2,3,7,8-Tetrachlorodibenzo-p-dioxin in Pregnant Long Evans Rats: Disposition to Maternal and Embryo/Fetal Tissues

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Prenatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) interferes with fetal development at doses lower than those causing overt toxicity in adult animals. In a multigeneration study (Murray et al., 1979), female rats that were administered 0.01 μg TCDD/kg/day in their diet did not experience reduced fertility; however, reduced fertility was seen in the F₁ and F₂ generations. Exposure to TCDD during development produces alterations in the reproductive system of the developing pups, such as delayed puberty and reduced sperm counts in males (Mably et al., 1992a; Gray et al., 1995) and malformations in the external genitalia of females (Gray and Ostby, 1995). Therefore, the objectives of this study were to determine maternal and fetal tissue concentrations of TCDD that are associated with the adverse reproductive effects seen by Gray and co-workers. Pregnant Long Evans rats received a single oral dose of 1.15 μg [3H]TCDD/kg on Gestation Day (GD) 8 and maternal as well as fetal tissue concentrations of TCDD were measured on GD9, GD16, and GD21. On GD9, the highest level of TCDD localized in the maternal liver (25.1% dose). In addition, the amount reaching all the embryos on GD9 was 0.01% of the administered dose, which resulted in a concentration of 0.02% dose/g. The amount of TCDD reaching the fetal compartment (fetuses + placentas) increased to 0.12% dose/tissue on GD16 and 0.71% by GD21. The concentration of TCDD within the fetal compartment (0.01% dose/g) on GD16 was comparable to that found in the maternal blood and spleen. Concentrations of TCDD in a single embryo/fetus were 39.6, 18.1, and 22.1 pg/g on GD9, GD16, and GD21, respectively. Estimates of hepatic half-life of elimination in pregnant rats suggested that TCDD may be eliminated faster in pregnant LE rats. Therefore, measurements of biliary elimination were made in pregnant and nonpregnant LE rats to compare rates of metabolism; however, biliary elimination of TCDD is not affected by pregnancy. In conclusion, this dose administered during a critical period of organogenesis causes adverse effects on the developing reproductive system of rodents. This dose produced a body burden of 22.1 pg TCDD/g within a single fetus on GD21. This indicates that low-level TCDD exposure during the perinatal stage of life can produce adverse effects within the developing pups. © 1998 Society of Toxicology.

Key Words: 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD; toxicokinetics; disposition; body burden; embryo; fetus.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), an unwanted contaminant of combustion and chlorine bleaching processes, is the most toxic member of the halogenated aromatic hydrocarbons. Experimental animals as well as fish, wildlife, and domestic animals that are exposed to this compound exhibit effects such as a wasting syndrome, immunosuppression, thy mic atrophy, chloracne, carcinogenicity, and other toxic and biochemical effects. Most of these responses are thought to be initiated through the binding of TCDD to the arylhydrocarbon (Ah) receptor (Nebert et al., 1982; Poland and Knutson, 1982; Whitlock, 1987, 1990). In addition, TCDD alters the levels of many hormones and/or their receptors. For example, TCDD modulates estrogens, androgens, glucocorticoids, and thyroid hormones as well as altering glucocorticoid receptors (Bastomsky, 1977; Moore et al., 1985; Gorski et al., 1988; Lin et al., 1991; Lucier et al., 1991). A review of TCDD’s effects on the endocrine system can be found in Birnbaum (1994). Because of this endocrine disruption, the potential for TCDD to impair fetal development is quite great.

TCDD adversely affects reproduction and development in several species of laboratory animals, as well as fish, birds, and mammalian wildlife (Cheung et al., 1981; Cooper, 1989). In
addition, these developmental effects are seen at doses several orders of magnitude lower than those causing overt maternal toxicity. For example, in a multigeneration study, Sprague–Dawley rats that received 0.01 μg TCDD/kg/day in the diet did not experience adverse effects on fertility; however, there was a significant decrease in fertility in the F₁ and F₂ rats (Murray et al., 1979). Mably and co-workers (1992a) showed that a single maternal dose of TCDD as low as 0.064 μg/kg/day on Gestation Day (GD) 15 decreased sperm production and epididymal sperm reserves, but produced no sign of overt toxicity in the male pups or the adult animal. Furthermore, Gray et al. (1997a,b) showed that 0.20 μg TCDD/kg on GD15 in Long Evans (LE) rats delayed the onset of puberty, reduced sperm counts, and produced malformations in the external genitalia of the female pups.

