EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) IN THE LACTATING RAT ON MATERNAL AND NEONATAL VITAMIN A STATUS AND HEPATIC ENZYME INDUCTION: A DOSE-RESPONSE STUDY

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ABSTRACT

Young male Sprague-Dawley rats exposed to TCDD via mother's milk show induced hepatic EROD activity at doses where no effects on growth and organ weights are observable, while effects on tissue vitamin A contents occur concurrent with these toxic symptoms. In contrast to the dams, offspring show decreasing vitamin A contents of the lung in response to TCDD.

KEY WORDS: TCDD, vitamin A, EROD, neonatal rat, lung, mother's milk

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) have been identified and quantified in human mother's milk (Nygren et al 1986). With the levels found, estimated intakes by the child during the breast feeding period will exceed the recommended tolerable intake several-fold (Ahlborg et al 1988). At birth and during the lactation period needs for vitamin A, which is required for general growth and cellular differentiation, are marked. Exposure to vitamin A-depleting compounds such as TCDD, the most toxic among the PCDD/PCDF-congeners, during this period, when both the intake and endogenous stores of vitamin A are low, may therefore be more serious than later in life. It has been shown that a single maternal dose of 10 μg TCDD/kg body weight (p.o.) on the day of delivery reduce the capability in offspring to accumulate hepatic vitamin A (Håkansson et al 1987). The present study was made to establish dose-response relationships in the young rat exposed to TCDD via mother's milk, for toxic indicators, tissue vitamin A contents, and hepatic enzyme induction, in order to compare at which dose-levels these effects occur.

METHODS

Animals: Pregnant and unmated female Sprague-Dawley rats, (ALAB Laboratorietjänst, Sollentuna, Sweden) were fed R3-stockdiet (Ewos AB, Södertälje, Sweden) and water without any restriction. The dams and their litters were housed individually in plastic cages. The illumination cycle (12 hours light, 12 hours dark) and temperature (≈22°C) were automatically controlled.

Chemicals: TCDD (CIL, Woburn, MA, USA, Lot MLB-682-49) for administration was prepared as previously described (Håkansson *et al* 1987).

Experimental design: Groups of five rats were given 0.1, 1 or 10 μg TCDD/kg body weight by gavage on the day of delivery (day 0). The negative control group received the vehicle (corn oil) only. The litter size was normalized to 4 female and 4 male pups at the time for administration. The adult rats and male pups were sacrificed on days 21 and 28, respectively. Female pups, which were sacrificed on day 14, were not used in the present study. All animals were sacrificed by blood

withdrawal under thiopental anesthesia (90 mg/kg i.p.). Liver, kidney, and lung were collected and stored for biochemical assays.

Biochemical assays: Tissue vitamin A levels were analyzed as retinol by high performance liquid chromatography (Håkanssson *et al* 1987). Hepatic ethoxyresorufin O-deethylase (EROD) activity in microsomes was determined as earlier described (Wærn *et al* 1989).

Statistics: Statistical analyses (Wilcoxon rank sum test (2-tailed) and linear regression analysis) were made by using the SPSS/PC+ (SPSS Inc. Chicago, Il, USA) statistical program.

RESULTS

In the offspring, all measures presented (Table 1) showed significant ($p < 0.01$) dose-response effects when tested by linear regression analysis. However, only the hepatic EROD-induction and the reduction in pulmonary vitamin A content showed a log-linear relationship over the whole dose-range (Figure 1). Tissue vitamin A contents in exposed pups were significantly different from control animals at doses where typical TCDD-related symptoms (i.e. reduced body weight, increased liver-to-body weight ratio, decreased thymus-to-body weight ratio) occurred, whereas the hepatic EROD activity was 7-fold induced already at the lowest dose-level (Table 1).

Table 1. Effect of maternal TCDD treatment (0.1, 1 and 10 µg TCDD/kg body wt, p.o. within 24 h of delivery) on body weight, liver- and thymus-to-body weight ratios, vitamin A content in liver, lung and kidneys, and hepatic EROD activity in 28 days old male rats.[1]

Measures	*Control*	*0.1 µg TCDD/kg*	*1 µg TCDD/kg*	*10 µg TCDD/kg*
Number of pups	*15*	*19*	*20*	*20*
Number of litters	*4*	*5*	*5*	*5*
Terminal body weight (g)	*102 ± 10.4*	*104 ± 6.9*	*100 ± 3.9*	*80 ± 8.7**
Relative weights (g/kg)				
Liver	*47.1 ± 2.5*	*49.8 ± 3.3*	*50.0 ± 3.1**	*62.2 ± 4.3**
Thymus	*4.50 ± 0.35*	*5.02 ± 0.66*	*4.76 ± 0.48*	*2.86 ± 0.60**
Total vitamin A (µg)				
Liver	*186 ± 31*	*182 ± 25*	*167 ± 25*	*88 ± 15**
Lung	*2.43 ± 1.10*	*2.01 ± 0.65*	*1.27 ± 0.49**	*0.53 ± 0.25**
Kidney	*2.10 ± 0.73*	*1.85 ± 0.69*	*2.08 ± 0.71*	*5.15 ± 2.07**
*EROD activity (nmol/mg MP*min)*	*0.07 ± 0.03*	*0.47 ± 0.20**	*1.84 ± 0.50**	*3.98 ± 1.17**

[1] Values are means ± standard deviation for 15 control and 19 or 20 exposed rats.
** indicates a statistically significant ($p < 0.05$) difference between control and TCDD treated groups.*

