Disruption of Thyroid Hormone Homeostasis at Weaning of Holtzman Rats by Lactational but Not In Utero Exposure to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin

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The purpose of this study is to clarify whether lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is entirely responsible for the perturbation in thyroid hormone homeostasis during the neonatal period. Pregnant Holtzman rats were given a single oral dose of 1.0 μg TCDD/kg body weight on gestational day 15. Half of the litters were cross-fostered with the half of the dams treated with vehicle on postnatal day (PND) 1 to make four groups of rats, control (C/C), prenatal TCDD exposure only (T/C), postnatal TCDD exposure only (C/T), and both prenatal and postnatal TCDD exposure (T/T). On PND 21, the T/T and T/C groups, but not the T/C and C/C groups, showed a significant decrease in serum total thyroxin (TT4) and free thyroxin (FT4) concentrations in both sexes and a significant increase in serum thyroid-stimulating hormone (TSH) levels, particularly male pups. These two groups of male and female pups had significantly higher concentrations of TCDD in the liver, with marked induction of cytochrome P450 (CYP) 1A1 mRNA and intense immunostaining of CYP1A1 in the liver. UDP glycosyltransferase 1 family, polypeptide A6 (UGT1A6) and UGT1A7 mRNAs were induced in their livers, with positive immunostaining of UGT1A6. The transfer of TCDD from dams to the pups was confirmed by the detection of TCDD in mother’s milk and UGT1A6. The present results demonstrate that lactational, but not in utero, exposure to TCDD was responsible for the disruption of thyroid hormone homeostasis.

Key Words: hypothyroxinemia; lactational exposure; TCDD; thyroxin.

INTRODUCTION

Dioxins are ubiquitous contaminants in the environment, including soil, air, water, and biota, and are accumulated ultimately in humans through the food chain. Many studies using adult rodents have shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a prototype of dioxins, produces morphological and functional disorders of the thyroid after prolonged exposure (Henry and Gasiewicz, 1987; Potter et al., 1986; Van Birgelen et al., 1995). Recently, we demonstrated that maternal administration of a single dose of TCDD to Holtzman rats on gestational day (GD) 15 resulted in hyperplasia of thyroid follicular cells and functional disruption of pup thyroid as a consequence of a disrupted pituitary–thyroid axis (Nishimura et al., 2003).

Thyroid hormone has been reported to play a crucial role in development of the fetal brain in laboratory animals and humans. During fetal and early neonatal periods, disorders of thyroid hormone may lead to the development of motor and cognitive disorders (Sher et al., 1998). While the neuronal defects related to thyroid function could be readily reversed with appropriate medical treatment in adults, effects on the developing brain are irreversible if damage due to thyroid malfunction occurs early in life (Porterfield, 1994).

The major source of human exposure to dioxins is through the diet (Porterfield, 1994). Dioxins have been shown to accumulate in human tissues rich in fat such as adipose tissues, blood lipids, and milk fat. The human fetus is exposed to dioxins through placental transport, and larger quantities of dioxins are transferred to the infant via breast milk. A Dutch cohort study investigated the adverse effects of background exposure to polychlorinated biphenyls (PCBs) and dioxins on growth and development of healthy full-term infants. While lower total serum thyroxin (TT4) levels and higher thyroid stimulating hormone (TSH) levels of infants in the second week after birth were associated with higher PCB and dioxin levels in breast milk, delay in neuronal development and immune status, rather than lactational exposure, were associated with prenatal exposure to PCBs and dioxins (Huisman et al., 1995a, 1995b; Sauer et al., 1994). Similarly, Dutch epidemiological studies suggest that in utero rather than...
TABLE 1
Polymerase Chain Reaction Primer Name, Sequence, and Product Size

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’ )</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Forward CCATGACCCAGAACATCTGGG</td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>Reverse TCTCTGACATCCAGGACA</td>
<td></td>
</tr>
<tr>
<td>UGT1A1</td>
<td>Forward TGTTGTGCGGGGAGCTCATGTTCG</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td>Reverse ACTCCGCCCAAGTTCCACAAAGCA</td>
<td></td>
</tr>
<tr>
<td>UGT1A6</td>
<td>Forward TGCTGACATTCTCGAGGATTTC</td>
<td>319</td>
</tr>
<tr>
<td></td>
<td>Reverse TTTCTTGAATCTTGATAGGAGCCA</td>
<td></td>
</tr>
<tr>
<td>UGT1A7</td>
<td>Forward CAGTGTCGCCAGTGGAAAAACCA</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>Reverse GAAGAAACCTGGGCGAAGGTCA</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward CCTCTATGCCAACACAGT</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Reverse AGCCACCAATCCACACAG</td>
<td></td>
</tr>
</tbody>
</table>

lactational exposure to dioxins and/or PCBs is critical in the development of cognitive functions (Patandin et al., 1999).

