Studies on the uptake and carry over of polychlorinated dibenzodioxins and dibenzofurans from contaminated citrus pulp pellets to cows milk

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Introduction

In late 1997, the agricultural ministry of the state of Baden-Württenberg, Germany found during routine controls elevated levels of dioxins in milk (1). The Dutch authorities were informed in March 1998 on this problem. RIKILT-DLO did confirm the results obtained by the scientists in Germany. Further analyses revealed that citrus pulp pellets (CPP) originating from Brazil and present in several European countries contained varying levels of dioxins. CPP is manufactured from orange peel, which is dried before being used as animal feed. Approximately 1.3 million tons of CPP were exported from Brazil to Europe during the 1997/98 season. Several hundred samples of CPP and approximately hundred samples of milk have been analysed by a.o. CLUA and RIKILT-DLO. From the analysed brazilian citrus pulp pellets approximately 75% was positive with an avearage level of 6500 pg i-TEQ/kg product (dry matter). The average dioxin contamination of dioxins in Dutch milk was twice the background. Especially higher amounts in milk were found for two congeners 2,3,7,8-TCDD and 1,2,3,7,8–PeCDD that also have the highest toxicity. In the absence of legislation on animal feed dioxin levels, producers and users, in agreement with the European Commission and authorities of the Member States, decided in the summer of 1998 to temporarily halt imports and use of Brazilian CPP. Based on o.a the experience of RIKILT-DLO the maximum residue level (MRL) was in September 1998 set on 500 pg I-TEQ/kg product assuring that no extra contribution of dioxin contamination in food will be caused by animal feed (2). This MRL is solely based only on analytical information and not on bioavailability . In order to get insight in the bioavailability of dioxins a pilot experiment was carried out in which five cows were fed with contaminated CPP.

Experimental

Five lactating (ca. 30 kg milk/day) Friesian cows were fed during 28 days each with 23,3 kg feed consisting of 5,4 kg contaminated CPP (8500 pg i-TEQ/kg). After this period cows were fed dioxin free feed for another 28 days. At distinct intervals milk samples were taken (total of 24 per cow). From all the cows samples were taken at day 0, 28, 34 and 56 were analysed. From two cows milk samples were analysed taken at distinct intervals (total of 13).

Analysis
Analytical: Prior to extraction the samples were fortified wit 13C labeled standards. After extraction of dairy products and animal feed an extensive clean up is carried out to prepare concentrated extracts for quantitation with gaschromatography-high resolution masspectrometry.
Toxicokinetics: Toxicokinetic analysis of the data was performed with the aid of a computer program for nonlinear regression analysis: WinNonlin. The data were analysed using a two-compartment open model.

Results
The mean dioxin levels (in pg i-TEQ/g fat) of the milk samples taken from all cows at selected time intervals is shown in table 1.

Table 1. The mean (± s.d.) levels of 2,3,7,8-TCDD and total i-TEQ in milk samples taken from all cows (n=5) at four different ime periods.

Time periods (days)	Mean (± s.d.) dioxin levels (in pg i-TEQ/g fat)	
	2,3,7,8-TCDD	Total i-TEQ
0	0,35 (0,11)	0,67 (0,15)
28	3,69 (0,33)	7,78 (0,41)
34	1,77 (0,27)	3,32 (0,53)
56	0,87 (0,12)	1,78 (0,27)

In figure 1 the accumulation of dioxins during exposure (from day 0 to 28) and the decrease of dioxin levels during the wash-out period (from day 29 to 56) is shown for one cow (no. 3512) for the most toxic congener (2,3,7,8-TCDD) and for total i-TEQ.

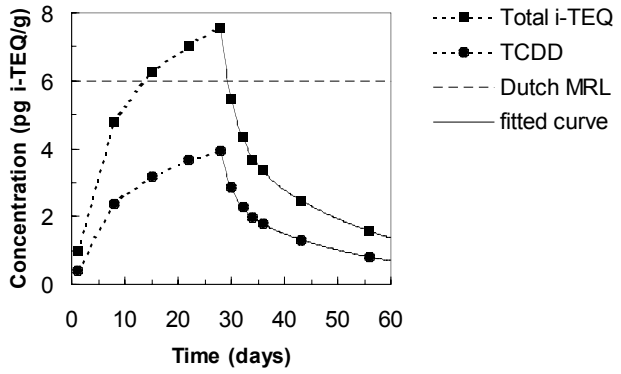

Figure 1. Dioxin levels (pg i-TEQ/g) in milk fat of cow 3512 during and after exposure to dioxin contaminated CPP.

After 2 to 3 weeks of continuous daily exposure the dioxin levels exceeded the Dutch tolerance level of dioxins in milk fat (6 pg i-TEQ/g). After 28 days of exposure the steady state was not achieved. The decrease of dioxin levels was characterized by a two-phase disposition profile. The half-life of elimination was approximately 20 days. For both cows the correlation coefficient of the curve fit of the data obtained during the wash-out period was good (> 0.999).

Discussion

The dioxin levels in milk analysed for all cows on four different time periods were very similar. This indicates a comparable accumulation and elimination of dioxins probably due to a similar intake (homogenously contaminated feed) and relatively small differences in milk yields for all cows.

The accumulation and elimination of dioxins after exposure to contaminated CPP was more rapid than reported by other authors (3,4). The reason for this phenomenon remains unclear. It might have been due to the matrix (CPP contains essential oil) or to the experimental setup (relatively short periods of exposure and wash-out). Although the curve fit of the data during the wash-out period was good the obtained toxicokinetic parameters (like rates of distribution and elimination) could not be used satisfactorily for the curve fitting of the accumulation phase. This could have been due to the limited data points during this phase and/or the uncertainty about the rate of absorption (fluctuating intake cannot be ruled out). Furthermore the assumption that distribution and elimination during the exposure are similar to those during the wash-out period may not be valid. Reviewing the available literature on the toxicokinetics of dioxins in lactating cows it becomes more and more evident that a biphasic disposition profile of dioxins can be found. Due to the fact that the distribution of dioxins during disposition (the first part of the curve) is relatively short, approximately one week, this rapid decline is overlooked in some toxicokinetic studies.

Conclusion

The exposure of lactating cows to a dioxin contaminated feed ingredient (CPP) leads to a rapid accumulation of dioxins in milk. Already after 2 to 3 weeks of exposure the dioxin levels in milk fat exceed the Dutch tolerance level (6 pg i-TEQ/g). On the other hand, the excretion of dioxins, after a relatively short period of exposure, is also rapid and dioxin levels decrease within one week below MRL. This is due to to a biphasic disposition profile of dioxins which is characterized by a rapid first phase (distribution) followed by a slower second phase (elimination).

References

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