Cl_5DF, and 2,3,4,7,8-Cl_5DF; 30−40% for 1,2,3,7,8-Cl_5DD; and <20% for the Cl_6DF. These results are in good agreement with the results obtained for lactating cows in the parallel experiment (*12*). Cow 8332 showed lower absorption rates. The fact that the fecal excretion of most congeners including the Cl_6DF exceeded the dietary uptake indicates that fecal excretion was overestimated and/or dietary uptake was underestimated. The uncertainty in the mass balance was greater in the experiment with the nonlactating cows because the feed samples were not collected directly from the material fed to the animals.

For 1,2,3,4,6,7,8-Cl_7DD and Cl_8DD in particular, and for the other higher chlorinated congeners to a lesser degree, the fecal excretion was far in excess of the dietary uptake. The variability of the concentrations in the grass silage was high for these congeners (CV of ~50% for the 10 samples analyzed). It is therefore possible that the grass silage fed to the nonlactating cows had distinctly higher levels of these congeners, even though it came from the same silo as the samples analyzed. It is worth noting that 1,2,3,4,6,7,8-Cl_7DD and Cl_8DD may be formed within the digestive tract of the cow (*21*). However, no evidence of this was found for the lactating cows.

Dietary Absorption during Phase 2. The upper panels in Figure 2 show the average fecal excretion of the PCDD/F congeners during phase 2 in the 2 nonlactating cows expressed as % of the dietary uptake. The fecal excretion of the higher chlorinated congeners exceeded the dietary uptake. Given that the fecal excretions of Cl_8DD and Cl_8DF were a factor of 11 and 25 higher, respectively, than during phase 1, these chemicals must have originated from the feed and not the cows. It follows that the dietary uptake of these chemicals was likely underestimated. This can be explained by the difficulty in collecting representative samples of feed where the major source of the contaminant is soil particles. Following the approach taken for the lactating cows (*12*), the consumption of grass silage was adjusted until the fecal excretion of Cl_8DD and Cl_8DF balanced the dietary uptake (the absorption of

