Postnatal Effects After Continuous Maternal Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in Three Generations of Rats

Elisabeth Koch, Renate Thiel, Ibrahim Chahoud, and Diether Neubert

Institute of Toxikologie and Embryopharmakologie, Freie Universität Berlin, Garystr. 5, D-1000 Berlin 33, Germany

ABSTRACT

A multigeneration study on the reproductive toxicity of TCDD in rats was conducted. We intended to expose the animals to a rather constant tissue concentration during the entire life cycle. To achieve this, we treated the animals with one initial high loading-dose of 50, 120 or 250 ng TCDD/kg body wt and attempted to keep a constant exposure level with weekly maintenance-doses (20% of the loading-dose). As the measurement of the TCDD concentration indicated, it was necessary to apply two loading-doses during the phase of rapid growth and in-between and thereafter we increased the maintenance-doses to 40% of the loading-dose. Among the offspring of the F_1-, F_2- and F_3-generation the following results were observed:

1. Litter size at birth and postnatal survival were not affected by the TCDD treatment.
2. The relative liver weight of the male rats on day 21 postnatally showed a statistically significant increase in all treated groups and generations.
3. The relative liver weight of the female rats on day 21 postnatally showed a statistically significant increase only in the middle and the highest dose group in all generations.
4. TCDD exposure via mother's milk in the ng/kg body-wt-range led to a tendency decrease in the relative thymus weight on day 21 postnatally in the groups with the two highest dose regimes.

KEYWORDS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; Wistar rats; Postnatal; Tissue concentration

INTRODUCTION

Effects which may be considered clearly toxic, such as body weight loss or reduction in thymus weight, may be triggered by TCDD in susceptible species with single doses of the lower μg/kg-body-wt-range (1, 2). This dose range has so far been preferred in the majority of studies with TCDD. A multigeneration study using the same daily dose (ng/kg body-wt-range) throughout the treatment period was performed by MURRAY et al. (3). A significant decrease in the average thymus weight (absolute and relative) and an increase of the relative liver weight was seen at 10 ng TCDD/kg body wt/day in the F_3-generation of 21-day-old male pups. The female pups showed a significant increase of the relative thymus weight and a trend for a decrease of relative liver weight was noted. Since considerable variations in the kinetics occur during such a dosing schedule and the body burden at different stages cannot be defined, we have performed a multigeneration study in which the body burden was kept rather constant and tissue levels were measured regularly during the entire experiment.

MATERIAL and METHODS

Animal maintenance. Male and female Wistar rats (Bor: Wisw/spf, TNO) weighing 350-450 g and 200-220 g, respectively, were purchased from Winkelmann (Borchen, FRG). During the experiment they were kept under spf 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® 1324) and water ad libitum.

Substance and treatment. ^{14}C-TCDD supplied by Cambridge Isotope Laboratories (Woburn, Mass., USA) had a radiochemical purity of 97 % and a specific activity of 107 mCi/mmol (according to the manufacturer).

The substances were dissolved in a toluene/DMSO mixture (1+2; vol/vol) (4). The solutions were injected subcutaneously into the back of the animal at a volume of 0.2 ml/kg body wt (at 3 weeks of age the juvenile rats received the first subcutaneous injection at a volume of 0.5 ml/kg body wt). The control animals received the vehicle only. To achieve rather constant tissue concentrations we treated the F_0-animals with one initial high loading-dose of 50, 120 or 250 ng TCDD/kg body wt and attempted to keep a constant exposure level with weekly maintenance-doses (20% of the loading-dose). After weaning (at 3 weeks of age) the F_1-generation was treated with the same dose regimen (loading-/maintenance-dose). During the phase of rapid growth this dosing schedule was insufficient to keep the tissue concentration constant. Therefore, we treated the rats of the F_1-generation with a second loading-dose. To achieve rather constant tissue concentrations the animals of the F_2- and F_3-generation were treated with two loading-doses during the phase of rapid growth and the weekly maintenance-doses were increased to 40% of the loading-dose. In this way a rather constant body burden was maintained.

Measurement of TCDD concentraions. ^{14}C-TCDD was measured after solubilization of the samples in 3 ml TS1® (Zinsser, Frankfurt, FRG) and sonication for 30 min three days later. After addition of 15 ml scintillation cocktail Hionic Fluor® (Packard, Frankfurt, FRG), the samples were analysed by scintillation counting. The data were corrected for quenching. It had been shown before that the tissues evaluated only contained the unchanged TCDD and that the radioactivity measurements gave the same values as direct chemical analysis of TCDD.

Evaluation of postnatal development. The pregnant dams were allowed to deliver. The litter weight as well as the number of viable offspring was recorded every day, the average weight of each offspring per litter was calculated. At the end of weaning (at 3 weeks of age) the weanlings which were not randomly selected as parents for the next generation were sacrificed. All major organs were weighed and the concentration of TCDD was measured in the liver and the subcutaneous white adipose tissue.

RESULTS and DISCUSSION

The litter size of viable newborn in all investigated generations was the same in the controls and TCDD treated groups. The average weight of the offspring at birth as well as on day 21 postnatally also showed no statistically significant difference when compared with the control group.

An apparent statistically significant decrease of the relative thymus weight was noted on day 21 postnatally among the female rats of the middle and the high dose group in the F_2- and F_3-generation, but not in the F_1-generation, but when compared with the pooled controls this effect was observed in the low and middle dose group in the F_1-generation and in the groups with the two highest dose regimes in the F_3-generation (Table 1). MURRAY et al. (3) observed a significant increase of the thymus weight (relative and absolute) after oral exposure to 10 ng TCDD/kg body wt/day among the male and female Sprague-Dawley rats in the F_3-generation on day 21 postnatally.