Several studies have investigated the toxicokinetic properties of TCDD to explain gender as well as species differences in the disposition of TCDD (Pohjanvirta et al., 1989; Li et al., 1995). Furthermore, tissue concentrations of TCDD associated with cleft palate and hyphophysis in developing mice have been measured (Abbott and Birnbaum, 1990; Abbott et al., 1996). The objective of the current study was to determine maternal and fetal tissue concentrations of TCDD after pregnant LE rats received a single oral dose of 1.15 μg TCDD/kg on GD8. A similar dose has been shown by Gray et al. (1995) and Gray and Ostby (1995) to produce genital malformations and premature reproductive senescence in female pups as well as a persistent reduction in sperm counts in male offspring. We focused on the disposition after GD8 (an early period of organogenesis) administration because administration of TCDD on this day produced a broader spectrum of developmental effects than treatment on GD15. Tissue concentrations of TCDD were measured to better understand the toxicokinetic properties of this compound in pregnant LE rats as well as embryos and fetuses during a critical period of organogenesis. In addition, the biliary elimination of TCDD in pregnant and nonpregnant LE rats was investigated to determine whether the pharmacokinetics of TCDD was altered during pregnancy.

**MATERIALS AND METHODS**

**Chemicals.** [³H]TCDD (sp act 34.7 Ci/mmol) was obtained from Radian Corporation (Austin, TX) and was purified by reverse-phase high-pressure liquid chromatography to ≥99% purity (Diliberto et al., 1995). Dosing solutions were prepared by adding [³H]TCDD (0.91 mCi/ml) to toluene as corn oil. Volatile compounds were removed by evaporation using a Savant Speed-Vac (Savant Instruments Inc., Farmingdale, NY).

**Animals and treatments.** Eight-week-old, time-pregnant LE rats (200–250 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC) on GD5. Animals were housed individually in clear plastic cages with hardwood bedding (Beta Chips, Northeastern Products, Warrensburg, NY). Animals were maintained during pregnancy on Laboratory Rodent Diet (No. 5001, PMI Feeds, Inc., St. Louis, MO) and unpurified tap water ad libitum in a room with a 12:12-h photoperiod and a temperature of 20–24°C, with a relative humidity of 40–50%.

**Experimental design and treatments.** Five pregnant rats were assigned to each treatment. Rats received a single oral dose of 1.15 μg [³H]TCDD/kg (~30.8 μCi/rat) in 5 ml corn oil/kg on GD8. The dosing solution was originally calculated to be 1.0 μg [³H]TCDD/kg; however, analysis of the dosing solution revealed that the actual concentration was 1.15 μg [³H]TCDD/kg. The day following overnight mating which resulted in a copulatory plug at the breeding facility was designated GD0. The dams were anesthetized with CO₂ on GD9, GD16, and GD21 and 5 ml of blood was removed via cardiac puncture. The animals were then terminated by cervical dislocation while under anesthesia. The following maternal tissues were removed and weighted: liver, lungs, kidneys, thymus, spleen, adrenals, muscle (left thigh), adipose tissue (perirenal fat), and skin (ears). On GD9 individual embryos were removed from the uterus and analyzed to determine TCDD concentration. On GD16 and GD21 each fetus was subdivided: first, the head was dissected from the body directly beneath the mandible; next, the fetal liver was removed followed by the fetal urogenital tract; the remaining tissue was analyzed as the fetal body. In addition, individual placentas were examined and fetal brains were analyzed on GD21.