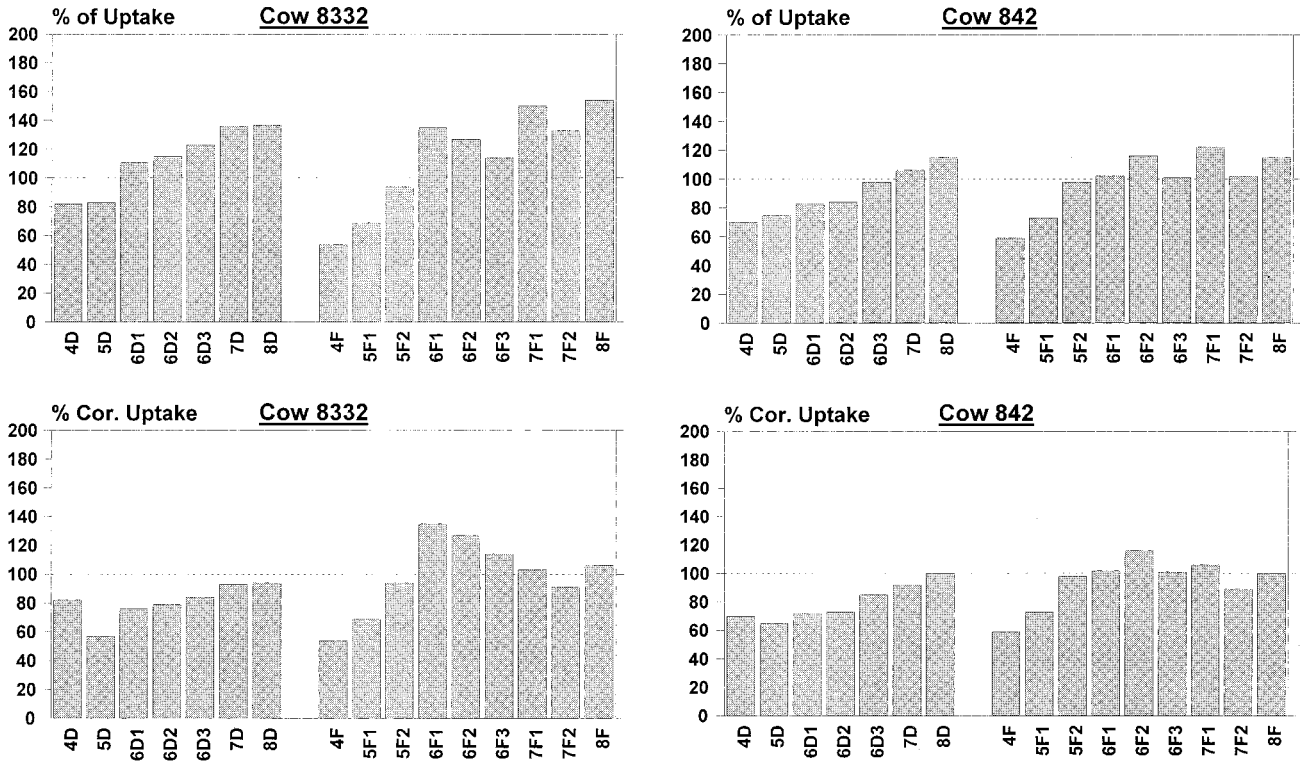

Figure 2. Average fecal excretion of PCDD/F congeners for each of the cows during phase 2 expressed as a percentage of the dietary uptake (upper panels) and the corrected dietary intake (lower panels). See text for details of the correction.

these compounds is typically < 1% (*12*)). The dietary uptake of the other congeners that were primarily associated with soil particles (i.e., for which the concentrations in the contaminated grass silage were at least twice the concentrations in the uncontaminated silage) were then corrected using this revised grass silage consumption rate. These corrected dietary uptake rates were used to recalculate the fecal excretion as a percent of dietary uptake (see Figure 2, lower panels). With the exception of the Cl_6DFs, the dietary absorption of the PCDD/Fs was comparable to that measured in the lactating cows fed the contaminated feed (*12*). A comparison of phase 1 and phase 2 for the nonlactating cows is difficult, as for phase 1 the absorption rate could not be determined for those congeners which were elevated during phase 2. Nevertheless, the good agreement between the dietary absorption measured in the lactating and nonlactating cows during phase 2 reinforces the conclusion of the earlier paper that the dietary absorption of PCDD/Fs from feed contaminated as a result of sewage sludge application is not substantially different from the dietary absorption from feed contaminated via atmospheric deposition.

Discussion of Dietary Absorption Behavior. The fact that the absorption rate did not rise when the dietary uptake was increased indicates that the absorption process is limited by the kinetics of contaminant exchange between the lumen and the body tissue. In contrast, for humans the uptake of most PCBs and PCDD/Fs is so effective that there is no such kinetic limitation to dietary absorption. The fecal excretion of chemical is the result of a partitioning process between human body tissue and the lumen of the gastrointestinal tract. The flux of chemical in the feces is proportional to the contaminant concentration in body tissue and is independent of the amount of chemical ingested, which means that with increasing ingestion rate the absorption rate rises (*22*). In the nonlactating cows, the fecal excretion of many congeners was higher during phase 2. Because the tissue concentrations of most congeners changed very little (see below), this excretion could not have been the result of equilibrium partitioning. Instead, the fecal excretion of the PCDD/Fs was directly proportional to the dietary uptake of PCDD/Fs. We interpret this as evidence that the kinetic limitations imposed by the digestive tract meant that only a fixed fraction of the ingested chemical could be absorbed and that a fixed fraction was excreted. This is consistent with a mathematical model of persistent organic pollutant behavior in cows (*23*).

The independence of the absorption rate from the dietary intake also indicates that the tissue concentrations in the organism were low compared to the levels in the food. When the levels in the tissue become so high that a partitioning equilibrium between the contaminant in the lumen and the tissue is approached, then the net absorption will decrease or a net excretion can even result (*24*). In this case an increase in the chemical uptake will cause the absorption rate to increase. This was not observed in this study. Because of the efficient excretion of lipophilic organic contaminants via the milk, they cannot accumulate in lactating cows to an extent sufficient to cause a decrease in dietary absorption. The nonlactating cows in this study had not been dry long enough for the PCDD/Fs to accumulate to much higher levels in their tissue. This is reflected in the concentrations measured in their tissue (see below), which on a lipid weight basis were similar to the concentrations measured in the milk of the lactating cows (*12*). A reduced absorption can only be expected in old nonlactating animals (e.g., bulls) or cattle that have previously been exposed to much higher contaminant levels in the feed (*23*).

