Toxicokinetic properties of trihalogenated dibenzo-*p*-dioxins in rats.

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Introduction

Very little information exists on the toxicokinetic properties of trihalogenated dibenzo-*p*-dioxins. Available data on the relative toxicities of these congeners are not relevant or insufficient for the risk assessment. Therefore, in our study we investigated the tissue distribution of 2,3,7-trichlorodibenzo-p-dioxin (Cl_3DD), 2,3,7-tribromodibenzo-p-dioxin (Br_3DD) and 2,3-dichloro-7-bromodibenzo-p-dioxin (Cl_2BrDD) in female Wistar rats following intravenous (i.v.) injection. Two types of experiments were performed:
(I) *time-dependence* of tissue concentrations measured at different times (6 hours - 14 days) after a single i.v.-injection of a mixture containing 50 µg/kg body wt of each congener, and
(II) *dose-dependence* of tissue distribution 30 minutes after i.v.-injection of a mixture containing 3, 10 or 50 µg/kg body wt of each congener.

Material and Methods

Purity of the congeners: The purity of trihalogenated congeners used in this study was analyzed in Bayreuth (Ökometric GmbH): 2,3,7-trichlorodibenzo-p-dioxin (Cl_3DD) was found to have a purity of 99.7%, 2,3-dichloro-7-bromodibenzo-p-dioxin (Cl_2BrDD) of 99.8% and 2,3,7,-tribromodibenzo-p-dioxin (Br_3DD) of 99.8%.

Animal treatment: The congeners were dissolved in toluene and applied as a suspension in peanut oil/0.9% NaCl (1+9, v/v), the suspension contained less than 5% toluene, the applied volume was 0.5 ml/kg body wt.
In the first experiment (time-course study), a group of female Wistar rats (n=3) weighing 160 - 220 g was treated intravenously (through the tail vein) with a mixture containing 50 µg/kg body wt of each congener. Tissue concentrations (liver, adipose tissue and thymus) were determined 6 hours and 1, 4, 7, and 14 days following treatment.
In the second experiment (dose-dependent study), groups of rats (n=3) were treated with mixtures containing 3, 10 or 50 µg/kg body wt of each congener. Tissue concentrations in liver, adipose tissue and thymus were determined 30 minutes following treatment.

Analytical Methods: The freeze-dried tissue samples (liver, adipose tissue and thymus) were homogenized with Na_2SO_4, packed in a glass column, and eluted with hexane/dichloromethane (1:1). The extract was concentrated and transferred to a combined clean up/column chromatography system. The first step was an acid-base-silica gel column, while on the subsequent alumina column the dioxins were chromatographically separated from other compounds. The procedure was performed on a modified FMS-100 clean up apparatus. The fraction containing the dioxins was reduced to dryness, taken up in 30 µl toluene and stored at -20° C until analysis. ^{13}C-labelled quantification and recovery standards were added after the homogenization step and before reducing the final eluate, respectively. The samples were analyzed by HRGC/HRMS (VG Autospec) or HRGC/MS (HP MS Engine) with detection limits in the range of 10 pg/g DW. The reproducibility of the method was better than ± 10%.

Results and Discussion

Time-dependent changes of tissue concentrations.

Time-courses of the concentrations of Cl3DD, Br3DD and Cl2BrDD were similar in all investigated tissues. However, we observed considerable differences in the toxicokinetics of these compounds between liver, adipose tissue and thymus (Figure 1).
The highest concentrations were found in the liver: 0.12 ± 0.04 ng/g for Cl3DD, 0.06 ± 0.01 ng/g for Cl2BrDD and 0.1 ± 0.03 ng/g for Br3DD measured six hours after treatment. In the following time all congeners revealed a rapid elimination from the liver and seven days after treatment, levels ranging from 0.001 ng/g to 0.002 ng/g were measured. However, during this time period the elimination was not linear (when using a half-log plot), thus it was not possible to use the concentration time-courses for the calculation of elimination half-lives of these congeners. Fourteen days after treatment, the concentrations in the liver were below the detection limit (<1 pg/g).

The concentrations in the thymus measured six hours after treatment (Figure 1) were considerably higher compared to concentrations in the liver (40, 117 and 76 times for Cl3DD Cl2BrDD and Br3DD, respectively). In the following time period (6 hours to 7 days), the concentrations declined in a non-linear manner (when using a half-log plot) as already described for the liver (see above). Fourteen days after treatment, the concentrations in the thymus were below the detection limit (<10 pg/g).

The highest concentrations measured in the adipose tissue were: 32.5 ± 2.1 ng/g for Cl3DD, 36.2 ± 2.8 ng/g for Cl2BrDD and 28.3 ± 2.4 ng/g for Br3DD (six hours following treatment; Figure 1). In contrast to liver and thymus, these concentrations remained at the same level for 24 hours after treatment. During the next days the concentrations of all congeners decreased and values of 0.41 ± 0.09 ng/g (Cl3DD), 0.56 ± 12 ng/g (Cl2BrDD) and 0.6 ± 0.1 ng/g (Br3DD) were measured 14 days after treatment.

