Excretion of Polychlorinated Dibenzo-*p*-dioxins and -furans in Milk of Cows Fed on Dioxins in the Dry Period

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A toxicokinetic experiment with four cows fed polychlorinated dibenzo-*p*-dioxins and -furans in the dry period for about 100 days is described. The excretion of the dioxins in milk after calving was characterized by a distribution and an elimination phase. The distribution phase lasted for about 7 days. The duration of the elimination phase depended on the kind of congener. The longest elimination half-life was found for hexachlorinated congeners. The elimination half-life in milk fat of the congeners 2,3,7,8-TCDD, 1,2,3,7,8-PECDD, and 2,3,4,7,8-PECDF, contributing predominantly to the I-TEQ value (international toxicological equivalent to 2,3,7,8-TCDD), varied between 63 and 76 days in three of the four cows. One cow deviated strongly, with an elimination half-life between 36 and 38 days for the predominant congeners, due to a low body fat content after calving twins and two molas. The I-TEQ elimination half-life, accounting for the composition of the congeners in the milk fat, varied for three cows between 78 and 87 days.

INTRODUCTION

An official tolerance level exists in The Netherlands for polychlorinated dibenzo-*p*-dioxins and -furans in milk fat. This level [6 pg of International Toxicological Equivalence (I-TEQ)/g of milk fat] was exceeded in a small area (Lickebaert) due to the presence of a waste incinerator plant. Contaminated milk was handled separately from noncontaminated milk until dioxin levels were again below the tolerance level.

Though many measures have been taken to reduce the exhaust of dioxins by incinerator plants, so that the problem is not likely to occur again, it was felt necessary to study the toxicokinetic behavior of these dioxins in the cow, especially as not much information is available in the literature.

An important question to be answered was how long it would take the cows, after being fed with dioxin-free feed, to produce milk acceptable for human consumption. In this paper results obtained with cows in calf fed dioxins in the dry period are described. Lactating cows excrete dioxins mainly via feces and milk, but cows used in our experiment did not produce milk during the period they were fed dioxins. Therefore, they reflect probably the worst-case situation, compared to cows normally producing milk, because the body burden will be higher compared to that of other cows.

EXPERIMENTAL PROCEDURES

Animals. Four clinical healthy cows in calf, originating from an area contaminated with dioxins (Lickebaert), and a control cow in calf, originating from a noncontaminated area (Lelystad), were used (see also Table I).

Feed. The already contaminated cows in calf received the whole accumulation period grass silage harvested in the contaminated area (Lickebaert) (8.6 kg/day dry matter). The day after milk production had been stopped, each cow ingested an extra feedstuff briquette contaminated with a dioxin mixture. The dioxin intake via these briquettes was 65 ng of I-TEQ/day. The dioxin pattern of this synthetic mixture (made at RIVM, Bilthoven NL; Table II) was more or less comparable to the original contamination pattern in the grass silage from the Lickebaert area. During the accumulation period the cows received also 2 kg of concentrated feedstuff/day.

The control cow received clean uncontaminated feed before and after calving, as did the four contaminated cows after calving.

Sampling. Immediately after calving, sampling of milk started. Cows were milked twice a day. From this, according to milk production ratio, every other day one sample was made (250 g total) for dioxin analysis.

Table I. Weight of Cows before and after Calving, Breed, Milk Production after Calving, Fat Content in Milk

cow	wt,[a] kg	wt,[b] kg	breed[c]	milk prod per day,[d] kg	fat content, %
1777[e]	748 (9)	673	75% FH-25% HF	31.3	5.53
5005	634 (1)	549	100% FH	24.4	4.42
5027	618 (12)	567	100% MRY	20.4	3.66
5038	741 (10)	664	100% MRY	23.2	3.86
5801	632 (0)	580	75% MRY-25% FH	23.2	4.74

[a] Days before calving given in parentheses. [b] Weight day after calving. [c] FH, Friesian Holstein; HF, Holstein Friesian; MRY, Meuse, Rhine, Isel. [d] Milk production after calving. [e] Control cow.