Using a cross-fostering protocol, Crofton et al. (Crofton et al., 2000) reported that lactation is the major route of Arochlor 1254, which is responsible for ototoxicity and hypothyroxinemia. A toxicokinetic study using 14C-labeled Arochlor 1254, which is responsible for ototoxicity and development of cognitive functions (Patandin et al., 1999). It is still uncertain, however, whether lactational exposure to TCDD is mainly responsible for the disruption of thyroid hormone homeostasis and morphological changes in the liver and thyroid during the early stages of development. In the present study, we used a cross-fostering protocol on newborn rats to investigate this aspect of TCDD toxicity.

MATERIALS AND METHODS

Animals and exposure to TCDD. Male and female Holtzman rats were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and bred at the National Institute for Environmental Studies (NIES, Tsukuba, Japan). They were maintained in a controlled room at 23° ± 1°C and humidity at 50 ± 10%, on a 12-h light–dark cycle. The animals received food and distilled water ad libitum and were handled with humane care under the guidelines for animal experiments at the NIES. Ten-week-old female rats in proestrus were mated 1:1 with males overnight, and females that had a vaginal plug the following morning were designated as GD 0. Pregnant rats were divided randomly into two groups, with 12 rats per group at the beginning of the experiment. Each group of pregnant rats was administered orally with 1 equivalent volume of corn oil as vehicle on GD 15.

After spontaneous delivery, pups were divided into the following four groups according to the cross-fostering protocol. The first group of pups was exposed to TCDD both in utero and via lactation and was designated T/T group. We switched the mothers of the second and third groups of pups on PND 1 so that they were exposed to TCDD either in utero only (T/C) or via lactation only (C/T). The fourth group of pups was born to dams and fostered by the same dams administered vehicle (C/C). Serum and tissues from each group of neonates (n = 6) were collected on postnatal day (PND) 21 and analyzed. Liver and thyroid glands with the trachea were excised, fixed in Zamboni’s solution for 24 h at 4°C, and processed for immunohistochemical and histopathological examinations. For biochemical examination, tissues were snap-frozen in liquid nitrogen and stored at −80°C until analysis.

Male and female rats (n = 4–6) from each group were killed on PND 49 for histopathological examinations.

Thyroid hormone analysis. Total thyroxin, free thyroxin (FT4), and total triiodothyronine (TT3) in the serum were determined with Amerlex radioimmunoassay kits (Amersham, Buckinghamshire, UK) according to the manufacturer’s instructions. Serum TSH concentrations were quantified with a rat TSH enzyme immunoassay kit (Amersham) as described elsewhere (Nishimura et al., 2002).

Immunohistochemistry. Cytochrome P450 (CYP) 1A1 and the UDP-glucuronosyltransferase 1-6 precursor UDP glycosyltransferase 1 family, polypeptide A6 (UGT1A6) in the liver were stained in tissue sections by an indirect immunohistochemical technique. Briefly, the deparaffinized and rehydrated sections were pretreated in 0.01 M sodium citrate buffer (pH 6.0) with microwave heating and washed in phosphate-buffered saline (PBS). To quench endogenous peroxidase activity, sections were covered with 0.3% H2O2 dissolved in 100% methanol. Goat polyclonal antibody against CYP1A1 (G-18; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was diluted 1:200 and rabbit antibody against rat UGT1A6-specific peptide (Ikushiro et al., 1995) was diluted 1:200 in phosphate buffered saline (PBS). The primary antibodies were incubated over the sections in a humidified chamber for 1 h at 37°C. Sections were subsequently washed with PBS, followed by incubation for 1 h with biotinylated rabbit anti-goat immunoglobulin G (IgG) (BA-5000, Vector Laboratories, Vector, Burlington, CA) or goat anti-rabbit IgG (BA-1000, Vector Laboratories), which were diluted 1:300 in PBS. They were incubated with an avidin–biotinylated peroxidase complex (PK-4000, Vector Laboratories). Immunoreactions were performed using hydrogen peroxide–activated 3,3′-diaminobenzidine-tetrachloride (Sigma, St. Louis, MO). Sections were counterstained for 10 s in Mayer’s hematoxylin. Negative controls, in which the primary antibody was replaced with normal rabbit IgG, did not show nonspecific staining.

