Maternal and fetal disposition of TCDD in Long Evans rats

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), an unwanted contaminant of combustion and bleaching processes, is one of the most potent toxicants known[1]. TCDD is an endocrine disrupter, which is able to alter the levels of many hormones and/or their receptors[2]. As a result of the altered homeostatic processes, TCDD adversely affects reproduction and development in several species of laboratory animals. These reproductive 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[3]. In addition, a single, maternal dose as low as 0.064 μg TCDD/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[4]. Further studies by Gray and coworkers showed that administration of 0.02 μg TCDD/kg on GD15 in Long Evans rats delayed the onset of puberty, reduced sperm counts and produced malformations in the external genitalia of the female pups[5].

These studies demonstrate that exposure to TCDD during the perinatal stage of life can produce reproductive alterations. In addition, these abnormalities can occur at concentrations much lower than those causing maternal toxicity and are dependent upon the time of exposure. Therefore, we chose to investigate the toxicokinetics of TCDD in Long Evans rats during critical periods of organogenesis. This data will provide insight into the risks associated with human exposure to this potent toxicant.

Materials and Methods

Chemicals: [^{3}H]-TCDD was obtained from Radian Corporation (Austin, TX) and was purified by reverse-phase high-pressure-liquid-chromatography to ≥ 99 % purity (Sp. Ac. 34.7 Ci/mmol).
Treatment of Animals: Eight week old time-pregnant Long Evans rats (200-250g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). The rats received a single oral dose of 1.0 μg [^{3}H]-TCDD/kg in 5ml/kg corn oil on GD 8 or GD15.
Tissue Isolation: Animals dosed on GD8 were terminated on GD9, GD16 and GD21 by CO_2 asphyxiation. Animals dosed on GD15 were terminated on GD16. Maternal liver, lungs, kidneys, thymus, spleen, adrenals, muscle, fat, skin and blood were excised. The total embryonic compartment was taken on GD9 and GD16. On GD16 and GD21, for each fetus, the

urogenital tract was removed followed by the fetal liver. The head was dissected from the body directly beneath the mandible. Placentas were also examined.

Oxidation and quantitation of maternal and fetal tissues: All tissues were oxidized using a Packard 307 Sample Oxidizer followed by counting in a Beckman Model LS6000 LL liquid scintillation spectrometer and the data was analyzed in the following dosimetric units: % dose/tissue, % dose/g tissue, ng TCDD/tissue and ng TCDD/g tissue.

Data Analysis: For calculation of % total dose, blood mass was assumed to be 7.4 % of body wt, skin (19%), muscle (40%), and fat (7%)[6].

Results/Discussion

Previous studies have shown that effects associated with exposure to 1.0 μg TCDD/kg on GD8 resulted in delayed puberty and decreased sperm counts in males and premature reproductive senescence with structural abnormalities in the external genitalia of female pups[5]. After a single administration of this dose on GD8, the highest % dose/tissue of [^{3}H]-TCDD was localized in the maternal liver (26.3) on GD9. This had decreased to 13% by GD16 and 6.4% by GD 21 (Table 1). This data suggests that TCDD is eliminated more rapidly from the liver in pregnant females than nonpregnant females[7]. On GD9, 0.01% of the administered dose was present in the entire fetal compartment, resulting in a concentration of 0.02 %dose/g. On GD16, however, 0.10% of the administered dose reached the fetal compartment (0.01 %dose/g). This increase may be explained by an increase in fetal mass. Thus, a maternal dose of 1.0 μg TCDD/kg on GD8, results in a tissue concentration within the fetal compartment on GD9 of 42 ppt. When analyzing these amounts on a concentration basis (%dose/g), the GD16 fetal compartment contained approximately the same concentration as maternal blood and spleen. Furthermore, there appears to be an even distribution of TCDD throughout the fetus (Table 2). This indicates that TCDD is not sequestered within the different fetal tissues.