Concurrently, four additional pregnant LE rats received a single, oral dose of 1.15 μg [³H]TCDD/kg (~30.8 μCi/rat; 5 ml/kg) on GD8. Feces were collected for 24, 48, and 72 h postdosing. The feces were then dried and ground and 100-ng triplicate samples were analyzed.

**Biliary excretion studies.** Nonpregnant and pregnant (GD8) LE rats were anesthetized with urethane (Sigma, St. Louis, MO) (125 mg/kg, ip) and the common bile duct was exposed and canulated. Animals received a dose of 1.0 μg [³H]TCDD/kg (~30.8 μCi/rat) in 1:1:3 Emulphor–ethanol:water; 2.2 μCi/rat) via the femoral vein and the bile was collected for 6 h. After bile collection the rats were terminated by perforation of the diaphragm. Radioactivity in the bile was determined by aliquoting 50-μl samples of the weighed bile into scintillant for analysis by liquid scintillation spectrometry.

**Oxidation and quantitation of samples.** All tissues (maternal and fetal) and feces were oxidized using a Packard 307 Sample Oxidizer with an

![FIG. 1. Amount of TCDD (% dose/tissue) found in maternal tissues on GD9, GD16, and GD21 after a single oral dose of 1.0 μg [³H]TCDD/kg on GD8. Tissue levels of [³H]TCDD were determined by sample combustion followed by analysis in a liquid scintillation spectrometer. All data are represented as the mean ± standard deviation (n = 4–5).](https://academic.oup.com/toxsci/article-abstract/45/2/129/1653863/130?download=true)
Log \( C \)o represents the intercept and \(-1^\text{2.303}\) is the slope of the line. The elimination half-life, which is the time required for the tissue concentration of a chemical to decrease by one-half. Assuming elimination from the liver occurs by a first-order process, a mathematical equation to describe this process is

\[
\frac{\text{d}C}{\text{d}t} = -k_{\text{el}}C
\]

where \( k_{\text{el}} \) is the apparent first-order elimination rate constant. \( k_{\text{el}} \) was obtained by fitting the time course liver distribution using linear regression analysis. The equation describing the line was \( y = -0.94x + 1.74 \). The tissue half-life was then calculated by the equation \( t_{1/2} = \frac{0.693}{k_{\text{el}}} \) (Klaassen and Rozman, 1991).

**Statistical methods.** The data on the percent dose per gram of tissue were analyzed in a step-down procedure. In the first multivariate analysis of variance (MANOVA), the four individual tissue compartments in the fetus (head, body, liver, and urogenital tract) were tested for statistically significant differences (Hotelling-Lawley trace, \( p < 0.05 \)) from the whole fetus. The independent variable in the analysis was gestation day. In the second stage of the multivariate analysis, the four individual tissue compartments were compared to one another. Finally, for the tissue pairwise comparisons, multivariate \( t \) tests were used (\( p < 0.05 \)). If statistical significance was not observed at any level of the analysis, none of the subsequent step-down analyses were performed. All data are represented as the mean ± standard deviation.

On GD16, whole fetus TCDD concentrations were analyzed to determine if uterine position affected TCDD concentrations using a one-way nested design with location and dams considered random effects (Snedecor and Cochran, 1967).

**RESULTS**

**Maternal LE rats.** One day after GD8 administration, the highest level of radioactivity in the dam was found in the liver (25.1% of dose/tissue), followed by adipose tissue (7.5%) (Fig. 1). The lowest levels were found in blood (0.11%), lung...
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FIG. 2. Concentration of TCDD (% dose/g tissue) found in maternal tissues on GD9, GD16, and GD21 after a single oral dose of 1.0 μg [3H]TCDD/kg on GD8. Tissue levels of [3H]TCDD were determined by sample combustion followed by analysis in a liquid scintillation spectrometer. All data are represented as the mean ± standard deviation (n = 4–5).

(0.10%), thymus (0.04%), and spleen (0.02%). Two other tissues with relatively high levels were the skin (2.8%) and muscle (1.7%). On GD9 the maternal liver contained the largest % dose, but by GD16 the amount had decreased to 15.0% and continued to drop to 9.1% by GD21 (Fig. 1). The apparent half-life of TCDD in the liver was approximately 8 days.