RNA extraction and reverse transcriptase–polymerase chain reaction. All enzymes and cofactors used for reverse transcription (RT) and polymerase chain reaction (PCR) were purchased from Takara (Otsu, Japan). All primers were purchased from Amersham Pharmacia Biotechnology (Piscataway, NJ). Sequences of PCR primers for amplification of CYP1A1, UGT1A1, UGT1A6, UGT1A7, and β-actin are summarized in Table 1. Total RNA was isolated from liver with Isogen (Nippon Gene, Tokyo, Japan) and reverse transcribed in an AMV reverse transcriptase, 0.125 M dNTP, 0.25 M/μl AMV reverse transcriptase, 0.125 M oligo dT-adaptor primer, 1 U/μl RNase inhibitor, and 1 μg of total RNA with the RNA LA PCR kit (Takara).

Polymerase chain reaction amplification was carried out in a 10-μl reaction mixture containing 2.5 mM MgCl2, 0.25 U Takara LA Taq, 0.2 μM of each forward and reverse primer, and 2 μl of the reverse transcription product as template (18 cycles for CYP1A1, 24 cycles for UGT1A1 and UGT1A6, 25 cycles for UGT1A7, and 23 cycles for β-actin) by denaturing at 94°C for 30 s, annealing for 56°C for 30 s, and extending at 72°C for 30 min. Polymerase chain reaction products were detected as a single band on a 1.5% agarose gel in 1× TBE containing 2 μg/ml of ethidium bromide. Band intensity was quantified by the EDAS 290 system (version 3.5.3, Kodak, Rochester, NY).

Measurement of TCDD concentration. The presence of TCDD in tissue specimens (serum, liver and milk) was determined by essentially the same method as described elsewhere (Nishimura et al., 2002). The TCDD concentrations were analyzed by high-resolution gas chromatography–mass
spectrometry procedures with selected ion monitoring. Quantified values were calculated by the internal standard method, as previously described.

**Data analysis.** StatView for Windows (version 5.0, SAS Institute, Cary, NC) was used for statistical analyses. Data are expressed as mean ± S.E.M. Differences in means among the groups were analyzed by one-way analysis of variance (ANOVA) followed by Scheffe’s test as a post-hoc comparison, and compared each treatment groups; *p* values < 0.05 were considered statistically significant.

**RESULTS**

**Tissue Weights in the Cross-Fostered Pups**

As a sensitive marker of response to TCDD, thymus and liver weights of the cross-fostered pups were measured on PND 21. As summarized in Table 2, a significant increase in liver weight was found in male and female pups of the C/T and T/T groups compared to those of the C/C group. This tendency was more apparent in females than in males. In the thymus, however, a marked trend of decreased weight was observed in both sexes of pups in the C/T and T/T groups compared to those of the C/C group.

**Serum Thyroid Hormone Concentrations in the Cross-Fostered Pups**

Serum thyroid status was examined for male and female pups on PND 21. While there were no effects of TCDD on the serum TT4 and FT4 concentrations in male and female pups that were not exposed to TCDD postnatally (T/C groups), the male and female pups from the C/T and T/T groups showed a significant reduction in serum TT4 concentrations compared to those of the C/C group. In addition, the male and female pups of the C/T group and the only female pups from T/T group had a significant decrease in serum FT4 concentrations (Fig. 1). The serum TT4 concentrations in the C/T and T/T groups were 40% lower than those of the C/C group. No significant difference in serum TT3 concentrations was observed among the four groups. Serum TSH levels in male pups, but not female pups, of the C/T and T/T groups were significantly higher compared to those of the C/C group.

