RELATIONSHIP OF STEADY-STATE EXPOSURE AND PHARMACOKINETICS OF [^{3}H]2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) IN THE FEMALE MOUSE

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INTRODUCTION

The polyhalogenated aromatic hydrocarbons (PHAHs) are some of the most persistent contaminants in the environment. These ubiquitous compounds have been detected in the food chain and subsequently have become a "natural" component of the general population diet. The predominant source of body burdens in humans is mainly via food consumption. Because of this major daily source for human exposure, information is needed to estimate steady-state concentrations at low, repeated exposures. Consequently, steady-state exposures may have important implications in human health risk assessments.

OBJECTIVES

The objectives of the present study were to determine the time to reach steady-state conditions in mice where the rate of administration of TCDD equals the rate of elimination of TCDD; to quantitate terminal tissue concentrations of TCDD; to determine the rate of elimination; and to compare the effects of dose on tissue concentrations at steady-state.

APPROACH

The approach was to administer [^{3}H]TCDD (low and high doses) and quantitate elimination for 91 days after the first dosing and quantitate terminal tissue concentrations.

MATERIALS AND METHODS

TCDD was obtained from Radian Corporation (Austin, TX) with purity >98% as determined by gas chromatography/mass spectrometry. [1,6-^{3}H]TCDD was synthesized by Chemsyn Science Laboratories (Lenexa, TX) with a specific activity of 39.5 Ci/mmole and purified by high-pressure liquid chromatography (HPLC). Purity of >99% was verified by HPLC and by a rat bioassay of biliary excretion.

Female B6C3F1 mice (8 wks old, ~20 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC) and allowed 1 week to acclimate in metabolism cages. They were maintained on a 12-hr light/dark cycle under conditions of

constant temperature and humidity and provided pellet feed (BioServe, Frenchtown, NJ) and water *ad libitum*.

Mice were treated orally by gavage 5 days/week for 13 weeks. The doses were 1.5 and 150 ng [^{3}H]TCDD/kg/day (0.003 and 0.03 μCi /day, respectively) in corn oil at 10 ml /kg. Feces and urine were collected daily. At the end of the study, mice were euthanized by CO_2 asphyxiation and tissues were collected. The following tissues were obtained: liver, perirenal fat, mesenteric fat, remaining dissectable fat, skin, lungs, muscle, blood, brain, thyroid, thymus, spleen, adrenals, kidneys, pancreas, stomach and contents, small intestines and contents, large intestines and contents, ovaries, uterus, bone, bone marrow, and heart. All tissues and feces were analyzed for total radioactivity by combustion followed by liquid scintillation. Urine was analyed directly by liquid scintillation.

Body composition estimates for blood and muscle tissue were 5 and 35%, respectively. Percentages body weight for adipose tissue and skin were calulated from total dissection of the carcasses.

RESULTS

The repeated dosing of TCDD in the present study caused no mortality or morbidity. There were no significant differences in body weight or changes in liver/body or thymus/body weight ratios between the low and high doses.

The high affinity of TCDD for hepatic tissue demonstrated dose-dependent kinetics. As the administered dose increased from 1.5 to 150 ng [^{3}H]TCDD/kg body wt, a larger percentage of the total administered dose was found in liver. Similar dose-dependent kinetics have been found in an acute study of [^{3}H]TCDD dose in the female B6C3F1 mouse (Diliberto *et al.*, submitted). In the present study, distribution of TCDD-derived radioactivity following subchronic exposure was dose-dependent. As the administered dose increased, relative concentration in extra-hepatic tissues decreased. In contrast the relative concentration in the liver increased supralinearly (Table 1). At the low dose, fat contained the highest concentrations of [^{3}H]TCDD-derived radioactivity (Table 1). While at the high dose, the total fat and liver depots of % dose were similar. Tissue concentrations were <1% dose/g in all tissues except for thyroid, skin, adrenals, liver, and fat (~1, 1, 2, 2, and 5% dose/g, respectively) at the low dose and were <1% dose/g in all tissues except for liver and fat at the high dose. Dose-related differences in liver-to-fat concentration ratios were noted as seen in Table 1. Similar ratios of 0.6 and 3.1 were found 7 days after dosing in a single oral dose study of 0.1 and 10 μg/kg in the mouse (Diliberto *et al.*, submitted).

Differences in fecal elimination appeared to be dose-related. Cumulative fecal excretions for the low and high doses were 34.90±3.35 and 46.07±5.53% dose, respectively. There was no statistical difference in the cumulative urine excretion for the low and high doses of 11.95±1.04 and 14.01±1.87% dose, respectively. Cumulative urine and fecal excretions profiles at the low dose suggested that TCDD was at steady-state; but the high dose profiles did not appeared to have reach steady-state conditions by 91 days.

CONCLUSIONS

In summary, the present study demonstrated that subchronic administration of low and high doses of TCDD reached or approached steady-state conditions. The dose-dependency of tissue distribution in the female B6C3F1 mouse was still evident, similar to the acute mouse study (Diliberto *et al.*, submitted). The enhanced sequestering of dioxin in the liver at high doses does not represent the true distribution seen environmentally under low exposure conditions. The concentration of dioxin in fat at low doses during steady-state may be similar to the levels seen in man at low environmental levels. Consequently, dioxin at steady state levels may be the more appropriate measure of dioxin concentration when extrapolating dose-response relationships between dioxin and possible adverse responses in humans. Therefore, steady-state status of dioxin and its congeners should be considered for high to low dose extrapolations in risk assessment.

(This abstract does not necessarily reflect EPA policy.)

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TABLE 1

DISTRIBUTION OF TCDD-DERIVED RADIOACTIVITY

(PERCENT CUMULATIVE DOSE PER GRAM TISSUE, MEAN ± SD)

	DOSE	
	1.5 ng/kg	150 ng/kg
LIVER	1.96 ± 0.30	6.63 ± 0.74
FAT	5.46 ± 0.81	1.62 ± 0.15
LIVER/FAT	0.36 ± 0.03	4.10 ± 0.34