Tissue distribution and toxicokinetics of 3,4,3´,4´-tetrachlorobiphenyl (PCB-77) in male rats after a single s.c. dose

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1. Introduction:

In connection with a study on possible adverse effects of a single dose of 3,4,3´,4´-tetrachlorobiphenyl (PCB-77) on the reproductive organs of male rats[1)], the toxicokinetics and corresponding tissue concentrations of this compound in various organs were investigated.

2. Materials and Methods

Animal maintenance: Male Wistar rats (Bor:spf, TNO; Fa. Winkelmann, Borchen, Germany) were kept under spf conditions at a constant day/night cycle (light from 9:00 a.m. to 9:00 p.m.), at 21 ± 1^0 C and 50 ± 5% relative humidity. The animals received a standard pellet feed (Altromin^R 1342) and tap water ad libitum. They were adapted to the conditions of the animal quarters for 3 weeks before starting experiments.
Chemicals and treatment: PCB-77 was purchased from Ökometric GmbH (Bayreuth, Germany). The purity of the substance was 99.2%. Levels of the toxicologically relevant PCDD/PCDF congeners were below the limit of detection. For the studies on adverse effects on reproduction, adult male rats were randomly divided into control and treatment groups. Fifty animals were treated with PCB-77 and the vehicle controls (n=50) were treated with the solvent. Treated as well as control groups were divided into five subgroups each. The animals received a single dose of 6 mg PCB-77/kg body wt. Based on a 2,3,7,8-TCDD toxicity equivalency factor (TEF) of 0.0005[2)], this dosage was calculated to be equipotent to a single dose of 2,3,7,8-TCDD of 3 µg/kg body wt which had been demonstrated to affect rat testis[3)].The substance was dissolved in oil (oleum arachidis) and subcutaneously applied in a volume of 1 ml/kg body wt.
Samples: Samples of adipose tissue (abdominal fat), liver and testis (one left testis per group) were taken from the animals of this study 1, 2, 4, 6 or 8 weeks after treatment. From an additional group of ten animals (two rats/date) corresponding samples (including whole blood) were taken 0.5, 1, 2, 3 and 5 days after treatment.

Analysis
Extraction (adipose tissue, liver, testis): Fat extraction (10 g liver, 3 g adipose tissue, 3 g testis) was carried out by grinding the material with equal amounts of sodium sulfate (30 g, for testis: 20 g) and sea sand followed by a 350 ml (for testis: 300 ml) column extraction with

hexane/acetone (2/1 = v/v)[4]. Aliquots of the extracts were used for gravimetric fat determination[4].
For whole blood 5 ml were diluted with 4 ml water and 0.75 ml ethanol. Samples were given on a column successively filled with a glass fiber (1 µm) disk, 5 g sodium chloride and 4 g ChemElut ™ followed by two additional layers in the same sequence. After complete infiltration of the samples, they were left to equilibrate for 10 minutes and then extracted with 85 ml hexane/i-propanol (3/2 = v/v)[5].
Clean-up (adipose tissue, liver, testis): Aliquots of the fat extracts (for adipose tissue 15 %, liver 60 %, testis 90 %) were reduced to a volume of 1 ml in i-octane and subsequently transferred to a column (1 cm i.d.; successively filled with 7 g silica/sulfuric acid and 1 g potassium silicate, separated by 0.5 g sodium sulfate) for elution with 60 ml hexane[6]. Then a small glass column filled with 1 g of silica was used for elution with 6 ml hexane and 6 ml toluene/hexane (35/65 = v/v)[7]. After evaporation the extracts were dissolved in 1 ml toluene and further diluted if necessary. The recovery rate for PCB-77 was between 80 and 110%.
For blood complete extracts were reduced to 1 ml i-octane solution and subsequently transferred to a small column (successively filled with 0.75 g silica/sulfuric acid and 0.25 g potassium silicate, separated by 0.2 g sodium sulfate)[6] for elution with 10 ml hexane. After evaporation, the extracts were dissolved in 0.1 ml toluene and further diluted, if necessary. The recovery rate for PCB-77 was between 70 and 110%.
Determination: Extracts were analyzed by using HRGC-ECD.
GC: Varian 3300, helium with 200 kPa as carrier gas, split/splitless injection (250 °C, 1 min closed) of 1 µl. Column : 60 m x 0.32 mm DB5 and DB1, df 0.1 µm. Temperature programme: 90 °C (4 min hold) to 220 °C at 20 °C/min, then to 280 °C at 2 °C/min (10 min hold). Electron capture detector (ECD): 350 °C, N_2 as make-up gas (35 ml/min). Quantification was achieved by use of external standards.