**Induction of CYP1A1 mRNA and Localization of CYP1A1 in the Liver of Cross-Fostered Pups**

Cytochrome P450 1A1 mRNA induction in the liver was analyzed by semi-quantitative RT-PCR (Fig. 2). The expression

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**TABLE 2**

Relative Tissue Weights of Offspring on PND 21 after Exposure to TCDD on Gestational Day 15

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Thymus</th>
<th>Liver</th>
<th>Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>3.67 ± 0.12</td>
<td>0.49 ± 0.04</td>
<td>3.70 ± 0.13</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td>T/C</td>
<td>3.87 ± 0.17</td>
<td>0.46 ± 0.04</td>
<td>3.93 ± 0.14</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td>C/T</td>
<td>4.22 ± 0.13*</td>
<td>0.39 ± 0.02*</td>
<td>4.29 ± 0.14*</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>T/T</td>
<td>4.27 ± 0.09*</td>
<td>0.36 ± 0.02*</td>
<td>4.71 ± 0.07*</td>
<td>0.39 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are given as percent body weight. Data represent the mean ± S.E.M. for six rats. *p < 0.05, significantly different from the vehicle-treated control.
of hepatic CYP1A1 mRNA was markedly induced in male and female pups of the C/T and T/T groups, in contrast to basal and intermediate levels in the C/C and T/C groups, respectively. Additional evidence of CYP1A1 induction was provided by immunohistochemical staining (Figs. 3A–D). The liver sections from the male pups of the lactationally exposed groups (C/T and T/T groups) showed strong immunostaining for CYP1A1 in the cytoplasm of hepatocytes around the central vein. In contrast, almost no immunoreactivity for CYP1A1 was detected in the C/C and T/C groups (Fig. 3A and 3B). A very similar immunostaining result was obtained for female pups. Not only the number but also the staining intensity for CYP1A1 in the four groups support the results of the RT-PCR analysis.

Quantification and Immunolocalization of UGTs in the Cross-Fostered Pups

To confirm whether induction of various UGT mRNAs had occurred in the cross-fostered pups, UGT1A1, UGT1A6, and UGT1A7 mRNA levels were determined on PND 21 by semi-quantitative RT-PCR analysis. Significant induction of UGT1A6 and UGT1A7 mRNAs were found in male and female pups in the C/T and T/T groups, but not in the C/C and T/C groups (Fig. 4). Interestingly, no difference was observed in UGT1A1 mRNA expression among the four groups of rats. These findings were supported by immunohistochemical staining for UGT1A6. The immunoreactivity for UGT1A6 protein was localized in the centrilobular region of livers in male (Figs. 5A–5D) pups of the C/T and T/T groups, whereas it was negligible in liver tissues from the T/C and the C/C groups. The immunohistochemical localization of UGT1A6 in female pups was found to be nearly identical to that of male pups.

Histopathological Examination of Thyroids in the Cross-Fostered Pups

We previously reported that perinatal exposure to TCDD produced proliferative lesions in pups of TCDD-exposed maternal rats (Nishimura et al., 2003). Here we studied whether lactational exposure to TCDD affects the morphological changes in thyroids of pups. We examined male and female rats for histopathological changes of thyroids on PND 49. The male (Figs. 6A–D) and female (data not shown) rats from the C/T and T/T groups had proliferative lesions of follicular cells, including hyperplasia; in contrast, thyroids of the C/C and T/C groups had normal appearance.

TCDD Measurements in Livers, Serum, and Stomach Milk Specimens from Cross-Fostered Pups

The TCDD levels in livers and serum of cross-fostered pups on PND 21 are summarized in Table 3. The concentrations of hepatic TCDD in male and female pups from the C/T and T/T groups were more than 25- to 30-fold higher than those of the T/C group. Similarly, serum showed a 10- to 12-fold higher concentration of TCDD in the C/T and T/T groups than in the T/C group. The TCDD concentrations in milk remaining in the stomachs of three male pups on PND 1 nursed by TCDD-exposed dams were quantified. A large amount of TCDD (979 pg/wet-g) was detected in the ingested milk.

DISCUSSION

The aim of the present study was to identify whether lactational exposure is responsible for TCDD-induced endocrine-disrupting effects on offspring thyroid. In a previous publication (Nishimura et al., 2003), we demonstrated that gestational and lactational exposure to TCDD resulted in perturbation of thyroid hormone homeostasis in offspring,
including a marked induction of genes by TCDD, as well as proliferative lesions of thyroid caused by an accelerated secretion of TSH. Furthermore, we suggested that these effects were a consequence of a disorder of feedback mechanism, because a significantly elevated secretion of TSH was confirmed to have been maintained in the blood circulation even after restoration of T4 levels.