Table II. Composition of Synthetic Dioxin Mixture Used To Spike One Feedstuff Briquette

^{12}C congeners	quantity per briquette, ng	I-TEQ, ng
2,3,7,8-TCDD	10.1	10.10
1,2,3,7,8-PECDD	10.0	5.00
1,2,3,4,7,8-HXCDD	20.0	2.00
1,2,3,6,7,8-HXCDD	56.0	5.60
1,2,3,7,8,9-HXCDD	32.0	3.20
1,2,3,4,6,7,8-HPCDD	735.0	7.35
OCDD	1944.0	1.94
2,3,7,8-TCDF	22.0	2.20
1,2,3,7,8-PECDF	30.0	1.50
2,3,4,7,8-PECDF	19.2	9.60
1,2,3,4,7,8-HXCDF	30.0	3.00
1,2,3,6,7,8-HXCDF	39.6	3.96
1,2,3,7,8,9-HXCDF	11.0	1.10
2,3,4,6,7,8-HXCDF	50.5	5.05
1,2,3,4,6,7,8-HPCDF	303.0	3.03
1,2,3,4,7,8,9-HPCDF	20.4	0.20
OCDF	401.6	0.40
total		65.23

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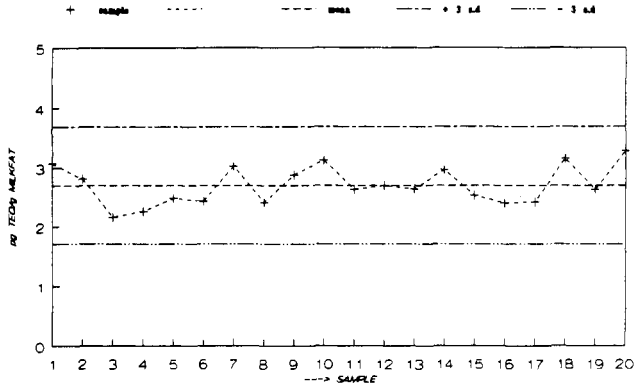

Figure 1. Reproducibility of the analysis of dioxins in a control sample butterfat.

Analysis. *Fat Extraction.* Milk fat was extracted according to the AOAC procedure (Helrich, 1990). Therefore, sodium oxalate and ethanol were added to the milk and then the fat was extracted with diethyl ether and petroleum ether.

Extraction and Determination of Dioxins. The method is extensively described elsewhere (van Rhijn et al., 1992). After the milk fat was spiked (Fürst et al., 1989) with appropriate ^{13}C-labeled dioxins and furans, the dioxins are extracted and cleaned with gel permeation chromatography on a Bio-Beads SX-3 column. After evaporation of the dioxin-containing fraction, the residue is dissolved in hexane and cleaned on a small basic alumina column.

Dioxins are separated from other nonplanar compounds on a porous graphitized carbon HPLC column, using backflush. The final eluent, containing the native and labeled dioxins, is evaporated and dissolved in 10 μL of toluene. An aliquot of 2 μL is splitless injected on a GC (capillary column DB5, length 60 m, inner diameter, 0.25 mm) connected to a VG Autospec mass spectrometer (resolution 10 000) operating in multiple ion mode.

The cleanup of the milk fat and the GC–MS determination are highly automated as described (van Rhijn et al., 1992).

Toxicokinetics. For calculation of the half-lives of dioxins, the software program PKCALC (Shumaker, 1986) was used.

RESULTS

The period of dioxin intake during the dry period was more or less the same for three of the four cows (105 days), while cow 5801 was exposed to the dioxin intake 2 weeks longer (119 days). Cow 5005 gave birth to twin calves and two molas and was, compared to the others, in a less good condition. This animal decreased about 10% in body weight during the sampling period (due to a relatively high milk production) and possessed a lower body fat content than the other animals.

During analysis of the collected milk samples, much attention was given to quality control. Two control samples were available, one being a low contaminated natural butterfat and the other a completely decontaminated milk fat thereafter spiked with all 17 native 2,3,7,8-dioxins at a level of 2 pg/g fat for each compound, resulting theoretically in 5.85 pg of I-TEQ/g of fat. For this spiked butter control sample a mean value of 6.01 pg of I-TEQ/g of fat was found (n = 28; sd = 0.48; CV = 8.1%).

