COMPARISON OF THE EFFECTS OF TCDD AND A DEFINED PCDD MIXTURE ON THE NUMBER OF SPERM IN RAT TESTES

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ABSTRACT

The number of spermatozoa was determined in the testes of rats after a fractionated dosage of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and a PCDD mixture.

Male Wistar rats were treated 16 times (every third day) with 75 ng 2,3,7,8-TCDD/kg body wt. The calculated dose at the end of the treatment was about 632 ng TCDD/kg body wt. Furthermore, one group of rats received a defined PCDD mixture. The applied dose of the mixture corresponded to 82 ng TCDD-equivalents/kg body wt using modified "TCDD-toxic-equivalency" factors (TEF) (UBA 1985; Schulz-Schalge, this issue).

The results showed that there was no significant reduction in sperm count in any treated group as compared to the controls.

KEYWORDS

TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; Wistar rats; Testes; Sperm count

ABBREVIATIONS

PCDD = Polychlorinated dibenzo-*p*-dioxins; TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

INTRODUCTION

In a previous study we investigated the number of sperm in the testes of rats after a single subcutaneous injection of 0.5, 1, 3 or 5 µg TCDD/kg body wt (Hartmann et al., 1990). The doses of 3 µg/kg and 5 µg/kg led to a clear-cut and statistically significant decrease in the number of sperm in the testes, as well as to morphological alterations in this organ. The lowest dose leading to just significant effects on the sperm count in the testes was 1 µg TCDD/kg body wt. No effect was found after an injection of 0.5 µg TCDD/kg body wt.

Here we report on the results of studies performed to answer two questions:
1. Are the fractionated doses leading to a total of 0.6 µg TCDD/kg body wt more effective in reducing the number of sperm than a single injection of this total dose ?
2. Is a PCDD mixture more effective in this respect than an equivalent dose of TCDD ?

MATERIAL AND METHODS

Animal maintenance and treatment
Male Wistar rats (Bor: Wisw/spf, TNO) weighing 350 - 450 g were purchased from Winkelmann (Borchen, FRG). During the experiment they were kept under conventional conditions at a constant day/night cycle (light from 9:00 to 21:00 h), at a temperature of 25 ± 1° C and 50% relative humidity. They received a standard pellet feed (Altromin^{R} 1324) and water ad libitum. Body weight and food consumption was measured every third day.

^{14}C-TCDD supplied by Cambridge Isotope Laboratories (Woburn, USA) had a radiochemical purity of 97% and a specific activity of 122 mCi/mmol (according to the manufacturer). The mixture of PCDDs was obtained by catalytic dechlorination/hydrogenation of OCDD (Hagenmaier et al., 1987; Wiesmüller, 1990). The amount of each of the single, repeated doses corresponded to 82 ng modified TE/kg body wt (composition of the mixture and calculation of the TE-factors according to Schulz-Schalge, cf. Table 2; this issue).

The substances were dissolved in a toluene/DMSO mixture (1+2; vol/vol). The solutions were injected subcutaneously under the skin of the back at a volume of 0.2 ml/kg body wt using a 100 µl-Hamilton^R-syringe (Bonaduz, Switzerland).

Male Wistar rats received 16 single doses of 75 ng TCDD/kg body wt, or 82 ng modified TE/kg body wt of the PCDD mixture. This dose was administered every third day. The number of sperm in the testes was determined after sacrificing the animals one day after the sixteenth treatment.

Dose calculation
The actual dose at the end of the treatment was calculated from the equation:

$$D_n = D \times \frac{1 - (2^{-t})^n}{1 - 2^{-t}}$$

D = Dose of single application
n = number of single doses
^{t} = dose interval/elimination half-life

The actual dose given up to the last treatment was calculated as 632 ng TCDD/kg body wt. Calculation of the actual dose of the repeated administrations of the PCDD mixture is more difficult since the t/2 are not precisely known and vary for the different components.

Sperm count
The testes were weighed and homogenized in a Potter-Elvejhem homogenizer using 10 ml 0.9% NaCl containing 0.05% Triton X-100 per testes. After further dilution of 1:10 the number of the homogenization resistant spermatozoa was counted in a Bürker hemocytometer, and the number of sperm/testes was calculated.