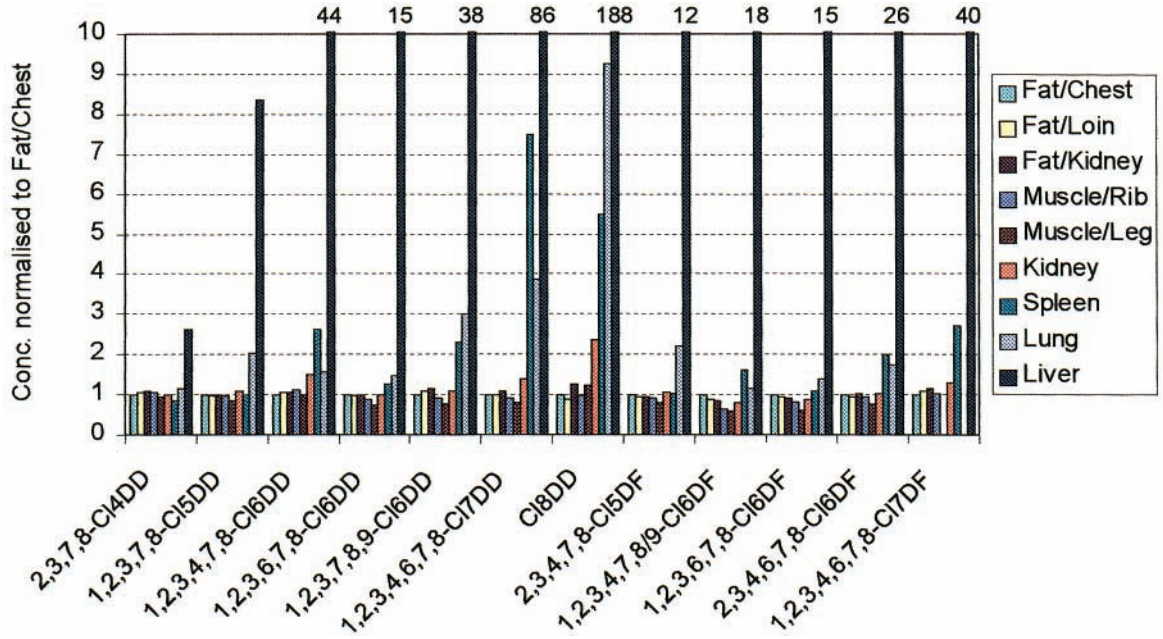

Figure 3. Concentrations of the persistent PCDD/F congeners in different tissues of the 2 nonlactating cows sacrificed at the end of phase 1. The average concentrations for the two animals are shown. The concentrations were all normalized to the concentration of the same congener in the chest fat sample to facilitate the illustration of the data. The concentrations of 1,2,3,4,7,8,9-Cl_7DF and Cl_8DF were below the level of quantification in many tissues and, hence, are not shown.

PCDD/F Distribution at the end of Phase 1. The concentrations measured in fat and muscle samples collected from the two animals sacrificed at the end of phase 1 contained background concentrations of the order of 0.4−0.6 pg of 2,3,7,8-Cl_4DD toxicity equivalents (TEQ) per g of lipid. The PCDD/F pattern found in the fat and muscle was similar to that which was found in the milk of the lactating cows. The majority of the toxicity equivalents were contributed by 2,3,4,7,8-Cl_5DF and 2,3,7,8-Cl_4DD. The PCDD/F distribution in these animals was judged to be close to steady state because their contaminant uptake and their body weight had been reasonably constant for at least several months and the animals were not lactating.

The lipid-normalized tissue concentrations in the two animals were averaged and plotted (Figure 3). To facilitate the viewing of data for the different congeners, the concentrations in the different tissues were normalized to the concentration in chest fat. The concentrations of 2,3,7,8-Cl_4DF, 1,2,3,7,8-Cl_4DF, the Cl_7DFs, and Cl_8DF were close to or below the level of quantification and they are not included in the figure.