In the present study, a significant decrease in TT4 and FT4 levels of both sexes was accompanied by a concomitant increase in TSH levels in the circulation only in newborn rats exposed to TCDD lactationally (C/Tand T/T groups) on PND 21. In contrast, controls (C/C group) and neonates exposed to TCDD only in utero (T/C group) were unaffected. The weights of thymus and liver, organs that are sensitive to TCDD toxicity, were decreased and increased, respectively, on PND 21 in the C/T and T/T groups, but not in the C/C and T/C groups. These effects of TCDD on the weight of these organs...
Concentrations of TCDD in the Liver and Serum from Offspring on PND 21 and Milk Specimens in the Stomach of Offspring on PND 1 Born to Dams Administered TCDD on Gestational Day 15

<table>
<thead>
<tr>
<th>Group</th>
<th>Male Liver (pg/g)</th>
<th>Male Serum (pg/ml)</th>
<th>Female Liver (pg/g)</th>
<th>Female Serum (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/C</td>
<td>44 ± 16</td>
<td>2.5 ± 0.6</td>
<td>43 ± 7</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>C/T</td>
<td>1461 ± 402</td>
<td>26.0 ± 4.0</td>
<td>1243 ± 422</td>
<td>20 ± 6.2</td>
</tr>
<tr>
<td>T/T</td>
<td>2350 ± 391</td>
<td>31.0 ± 3.1</td>
<td>1725 ± 220</td>
<td>20 ± 1.2</td>
</tr>
</tbody>
</table>

Milk from male offspring on PND 1 = 979 ± 136 (TCDD pg/g)

The amounts of TCDD were determined by high-resolution gas chromatography–mass spectrometry. Lower detection limit for TCDD was 0.1 pg/g tissue. Data represent the mean ± S.E.M. for 3 or 4 rats.

are in good agreement with previous studies indicating that TCDD toxicity occurred in offspring.

The most consistent effect of TCDD is a decrease in TT4 and FT4 in the blood circulation, an effect studied in adult mammals (Bastomsky, 1977), newborn animals (Seo et al., 1995), and humans (Brucker-Davis, 1998). Both chronic administration (Capen, 1992; Sewall et al., 1995) and a single dose of TCDD (Nishimura et al., 2002; Seo et al., 1995) increased secretion of pituitary TSH, as a compensatory response to a lower concentration of circulating T4, which led to thyroid follicular cell hyperplasia and hypertrophy. In support of this observation, we showed that the proliferative changes of thyroid follicular cells on PND 49 were produced only via lactational exposure to TCDD (Fig. 6). These pathological lesions show a remarkable consistency with those observed in adult thyroids given a single dose of TCDD (Nishimura et al., 2002) and in pup thyroids exposed to TCDD in utero and lactationally (Nishimura et al., 2003).

In the present study, we observed a slight but statistically insignificant increase in serum TSH in female pups, in contrast to a significant increase in serum TSH levels in male pups in the C/T and T/T groups. The underlying mechanism of this sex difference in TSH response to TCDD is not clear at present. We observed previously (Nishimura et al., 2003) that the serum TSH levels were significantly elevated in both sexes in the highest TCDD dose group, and the concentrations of TSH in male rats tended to be higher than those in the female rats at all developmental stages examined. In fact, the presence of a sex difference in hypothalamic-thyroid function was suggested by McClain et al. (1988). Phenobarbital, a hepatic microsomal enzyme inducer, promoted thyroid tumours more in male rats than in female rats, mediated via increased TSH secretion as a compensatory response to the T4 metabolism and excretion. Higher TSH responsiveness to thyrotropin releasing hormone (TRH) was observed in male rats but not in female rats, and the presence of testosterone was suggested to cause this difference (Christianson et al., 1981). Therefore, one possible explanation for the difference in response to TCDD between male and female rats might be that male rats are more susceptible to TCDD than female rats in terms of TSH response.