Fecal excretion of TCDD-derived radioactivity was 16.1% (41 ng), 6.2% (16 ng), and 3.7% (9.4 ng) of the dose 24, 48, and 72 h postadministration. Within 3 days after dosing, 26% of the administered dose was eliminated via the feces.

The liver contained the highest concentration of TCDD, which was 2.05% dose/g on GD9. This dropped to 1.00% on GD16 and to 0.64% dose/g by GD21. Maternal adipose tissue also contained relatively high concentrations of TCDD: 0.42 (% dose/g) (GD9), 0.61 (GD16), and 0.62% dose/g (GD21) (Table 1). The distribution of TCDD in other maternal tissues is illustrated in Fig. 2 and Table 1.

Embryonic data. Embryonic (GD9) and fetal (GD16 and GD21) samples were oxidized to determine concentrations of TCDD that reached the developing embryo/fetus. The amount of TCDD found in all the fetal samples was summed to determine the amount of TCDD that reached the embryonic/fetal compartment (Table 2). On GD9, 0.01% of the administered dose was present in the entire embryonic compartment, which includes all the embryos as well as the placentas. This resulted in a tissue concentration of 0.02% dose/g tissue. However, by GD16 the % dose reaching the fetal compartment had increased to 0.11% (0.01% dose/g) and had further increased to 0.71% (0.01% dose/g) by GD21 (Fig. 2 and Table 2). Although the later time points contain a greater percentage of the total dose, this increase in the amount of compound reaching the fetuses can be explained by the large increase in fetal weight during this time period. When analyzing these amounts on a concentration basis (% dose/g), the GD16 fetal compartment contained approximately the same concentration as maternal blood and spleen and tended to decrease over time (Fig. 2).

On GD16 and GD21 the individual fetuses were subdivided into four different tissues: liver, urogenital tract, head, and remaining tissue (body). On GD16 the liver, urogenital tract, head, and body had 0.01, 0.01, 0.007, and 0.008% dose/g, respectively (Fig. 3). On GD21 the concentration had increased in the liver, urogenital tract, head, and body (0.02, 0.02, 0.01, and 0.01% dose/g, respectively). Fetal brains were dissected out on GD21 and had considerably lower TCDD concentrations (0.003% dose/g).

At each time point following administration on GD8, whole embryos/fetuses were also oxidized to determine the amount of TCDD that reached a developing embryo/fetus. On GD9, 0.0005% of the administered dose reached a single embryo, resulting in a tissue concentration of 39.6 pg TCDD/g tissue (Table 2). The concentration in an individual fetus dropped to 18.1 pg/g by GD16 but significantly increased to 22.1 pg/g on GD21.

On GD16 there was no significant difference in whole fetus concentrations within a dam (p = 0.973), which implies that the responses are equal for pups from the same dam. The variance among dams was estimated to be 0.00000766 and the variance among pups within dams was estimated to be 0.00000541. Because the variance among pups was essentially 0, we concluded that pups from the same dam contained equivalent concentrations of TCDD. This indicates that fetal tissue samples could be pooled and that the litter mean would give an accurate representation of individual fetal concentrations from the same dam. There was a significant difference in the level of TCDD among dams (p < 0.001). Since we did not determine whole body burden for the dams, we did not relate tissue concentrations found in the dams to those in the pups.

Biliary studies. Pregnant and nonpregnant LE rats received a dose of 1.0 μg [3H]TCDD/kg body weight and biliary elimination was measured for 6 h. During the 6 h the nonpregnant rats eliminated 1.51% of the total administered dose, whereas the pregnant rats eliminated 1.62% of the dose (Fig. 4). These results indicate that there is no difference in the biliary elimination rate of TCDD, which is an indirect measure of metabolism, in pregnant and nonpregnant LE rats.
The toxicity of TCDD in the chicken embryo is 0.25 μg/kg egg weight. This determinant in a chemical's ability to cause adverse effects is determined by sample combustion followed by analysis in a liquid scintillation spectrometer. Fetal concentrations were determined for at least four fetuses from each of five dams; NA, not assayed. Statistical significance includes GD16 and GD21.