Three compounds (2,3,7,8-TCDD, 2,3,4,7,8-PECDF, and 1,2,3,7,8-PECDD) contribute about 75% to the total dioxin contamination of milk expressed as I-TEQ. The accuracy for these three compounds was, respectively, 100.4, 104.0, and 101.0%. For the total I-TEQ the accuracy was 102.7%. The reproducibility of the analysis of dioxin in the second control sample is shown in Figure 1. Recoveries for all labeled ^{13}C internal standards were between 25 and 150% with a mean recovery between 57 and 82%.

In Figure 2 the concentration–time curve of total dioxins

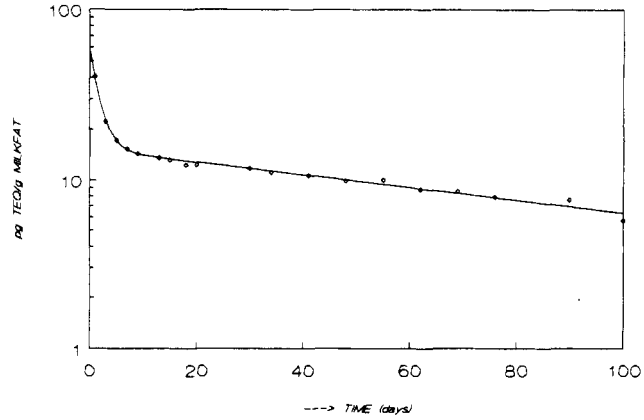

Figure 2. Excretion curve of total dioxins (picograms of I-TEQ per gram of fat) in milk of cow 5801 after calving.

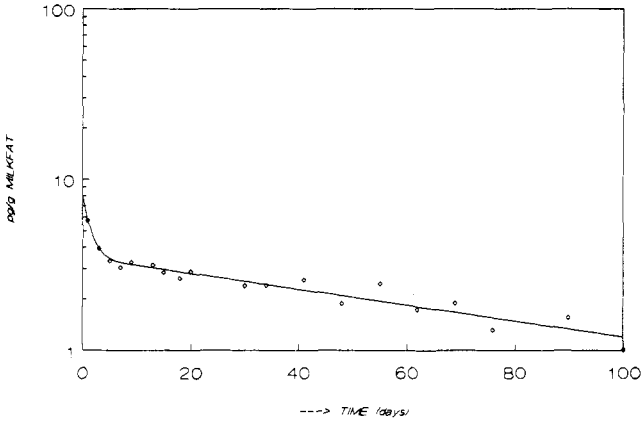

Figure 3. Excretion curve of 2,3,7,8-TCDD (picograms per gram of fat) in milk of cow 5801 after calving.

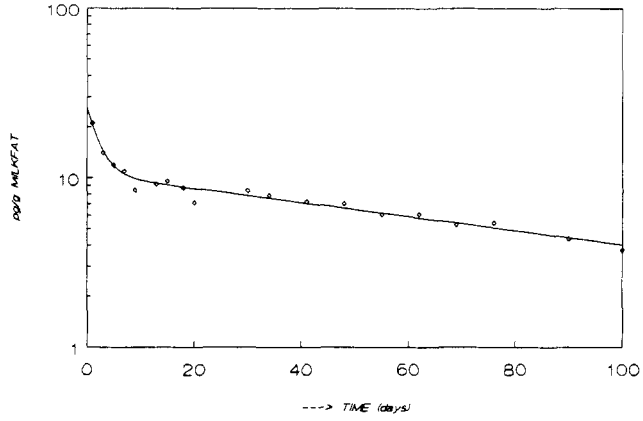

Figure 4. Excretion curve of 2,3,4,7,8-PECDF (picograms per gram of fat) in milk of cow 5801 after calving.

in milk (expressed as picograms of total I-TEQ per gram of fat) of cow 5801 after calving is shown. The excretion curves are shown in Figures 3–5 for the compounds 2,3,7,8-TCDD, 2,3,4,7,8-PECDF, and 1,2,3,7,8-PECDD (being the three most important compounds from a toxicological point of view). For cows 5027 and 5038 similar curves are obtained. In Figure 6 the excretion curve of total dioxins in milk (picograms of I-TEQ per gram of fat) of cow 5005 is shown. The disposition of dioxins in milk of cows after calving is characterized by a biphasic excretion profile. An initial disposition phase of approximately 7 days, in which the dioxin concentration quickly decreases, is followed by a second disposition phase which is characterized by a very slow excretion of dioxins in milk. The excretion curve can be described by the equation (Ritschel,

