Disposition of TCDD in pregnant C57BL/6N mice from 0.5 to 24 hours post-exposure.

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1. Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin is a persistent environmental contaminant. This compound is teratogenic in C57BL/6N mice and induces cleft palate and hydronephrosis at doses which are not overtly maternally toxic[1]. The mechanism of induction of clefting involves effects on proliferation and differentiation of the palatal epithelial cells[2]. TCDD exposure alters expression of several growth factors and down-regulates the Ah receptor (AhR) in the palatal cells[3,4]. Previous studies demonstrated that TCDD reaches the mouse fetus in a dose and time-dependent manner[5,6,7]. However, these studies did not examine distribution within the first 24 hours following dosing. In an earlier study, TCDD was in the placenta, fetal head, and body at 3 hours post-dosing. These embryos were exposed on GD 11 to 30 μg/kg body weight and it was not possible to disect the palatal shelves as these structures form at a later developmental stage (GD12). In the present study, the exposure to [^{3}H]-TCDD was performed using the dosing regimen which is routinely used in studies of the mechanism of induction of cleft palate. In this dosing protocol, exposure on GD 12 to 24 μg TCDD/kg body weight induces 85-100% clefting[1,2,8]. The results of this study will be important for supporting investigations of TCDD-induced gene regulation in fetal target tissue.

2. Methods and Materials

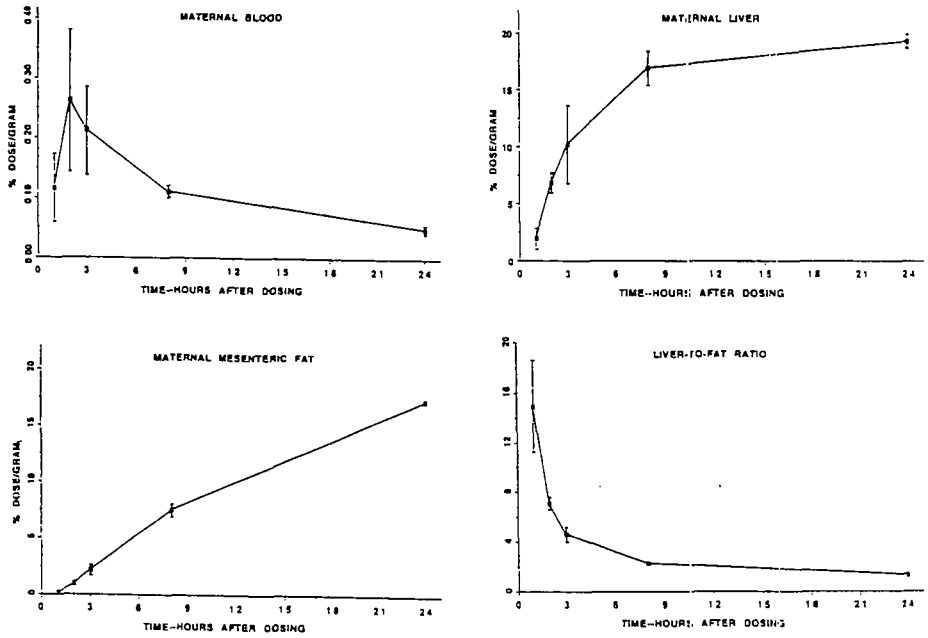
C57BL/6N pregnant female mice were obtained from Charles River Laboratories (Raleigh, NC) on GD 8 (plug day=gestation day [GD] 0). Animals were housed under controlled conditions of temperature (72$\pm$2° F), humidity (40-60%), and lighting (12/12 hour light/dark cycle), and provided food (Agway rat, mouse and hamster 3000) and water ad libitum. At 7:45 AM on GD 12, animals were weighed and dosed by oral gavage with [^{3}H]-TCDD in corn oil (24 μg/kg, 5ml/kg, approximately 40 μCi/mouse). Purity of the [^{3}H]-TCDD (Radian Corporation, Austin, TX) was confirmed by HPLC and by rat biliary assay. The dosing solution was prepared by adding the purified [^{3}H]-TCDD in methanol to corn oil and then evaporating the methanol to give 29.9 μCi/0.1 ml solution. Unlabeled TCDD was added (1.58 μg TCDD to 7.02 μg [^{3}H]-TCDD) to attain a concentration of 4.8 μg TCDD/ml. This dose given on GD 12 has been previously shown to induce cleft palate in 85-100% of the exposed fetuses. Tissues were collected at 0.5-1, 1.5-2, 2.5-3, 7.5-8, and 24-24.5 hours after dosing. A total of 13 mice were dosed and 3 were killed at each of the first 3 time points and 2 each at the last collection times. Tissues collected included maternal liver, blood, and mesenteric fat; placenta; fetal liver and secondary palate. Animals were killed by CO_2 inhalation and maternal blood was collected by cardiac puncture. Uteri and contents were removed and weighed and number of fetuses recorded. Maternal liver and mesenteric fat were collected and weighed. Placenta, fetal livers, and palates were pooled for each litter and combined weight recorded for each tissue. Palatal tissues were frozen and then sonicated in water to disrupt cells.