**DISCUSSION**

Exposure to TCDD during pregnancy causes prenatal mortality in several species of animals including the monkey, hamster, rat, and mouse (Sparschu et al., 1971; Courtney, 1976; McNulty, 1984; Olson and McGarrigle, 1992). In addition, these adverse developmental effects occur at doses lower than those causing overt maternal toxicity (Khera and Ruddick, 1973; Murray et al., 1979; Mably et al., 1992b; Gray and Ostby, 1995). For example, the LD₅₀ of TCDD in rainbow trout sac fry (0.04 μg/kg egg weight) is 25 times less than the LD₅₀ in juvenile rainbow trout (10 μg/kg body weight) (Kleeman et al., 1988; Walker and Peterson, 1991). Furthermore, bird embryos are more sensitive to TCDD toxicity than adults. The LD₅₀ of TCDD in the chicken embryo is 0.25 μg/kg egg weight compared to an LD₅₀ of 25–50 μg/kg body weight in an adult chicken (Grieg et al., 1973; Allred and Strange, 1977). Although the administered dose plays an important role in determining the extent of embryo/fetus lethality, another critical determinant in a chemical's ability to cause adverse effects is the time period during which exposure to TCDD occurs. Therefore, it is important to understand the relationship between exposure during a critical window of sensitivity and the concentration of chemical reaching the target tissue.

Recently, Gray et al. (1995) and Gray and Ostby (1995) demonstrated that 1.0 μg TCDD/kg administered on GD8 produced adverse reproductive and developmental effects in LE rat pups. These effects include reduced sperm counts and alterations in the sexual behavior of males as well as a partial clefting of the phallus, a vaginal thread, reduced ovarian weight, increased incidence of cystic hyperplasia of the endometrium, and premature reproductive senescence in female offspring. Therefore, to explain the adverse developmental effects, Gray and co-workers hypothesized that the developing urogenital tract may be a target tissue for TCDD and that exposure during development altered urogenital differentiation in fetal rats (Gray et al., 1995). In fact, concentrations of TCDD found in the urogenital tract were significantly greater (p < 0.05) than those found in the head and body on GD16 and GD21. However, urogenital tract concentrations were not different from those of fetal liver. Previous studies have inves-