Due to the finding that the time-courses of the concentrations were not linear during the investigated time period, our data could not be used for the calculation of half-lives of the trihalogenated congeners in the examined tissues. To demonstrate the non-linearity of the concentration time-courses, we calculated "half-lives" for various time-periods. Considering the data presented in Table 1, different "half-life"-values were obtained depending on the time period taken for the calculation. A rapid elimination at the beginning ("half-lives" ranging from 9.7 to 11.7 hours in the liver or from 2.9 to 3.1 hours in the thymus) gradually slow down and values of 52, 72 and 47 hours (liver), 67, 92 and 91 hours (Thymus) and 33, 36 and 43 hours (adipose tissue; Cl3DD, Cl2BrDD and Br3DD, respectively) were calculated from day 4 to day 7 after treatment.

Dose-dependent tissue concentrations.
In all dose groups (3, 10 and 50 µg/kg body wt) lowest concentrations were found in the liver and highest in the adipose tissue, concentrations in the thymus were in between. A dose-dependent increase of the concentration was observed in all tissues. However, an increase of the dose from 3 to 50 µg/kg body wt resulted in a more than 20-fold increase of the tissue concentrations in liver and in thymus, whereas a less than 10-fold increase was observed in the adipose tissue. To elucidate this phenomenon, we calculated the adipose tissue/liver, adipose tissue/thymus and thymus/liver concentration-ratios (Table 2). The adipose tissue/liver concentration-ratio decreased dose-dependently for all congeners. In contrast, the thymus/liver concentration-ratio did not reveal any dose dependent changes.

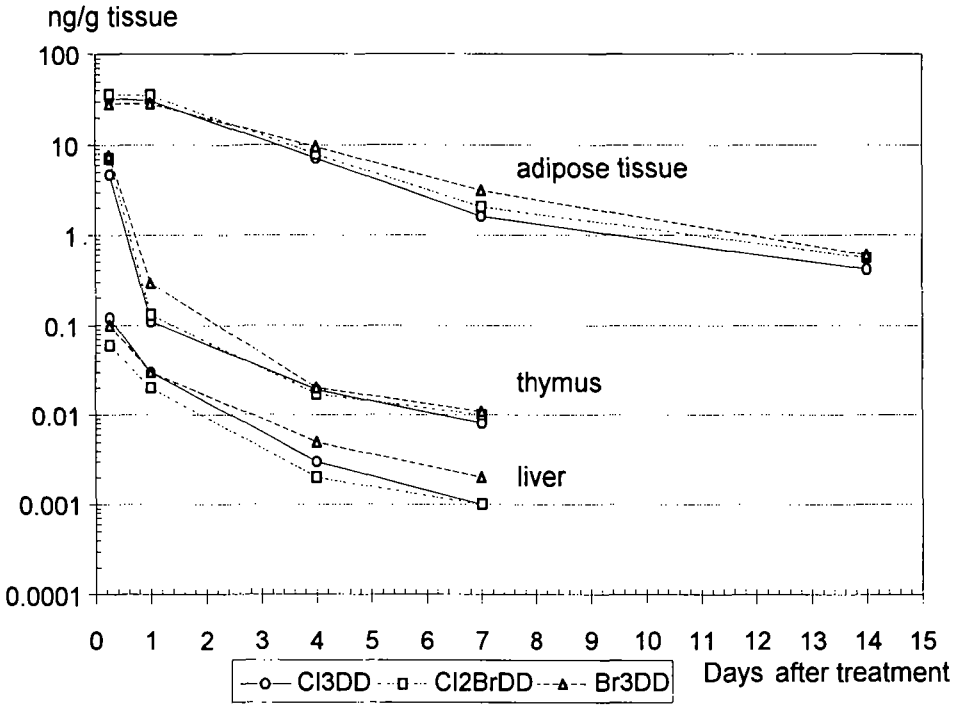

Figure 1: Time-course of the concentrations of Cl3DD, Cl2BrDD and Br3DD in the liver, adipose tissue and thymus following a single i.v.-injection of a mixture containing 50 µg /kg body wt of each congener (mean values, n=3).

Table 1: "Half-lives" of Cl3DD, Cl2BrDD and Br3DD in rat tissues. Values were calculated from data shown in Figure 1.

Time	Liver			Adipose tissue			Thymus		
period	Cl3DD	Cl2BrDD	Br3DD	Cl3DD	Cl2BrDD	Br3DD	Cl3DD	Cl2BrDD	Br3DD
days	(hours)			(hours)			(hours)		
0.25 - 1	9.3	10.2	11.7	*	*	*	3.1	2.9	3.1
1 - 4	32	24	27	34	33	45	31	27	19
4 - 7	52	72	47	33	36	43	67	92	91
7 - 14	n.d.	n.d.	n.d.	86	92	72	n.d.	n.d.	n.d.

Table 2: Concentration-ratios of Cl3DD, Cl2BrDD and Br3DD between adipose tissue, liver and thymus. Data were calculated from concentrations measured 30 minutes after i.v.-injection of a mixture containing 3 µg/kg, 10 µg/kg or 50 µg /kg body wt of each congener (mean values, n=3).

Dose	Adipos tissue / liver			Adipose tissue / thymus			hymus / liver		
(µg/kg)	Cl3DD	Cl2BrDD	Br3DD	Cl3DD	Cl2BrDD	Br3DD	Cl3DD	Cl2BrDD	Br3DD
3	9.3	11.9	6.6	3.7	4.2	2.7	2.5	2.9	2.4
10	9.7	6.4	6.1	3.7	3.0	2.8	2.7	2.1	2.2
50	4.9	4.2	1.9	2.1	1.4	1.2	2.3	2.9	1.6