3. Results and Discussion

The various tissue concentrations of PCB-77 in liver, adipose tissue, whole blood and testis during five days after application are given in Table 1 (whole weight basis) and Table 2 (fat weight basis).
After application of a single dose of 6 mg/kg body wt measurable concentrations of PCB-77 were found in testis (on whole weight basis) as early as 12 h after application. During the first week after application, the concentrations in this organ were about one to two orders of magnitude lower than the corresponding levels in adipose tissue and liver but were approximately in the same range as the corresponding PCB concentrations in whole blood.
On a fat weight basis, comparable PCB concentrations were determined in whole blood and testis which were about in the same range as the PCB concentrations in adipose tissue. In liver fat the accumulation of PCB-77 was highest by far.

The time courses of PCB-77 tissue concentrations measured during 8 weeks after application are given in Fig. 1 - 3 for liver, adipose tissue and testis.
The time course of PCB-77 concentrations after application of a single s.c. dose showed different kinetics in liver and adipose tissue. Initially, high concentrations were found in liver which continuously declined over the whole investigation period. In adipose tissue, considerable variations in the concentrations were observed during the first 24 hours after application which might be related to differences in absorption. Concentrations did not decline for 2 weeks after application indicating a certain (re)distribution during this period which

Table 1: PCB-77 Tissue concentrations (ng/g) in different organs (whole weight basis)

Days after treatment	Adipose tissue	Liver	Testis	Whole blood
0.5	2400	660	122.0	6.1
	480	310	20.7	n.d.
1	2690	740	19.4	8.6
	420	300	9.4	4.3
2	1990	270	18.3	5.4
	940	280	13.6	5.3
3	1340	390	10.4	3.8
	730	510	19.3	3.2
5	1420	180	7.4	2.6
	1690	110	7.0	1.8

n.d.:not determined

Table 2: PCB-77 Tissue concentrations (ng/g) in different organs (fat weight basis)

Days after treatment	Liver	Adipose tissue	Whole blood *)	Testis
0.5	12200	2670	1220	5690
	6940	520		450
1	18200	2930	1710	1120
	6530	450	860	470
2	6330	2160	1080	700
	5720	1020	1060	660
3	8080	1460	760	600
	9620	830	640	1110
5	3320	1530	520	430
	2330	1900	360	410