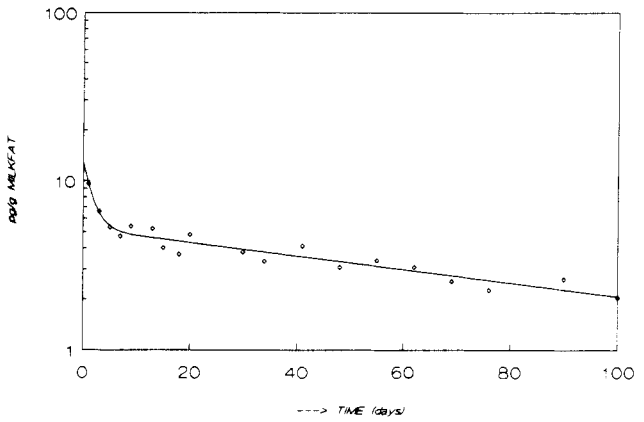

Figure 5. Excretion curve of 1,2,3,7,8-PECDD (picograms per gram of fat) in milk of cow 5801 after calving.

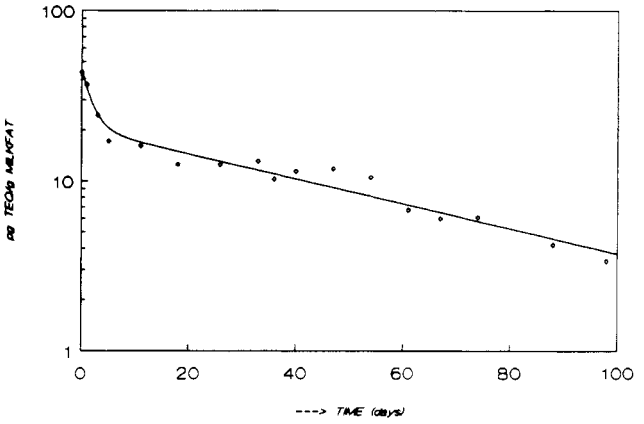

Figure 6. Excretion curve of total dioxins in milk (picograms of I-TEQ per gram of fat) of cow 5005 after calving.

1986)

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where α in the distribution (fast disposition) slope (days^{-1}), β is the elimination (slow disposition) slope (days^{-1}), A is the intercept of the monoexponential distribution slope α with the ordinate (pg/g of fat), B is the intercept of the back-extrapolated monoexponentional elimination slope β with the ordinate (pg/g of fat), and t is time (days).

The mean (±sd) disposition half-life of total dioxins expressed as I-TEQ during the initial phase, $t_{1/2\alpha}$, often referred to as the distribution phase, was 1.6 ± 0.35 days ($n = 4$). The disposition half-life of total dioxins expressed as I-TEQ during the second phase, referred to as the elimination phase, $t_{1/2\beta}$, of cow 5005 was considerably shorter than the $t_{1/2\beta}$ of total dioxins of the other three cows (see Table III). The mean $t_{1/2\beta}$ of total dioxins of cows 5027, 5038, and 5801 was 84 ± 5.0 days. The elimination half-life in milk fat of the congeners 2,3,7,8-TCDD, 1,2,3,7,8-PECDD, and 2,3,4,7,8-PECDF, contributing predominantly to the I-TEQ value, varied between 63 and 76 days in these three cows. In Table III the $t_{1/2\beta}$ of the individual dioxins as well as the total I-TEQ is given for each cow. Calculation of the initial phase was based on only three or four data points. Therefore, this part of the curve is not very well defined and individual $t_{1/2\alpha}$ are not given, although all data in Table III are obtained using a two-compartment open model. From Table III it is clear that for cow 5005 $t_{1/2\beta}$ of all compounds is considerably shorter than for the other animals. For cow 5005 $t_{1/2\beta}$ of all congeners was at least 36 days; for the other three animals $t_{1/2\beta}$ was at least 63 days. Longest elimination half-lives were obtained for hexachlorodioxins and -furans. There was no obvious correlation between the elimination half-lives of the different congeners and the degree of chlorination.

