Toxicity of a single dose of 3,4,3',4'-tetrachlorobiphenyl (PCB 77) in male rats

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Abstract
The effects on male fertility and the general toxicity of 3,4,3',4'-tetrachlorobiphenyl (PCB 77) were determined.

Male Wistar rats were treated with a single dose of 6 mg PCB 77/kg body wt and investigated 1, 2 and 4 weeks after treatment.

No general toxic effect could be detected. Liver weight was increased from the second week after treatment onwards. The activities of the hepatic microsomal enzymes EROD and MROD were induced only during the first two weeks after treatment.

1. Introduction

In connection with a study on the adverse effects of a single dose of 3,4,3',4'-tetrachlorobiphenyl (PCB 77) on the reproductive organs (Chahoud, this issue) of male rats we also investigated the toxic effects on body weight gain and (non-reproductive) organ weights and the induction of hepatic drug metabolizing enzymes.

2. Material 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° C and 50 ± 5% relative humidity. The animals received a standard pellet feed (Altromin® 1342) and tap water ad libitum. They were adapted to the conditions of our animal quarters for 3 weeks before starting the experiment.

Adult male rats were randomly divided into control and treatment groups. Thirty animals were treated with PCB 77 and the vehicle controls (n = 30) were treated with the solvent. The animals received a single dose of 6 mg PCB 77/kg body wt. The substance was dissolved in oil (oleum arachidis) and subcutaneously applied in a volume of 1 ml/kg body wt. Treated as well as control groups were assigned to three subgroups each. Dosed and corresponding control subgroups (n = 10) were investigated 1, 2 or 4 weeks after treatment.

Chemicals:
PCB 77 was purchased from Ökometric GmbH (Bayreuth, Germany). The purity of the substance was 99.2 %. Toxicologically relevant PCDD- and PCDF congeners were under the detection level.

Body weight gain and organ weights:
Treated as well as control animals were weighed 1 and 2 days as well as 1, 2 and 4 weeks after treatment. Also the weight of different organs (Table 3) was recorded.

Enzyme activities:
Alkoxyresorufin O-dealkylase activities (EROD, MROD, BROD, PROD) were determined spectrofluorometrically (SFM-25, Kontron) in liver microsomes according to the method of Burke and Mayer[1], modified by using an NADPH-regenerating system. Microsomal protein concentrations were determined using the Biorad® protein assay with a BSA standard (Sigma).

3. Results
Body weight gain and organ weights:
The relative body weight gain as well as the weekly food intake were not different between PCB 77-treated and control groups (Tables 1 and 2). The organ weights were recorded 1, 2 and 4 weeks after treatment. The absolute as well as the relative weight of liver was significantly higher in the PCB 77-treated groups 2 and 4 weeks after treatment (Table 3).

Table 1: Body weight gain 1 and 2 days after treatment

n	Control 30	PCB 77 30
Initial weight (mean ± SD)	368 ± 18	388 ± 20
Weight after treatment:		
1st day (mean ± SD)	369 ± 18	389 ± 19
weight gain (%)	0.56 ± 1.0	0.40 ± 1.1
2nd day (mean ± SD)	372 ± 18	391 ± 20
Weight gain (%)	1.18 ± 1.2	0.90 ± 1.18

Table 2: Body weight gain 1, 2 and 4 weeks after treatment

n	1st week 30	2nd week 20	4th week 10
Control:			
Body weight (mean ± SD)	378 ± 19	383 ± 17	392 ± 21
Weight gain (%)	2.75 ± 2.76	3.80 ± 3.14	7.03 ± 2.80
PCB 77:			
Body weight	393 ± 24	407 ± 28	426 ± 25
Weight gain (%)	1.45 ± 3.13	4.64 ± 3.49	9.05 ± 2.48
Weekly food intake (g; mean ± SD):			
Control	174.1 ± 12.6	160.6 ± 14.0	142.5 ± 11.9
PCB 77	179.4 ± 18.3	170.7 ± 20.1	158.0 ± 13.1

Table 3: Absolute and relative organ weights (mean ± SD) 1, 2 and 4 weeks after treatment

