Acute administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Long Evans rats: Comparison of fetal tissue levels and adverse developmental effects

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a contaminant of combustion and bleaching processes, is one of the most potent toxicants known. TCDD is an endocrine disrupter, which is able to alter the levels of many hormones and/or their receptors [1]. 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 [2]. In addition, a single, maternal dose as low as 0.064 μg TCDD/kg/day on gestation day (GD) 15 decreased epididymal sperm reserves, but produced no sign of overt toxicity in the male pups or the adult animal [3]. Further studies by Gray and coworkers showed that administration of 0.20 μg TCDD/kg on GD15 in Long Evans rats delayed the onset of puberty in male pups and produced malformations in the external genitalia of the female pups [4, 5]. A dose of 1.0 μg TCDD/kg on GD8 resulted in female offspring that were subfertile compared to controls [6]. Thus, exposure to TCDD during the perinatal stage of life can produce persistent reproductive alterations. These abnormalities can occur at concentrations much lower than those causing maternal toxicity and are dependent upon the time of exposure. The objectives of our studies were to determine whether tissue concentration is the appropriate dose metric to assess the potential for the development of adverse effects in developing LE pups. Comparison of fetal TCDD concentrations and adverse reproductive effects after different acute exposures, administered during critical periods of development, can provide valuable insight into the appropriate dose metric and can aid in assessment of potential risks associated with human exposure to this chemical.

Materials and Methods

Chemicals

2, 3, 7, 8-Tetrachloro [1, 6-^{3}H] dibenzo-*p*-dioxin (MW=322) was synthesized by Radian Corp. and purchased from Cambridge Isotope Laboratories (Wolburn, MA). Radiopurity was ≥ 98 % by RP-HPLC.

Treatment of Animals

Female, time-pregnant, Long Evans rats (8-12 weeks old, ~225g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were dosed by oral gavage on gestation day (GD) 8 with 1.15 μg ^{3}H-TCDD/kg or on GD15 with 0.05, 0.20, 0.80 or 1.0 μg ^{3}H-TCDD/kg in 5 ml corn oil/kg (n=5).

Tissue Isolation

For the study of disposition following GD8 and GD15 dosing, dams were euthanized on GD16 and GD21 by CO_2 asphyxiation. The following maternal tissues were removed for TCDD analysis: maternal liver, thymus, muscle, skin, fat and blood. On GD16 and GD21, the fetal liver was removed followed by the urogenital tract from each fetus. The head was dissected from the body directly beneath the mandible.

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 units: % dose/tissue, % dose/g tissue, ng TCDD/tissue and ng TCDD/g tissue.

Data Analysis

On GD16 and GD21, dose-response relationships were plotted showing fetal tissue concentration of TCDD versus the incidence of adverse developmental effects found in the published literature [4-7]. For the disposition study on GD8, LE rats were dosed with 1.15 μg ^{3}H-TCDD/kg, whereas Gray used a dose of 1.0 μg TCDD/kg on GD8 [6, 7]. When comparing tissue concentration and response data, the tissue concentrations following GD8 exposure were corrected for differences in dose assuming that tissue concentration increases linearly with administered dose. All graphs show the mean ± standard deviation for tissue concentration and response.

Results and Discussion

Administration of 1.0 μg TCDD/kg during a period of major organogenesis (GD8) produced fetal concentrations of TCDD on GD16 of approximately 18-22 pg/g. Exposure on GD15 to 0.05, 0.20, 0.80 or 1.0 μg ^{3}H-TCDD/kg produced fetal concentrations ranging from 7-58 pg/g on GD16, respectively. For the four end-points examined (ejaculated sperm counts and puberty delay in males and urethra-phallus distance and vaginal thread incidence in females), the dose-response curves on GD16 show that similar tissue concentrations result in comparable responses (Fig. 1). It is interesting that a dose of 1.0 μg TCDD/kg on GD8 produced similar tissue concentrations and responses as a dose of 0.20 μg TCDD/kg on GD15. Although the concentration in a single fetus appears to underpredict the incidence of vaginal thread in the females (Fig. 1D), this may be due to the variability in this response. Overall, the fetal concentration appears closely associated with the responses examined.

Figure 2 illustrates the relationship between concentration in different fetal tissues and a single response (urethra-phallus distance in females) on GD21. It is clear from these two acute dosing regimens that the concentration in a urogenital tract, a single fetus, and placenta reasonably predict TCDD's affect on the external genitalia of females as measured by urethra-phallus distance. At this time-point (GD21), concentrations after exposure to 1.0 μg TCDD/kg on GD8 are similar to those seen after exposure to 0.20 μg TCDD/kg on GD15. There was a large degree of variability in fetal liver concentrations, which may explain why concentrations in this tissue did not predict the response. However, TCDD concentrations found in three different fetal tissues adequately predict TCDD's affect on urethra-phallus distance.

In conclusion, these two different acute exposures produce equivalent tissue concentrations at a critical period of sensitivity that is sufficient to produce the same magnitude of adverse, developmental effects. It should be noted that GD16 fetal concentrations following GD15 exposure

would not be appropriate to predict risk for those responses whose critical period of sensitivity is prior to GD15 e.g., premature reproductive senescence. Hence, some of the observed effects in females cannot be predicted from the GD15 data. These dispositional studies provide a better understanding of the toxicokinetics of TCDD in pregnant rats after acute exposures and demonstrates that tissue concentration is the appropriate dose metric to predict adverse reproductive and development effects. (This abstract does not necessarily represent EPA policy.)