On day 21 postnatally we observed an increase in relative liver weight among the male offspring in all TCDD treated groups and among the female offspring in the groups treated with the two higher dose regimes.

The corresponding concentrations of TCDD in the liver and the subcutaneous adipose tissue on day 21 postnatally are shown in Table 2. A concentration of 500 pg/g liver for the male rats and a concentration of 1,000 pg/g liver for the females led to a statistically significant increase of the relative liver weight. According to the data of KOCIBA et al. (5) and PITOT et al. (6), the no-observed-adverse-effect-concentration (NOAEC) for the induction of liver adenomas would be > 1000 ppt in the target tissue.

Table 1: **Relative weight of liver and thymus on day 21 postnatally**

% of body wt *(Number of animals)*

Tissue	Sex	Control	TCDD 50/10*(20#)	TCDD 120/24*(48#)	TCDD 250/50*(100#)
F_1-generation					
liver	male	3,56 ± 0,23*(19)*	3,76 ± 0,33[a]*(49)*	3,88 ± 0,37[a]*(63)*	4,03 ± 0,22[a]*(42)*
liver	female	3,73 ± 0,22*(26)*	3,88 ± 0,33 *(41)*	4,05 ± 0,35[a]*(39)*	4,01 ± 0,37[a]*(12)*
thymus	male	0,37 ± 0,08	0,39 ± 0,12	0,36 ± 0,09	0,37 ± 0,10
thymus	female	0,40 ± 0,09	0,40 ± 0,10	0,39 ± 0,07	0,41 ± 0,06
F_2-generation					
liver	male	3,56 ± 0,41*(52)*	3,86 ± 0,44[a]*(55)*	3,82 ± 0,41[a]*(74)*	3,91 ± 0,40[a]*(57)*
liver	female	3,74 ± 0,38*(76)*	3,89 ± 0,51 *(54)*	4,05 ± 0,52[a]*(77)*	4,13 ± 0,34[a]*(39)*
thymus	male	0,43 ± 0,07	0,43 ± 0,09	0,41 ± 0,09	0,40 ± 0,05
thymus	female	0,47 ± 0,07	0,45 ± 0,10	0,43 ± 0,10[a]	0,43 ± 0,06[a]
F_3-generation					
liver	male	3,71 ± 0,32*(53)*	3,97 ± 0,33[a]*(62)*	4,07 ± 0,61[a]*(56)*	4,21 ± 0,34[a]*(75)*
liver	female	3,87 ± 0,36*(62)*	3,94 ± 0,39 *(44)*	4,20 ± 0,32[a]*(51)*	4,27 ± 0,37[a]*(45)*
thymus	male	0,39 ± 0,08	0,40 ± 0,12	0,35 ± 0,08[a]	0,36 ± 0,06
thymus	female	0,43 ± 0,07	0,42 ± 0,08	0,39 ± 0,09[a]	0,38 ± 0,07[a]

* = **(loading-/maintenance-dose)** *ng TCDD/kg* body wt
\# = weekly maintenance-dose in the F_2- and F_3-generation
[a] = $p<0.05$ (Dunnett-Test)

CONCLUSION

After continuous exposure of female Wistar rats during three generations we observed the following effects among their offspring:

1. TCDD exposure via mother's milk in the ng/kg body-wt-range led to a tendency decrease in a relative thymus weight on day 21 postnatally in the groups with the two highest dose regimes.
2. The increase in liver weight occured in the male offspring at concentrations of 500 pg TCDD/g liver and higher, and at 1,000 pg/g liver in the female offspring at weaning.

3. A no-observed-adverse-effect-concentration (NOAEC) for the decrease of relative thymus weight for the female offspring was found to be about 1,000 pg TCDD/g wet liver and must be greater than 2,500 pg/g liver for the male offspring at weaning.

Table 2: Concentration of TCDD in liver and subcutaneous white adipose tissue on day 21 postnatally

	pg TCDD/g wet tissue					
	50/10* (20#)		120/24* (48#)		250/50* (100#)	
Generation	Liver	Adipose tissue	Liver	Adipose tissue	Liver	Adipose tissue
F_1	517	-	1379	-	2015	-
F_2	477	159	922	265	1671	413
F_3	729	193	1403	295	2505	405

*=(loading-/maintenance-dose) *ng TCDD/kg* body wt
#=weekly maintenance-dose in the F_2- and F_3-generation

REFERENCES

1) Harris MW, Moore JA, Vos JG, Gupta BN. General biological effects of TCDD in laboratory animals. *Environ Health Perspect* 1973;5:101-109
2) Thompson TN, Watkins JB, Gregus Z, Klaassen CD. Effect of microsomal enzyme inducers on the soluble enzymes of hepatic phase II biotransformation. *Toxicol Appl Pharmacol* 1982;66:400-408
3) Murray FJ, Smith FA, Nitschke KD, Humiston CG, Kociba RJ, Schwetz BA. Three-generation reproduction study of rats given 2,3,4,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the diet. *Toxicol Appl Pharmacol* 1979;50:241-252
4) Abraham K, Krowke R, Neubert D. Absorption of TCDD following parenteral application in rats using various vehicles. *Chemosphere* 1989;19:893-898
5) Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CN, Barnard SD, Hummel RA, Humiston CG. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol Appl Pharmacol* 1978;46:279-303
6) Pitot HC, Goldworthy TL, Moran S, Kennan W, Glauert HP, Maronpot RR, Campbell HA. A method to quantitate the relative initiating and promoting potencies of hepatocarcinogenic agents in their dose-response relationship to altered hepatic foci. *Carcinogenesis* 1987;8:1491-1499