It can be seen that the lipid-normalized concentrations were similar in most of the tissues studied. The most notable exception was the liver which had consistently higher concentrations. The degree of elevation of the liver concentration increased with increasing degree of chlorination of the PCDD/F congeners, ranging from a factor of 2.5 for 2,3,7,8-Cl_4DD to a factor of ∼200 for Cl_8DD. The concentrations in the spleen and lung were also elevated, albeit to a lesser extent. Again, it was the higher chlorinated congeners that showed the most pronounced elevation.

The total lipid mass of the different tissues and organs in each of the cows was estimated using the mass of the different tissues and organs determined after sacrificing the animals and representative fat contents of the different tissues as determined in oxen (650 kg live wt.) by refs *25*−*27*. The tissue lipid mass was multiplied by the lipid-normalized PCDD/F concentration in the tissue to obtain the quantity of PCDD/F in the tissue, and this was tallied up for the different tissues to obtain an estimate of the body burden in the cows. Because the lipid masses of the different nonorgan tissues could not be estimated, this was treated as a single compartment in which the concentrations were assumed to be equal to the average of the lipid-normalized concentrations in the five fat and muscle tissues analyzed.

Of the tissues with elevated concentrations, only the liver made a significant contribution to the PCDD/F body burden in the cows. This contribution increased with increasing degree of chlorination, ranging from <1% for 2,3,7,8-Cl_4DD to 25% for Cl_8DD. Only 2−3% of the TEQ burden in the cow was present in the liver. Thus, for an animal close to steady state the body burden of PCDD/Fs can be well approximated by multiplying the lipid-based concentration in fat or muscle tissue by the total lipid mass of the animal. Furthermore, the risk in consuming beef can be assumed to be directly proportional to the fat content of the meat. The exception is liver, for which the lipid-weight-normalized concentrations in animals with background contamination (5.4 and 8.2 pg WHO-TEQ/g in this study) can exceed the standards for dairy or meat products set in some countries. However, the effect of the high lipid-normalized concentrations in liver is compensated to some extent by the low lipid content of this organ. The consumption of 100 g of liver from the animals studied here by a 7-kg individual would result in an uptake of 0.23 (cow 827) or 0.31 (cow 835) pg WHO-TEQ/kg body weight. This can be compared with the WHO guideline for daily intake of 1−4 pg TEQ/kg body weight.

PCDD/F Distribution at the end of Phase 2. Given the homogeneous distribution of the PCDD/Fs in the different fat and muscle tissues observed during phase 1, one tissue (chest fat) was initially chosen to illustrate the differences between the concentrations in the two animals sacrificed at the end of phase 1 and the two animals sacrificed at the end of phase 2 (Figure