When it appears that a cow, after calving, produces too highly contaminated milk, it is possible to predict the time (withdrawal time), by using the half-lifes for total I-TEQ, needed by the cow after being put on a clean diet to produce milk below the tolerance level. The accepted tolerance level in The Netherlands is 6 pg of I-TEQ/g of milk fat. If the cow is put on a clean diet immediately after calving, the withdrawal period can be calculated using a monophasic or biphasic disposition profile. Based on a biphasic disposition profile a graphic plot of initial I-TEQ concentration vs withdrawal period also results in a biphasic curve which can be derived from eq 1 and is described as follows:

$$C(0) = C(t)(\alpha - \beta)/[(k_{21} - \beta)e^{-\beta t} - (k_{21} - \alpha)e^{-\alpha t}] \quad (2)$$

The following parameters were derived from eq 1:

$$k_{21} = (A\beta + B\alpha)/(A + B) \quad (3)$$

and

$$C(0) = A + B \quad (4)$$

In eqs 2–4 $C(t)$ is the total dioxin concentration (expressed as I-TEQ) in milk fat at time t (withdrawal time) which equals 6 pg of I-TEQ/g of milk fat for the Dutch situation, $C(0)$ is the total dioxin concentration in milk fat at day 1 (initial concentration), and k_{21} is the distribution rate constant for transfer from peripheral to central compartment. The calculation of the withdrawal period can also be based on the dioxin concentration in milk sampled 7 days after the cow has been put on a clean diet. The obtained monophasic curve can be described by

$$C(8) = C(t)e^{\beta t} \quad (5)$$

where $C(t)$ = 6 pg of I-TEQ/g of milk fat and $C(8)$ is the total dioxin concentration in milk at day 8. To determine the withdrawal period based on different moments of milk sampling (0 and 7 days after the cow has been put on a clean diet), the mono- and biphasic curves are given in Figure 7. For instance, when the milk is sampled at day 8 (monophasic curve) and the dioxin concentration measured, equal to 10 pg of I-TEQ/g of fat, it will take about 60 days before the concentration has decreased to 6 pg of I-TEQ/g of fat under the condition that the cow is put on a clean diet immediately.

DISCUSSION

The information in the literature on the accumulation and/or disposition of dioxins in cow's milk is limited. In a feeding experiment Firestone et al. (1979) determined levels of dioxins and polychlorinated biphenyls (PCBs) in milk of lactating dairy cows during and following oral administration of commercial grade pentachlorophenol contaminated with dioxins and furans. From the presented dioxin levels in composite milk fat of treated cows during the accumulation period it can be concluded that the steady-state plateau (90% of the true theoretical value) is achieved at the end of the treatment after 69 days. Therefore, the time to reach steady state in milk, based on a mean elimination half-life of total dioxins of approximately 46 days, was 1.5 times this half-life. This clearly indicates that significant elimination occurs before a distribution equilibrium is achieved because only when a compound distributes rapidly (instantaneously) compared with elimination is 90% of the steady-state plateau reached after 3.3 terminal half-lives (Roland and Tozer,

Table III. Elimination Half-Life (Days) and Correlation Coefficients for Individual Dioxins and for Total I-TEQ in Milkfat of Cows after Calving, Obtained with PKCALC (Shumaker, 1986)[a]