No clinical signs were observed in the treated adult rats. However, one of the dams in the control group died a few days after the administration of corn oil. The reason for this death could not be identified. Adult rats, both nursing (Table 2) and unmated (data not shown), showed essentially the same responses, except for the slightly more pronounced decrease in relative thymus weight and increase in EROD activity at the 1.0 µg/kg body weight dose in unmated rats (data not shown). Significant ($p < 0.01$) dose-response relationships were found for renal vitamin A and hepatic EROD activity in the dams, while in unmated rats a significant response was found also for the relative thymus weight. Of these responses, only the effect on thymus and EROD activity in the unmated

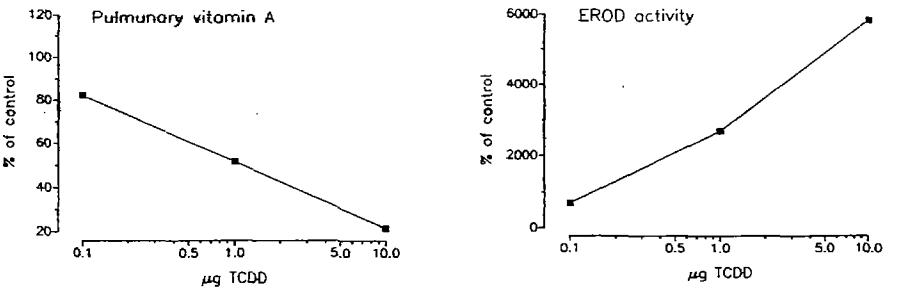

Figure 1. Effect of maternal TCDD treatment (0.1, 1 and 10 µg TCDD/kg body wt, p.o. within 24 h of delivery) on the vitamin A content in the lung and on the hepatic EROD activity in 28 days old male rats. Data is presented as percent of control values.

rats showed a log–linear relationship over the whole dose–range (data not shown). In contrast to the offspring, adult rats showed no effect of TCDD on the relative liver weight and the vitamin A content in the lung (Tables 1 and 2). Furthermore, there was no treatment–related decrease in the body weight of adult rats (Table 2).

Table 2. Effect of a single oral dose of 0.1, 1, and 10 µg TCDD/kg body wt given within 24 h of delivery on body weight, liver– and thymus–to–body weight ratios, vitamin A content in liver, lung and kidneys, and hepatic EROD activity in dams 21 days after treatment.[1]

Measures	*Control*	*0.1 µg TCDD/kg*	*1 µg TCDD/kg*	*10 µg TCDD/kg*
Terminal body weight (g)	*296 ± 19*	*321 ± 13*	*304 ± 41*	*344 ± 7**
Relative weights (g/kg)				
Liver	*41.0 ± 4.0*	*40.3 ± 2.9*	*40.8 ± 4.0*	*47.0 ± 2.5*
Thymus	*0.79 ± 0.17*	*0.78 ± 0.14*	*0.83 ± 0.08*	*0.46 ± 0.13**
Total vitamin A (µg)				
Liver	*5240 ±568*	*4071 ±960*	*3889 ±882*	*3887 ±377**
Lung	*0.96 ± 0.16*	*1.19 ± 0.32*	*1.11 ± 0.48*	*1.04 ± 0.16*
Kidney	*0.92 ± 0.16*	*1.16 ± 0.46*	*1.77 ± 0.49**	*7.38 ± 3.51**
*EROD activity (nmol/mg MP*min)*	*0.06 ± 0.01*	*0.13 ± 0.04**	*0.52 ± 0.28**	*3.54 ± 0.50**

[1] *Values are means ± standard deviation for 4 control and 5 exposed dams at each dose–level.*
** indicates a statistically significant ($p < 0.05$) difference between control and TCDD treated groups.*

DISCUSSION

The most sensitive response to TCDD exposure, in the present study, is hepatic EROD induction, both for neonatal and adult rats. A statistically significant difference in EROD activity compared to control rats is present at a dose at least two orders of magnitude below the dose where effects on growth and tissue weights are significant.
Significant effects of TCDD on tissue vitamin A contents occur in the dose–range where the toxic

symptoms are observed. Effects of TCDD, obtained in this study, on hepatic and renal vitamin A, both in dams and offspring, confirm and extend previous findings (Håkansson et al 1987). In contrast to previous findings in adult male rats (Håkansson et al 1990), dams and unmated rats in the present study show no effect of TCDD on pulmonary vitamin A (Table 2). Furthermore, adult rats in the present study have a markedly lower vitamin A content of the lung. There is presently no explanation for this discrepancy, although it may be related to sex. In contrast, pulmonary vitamin A in the offspring is markedly reduced at a dose, which produced no significant effect on hepatic or renal vitamin A. This data may suggest a direct deprivation of vitamin A in the lung of the young rat as a result of TCDD exposure. Such an effect could occur either by depletion of the existing stores or by limiting the availability of vitamin A for storage and/or utilization in the lung.
The more pronounced effects of TCDD on liver-to-body weight ratio, terminal body weight, and vitamin A content in the lung of the offspring as compared to the adult rat, indicate that the neonatal rat either is more sensitive to TCDD with respect to these responses or received a higher dose of TCDD per kilogram body weight. In the present study, no attempts were made to measure the actual dose received by the pups. However, it is known from studies in mice, that the dose per kilogram body weight of TCDD received by nursed pups is similar to the dose given to the dam (Nau et al 1986). This data would thus further support the notion that the neonatal rat is more sensitive to TCDD-exposure than the adult rat.

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