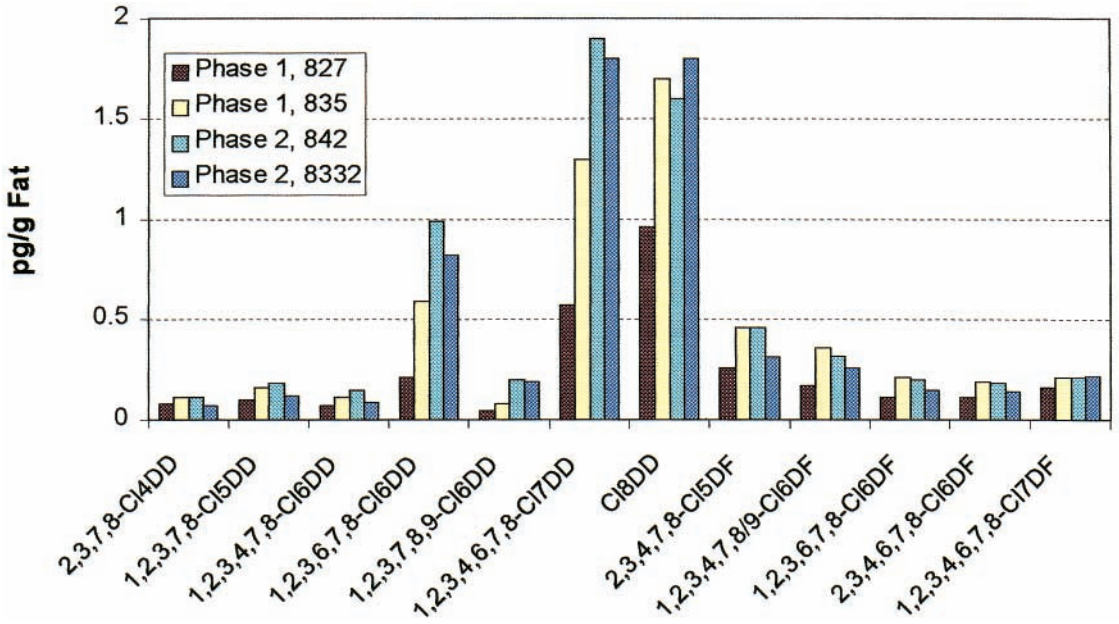

Figure 4. Concentrations of the persistent PCDD/F congeners in chest fat of the 2 nonlactating cows sacrificed at the end of phase 1 and the two nonlactating cows sacrificed at the end of phase 2. The concentrations of 1,2,3,4,7,8,9-Cl_7DF and Cl_8DF were below the level of quantification in many tissues and hence are not shown.

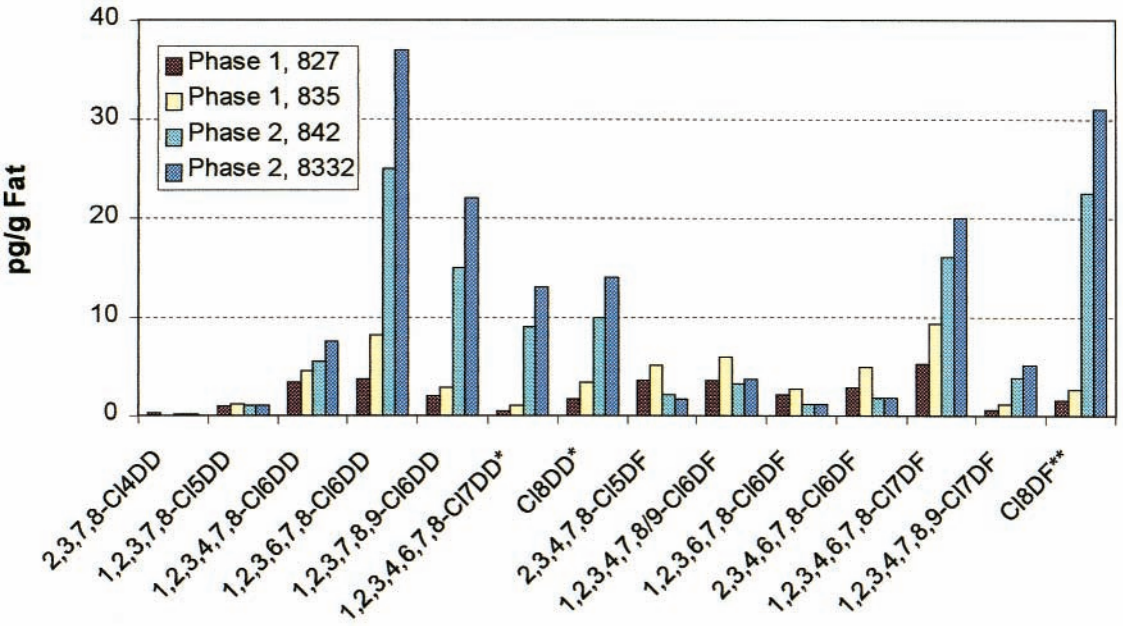

Figure 5. Concentrations of the persistent PCDD/F congeners in the liver of the 2 nonlactating cows sacrificed at the end of phase 1 and the two nonlactating cows sacrificed at the end of phase 2. Asterisk (*) indicates concentrations were divided by 100; double asterisk (**) indicates concentrations were divided by 2.

4). Concentrations of most of the congeners were similar in all four of the cows. For 2,3,7,8-Cl_4DD and the Cl_{4-6}DFs there was little change in the dietary uptake between phase 1 and phase 2. The similarity in the concentrations of these congeners is evidence that the initial concentrations in all 4 animals were comparable. For 3 of the congeners which were most elevated in the feed during phase 2, namely 1,2,3,6,7,8-Cl_6DD, 1,2,3,7,8,9-Cl_6DD, and 1,2,3,4,6,7,8-Cl_7DD, there is some indication of higher levels in the phase 2 animals. However, the effect of ingesting feed with ~50 times higher levels of these congeners for 27 days on the concentrations in chest fat was relatively small.