References

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GD16

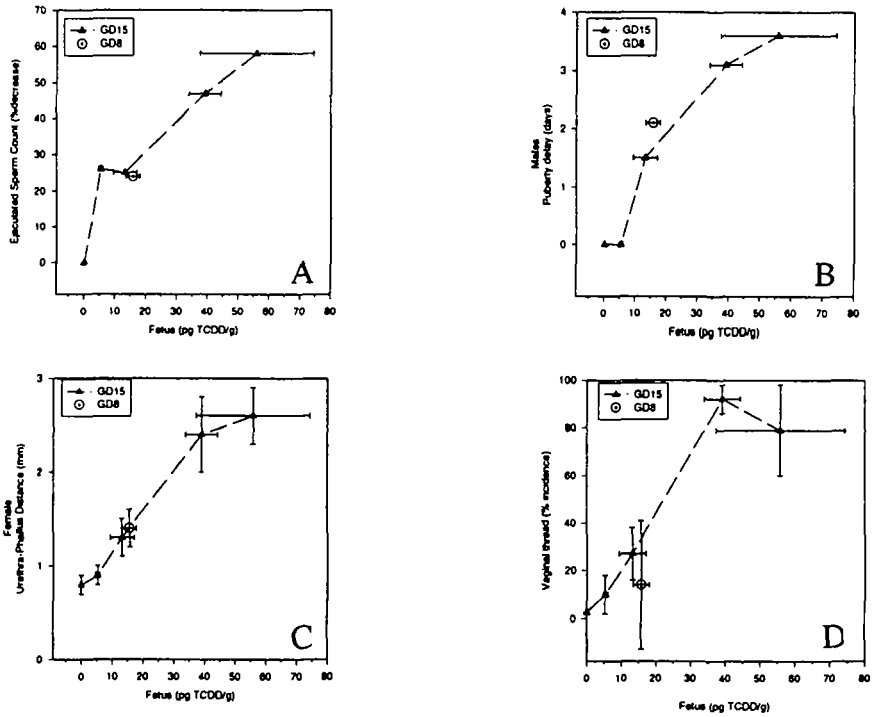

Figure 1. Dose-response relationship between fetal TCDD concentration and four responses (ejaculated sperm counts and puberty delay in males and urethra-phallus distance and vaginal thread incidence in females).

GD21

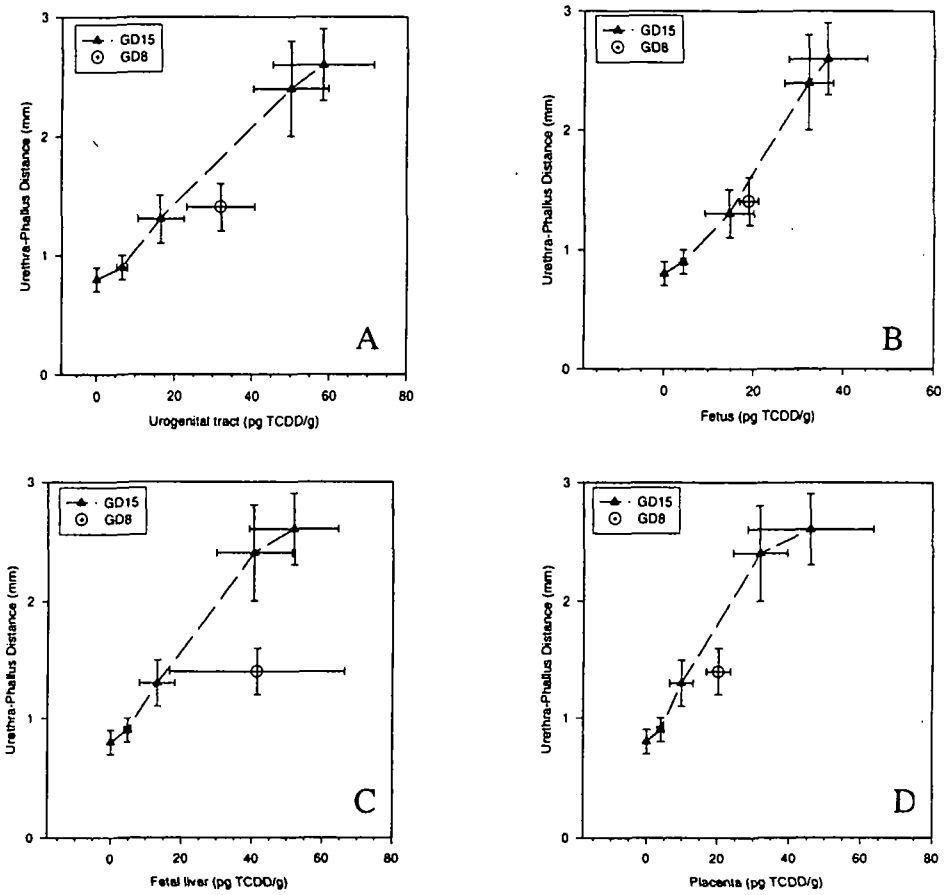

Figure 2. Dose-response relationship between tissue concentration and urethra-phallus distance in females on GD21.