The reproductive effects seen after administration on GD15 are similar to those seen after GD8 administration; however, the severity of some of the effects are greater. For example, there was a greater decrease in sperm numbers in the male pups and 80% of the female pups displayed abnormalities in the external genitalia after dosing on GD15[5]. On GD16, the highest % dose/tissue after dosing on GD 15 with 1.0 μg TCDD/kg is again found in the maternal liver (29.8) (Table 3). On GD16, the fetal compartment contained 0.15 % of the dose. This resulted in a concentration (%dose/g) of 0.02 (Table 4). Thus, the fetal compartment on GD16 contained 480 pg TCDD, resulting in a tissue concentration of 59 ppt. Again, there appears to be an even distribution of TCDD within the fetus, which indicates that TCDD is not sequestered within the fetus.

Conclusions

Administration of 1.0 μg TCDD/kg during a critical period of organogenesis causes adverse developmental effects in the Long Evans rat. This indicates that the perinatal stage of life is extremely sensitive to TCDD exposure and that very low levels of this chemical can produce developmental effects within the pups. Therefore, a better understanding of the toxicokinetic properties of TCDD within pregnant animals may help to assess the potential risks associated with human exposure to TCDD.

This abstract does not necessarily reflect EPA policy

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Table 1. Tissue content of [^{3}H]-TCDD reaching maternal tissues after administration of 1.0 μg TCDD/kg on GD8. Rats were treated and tissues were collected as described. [^{3}H]-TCDD levels were determined by fraction oxidation and the data are expressed as % of administered dose reaching the maternal tissues (n=5). Values are mean ± standard deviation. NA=Not Assayed

% DOSE/TISSUE			
TISSUE	GD9	GD16	GD21
Maternal liver	26.3 ± 7.1	13.3 ± 2.9	6.41 ± 2.0
Maternal blood	0.124 ± 0.06	0.15 ± 0.04	0.07 ± 0.03
Maternal fat	7.8 ± 2.7	11.3 ± 2.7	11.2 ± 2.78
Maternal kidney	0.12 ± 0.03	0.05 ± 0.01	0.03 ± 0.01
24 hr feces	15.4 ± 6.5	NA	NA

Table 2. Concentration of [^{3}H]-TCDD found in fetal tissues on GD 16 after GD8 dosing. Values reported are the concentration reaching a single tissue. At least five tissues were analyzed from each litter from three dams. To calculate the amount reaching the entire fetal compartment, the amount in the fetal tissues was summed. Values are mean ± standard deviation.

% DOSE/g	
	GD16
fetal compartment	0.009 ± 0.0001
placenta	0.011 ± 0.002
head	0.007 ± 0.001
body	0.007 ± 0.001
liver	0.010 ± 0.001
urogenital tract	0.009 ± 0.005

Table 3. Tissue content of [^{3}H]-TCDD reaching maternal tissues after administration of 1.0 μg TCDD/kg on GD15. Rats were treated and tissues were collected as described. [^{3}H]-TCDD levels were determined by fraction oxidation and the data are expressed as % of administered dose reaching the maternal tissues (n=4). Values are mean ± standard deviation.

% DOSE/TISSUE	
TISSUE	GD16
Maternal liver	29.8 ± 2.6
Maternal blood	0.36 ±0.15
Maternal fat	6.8 ± 4.0
Maternal kidney	0.13 ± 0.08
24 hr feces	22.6 ± 7.3

Table 4. Concentration of [^{3}H]-TCDD found in fetal tissues on GD 16 after GD15 dosing. Values reported are the concentration reaching a single tissue. At least five tissues were analyzed from each litter from four dams. To calculate the amount reaching the entire fetal compartment, the amount in the fetal tissues was summed (n=4 dams). Values are mean ± standard deviation.

% DOSE/g	
	GD16
fetal compartment	0.02 ± 0.007
placenta	0.026 ± 0.01
head	0.014 ± 0.005
body	0.015 ± 0.005
liver	0.02 ± 0.01
urogenital tract	0.019 ± 0.007