DOSE- AND TIME-DEPENDENT TISSUE DISTRIBUTION AND INDUCTION OF CYP1A1 AND CYP1A2 IN FEMALE B6C3F1 MICE FOLLOWING ACUTE EXPOSURE TO [^{3}H]TCDD

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Animal and human studies strongly suggest the need for appropriate animal dose-response data sets with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, "dioxin") in order to extrapolate the dose-response relationships between dioxin and possible adverse responses in humans. Dioxin's dose-response relationship using the endpoint of tissue disposition has been documented to be dose- and time-dependent in rats, with a focus on liver and adipose tissue. Until now, there have been no reported studies of tissue disposition in mice looking at dose- and time-dependent distribution of TCDD as well as the potential for dose- and time-dependent sensitivities of target tissues to enzyme induction and the correlation of enzyme activity with the corresponding TCDD tissue concentrations.

The objectives of this study were to determine theeffects of dose and time on tissue distribution of TCDD as measured by radioactivity in 18 different tissues and for induction of cytochrome P450 1A1 (CYP1A1) and 1A2 (CYP1A2) over multiple time points and to determine potential differences in tissue sensitivity by comparing effects of TCDD on CYP1A1 induction.

The approach to the study was to administer [^{3}H]TCDD (0, 0.1, 1, or 10 µg/kg body wt) by oral gavage at a dosing volumue of 10 ml/kg to female B6C3F1 mice. The delivered dose was quantitated in blood, liver, lung, kidneys, adrenals, thymus, skin, adipose tissue, muscle, brain, spleen, thyroid, heart, mesenteric lymph nodes, nasal tissue, pancreas, bone marrow, and bone. Also the induction of CYP1A1 (in liver, skin, and lungs) and CYP1A2 (in liver) activities (DeVito *et al.*, 1993) were measured at 7, 14, 21, and 35 days following dosing.

TCDD was obtained from Radian Corporation (Austin, TX) with purity >98% as determined by gas chromatography/mass spectrometry. [1,6-3H]TCDD was synthesized by Chemsyn Science Laboratories (Lenexa, TX) with a specific activity of 39.5 Ci/mmole and purity of ≥98%. Purity was verified by reverse-phase high-pressure liquid chromatography (HPLC) (System Gold, Beckman Instruments, Inc., Fullerton, CA) using a C_{18} µBondapak stainless-steel column with a Guard-PAK™ precolumn inset (Waters, Milford, MA) and an isocratic solvent system of 85%

methanol/15% water. A radioactive flow detector (Beckman Model 171, Beckman Instruments, Fullerton, CA) with Flo-Scint III cocktail (Radiomatic Instruments, Tampa, FL) was used to monitor radioactivity.

Female B6C3F1 mice (8 wks old, ~20 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC) and allowed 1 week to acclimate. They were maintained on a 12-hr light/dark cycle under conditions of constant temperature and humidity. Mice were randomly assigned to treatment groups (five animals per group--each group housed in a mouse cage) with free access to food (Purina 5001 Rodent Chow, Ralston-Purine, St. Louis, MO) and water. At 7, 14, 21, and 35 days post-treatment, mice were euthanized by CO_2 asphyxiation and tissues were collected. At the time of sacrifice, liver, skin, and lungs were removed and processed for enzyme activity according to the method of DeVito *et al.* (1993). All tissues were analyzed for total radioactivity by combustion.

In this study, the high affinity of TCDD for hepatic tissue demonstrated dose-dependent kinetics. As the administered dose increased from 0.1 to 10 µg [^{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 the rat (Abraham *et al.*, 1988). The percent administered dose/g in liver increased with dose (from low- to mid-dose) but then did not increase further with a 10-fold greater dose. Hepatic concentrations of TCDD decreased with time at all doses correlating with the half-life of TCDD. Distribution of TCDD-derived radioactivity throughout other tissues in the body was dose- and time-dependent: as the administered dose increased, concentration in extra-hepatic tissues decreased. The highest concentration of per cent administered dose in each tissue, with the exception of liver, was found at the lowest dose on day 7; after that, the concentrations of percent administered dose decreased with dose and time. Liver and fat contained the highest concentrations of [^{3}H]TCDD-derived radioactivity. Although the high dose was 100 times greater than the low dose, liver concentrations were about 180 times greater in the high versus the low dose at 7 days indicating dose-related differences in disposition. Adipose tissue concentrations were only approximately 40 fold greater in the high versus the low dose group. For the lowest dose at 7 d, tissue levels were <3% dose/g in all tissues except for thyroid, adrenals, skin, liver, and fat with ~3, 6, 6, 15, & 24% administered dose/g, respectively. Distribution of TCDD-derived radioactivity in all tissues appeared to be non-linear with the percentage administered dose. In all tissues except liver, the relative percentage of total dose decreased while it increased for liver with higher doses.

EROD activity can be used as a biomarker for CYP1A1 levels, correlating well with the amount of CYP1A1 protein (Kedderis *et al.*, 1991; Tritscher *et al.*, 1992). EROD activity was measured in microsomes isolated from liver, lung, and skin in control and TCDD-treated mice. CYP1A1 induction was dose-dependent in liver, lung, and skin. However, induction of EROD activity in liver, lung, and skin

was not direcly correlated with tissue concentrations of TCDD. While fold induction of EROD activity was 2 times greater in the lung than in liver at 10 μg [^{3}H]TCDD/kg body wt, concentration of TCDD in liver was 100 times greater than in the lung. In contrast, the fold inductions of EROD activity in skin and liver were similar; but the concentration of TCDD in liver was 20 times greater than in skin.

Acetanilide-4-hydroxylase (ACOH) activity is a marker for CYP1A2 levels (Liu *et al.*, 1991). CYP1A2 activity in control animals was 3,390 ± 340 pmoles/min/mg microsomal protein which was approximately 5.2 times the activity of CYP1A1 in hepatic tissue. Induction of hepatic ACOH activity was dose-dependent: ACOH activity increased with increasing dose.

Dose- and time-dependent expression of CYP1A1 and CYP1A2 induction was demonstrated in the liver. While liver EROD activity was 5.2 times lower than ACOH activity in control animals, at the highest dosage tested, liver EROD activity and ACOH activity were similar. Over the time period of 35 days studied and unlike EROD activity, there was only a minimal decreased in the induced ACOH activity for the three dosages tested.

The results of the present study demonstrated the dose-and time-dependency in tissue distribution and induction of CYP1A1 and CYP1A2 as well as tissue sensitivies for enzyme induction in the female B6C3F1 mouse. These sensitive biomarkers of enzyme induction may be used as surrogates for exposure and in estimating risk. The liver sequestered administered dioxin at high doses. However at low environmental levels, dioxin in man may preferentially deposit in extrahepatic tissues. Therefore, dose-dependent tissue distribution of dioxin and its congeners should be taken into consideration for high to low dose extrapolations in risk assessment.

(This abstract does not necessarily reflect EPA policy.)

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