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<th>Tissue</th>
<th>GD9</th>
<th>GD16</th>
<th>GD21</th>
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<tbody>
<tr>
<td>Whole embryo/fetus</td>
<td>0.5 ± 0.1</td>
<td>2.9 ± 0.6</td>
<td>49.4 ± 5.2</td>
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<tr>
<td></td>
<td>(1.4 ± 0.2)</td>
<td>(7.1 ± 1.5)</td>
<td>(115.0 ± 12.9)</td>
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<tr>
<td></td>
<td>[39.6 ± 10.0]</td>
<td>[18.1 ± 2.6]</td>
<td>[22.1 ± 2.6]</td>
</tr>
<tr>
<td>Head</td>
<td>1.2 ± 0.3</td>
<td>9.6 ± 0.6</td>
<td>(22.0 ± 2.6)</td>
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<tr>
<td></td>
<td>(3.0 ± 0.7)</td>
<td>[17.5 ± 3.1]</td>
<td>[21.8 ± 3.1]</td>
</tr>
<tr>
<td>Body</td>
<td>1.5 ± 0.4</td>
<td>34.0 ± 4.0</td>
<td>9.4 ± 2.5</td>
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<td>(3.6 ± 0.9)</td>
<td>(79.4 ± 9.0)</td>
<td>9.4 ± 2.5</td>
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<tr>
<td>Liver</td>
<td>0.3 ± 0.1</td>
<td>7.5 ± 4.5</td>
<td>[26.1 ± 5.0]</td>
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<tr>
<td></td>
<td>(0.8 ± 0.3)</td>
<td>(17.8 ± 11.0)</td>
<td>[26.1 ± 5.0]</td>
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<td></td>
<td>[20.1 ± 8.0]</td>
<td>[23.7 ± 3.1]</td>
<td>[23.7 ± 3.1]</td>
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<tr>
<td>Urogenital tract</td>
<td>0.04 ± 0.01</td>
<td>1.1 ± 0.2</td>
<td>0.10 ± 0.03</td>
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<tr>
<td></td>
<td>(2.5 ± 0.6)</td>
<td>(36.8 ± 10.1)</td>
<td>(36.8 ± 10.1)</td>
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<td></td>
<td>[23.2 ± 6.7]</td>
<td>[26.7 ± 28.6]</td>
<td>[26.7 ± 28.6]</td>
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<tr>
<td>Placenta</td>
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<td>6.7 ± 1.3</td>
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<td>(12.6 ± 2.5)</td>
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<td></td>
<td>(32.2 ± 3.7)</td>
<td>(23.2 ± 3.9)</td>
<td>(23.2 ± 3.9)</td>
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<tr>
<td>Fetal compartment</td>
<td>9.4 ± 2.5</td>
<td>114.7 ± 29.2</td>
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<td>(22.7 ± 4.2)</td>
<td>(278.5 ± 72.6)</td>
<td>(1646.0 ± 306.0)</td>
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<td></td>
<td>(38.6 ± 9.7)</td>
<td>(26.1 ± 2.9)</td>
<td>(26.1 ± 2.9)</td>
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</table>

**Note.** Multiple comparisons were made using only the following tissues in the statistical analyses: fetal head, body, liver, and urogenital tract.

*Mean ± SD (N = 4-5).

Fetal concentrations were determined for at least four fetuses from each of five dams; NA, not assayed.

*Significantly greater than fetal head (p < 0.05).

*Significantly greater than fetal body (RM ANOVA, p < 0.05).

**FIG. 3.** The concentration of TCDD (% dose/g tissue) found within different fetal tissues after a single oral dose of 1.0 μg [³H]TCDD/kg on GD8. On days GD16 and GD21, fetuses were subdivided into liver, urogenital tract, head, and remaining tissue (body). Tissue concentrations of TCDD were determined by sample combustion followed by analysis in a liquid scintillation spectrometer. Fetal concentrations were determined for at least five fetuses from five dams. *Statistically different from fetal head (RM ANOVA, p < 0.05). (•) Statistically different from fetal body (RM ANOVA, p < 0.05). Statistical significance includes GD16 and GD21.
TCDD/kg in pregnant rats causes adverse effects in female effects of TCDD exposure since a single dose of 1.0 /ig the rat is more sensitive than the mouse to the developmental also occur in the urogenital tract of developing rats; therefore, induced alterations in growth factors and their receptors may EGF receptors as well as epithelia cell hyperplasia. TCDD-GD10 or 6 /ig TCDD/kg on GD12 caused up-regulation of mouse fetuses exposed to a single dose of 24 /xg TCDD/kg on (1990) showed that the developing ureteric epithelium of epithelium of fetal mice. Studies by Abbott and Birnbaum (20.1 pg/g) appears to be sufficient to alter ureteric differenti- tion since female LE pups prenatally exposed to TCDD ex- periment as well as others that are currently being performed in our laboratory will provide a better understanding of the toxi- cokinetic properties of TCDD within pregnant animals after acute and subchronic exposures. Furthermore, it will enable us to determine whether tissue concentration is the appropriate
tigated the disposition of TCDD in fetal mice during different periods of gestation (Nau and Bass, 1981; Abbott et al., 1989). Distribution profiles were similar; however, in our studies the difference in TCDD concentration between fetal liver and extrahepatic tissues was not as great as that seen in fetal mice. Differences in both timing and dose level as well as in the species are likely responsible for the apparent discrepancy. Although TCDD does not appear to be preferentially sequestered within the urogenital tract compared with the liver, the urogenital tract is a target tissue and this low concentration (20.1 pg/g) appears to be sufficient to alter ureteric differen- tiation since female LE pups prenatally exposed to TCDD exhibit malformations in their external genitalia (Gray and Ostby, 1995; Gray et al., 1997b).

