Effects of a single dose of 3,3',4,4'-tetrachlorobiphenyl (PCB 77) on rat testis

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Abstract
The aim of this study was to investigate the toxic effect of a single dose of PCB 77 on reproductive organs at a dose level of 6 mg PCB 77/kg which should correspond to 3 μg TCDD/kg. The treatment caused a marginal toxic effect on the reproductive organs in male adult rats. Four weeks after treatment the relative weights of testis, epididymis, and prostate were significantly reduced. Furthermore, the number of spermatids/testis was significantly lower. One week after treatment the percentage of abnormal sperm was increased and altered seminiferous tubules could be observed. Manifestation of these effects was not comparable to the effects caused by 3 μg TCDD/kg. There was a clear-cut manifestation of the effects of TCDD as early as one week after treatment.

1. Introduction

A single dose of TCDD (3 μg/kg body wt., s.c.) induced a severe effect on rat testis[1]. 3,3',4,4'-tetrachlorobiphenyl (PCB 77) is one of the most toxic PCB congeners. We performed this study to investigate whether this congener has an adverse effect on the testis and whether manifestion of this effect is comparable to the toxicity of TCDD.

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.

Chemicals and Treatment:
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.
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.
Since a single dose of 3 μg TCDD/kg affects rat testis and the toxic equivalency factor (TEF)[2] of 0.0005, the animals were treated with 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 respective control subgroups (n = 10) were investigated 1, 2 or 4 weeks after treatment.

Number of spermatids in the testis: The tunica albuginea was removed. The testis was minced, homogenized in 10 ml 0.9% NaCl containing 0.05% of Triton X-100 at medium speed in an IKA-RW 15-tissuemizer, (Janke und Kunkel, Staufen im Breisgau, Germany) for 2 min. The number of homogenization-resistant spermatids was counted in a hemocytometer (Buerker).

Morphology of the sperm: The ductus deferens was rinsed with 1 ml 0.9% NaCl. To assess the ratio of morphologically abnormal sperm, a dried smear of the sperm suspension was stained with 2% eosine. 200 sperm per slide were studied microscopically at a magnification of 1:1000 and the normal or abnormal number of sperm was recorded. Sperm were scored as either normal or abnormal with the method described by Soares[2]. Sperm abnormalities manifest themselves in the head and/or in the tail of sperm. Sperm with head abnormalities were calculated separately.

Morphology of the testes:
Rat testes were investigated by light and electron microscopy. For the light microscopic investigation fixation was performed with 4% buffered formaldehyde; embedding was done in paraplast, staining with haematoxylin/eosine or Azan. For electron microscopy, the specimens were fixed in Karnovsky's solution (3% glutaraldehyde + 3% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2), post-fixed in buffered 1% OsO_4, dehydrated in the ascending alcohol series, embedded in Epon and contrasted with uranyl acetate/lead citrate. A Zeiss EM 10 served for evaluation of the pictures.

3. Results

Weights of reproductive organs and number of spermatids/testis:
In comparison to the controls the relative weight of the reproductive organs decreased time-dependently. Four weeks after treatment the differences were statistically significant for testis, epididymis and prostate. The number of spermatids/testis was also significantly reduced at this time point (Table 1).

Morphology of the sperm: In Table 2 the percentage of abnormal sperm (total) as well as sperm with head abnormalities (head abnormal) are presented. The ratio of abnormal sperm was significantly increased 1, 2 or 4 weeks after treatment as compared to the controls, while the ratio of sperm with head abnormalities was significantly increased only 4 weeks after treatment.

Light microscopy of the testis:
In the first week after treatment some seminiferous tubular segments appeared to be normal, others showed changes, such as loosening of the cell unit, necrotic cells in the lumen and reduction of the cell number. Tubules with a lower cell number had shrunk, their contour resembling a thorn-apple. Two weeks after treatment almost all tubules exhibited changes. In addition to the above-described changes vacuoles occurred which might represent larger lipid inclusions. The same effect was observed 4 weeks after treatment.