The liver, in contrast, contained distinctly higher concentrations of all congeners that were elevated in the feed during phase 2, with the exception of 1,2,3,7,8-Cl_5DD (Figure 5). For 1,2,3,4,6,7,8-Cl_7DD and Cl_8DF the levels in liver at the end of phase 2 were more than an order of magnitude greater than the levels at the end of phase 1. This indicates that much more of the contaminant taken up during phase 2 was sequestered into the liver than into chest fat.

Distinct differences in the degree of preferential sequestration into the liver were observed for different congeners. Consider for instance 1,2,3,6,7,8-Cl_6DD and Cl_8DD: in liver, the average concentrations of both compounds were 5 times higher in phase 2 cows than in phase 1 cows. For 1,2,3,6,7,8-Cl_6DD, this was accompanied by an increase in the average concentrations in chest fat by a factor of 2.25. However, for Cl_8DD the

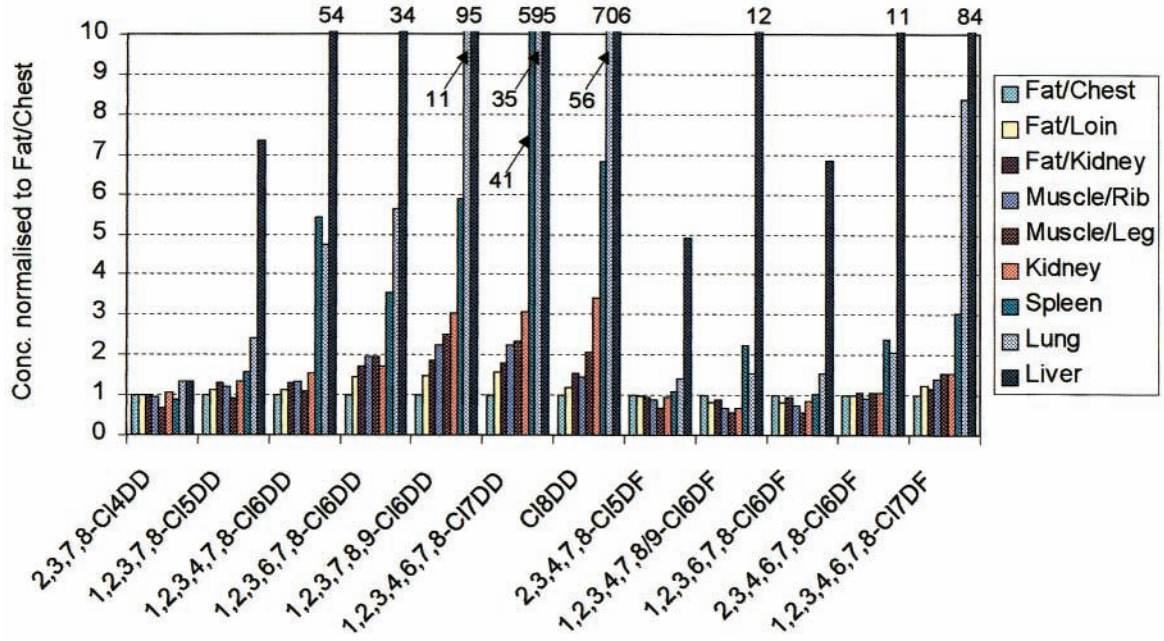

Figure 6. Concentrations of the persistent PCDD/F congeners in different tissues of the 2 nonlactating cows sacrificed at the end of phase 2. The average concentrations for the two animals are shown. The concentrations were all normalized to the concentration of the same congener in the chest fat sample to facilitate the illustration of the data. The concentrations of 1,2,3,4,7,8,9-Cl_7DF and Cl_8DF were below the level of quantification in many tissues and, hence, are not shown.

corresponding increase in concentration in chest fat was only marginal (Figure 4). Overall, the degree of preferential sequestration into the liver increased with increasing degree of chlorination.

The PCDD/F sequestration is further explored in Figure 6, in which the lipid-based concentrations in the different tissues, averaged for the two cows sacrificed at the end of phase 2, are plotted. The concentrations were again normalized to the levels in chest fat to facilitate comparison of the different congeners. In contrast to the phase 1 cows (Figure 3), a systematic gradient in the concentrations between different tissues was observed for those congeners that were elevated in the feed during phase 2. The lipid-based concentrations increased in the order chest fat, loin fat, kidney fat, rib muscle, leg muscle, kidney, spleen, lung, and liver. In the phase 1 animals only the spleen, lung, and liver showed higher levels, and the degree of elevation was considerably lower than that in the phase 2 animals.

