EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ON PITUITARY AND THYROID HORMONE LEVELS IN MALE RATS

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces a variety of toxic effects such as immunosuppression, teratogenicity, carcinogenicity and disrupting endocrine systems (1, 2). Thyroid is one of the target organs of TCDD toxicity. Exposure to TCDD has been reported to cause an alteration in thyroid hormone metabolism in rodent (3,4) and human babies (5) through lactation. Thyroid hormones play a very important role in brain development, especially during the perinatal period (6). It is believed that the embryonic stage of development is highly susceptible to TCDD toxicity (7, 8). Specific attention should be given to the biological influence on embryos following maternal exposure to TCDD.

The aim of the present study was to evaluate the influence of maternal exposure of TCDD on endocrine function of offspring, especially thyroid function and morphology. Rat dams were dosed to TCDD via gavage on gestational Day 15. Thyroid and pituitary hormone levels were measured in serum from the male offspring on different developmental stages. Because induction of UDP- glucuronosyltransferase (UDP-GT) is believed to be the mechanism that contributes to decrease in serum T4 concentrations following TCDD exposure (9), the activities of UDP-GT were analyzed. Liver microsomal ethoxyresorufin-O-deethylase (EROD) induction was also measured as an indicator of the degree of Ah receptor-mediated response in the offspring (1).

Materials and Methods

Animals and treatments: On Day 15 of gestation, pregnant Holtzman rats (5-6 per group) were given a single oral dose of TCDD (12.5, 50, 200 or 800 ng TCDD/kg) or equivalent volume of corn oil.
On Day 2, the pups were examined for gross abnormalities. Body weights and number of each sex were also recorded. The litters were culled to 8, 5 males and 3 females when possible. The pups were kept with their natural mothers until weaning. From each litter male pups were randomly sacrificed on Day 49s (n=10-12), 63 (n=3-5) and 120 (n=10-12) to examine the effects of perinatal TCDD exposure.
Sample collection and processing: Body weight, liver, brain, pituitary, thymus, and

thyroid weights were recorded and blood was collected on Days 49, 63 and 120. The blood was centrifuged at 900x *g* for 15 min and the plasma was stored at -80°C until hormone analyses. Portions of the liver were frozen in liquid N_2 and then stored at -80°C until enzyme activity measurements. Thyroid gland tissues were fixed in HistoChoice fixative embedded in paraffin, and sectioned at 5 μm. Sections were stained with hematoxylin and eosin.

Thyroid hormone analysis: Serum levels of thyroxin (T4) and triiodothyronine (T3) were determined using radioimmunoassay (RIA) kits (Amerlex-M: Amersham LIFE SCIENCE).

Pituitary hormone analyses: Thyroid stimulating hormone (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were determined using enzyme immunoassay (EIA) system, rTSH EIA, rLH EIA and rFSH EIA system (Amersham LIFE SCIENCE), respectively.

EROD and UDP-GT activity: Microsomal EROD activity was fluorometrically measured according to a modified method of Lu and Levin (10). Liver microsomes were prepared using the protocol of Seo *et al* (11). UDP-GT activity was assayed according to a modification of the method of Watanabe *et al* (12).

TCDD analysis: Livers, adipose tissues and serum from offspring were analyzed for TCDD concentration on Day 120 by using gas chromatography-mass spectrometry procedures with specific ion monitoring.

Statistics: Values are expressed as the mean ± SEM. One-way ANOVA was employed to assess dose effects and post hoc comparisons were made by Fisher PLSD test.

Results and Discussion

No TCDD effect was observed in litter size, birth weight and sex ratios of offspring for all groups at any developmental stages. The highest maternal dose of TCDD caused an increase in liver weight (15.3%) as well as significant thymic atrophy (20.1%) on Day 63 but not Day 49 and Day120 with no significant changes in thyroid and brain weights.

A significant decrease in serum T4 levels (14.7%) was induced at the highest dose of TCDD on Day 63 (Table 1), but not on Day 49 and Day 120. The effects of TCDD on serum T4 levels would be reversible. Serum T3 and TSH levels were not affected in these offspring at any stages (Table 2). Serum levels of LH in the offspring in all groups were not affected by TCDD. Although an increase in serum FSH levels on Day 120 was observed in all experimental groups including control, no significant differences between TCDD-exposed and control rats were found (Table 3). EROD and UDP-GT activities in liver microsome from offspring were not statistically significant.

The analysis of TCDD in tissues showed the greater amounts of TCDD accumulated in adipose tissue rather than liver on Day 120. The cellular hyperplasia of follicle and hypertrophy of thyroid glands (in the 2/12 rats) and the significant decrease in serum T4 level in the highest - dose (800 ng/kg) group suggest that this dose would be the minimum dose required to affect thyroid hormone system. An increase in liver weight and thymic atrophy observed at the highest dose group, also supported this notion.

Earlier publications that TCDD exposure in utero produces morphological and

reproductive alterations in female rat offspring at dose of 200 ng/kg TCDD (8), and alterations on spermatogenesis and reproductive capability in male offspring at dose of 64 ng/kg TCDD (7). However, in the present study, changes in thyroid hormone status after gestational and lactational exposure to TCDD are not so sensitive as reported., Considering the finding by Seo et al (11) that maternal exposure of TCDD on gestational days 10-16 affected T4 levels in female offspring, further studies are needed to determine critical window for TCDD toxicity on hypothalamic-pituitary thyroid system.

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Table 1. Serum T4 levels from offspring of dams exposed to TCDD on gestational Day 15

TCDD dose (ng/kg)	T4 (n mol/L) PND 49	PND 63	PND 120
0	67.1 ± 8.8	47.1 ± 2.1	50 ± 7.9
12.5	57.3 ± 8.5	49.0 ± 7.2	47 ± 3.5
50	69.7 ± 8.6	47.7 ± 8.8	49 ± 4.2
200	67.6 ± 15.0	49.1 ± 1.5	47 ± 7.4
800	61.6 ± 11.0	40.2 ± 1.5*	47 ± 7.3

Data are means ± S.E.M.
* Significant Difference from the controls, $P<0.05$.

Table 2. Serum TSH levels from offspring of dams exposed to TCDD on gestational Day 15

TCDD dose (ng/kg)	TSH (n mol / L) PND 49	PND 63	PND 120
0	7.1 ± 2.5	10.4 ± 1.2	11.2 ± 1.0
12.5	8.0 ± 3.8	10.0 ± 1.4	9.5 ± 0.9
50	9.1 ± 3.2	10.2 ± 1.2	11.9 ± 2.5
200	9.0 ± 3.1	10.8 ± 0.6	10.4 ± 1.6
800	8.2 ± 3.7	10.4 ± 0.2	11.3 ± 2.9

Data are means ± S.E.M.

Table 3. Serum FSH levels from offspring of dams exposed to TCDD on gestational Day 15

TCDD dose (ng/kg)	FSH (ng / ml) PND 49	PND 63	PND 120
0	62 ± 10	55 ± 15	97 ± 18
12.5	62 ± 13	53 ± 9	88 ± 18
50	59 ± 7	53 ± 7	97 ± 14
200	56 ± 7	56 ± 7	91 ± 15
800	57 ± 10	54 ± 6	90 ± 9

Data are means ± S.E.M.