Electron microscopy of the testis:
One week after injection only some seminiferous tubules were affected by the changes described below. Two and four weeks after treatment almost all tubules showed changes. These changes were due to a loosening of the connections between Sertoli cells and spermatozoa precursors and the connections among spermatozoa themselves as well as to necrosis and vacuolisation of Sertoli cells, densification of spermatid nuclei and vacuole-like dilatation of intercellular spaces. Changes could not be observed in vessels and interstitial cells of Leydig.

Table 1: Relative weights of reproductive organs (mean ± SD) and number of spermatids/testis (millions)

	1st week	2nd week	4th week
Body weight: (g; mean ± SD)			
Control:	364 ± 20	377 ± 13	392 ± 21
PCB 77:	376 ± 19	390 ± 16	426 ± 25
Relative testis weight:			
Control:			
left	0.46 ± 0.04	0.44 ± 0.02	0.46 ± 0.03
right	0.46 ± 0.04	0.46 ± 0.03	0.44 ± 0.06
PCB 77			
left	0.46 ± 0.02	0.45 ± 0.03	0.41 ± 0.03*
right	0.46 ± 0.02	0.45 ± 0.03	0.41 ± 0.03
Relative epididymal weight:			
Control:			
left	0.160 ± 0.01	0.156 ± 0.01	0.157 ± 0.01
right	0.161 ± 0.01	0.156 ± 0.01	0.153 ± 0.01
PCB 77			
left	0.166 ± 0.01	0.160 ± 0.01	0.143 ± 0.01*
right	0.166 ± 0.02	0.148 ± 0.03	0.141 ± 0.01*
Relative prostate weight:			
Control:	0.107 ± 0.02	0.100 ± 0.02	0.109 ± 0.02
PCB 77:	0.113 ± 0.02	0.094 ± 0.02	0.088 ± 0.02*
Relative seminal vesicle weight:			
Control:	0.332 ± 0.04	0.346 ± 0.05	0.340 ± 0.07
PCB 77:	0.343 ± 0.06	0.334 ± 0.04	0.306 ± 0.07
Number of spermatids/testis:			
Control:			
Mean ± SD	183 ± 38	335 ± 57	314 ± 24
Max	267	488	364
Min	136	270	277
PCB 77			
Mean ± SD	315 ± 18	307 ± 37	213 ± 62*
Max	361	364	338
Min	286	252	130

* = T-Test, $p < 0.05$

Table 2: Percentage of abnormal sperm

	1st week	2nd week	4th week
Control:			
total (%):			
Median	5	4.25	3.75
Max	7	6.5	7.0
Min	2	1.5	2.5
Head abnormal (%)			
Median	3.25	2.5	2
Max	5	5	6
Min	1.5	0.5	1.5
PCB 77:			
total: (%)			
Median	7*	7*	5*
Max	8.5	8	12.5
Min	4	3	3
Head abnormal (%)			
Median	3	3	4.25*
Max	5.5	4.5	11.5
Min	2	1	2

* = Mann-Whitney-Test, $p < 0.05$

4. Discussion

A single dose of 6 mg PCB 77/kg body wt. affected reproductive organs of male rats. Four weeks after treatment the relative weight of all testes (right and left), the relative epididymal weight as well as the relative prostate weight were significantly reduced in comparison to the control. The relative weight of the seminal vesicle was also reduced but not statistically significantly. The number of the spermatids/testis was significantly lower in the PCB 77 treated animals. Considering the percentage of abnormal sperm a significant increase could be observed at all stages of investigation. In respect to sperm head abnormalities there was a significant increase 4 weeks after treatment. The morphological investigations of the testes showed changes in some tubules 1 week after treatment. Two weeks and 4 weeks after treatment a more pronounced effect could be recorded, almost all seminiferous tubules exhibited changes. It can be concluded that a single dose of 6 mg PCB 77/kg body wt. induced a marginal toxic effect on the reproductive organs in the male adult rat.

The aim of this study was to investigate the toxic effect of PCB 77 on reproductive organs at a dose level which should correspond to 3 μg TCDD/kg. A dose of 6 mg PCB 77/kg was used, calculated on the base of the TE-factor 0.0005. The applied dose caused an adverse effect on the testis. Manifestation of these effects was not comparable to the effects caused by 3 μg TCDD/kg. There was a clear-cut manifestation of the effects of TCDD as early as 1 week after treatment.

5. References:

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