The quantity of PCDD/F sequestered was calculated by taking the difference between the inventories in cows 8332 and 842 at the beginning and at the end of phase 2. The inventories at the end were calculated using the measured tissue concentrations and tissue lipid masses which were estimated as described for the phase 1 cows. The inventories at the beginning of phase 2 were estimated as follows below.

It was observed that, although the concentrations in the two phase 1 cows were different, the PCDD/F congener pattern (the relative concentrations) in the two animals was very similar. This can be seen in Figures 4 and 5 for chest fat and the liver. This observation was not unexpected, as the absolute concentrations depend on the animal's body lipid mass and the total amount of feed consumed, which vary from cow to cow, whereas the PCDD/F pattern in the cow depends largely on the PCDD/F pattern in the feed that the cow has consumed, which, up until the end of phase 1, was similar for all four cows. On the basis of this evidence, it was assumed that the PCDD/F pattern in cows 8332 and 842 at the beginning of phase 2 was the same as the average pattern in cows 827 and 835 at the end of phase 1. It was furthermore assumed that the body burdens of 2,3,4,7,8-Cl_5DF, 1,2,3,4,7,8-Cl_6DF, 1,2,3,6,7,8-Cl_6DF, and 2,3,4,6,7,8-Cl_6DF changed only negligibly during phase 2, because the concentrations of these congeners were not elevated in the contaminated feed. The average pattern in cows 827 and 835 and the body burden of 2,3,4,7,8-Cl_5DF, 1,2,3,4,7,8-Cl_6DF, 1,2,3,6,7,8-Cl_6DF, and 2,3,4,6,7,8-Cl_6DF in cows 8332 and 842 at the end of phase 2 were used to calculate the body burden of the remaining congeners in cows 8332 and 842 at the beginning of phase 2.

The change in the inventories during phase 2 are listed in Table 3. Note that data are given only for those congeners which were elevated in the contaminated feed. The differences in sequestration among the different congeners are apparent. Whereas virtually all of the Cl_5DD and Cl_6DD taken up was sequestered into fat, about one-half of the Cl_7DD/F and three-quarters of the Cl_8DD was still in the liver at the end of phase 2.

The data support the following interpretation of PCDD/F pharmacokinetics in the cow. Following dietary absorption the PCDD/Fs are first sequestered primarily to the liver. From there they are redistributed into the other tissues. The rate of redistribution is related to the perfusion rate of the different tissues: it is most rapid to the highly perfused tissues − the lung and the spleen − whereas the redistribution to the poorly perfused fat tissue is slowest. The redistribution is more rapid for the lower chlorinated congeners, while the Cl_{7-8}DD/F are retained in the liver for longer periods of time. Eventually a steady state is approached in which the PCDD/F are homogeneously distributed in body lipids and the contribution of the organs to the body burden is low. This scenario is in agreement with the pharmacokinetic model of PCDD/F behavior in cows proposed by the authors of ref *28*.

It is interesting to speculate about the reason for the slower redistribution of the higher chlorinated PCDD/

Table 3. Change in the Chemical Inventory in Different Tissues during Phase 2 for Those PCDD/F Congeners that Were Elevated in the Contaminated Feed, and Comparison with the Estimated Total Dietary Absorption during Phase 2