RESULTS and DISCUSSION

High doses (in the µg/kg body wt-range) of 2,3,7,8-TCDD induce a reduction in the sperm count in testes of rats as well as typical histological changes in this organ (Chahoud et al., 1989). These findings are consistent with results of morphological studies of Mittler et al. (1984) performed after single i.p. injections of TCDD doses between 0.2 and 5 µg/kg body wt.

Using single subcutaneous doses of 2,3,7,8-TCDD and evaluating the effect 7 days later (at the time of maximum absorption) a LOEL (lowest-observed-effect-level) of 1 µg TCDD/kg body wt was found and a NOEL (no-observed-effect-level) of 0.5 µg TCDD/kg body wt (Hartmann et al., 1990). Since the concentrations of TCDD are very low in homogenates of testes (Krowke et al., 1989) it is not clear whether the effect of TCDD observed on the testes is due to a direct toxic action.

In this investigation it was studied whether 2,3,7,8-TCDD, when given as fractionated doses over a 5 to 6 week period exhibits a more pronounced effect when compared with the same total dose given once. For this purpose an actual, effective dose was selected (0.6 µg TCDD/kg body wt) which was just below the threshold of an effect seen by a single dose. This experimental set-up would permit the observation whether the fractionated dose regime would lead to an effect double as pronounced as that of a corresponding single dose. With the dose regime used no effect on body weight (wasting syndrome) or on testes weight was induced. The results of our studies indicate that the effect of repeated doses of 75 ng TCDD/kg body wt (leading to about 80% of a steady-state) on the testes does not considerably exceed that of a corresponding single dose.

We were furthermore interested in testing whether the TE-factors suggested (UBA, 1985; after modification for the 2,3,7,8-substituted pentachloro-dibenzodioxins) rightly predict the biological effects of a PCDD mixture. Therefore, we repeated the study with a defined PCDD mixture given at 3-day intervals for a total of 16 doses. The individual dose given represented 82 ng modified TE/kg body wt, i.e. an equivalent dose to the repeated doses of 2,3,7,8-TCDD used in the first experimental series. Again, no effect on the sperm count or on testes morphology was seen when compared with controls. This indicated that the use of these TE-factors does not clearly underestimate the effects on the testes in the rat.

Figure 1: **Comparison of statistical values of control males (box-plot of median, Q1, Q3, min, max) and the individual data of treated males**

Rats received 16 subcutaneous doses (every third day) of TCDD (75 ng /kg body wt) or of a PCDD-mixture (75 ng/TE/kg body wt). Measurement of the number of sperm was performed one day after the sixteenth injection. Controls: n = 19.

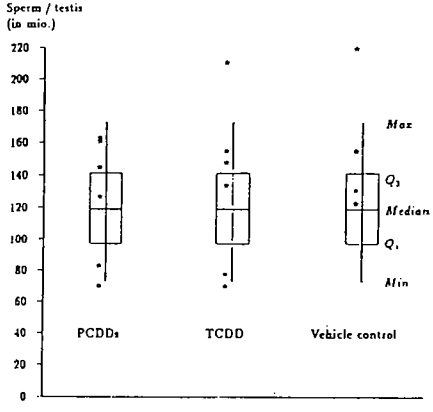

Table 1. Body and testes weights (g) after treatment with TCDD and a PCDD-mixture

Rats received 16 subcutaneous doses (every third day) of TCDD (75 ng /kg body wt) or a PCDD-mixture (75 ng/TE/kg body wt). Body weight and testes weights were measured one day after the sixteenth injection.

	Control	Vehicle-control	TCDD	PCDD
n	19	4	6	6
Body Weight				
	387 ± 35	393 ± 32	380 ± 38	384 ± 3
Testes Weight				
left	1.7 ± 0.2	1.8 ± 0.1	1.9 ± 0.1	1.7 ± 0.2
right	1.7 ± 0.3	1.8 ± 0.1	1.9 ± 0.1	1.7 ± 0.2

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