	cow 5005[b]			cow 5027			cow 5038			cow 5801		
congener	$t_{1/2\beta}$	r	n	$t_{1/2\beta}$	r	n	$t_{1/2\beta}$	r	n	$t_{1/2\beta}$	r	n
2,3,7,8-TCDD	37.8	0.8306	14	63.4	0.9313	9	71.6	0.9757	7	64.9	0.9604	17
2,3,4,7,8-PECDF	35.9	0.9345	14	75.4	0.8887	11	66.8	0.7922	6	72.7	0.9783	16
1,2,3,7,8-PECDD	36.0	0.8823	14	70.6	0.9218	12	67.9	0.8381	7	75.9	0.9511	15
1,2,3,4,7,8-HXCDF	44.4	0.9596	14	89.4	0.9868	12	[c]			71.9	0.9934	17
1,2,3,6,7,8-HXCDF	40.6	0.9357	14	111.9	0.9673	12	[c]			106.4	0.9932	17
2,3,4,6,7,8-HXCDF	39.3	0.9447	15	138.3	0.9847	11	97.5	0.9288	7	94.5	0.9873	17
1,2,3,4,7,8-HXCDD	83.8	0.9523	14	220.2	0.9761	11	102.4	0.9916	7	99.2	0.9896	16
1,2,3,6,7,8-HXCDD	77.4	0.9058	14	204.7	0.9121	11	190.7	0.9784	8	164.9	0.9866	17
1,2,3,7,8,9-HXCDD	48.5	0.9262	13	132.9	0.9714	10	128.6	0.9760	8	77.9	0.9853	17
1,2,3,4,6,7,8-HPCDF	37.1	0.9586	15	93.6	0.9985	12	137.1	0.9715	6	85.3	0.9976	16
1,2,3,4,7,8,9-HPCDF	64.2	0.9868	13	50.4	0.9967	12	60.2	0.9398	7	44.5	0.9941	17
OCDD	[c]			[c]			[c]			63.0	0.9866	14
total I-TEQ	40.8	0.9775	14	87.1	0.9667	11	86.8	0.8823	7	78.3	0.997	16

[a] $t_{1/2\beta}$, elimination half-life; r, correlation coefficient; n, number of data points used for calculation of elimination half-lives. [b] Animal with twins. [c] No or too few data.

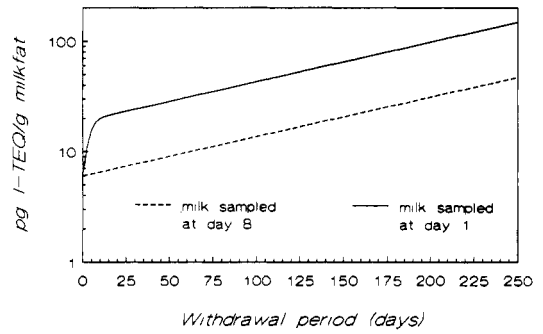

Figure 7. Withdrawal period until the total dioxin concentration (picograms of I-TEQ per gram of milk fat) in milk of a cow after calving has decreased to the Dutch tolerance level (6 pg of I-TEQ/g of milk fat) based on two different moments of milk sampling (0 and 7 days) after the cow has been put on a clean diet.

1989). The same phenomenon can also be observed after multiple dosing of PCBs to lactating cows. In a long-term study described by Fries et al. (1973) steady-state plateau in milk fat was achieved in a time period shorter than one elimination half-life ($t_{1/2\beta}$ = 69 days). However, steady state in body fat was not achieved at the end of the treatment after 60 days. Because of the highly lipophilic character of PCBs, dioxins, and furans, it is suggested that perfusion limited distribution occurs in tissues. Because the udder can be regarded as a richly perfused organ, a pseudosteady state is achieved in milk fat long before steady state is achieved in a slowly equilibrating tissue like body fat. The time to achieve plateau in adipose tissue will be determined by the elimination half-lives of dioxins and furans, irrespective of the time required to reach plateau in milk fat (Roland and Tozer, 1989). Due to the duration of dioxin intake in our experiment (≥103 days) it is assumed that this pseudosteady state in milk was achieved.

The disposition of dioxins in milk of lactating cows after calving was characterized by a biphasic profile. During the initial disposition phase, often referred to as distribution phase, a considerable decline in dioxin concentration occurs ($t_{1/2\alpha}$ = 1–2 days). This can also be observed in two experiments performed by Jones and co-workers in which single doses of 2,3,7,8-TCDD in different matrices were orally administered to lactating cows (Jones et al., 1987, 1989). From both experiments distribution half-lives of approximately 1–2 days can be deduced.