	change in inventory (ng)						dietary absorption (ng)
	fat	kidney	spleen	lung	liver	total	
				cow 8332			
1,2,3,7,8-Cl_5DD	3.3	0.02	0.00	0.02	0.14	3.4	
1,2,3,4,7,8-Cl_6DD	4.3	0.02	0.01	0.03	1.5	5.9	
1,2,3,6,7,8-Cl_6DD	134	0.26	0.09	0.52	9.3	145	184
1,2,3,7,8,9-Cl_6DD	35	0.15	0.03	0.21	5.7	41	58
1,2,3,4,6,7,8-Cl_7DD	309	1.2	2.1	4.6	346	662	708
Cl_8DD	151	0.92	0.10	4.8	348	505	290
1,2,3,4,6,7,8-Cl_7DF	13	0.04	0.02	0.15	4.4	17	
				cow 842			
1,2,3,7,8-Cl_5DD	3.5	0.01	0.00	0.00	0.13	3.6	
1,2,3,4,7,8-Cl_6DD	4.2	0.01	0.01	0.02	0.89	5.2	
1,2,3,6,7,8-Cl_6DD	120	0.20	0.09	0.20	5.8	126	235
1,2,3,7,8,9-Cl_6DD	32	0.07	0.03	0.09	3.6	36	56
1,2,3,4,6,7,8-Cl_7DD	240	0.73	2.4	3.2	228	475	583
Cl_8DD	63	0.76	0.14	4.7	231	300	257
1,2,3,4,6,7,8-Cl_7DF	3.4	0.01	0.02	0.09	3.2	6.7	

Fs. A higher sorption capacity of the liver for these compounds cannot, by itself, explain why the redistribution was slower. The rate of redistribution is governed by the blood/liver partition coefficient. If the blood/liver partition coefficient is lower for the more chlorinated congeners, they will be redistributed to other tissues more slowly.

The tissue distribution results for the lower chlorinated PCDD/Fs are consistent with observations for PCB 153 and Malish's study of PCDD/Fs in two cows which showed similar lipid-normalized concentrations in different tissues and organs, with higher levels in the liver (*10*, *16*). However, they stand in contrast to the results of Thorpe et al. (2001), who studied the distribution of 2,3,7,8-Cl_4DD, 1,2,3,7,8-Cl_5DD, 1,2,3,6,7,8-Cl_6DD, 2,3,4,7,8-Cl_5DF, and 1,2,3,4,7,8-Cl_6DF in two groups of Holstein−Friesian heifers: one which had been raised on an "uncontaminated" diet and a second which was fed the PCDD/F congeners for 28 days. In both the uncontaminated animals and in animals slaughtered 1, 14, and 27 weeks following the end of the PCDD/F feeding, the lipid-normalized concentrations in muscle were markedly higher than in sub-cutaneous and perirenal fat, and in many cases similar to those in liver. For instance, in the uncontaminated animals, the average ratio for liver/muscle/fat for 1,2,3,6,7,8-Cl_6DD was 2:3.7:1, whereas in our study the ratio for the Phase 1 animals was 15:0.83:1. They reported an average ratio of 17.2:5.6:1 for the animals slaughtered 1 week after the end of the 28-day PCDD/F feeding, whereas the Phase 2 animals in our study slaughtered immediately following a 27-day feeding showed a ratio of 25:1.4:1. The reasons for this discrepancy are unclear; they may be related to the extraction procedures employed.

Comparison of Increase in Body Burden with Estimated Absorption. The internal consistency of the results can be evaluated by comparing the total amount of chemical absorbed during phase 2 with the change in the contaminant inventory in the cow. Because the calculation of small relative changes in inventory is associated with a high degree of uncertainty, this comparison was undertaken only for those congeners for which the inventory in the cow increased by at least a factor of 2 (1,2,3,6,7,8-Cl_6DD, 1,2,3,7,8,9-Cl_6DD, 1,2,3,4,6,7,8-Cl_7DD, and Cl_8DD).

The quantity absorbed was determined by subtracting the fecal excretion from the corrected dietary uptake. This procedure could not be applied to Cl_8DD and was unreliable for 1,2,3,4,6,7,8-Cl_7DD as a result of the method used to determine the corrected dietary uptake. For these congeners the absorption was estimated by multiplying the corrected dietary uptake by the carryover rate measured in the lactating cows during phase 1 (at steady state the carryover rate is equal to the absorption rate for persistent chemicals in lactating cows).

The estimated absorption and the calculated change in inventory agree with each other within a factor of 2 (Table 3). Given the uncertainties in the calculations, this result is satisfactory and supports the internal consistency of the data and the interpretation presented here. Although the estimated absorption was higher than the change in inventory for 3 of the 4 congeners, this cannot be interpreted as evidence for the degradation of these congeners within the cows. The difference in the fluxes lies within the range of uncertainty of the calculated values. Other experiments have shown these substances to be very persistent in nonlactating cows with elimination half-lives of 100−200 days (*13*, *14*, *18*).

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