Although receptor expression in the urogenital tract of developing rat pups has not been extensively characterized, more information exists on receptor regulation within the ureteric epithelium of fetal mice. Studies by Abbott and Birnbaum (1990) showed that the developing ureteric epithelium of mouse fetuses exposed to a single dose of 24 /g TCDD/kg on GD10 or 6 /g TCDD/kg on GD12 caused up-regulation of EGF receptors as well as epithelia cell hyperplasia. TCDD-induced alterations in growth factors and their receptors may also occur in the urogenital tract of developing rats; therefore, the rat is more sensitive than the mouse to the developmental effects of TCDD exposure since a single dose of 1.0 /g TCDD/kg in pregnant rats causes adverse effects in female pups. The low concentration within the developing urogenital tract observed in this study, which was administered during a critical window of sensitivity, is enough to be associated with severe adverse effects in the offspring of LE rats.

Another interesting finding is that the embryonic/fetal com- partment may be viewed as a nonsequestering maternal organ. Tables 1 and 2 show that the concentrations of TCDD found in maternal organs are similar to those seen in fetal tissues. On GD16, the fetal compartment contained 26 ppt. This is similar to concentrations in maternal blood (18 ppt) and spleen (42 ppt). Although very little mass of chemical reaches the fetal compartment, the small size of the fetuses results in concentra- tions of TCDD that are adequate to induce adverse repro- ductive and developmental effects.

The highest concentration of TCDD was found in the maternal liver, with the second highest levels observed in the maternal adipose tissue. The half-life of TCDD in the liver was estimated as approximately 8 days. This is considerably shorter than values for the whole body half-life of elimination in male Long Evans rats (Pohjanvirta et al., 1990; Viluksela et al., 1996). It was originally thought that pregnancy may increase the rate of elimination of TCDD from the liver. However, results of the biliary studies indicate that there is no difference in the rate of hepatic elimination between nonpregnant and pregnant LE rats (Fig. 4). Furthermore, studies by Wang et al. (1997) show that female Sprague–Dawley rats exhibit a hepatic half-life of elimination similar to that observed in these LE rats.

Administration of 1.15 /g TCDD/kg during a critical period of organogenesis produces concentrations that range from 20 to 40 pg TCDD/g. Gray and Osby (1995) and Gray et al. (1995) have shown that a dose of 1.0 /g TCDD/kg administered on GD8 produces adverse reproductive and developmental effects in LE rat pups. Therefore, these tissue concentrations in the parts per trillion range are associated with adverse effects. This indicates that the perinatal stage of life is extremely sensitive to TCDD exposure and that very low fetal concentrations of this chemical can produce developmental effects within the pups.

Human populations poisoned by dioxins have body burdens between 96 and 7000 ng TEQ/kg body weight (DeVito et al., 1995). Human placentas from individuals exposed to PCDFs/PCBs in the Yu-Cheng cohort contained concentrations as high as 89 ng TEQ/kg wet weight (Schecter et al., 1996). We have shown that a dose of 1.15 /g TCDD/kg administered on GD8 results in 40 ppt in a single embryo. This indicates that this dose is able to induce adverse effects at fetal concentrations that are below those associated with accidental human exposure (Rogan et al., 1988; Chen et al., 1992). This disposition study as well as others that are currently being performed in our laboratory will provide a better understanding of the toxicokinetic properties of TCDD within pregnant animals after acute and subchronic exposures. Furthermore, it will enable us to determine whether tissue concentration is the appropriate
dose metric to predict adverse developmental and reproductive effects. This information may help to determine whether human exposure to low doses of PCDDs/PCDFs for long periods of time could result in detrimental developmental effects.

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REFERENCES


Murray, F. J., Smith, F. A., Nitschke, K. D., Humiston, C. G., Kokica, R. J.,