The results obtained for the three (normal) cows show a longer elimination half-life than Firestone et al. (1979) and Jensen and Hummel (1982) reported for 2,3,7,8-TCDD, 1,2,3,6,7,8-HXCDD, 1,2,3,4,6,7,8-HPCDD, and OCDD. Jensen and Hummel found an elimination half-life of 41 days for TCDD in milk, and Firestone et al. reported elimination half-lives for 1,2,3,6,7,8-HXCDD, 1,2,3,4,6,7,8-HPCDD, and OCDD of 51, 47, and 41 days, respectively. However, in both studies the one-compartment open model was used and data points during the distribution phase were included, resulting in shorter half-lives. In our view data from the distribution phase should not be included in the calculation of half-lives of the elimination phase.

The fact that the elimination half-lives of all congeners in milk fat obtained from cow 5005 were all significantly shorter compared to those of the other cows could be due to the relatively low amount of body fat and the high milk production after calving, which in turn was accompanied by a decrease in body weight. The lower amount of body fat was reflected by relatively higher initial concentrations in milk fat for cow 5005. This indicates a smaller volume of distribution, which could lead to a relatively fast transfer from body fat into milk fat. The influence of different depot tissues on the distribution of dioxins is not yet fully understood. The initial distribution of dioxins will be largely determined by factors such as tissue volume, tissue/blood partition ratios, protein binding, and perfusion rate. Analogous to the disposition of PCBs, liver and muscle tissue can serve as the primary early depots and from these depots dioxins are redistributed to tissues of greater affinity and lower perfusion like adipose tissue and skin (Matthews and Dedrick, 1984). Therefore, a slower process of redistribution begins simultaneously with the initial distribution. It should be kept in mind that at the beginning of lactation a new lipid depot is created by de novo synthesis of triglycerides in the mammary gland, which results in passive transfer of dioxins from blood to milk and a corresponding redistribution of dioxins from all tissues to blood to maintain their respective blood/tissue ratios (Bauman and Davis, 1974). Therefore, the fast decline in dioxin levels in milk immediately after calving might be different during another stage of lactation. However, the distribution and elimination of PCBs in milk fat were not related to the stage of lactation or the milk production level of lactating dairy cows (Fries et al., 1973). As observed by Firestone et al. (1979), we also found fluctuations of the dioxin concentration in milk during the elimination phase, resulting in an increase of the elimination half-life. This could be due to redistribution processes occurring simultaneously with the elimination process.

Strictly speaking, disposition curves based on I-TEQ

values cannot be used to describe the disposition behavior of congeners because I-TEQ values are composed of different congeners with different contributions to the I-TEQ value. The major contribution to the reported I-TEQ half-lives is caused by 2,3,7,8-TCDD, 2,3,4,7,8-PECDF, and 1,2,3,7,8-PECDD, and because the half-lives of these congeneers are more or less equal for all cows (except $t_{1/2\beta}$ of cow 5005) the half-lives of the I-TEQ levels can be used for practical purposes. The length of time required to restore cows' milk to a level below the Dutch tolerance level of 6 pg of I-TEQ/g of milk fat depends on the disposition rate constants, α and β, the intercompartmental transfer rate constant, k_{21}, and the initial concentration in the milk fat, $C(0)$ (see eq 2). However, the length of the distribution phase was not established for cows at different stages of lactation. A more conservative determination of the withdrawal period can be based on the dioxin concentration in milk sampled 7 days after the cow has been put on a clean diet. In this case the withdrawal period is determined by the elimination rate constant, β, and the total dioxin concentration at day 8, $C(8)$. On the basis of our results and those from other investigators (Ritschel, 1986; Jones et al., 1987, 1989) it can be assumed that after this initial waiting period the contribution of an initial phase is negligible and can be ignored.

In conclusion, the disposition of dioxins in lactating cows just after calving is characterized by a biphasic excretion profile. After long-term feeding of dioxins was stopped, a rapid initial decline in the dioxin level was followed by a very slow excretion of dioxins in milk. Processes occurring during disposition are complex due to concurrent initial distribution, redistribution, and elimination of dioxins. The withdrawal period of dioxins in milk of cows after calving can be determined from a biphasic curve applying initial dioxin concentrations in milk. To calculate the withdrawal period of dioxins in milk of cows at different stages of lactation, the application of a monophasic curve based on the dioxin concentration in milk sampled 7 days after the cow has been put on a clean diet is advised.

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