*) calculated on the basis of an assumed fat content of 0.5 %

Figure 1: Time course of PCB-77 concentrations in liver

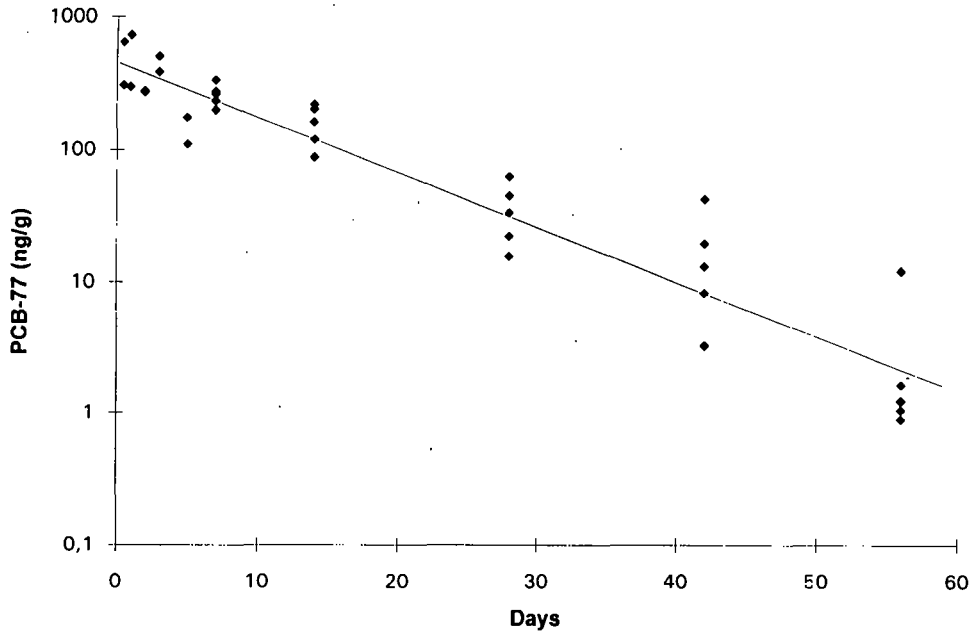


Figure 2: Time course of PCB-77 concentrations in adipose tissue

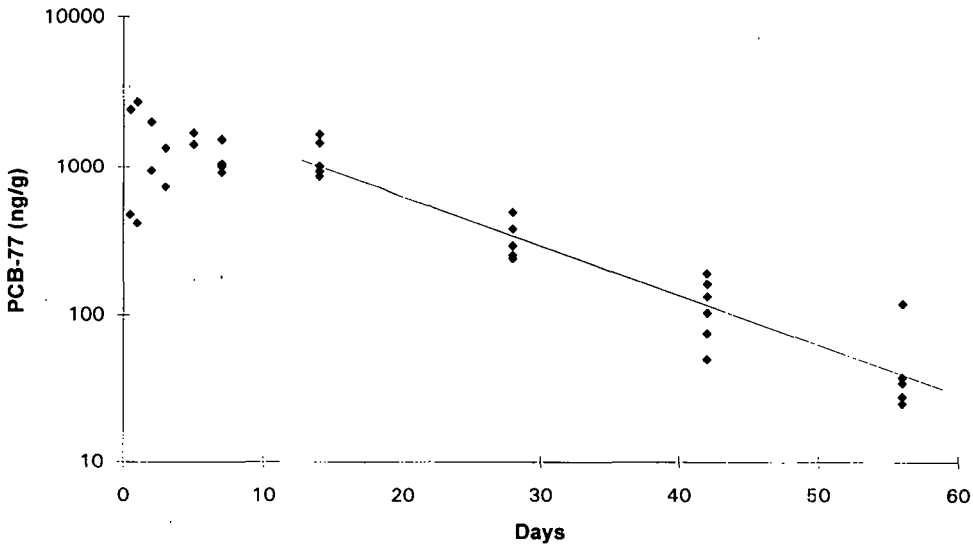
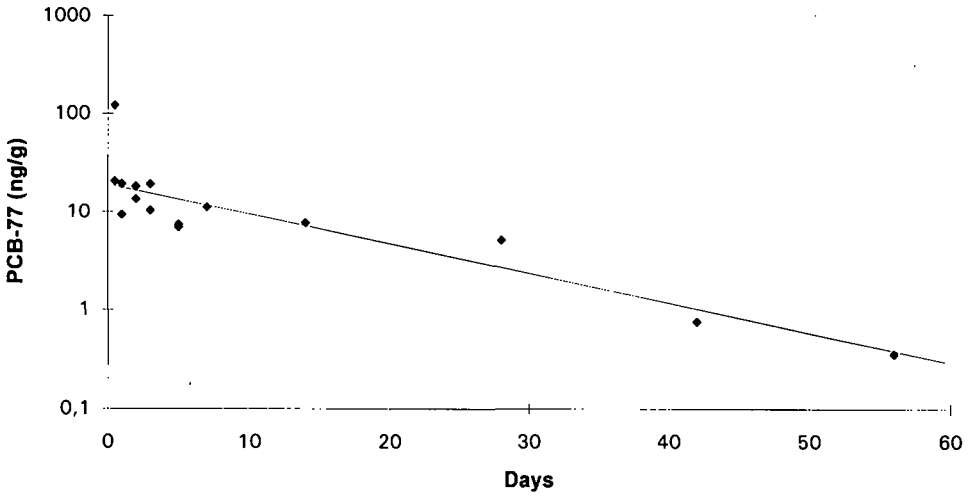


Figure 3: Time course of PCB-77 concentrations in testis



might, at least to some extent, be also due to the administration method.
For the experimental conditions of this fertility study, an elimination half-life of 7.2 days was calculated during the period of linear decline for liver and of 8.7 days for adipose tissue. These findings are different to data from studies on PCB-77 in mice which had been performed under steady state conditions[8]. From the PCB concentrations in the limited samples of testis, an elimination half-life of approximately 10 days was derived.
In contrast to the findings of related fertility studies in rats with 2,3,7,8-TCDD[3] our results clearly indicate the transfer of PCB-77 to the target organs of male fertility in rats.

4. References

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