	1st week	2nd week	4th week
Brain (g; %)			
Control	1.92 ± 0.08 (0.53 ± 0.04)	1.89 ± 0.08 (0.50 ± 0.02)	1.99 ± 0.08 (0.51 ± 0.03)
PCB 77	1.88 ± 0.13 (0.50 ± 0.04)	1.92 ± 0.05 (0.49 ± 0.02)	1.96 ± 0.08 (0.46 ± 0.2)
Thymus (mg; %)			
Control	398 ± 64 (0.11 ± 0.02)	416 ± 70 (0.11 ± 0.02)	358 ± 70 (0.09 ± 0.01)
PCB 77	374 ± 28 (0.10 ± 0.02)	421 ± 79 (0.11 ± 0.02)	425 ± 106 (0.10 ± 0.02)
Heart (g; %)			
Control	1.10 ± 0.14 (0.30 ± 0.03)	1.12 ± 0.18 (0.31 ± 0.04)	1.10 ± 0.15 (0.28 ± 0.03)
PCB 77	1.17 ± 0.04 (0.31 ± 0.03)	1.16 ± 0.11 (0.30 ± 0.02)	1.33 ± 0.11* (0.31 ± 0.02)*
Spleen (mg; %)			
Control	668 ± 96 (0.18 ± 0.02)	693 ± 11 (0.18 ± 0.03)	643 ± 92 (0.16 ± 0.02)
PCB 77	669 ± 73 (0.18 ± 0.02)	729 ± 13 (0.19 ± 0.03)	695 ± 124 (0.16 ± 0.02)
Liver (g; %)			
Control	10.5 ± 1.0 (2.89 ± 0.20)	10.3 ± 0.62 (2.74 ± 0.10)	10.0 ± 0.86 (2.55 ± 0.14)
PCB 77	10.8 ± 1.1 (2.87 ± 0.18)	11.3 ± 0.72* (2.88 ± 0.14)*	11.7 ± 1.08* (2.74 ± 0.15)*
Kidney r (g; %)			
Control	1.14 ± 0.09 (0.31 ± 0.02)	1.19 ± 0.13 (0.32 ± 0.03)	1.18 ± 0.12 (0.30 ± 0.02)
PCB 77	1.26 ± 0.09* (0.33 ± 0.02)*	1.24 ± 0.12 (0.32 ± 0.02)	1.29 ± 0.10* (0.30 ± 0.02)
Kidney l (g; %)			
Control	1.12 ± 0.08 (0.31 ± 0.01)	1.15 ± 0.10 (0.31 ± 0.02)	1.16 ± 0.13 (0.29 ± 0.02)
PCB 77	1.21 ± 0.11* (0.32 ± 0.02)	1.23 ± 0.11 (0.32 ± 0.03)	1.28 ± 0.20* (0.30 ± 0.03)
Ad. glands r (mg; %)			
Control	23 ± 2 (0.006 ± 0.0007)	21 ± 2 (0.006 ± 0.0004)	21 ± 3 (0.005 ± 0.0006)
PCB 77	26 ± 4* (0.007 ± 0.0009)	22 ± 3 (0.006 ± 0.0005)	23 ± 3 (0.005 ± 0.0005)
Ad. glands l			
Control	25 ± 3 (0.007 ± 0.0008)	23 ± 3 (0.006 ± 0.0007)	22 ± 3 (0.006 ± 0.0007)
PCB 77	27 ± 4 (0.007 ± 0.0009)	24 ± 5 (0.006 ± 0.0012)	25 ± 3* (0.006 ± 0.0007)

() = percentage
* = t-test, $p < 0.05$

Enzyme activities
EROD and MROD activities, indicative of specific induction of CYP1A1[2] and CYP1A2[3)5)] as well as PROD and BROD activities, indicative of induction of CYP2B[4)5)] are presented in Table 4.

Basic activities could be determined in the vehicle controls for all enzymes measured. After treatment with PCB 77 a reversible effect on enzyme induction could be detected. A distinct induction of EROD (approx. 8-9 fold) and MROD (approx. 4-5 fold) activities was observed during the first 2 weeks after treatment. PROD and BROD activities were not significantly induced. Four weeks after treatment all enzyme activities had diminished to values generally comparable to those of the controls.

Table 4: Induction of hepatic microsomal EROD, MROD, PROD and BROD activities

	control (n=12)	1st week (n=6)	2nd week (n=6)	4th week (n=6)
EROD	236 ± 50	2093 ± 398	1878 ± 1217	420 ± 222
MROD	140 ± 26	673 ± 117	500 ± 105	182 ± 58
BROD	106 ± 26	270 ± 50	236 ± 88	91 ± 20
PROD	18 ± 3	49 ± 7	38 ± 9	18 ± 3

Enzyme activities (mean ± SD) are given in pmoles resorufin/mg protein/min

4. Discussion

No general toxic effect of a single dose of 6 mg PCB 77/kg body wt could be observed. Neither body weight gain nor food consumption were reduced at any time. This dose induced, however, a hepatotoxic effect. The absolute as well as the relative weights of the livers were significantly elevated 2 and 4 weeks after treatment. During the first 2 weeks after treatment the activities of some hepatic microsomal enzymes (EROD, MROD) were induced. This effect was reversible 4 weeks after treatment. Data of toxicokinetics (not shown) indicated that PCB 77 had been eliminated almost completely from the liver within 1 week. This might explain the time course of induction of enzyme activities.

5. References

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