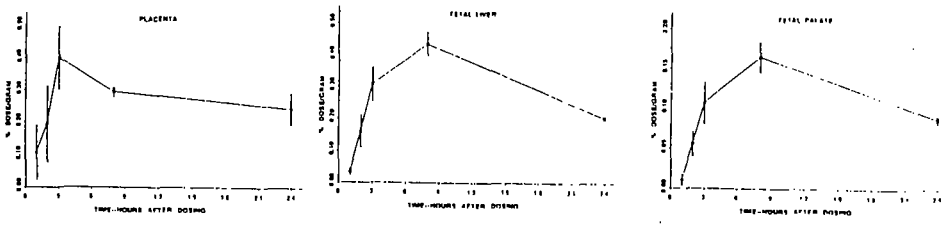
Aliquots of the palatal homogenate were taken for scintillation counting and determination of total protein and DNA. All other tissue samples were analyzed by oxidation in a Packard Oxidizer.

3. Results

Maternal Tissues: The [^{3}H]-TCDD was given by oral gavage in corn oil in the morning and was detected in maternal blood within 30 minutes. The % dose/gram continued to rise through the second hour and then declined, although TCDD was detected at 24 hours. In maternal liver the %dose/gram of tissue increased from 0.5 to 8 hours. The levels at 24 hours were similar to those observed at 8 hours. In contrast to the distribution to blood and liver, TCDD accumulated in maternal fat at a steady rate during the 0.5 to 24 hour period. The liver-to-fat ratio declined sharply during the first 3 hours. The decline was slower from 3-8 hours with only minor change thereafter. Plotting % of total dose (data not shown) gave profiles like those of % dose/gram for both maternal fat and blood. However % total dose increased in liver from 8 to 24 hours.

Placenta and Fetal Tissue: The placenta is a highly perfused tissue and the distribution of TCDD in the first 8 hours is very similar to that of maternal blood. A sharp increase in the first 3 hours is followed by a gradual decline from 8-24 hours, whether the % dose or % dose/gram is plotted. The % dose/gram in fetal liver increased from 0.5-8 hours and a decline was noted at 24 hours. During this period the fetus is rapidly increasing in size and the fetal liver weight increased from 4.5 mg at 0.5 hours post-dosing to 8.7 mg at 24 hours. TCDD reached the secondary palates within the first 30 minutes and levels increased as either % dose or %dose/gm up to 8 hours. From 8 to 24 hours a decline was observed, similar to that occurring in fetal liver. The profile of distribution to the palate was similar when examined as pg/palate, pg/wet weight, pg/mg total protein or DNA. The latter units were determined to facilitate comparisons of in vivo exposure data with previously





determined in vitro exposure and distribution of [^{3}H]-TCDD in palatal organ cultures.

4. Discussion

TCDD reaches the developing embryonic secondary palate within 30 minutes of administration of an oral dose to the pregnant female in the early morning of gestation day 12. The distribution to maternal blood and placenta was similar and very rapid, considering that the animals were at the start of the light cycle and fully fed. Similar profiles of rapid distribution followed by a slower accumulation, or plateau in the distribution curve of % dose/gram tissue, occurred in the maternal liver, fetal liver and palatal shelves. This pattern of TCDD disposition is in agreement with the data of Abbott et al.[7], in which concentrations in maternal liver and placenta (expressed as pg TCDD/wet weight of tissue) remained relatively constant from 3 to 72 hours. This suggests that the growing embryo continues to accumulate TCDD, however the increasing mass of the organs and tissues results in a relatively constant tissue concentration during the first 3 days following dosing. This outcome will be of importance in evaluating the responses of embryonic tissues in culture models. Modeling this distribution in palatal organ culture is fairly simple with the assurance that TCDD reaches the tissues rapidly and that a subsequent constant level of exposure *in vitro* models the situation of embryos exposed *in vivo*. C57BL/6N embryonic palatal shelves respond to TCDD in palatal organ culture and 100% of the shelves are affected at 5 x 10^{-11} M TCDD[7]. This concentration in medium results in an average accumulation after 24 hours in culture of 45 pg TCDD/mg DNA (unpublished data from a study of [^{3}H]-TCDD distribution in palatal organ culture). This is in good agreement with the 53.5 pg TCDD/mg DNA detected in palates 24 hours post-exposure in the present *in vivo* study.

The dosing regimen for this *in vivo* study is identical to that used to induce cleft palate in these mice. Therefore future studies of effects of TCDD on gene expression can be conducted with the knowledge that the ligand (TCDD) is present in the cells of the embryo within 30 minutes and may be there even earlier. This data also confirms that the *in vitro* models are reasonable facsimile of the distribution ocurring following an *in vivo* exposure. This study provides valuable new information regarding distribution in the pregnant mouse.

5. References

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