Gene expression of CYP1A1 and UGT mRNAs in the liver was induced markedly in the C/T and T/T groups, in contrast to no induction in the C/C and T/C groups. Induction of these genes was also confirmed by immunohistochemical examinations, which showed that an expression of immunoreactivity was localized in centrilobular hepatocytes (Fig. 3). The finding that TCDD-induced expression of CYP1A1 was restricted to the centrilobular region is consistent with the observations of Tritscher et al. (1992) of the liver from a rat chronically exposed to TCDD for 30 weeks. The distribution pattern of UGT1A6 protein after TCDD treatment in the present study was in good agreement with that observed in an in situ hybridization study by Saarikoski et al. (1998), which revealed that UGT1A6 mRNA was induced in the restricted area of the central veins of rats after 3-methylcholanthrene (MC) treatment. The region-specific induction of CYP1A1 and UGT1A6 proteins in the liver might be related to differences in TCDD localization. As the concentration of TCDD was reported to be significantly higher in centrilobular cells than in periportal hepatocytes (Santostefano et al., 1999), in the present study the differential expression of the genes observed might depend on the tissue concentration of TCDD.

To our knowledge, the present study provides the first evidence of localization of hepatic UGT1A6 protein in response to TCDD. In addition, TCDD did not affect gene induction of UGT1A1, whereas both UGT1A6 and UGT1A7 mRNAs were induced significantly. Extensive studies have suggested that UGTs are involved in glucuronidation of xenobiotics and endogeneous compounds, including T4 and T3. Mammalian UGT isoforms can be grouped into two major families, the UGT1 family and the UGT2 family, according to nucleotide and amino acid sequences. The UGT1 family is classified into the bilirubin cluster (UGT1A1 and UGT1A5) and the phenol cluster (UGT1A6 and UGT1A7) (Ikushiro et al., 1995; Luquita et al., 2001). Thyroid hormones are likely to be glucuronidated by different UGT enzymes (Visser et al., 1993). A member (or members) of the UGT1A subfamily is believed to glucuronidate T4, specifically UGT1A1 and UGT1A6, whereas the UGT2 subfamily is likely to glucuronidate T3 (Vansell and Klaassen, 2002). Bank et al. (1989) found that TCDD increased the UGT activity in rats. Among all rat UGT families, only UGT1A6 and UGT1A7 mRNAs are known to be induced selectively by MC and PCB (Bank et al., 1989; Emi et al., 1995, 1996). Furthermore, the possible involvement of UGT1A6 in glucuronidation of T4 is supported by evidence that UGT1A6 is responsible for T4 conjugation in cultured rat hepatocytes treated with MC (Jennitz et al., 2000). Our findings of a significant decline in serum levels of T4 with concomitant induction of UGT1A6 and UGT1A7, but not UGT1A1, strongly suggests a contribution of UGT1A6 and UGT1A7 in T4 glucuronidation. The role of UGT1A7 in addition to UGT1A6 in T4 glucuronidation needs further investigation.
A large amount of TCDD was detected in milk remaining in stomachs of newborn pups on PND 1 nursed by TCDD-exposed dams. Li et al. (1995) showed in a cross-fostering study that only 0.01% of total dose of TCDD given to the dams was transferred to fetal liver through the placenta. In our study, however, the concentration of hepatic and serum TCDD in the C/T and T/T groups was more than 25- to 30-fold higher as compared to the T/C group. Hepatic concentrations of TCDD in the rats from C/T and T/T group were 1461 and 2350 pg/g tissue, much higher than that of the T/C group value of 44 pg/g tissue. According to Dutch epidemiological cohort studies, the median dioxin toxicity equivalent (TEQ) concentration in tissue much higher than that of the T/C group value of 6,600 to 10,000 pg TEQ/g lipid, with the assumption that the serum contains approximately 0.3% fat. Although dioxin concentrations in the serum of infants and mothers were not available for the Dutch studies, some of the available data on average dioxin TEQ concentrations in the serum in adult humans without having known accidental dioxin exposure were reported to be approximately between 15 and 25 pg/g fat (Papke et al., 2003). The comparison for these data would allow us to estimate that the margin of exposure for the disruption of thyroid effects ranges from 250 to 670 for serum and 900 for milk, respectively. Thus, from the public health point of view, the suggestion of nourishing breast milk is valid in terms of benefits of nutrition and skin contact between mothers and babies, at least for possible deleterious effects on thyroid hormone homeostasis by dioxins. Based on the present cross-fostering study, we conclude that exposure to TCDD via breast milk, not by placental transfer, was entirely responsible for disturbance of thyroid hormone homeostasis in pups.

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