Toxicokinetics of eight ^{13}C-labelled polychlorinated dibenzo-p-dioxins and -furans in lactating cows.

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Abstract

Eight ^{13}C-labelled PCDD's and PCDF's dissolved in olive oil were administered intraruminally to four lactating cows as a single dose. Milk and body fat concentrations were monitored for 93 days. Mean elimination half lifes of most congeners varied from 27-49 days. Bioavailabilities varied with degree of chlorination from 36-2%.

Introduction

Since 1989 elevated levels of polychlorinated dibenzo-p-dioxins (PCDD's) and -furans (PCDF's) have been found in the milk of dairy cows kept on pastures in the immediate vicinity of some waste incinerators in the Netherlands. In connection with an extensive monitoring program of these compounds instituted on behalf of Dutch Public Health, Agricultural and Environmental Protection Authorities it was decided to investigate the toxicokinetics of PCDD's and PCDF's in lactating cows. From the literature [2-4] some kinetic data were available on a limited number of congeners only and in view of appropriate risk assessment it was considered necessary to obtain additional data on a larger set of compounds. In this report a toxicokinetic experiment with eight ^{13}C-labelled congeners in four lactating cows is reported. Apart from elimination half lifes for milk fat, data on bioavailability and distribution to body fat are given.

Materials and Methods

Four lactating 4-9 yr old Frisian cows (FH*HF), two of which were pregnant,

were purchased from farms afflicted by milk contamination problems. All animals were in good health, weighed between 529 and 551 kg and had calved 4-6 months before. They were housed in a well ventilated and thermally isolated room in which the temperature was approximately 15 °C. The animals were fed a 1/1 (dry matter) mixture of corn silage and artificially dried grass obtained from a non-contaminated area. In addition concentrate was supplied to compensate for milk production exceeding 9 l/day. Feeding protocols complied with Dutch Feeding Requirements. ^{13}C-labelled PCDD's and PCDF's (table 1) dissolved in 30 ml olive oil were administered intraruminally under light sedation. Milk samples were collected twice daily for 6 days following administration and twice weekly afterwards. Sodium azide was added to the samples for preservation. Fat biopsies were taken under local anesthesia 7, 27, 55 and 93 days after administration. At day 93 the cows were slaughtered and fat and tissue samples were taken from various parts of the carcass. Milk and body fat samples were analysed by gas chromatography-mass spectrometry as described previously [1] with appropriate modifications in connection with the use of ^{13}C-labelled congeners.
Half lifes were derived from the data points at 27, 55 and 93 days. *Bioavailability was calculated by determining* the area under the excreted amount/day versus time curve to which was added the amount remaining in the body fat at the day the animals were slaughtered (day 93). The weight of the fat compartment of these rather lean cows was estimated to be 35 kg. No correction was made for metabolic elimination and for any residues present in non-fat tissues on day 93.

Results

During the experiment no adverse health effects were observed in the cows and feed consumption complied fully with Dutch standards. Soon after administration the labelled PCDD's and PCDF's appeared in milk fat reaching maximum concentrations in approximately one day. During the distribution phase the levels dropped rapidly within 5-6 days and from then on elimination was much slower and followed approximately first order kinetics from day 27 to 93 for most congeners. Mean half lifes calculated using the data from days 27, 55 and 93 are shown in table 1.
Most PCDD's and PCDF's were eliminated with half lifes between 40 and 50 days but both heptachlorinated congeners had appreciably shorter half lifes. Another notable exception was 2,3,7,8-TCDF which was eliminated so rapidly (half life: 0.8 d) that distribution and elimination phase were indistinguishable. The concentration data for subcutaneous fat sampled on days 7, 27, 55 and 93 suggested that the distribution phase continued beyond day 27 and this was most obvious for the highly chlorinated congeners. No terminal half lifes could reliably be calculated from the fat concentration data but between days 27 and 55 fat concentrations tended to drop faster than those in

milk fat.
Mean bioavailabilities (in % of the dose) of the various congeners are also shown in table 1. In general bioavailability tended to decrease with the degree of chlorination which is most obvious for the heptachlorinated compounds. Again 2,3,7,8-TCDF appears to be in a special position because of its extremely fast elimination rate.

Table 1. Mean Elimination Half Lifes and Bioavailabilities of PCDD's and PCDF's in Lactating Cows.

^{13}C-labelled congener	Dose (ng/kg)	Mean half life[1] (days)	Bioavailability[2] (% dose)
2,3,7,8-TCDD	3.7	40.3	29.5
1,2,3,7,8-PeCDD	3.7	42.7	28.0
1,2,3,6,7,8-HxCDD	3.7	45.0	26.5
1,2,3,4,6,7,8-HpCDD	37.1	27.2	1.6
2,3,7,8-TCDF	3.7	0.8	1.3
2,3,4,7,8-PeCDF	3.7	48.5	35.6
1,2,3,4,7,8-HxCDF	3.7	48.5	17.8
1,2,3,4,6,7,8-HpCDF	37.1	33.9	1.7

1 n = 4
2 n = 3 (insufficient data for one cow)

Discussion.

In general the half lifes found in this study for the elimination of PCDD's and PCDF's in cow's milk are in excellent agreement with those reported by Jensen et al [2] and Firestone et al [3] for various congeners. This constitutes strong evidence that in lactating cows these compounds are distributed and eliminated by common non-discriminating processes. The underlying principle for such processes probably is that in the absence of significant metabolic clearance the kinetics of PCDD's and PCDF's are mainly governed by passive partitioning over tissues and body fluids according to their respective fat content. The fact that in cows the terminal elimination rate constant of many PCDD's and PCDF's is approximately equal to the excretion rate of milk fat divided by the total body fat weight probably is not mere coincidence. However, rates of transfer between the various compartments might complicate the picture considerably. Uptake of PCDD's and PCDF's from blood by the mammary secretory cells most probably is very fast as evidenced by the rapid appearance of these compounds in milk and the parallelism between blood and milk concentrations (cf. Jones et al [4]). A

different situation might exist for adipose tissue or at least for some sorts of adipose. From the present study it appears that the distribution rate to subcutaneous fat was low and inversely related with the degree of chlorination. Most notably the heptachlorinated congeners seem to distribute very slowly to body fat which might also explain the faster rate of elimination in milk fat (table 1). The extremely fast disappearance of 2,3,7,8-TCDF must be ascribed to its high rate of metabolic elimination. This phenomenon has also been observed in several other animal species [5].
The inverse relationship between bioavailability and degree of substitution as indicated by table 1 has been reported by Firestone et al [3] as well. For two of the three cows investigated here similar bioavailabilities were found but the third cow had virtually no residues in its body fat at slaughtering giving rise to a relatively low apparent bioavailability. The reason for this phenomenon is unknown.
It must be pointed out that bioavailability from the dosage form used here most probably is higher than from e.g. flue ash from incinerators. In rats bioavailability from flue ash was previously found to be three times lower than from a solution applied to the chow fed to the animals [6]. While this probably is due to the restricted accessibility of PCDD's and PCDF's contained in flue ash particles - which might be expected to affect bioavailabilities in both species equally - it cannot be excluded that the large differences in gastrointestinal physiology between cows and rats nevertheless give rise to significant differences in bioavailibilities in these species. For this reason further research into this subject is now under way in our Institutes.

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