Time-Course and Dose-Response Relationships of Subchronic Dosing with [^{3}H] 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) on Dosimetry and CYP1A1 and CYP1A2 Activities in Mice

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) is the prototype and most toxic member of a family of polyhalogenated aromatic hydrocarbons (PHAHs) sharing a common mechanism of toxicity[1]. These ubiquitous environmental contaminants concentrate in biological systems as a result of both their extreme lipophilicity and their resistance to biotransformation. The considerable bioconcentration of TCDD implicates food consumption as the chief source of environmental exposure to TCDD for animals in higher trophic levels; in fact, diet accounts for an estimated 90% of daily exposure in humans[2]. Moreover, gastrointestinal absorption of low doses of TCDD appears to be quite efficient (>80%) in both mice and men[3,4].

Studies in our laboratory have examined the effects of subchronic exposure on the dose-response relationships of various PHAHs in the female B6C3F1 mouse. Repeated low-level exposures mimic the most probable modes for human exposure, including dietary consumption. In addition, the steady-state kinetics resulting from repeated dosing permits more accurate assessment of the relative potency of these compounds. Thus, accurate risk assessment of human environmental exposure to TCDD requires information regarding the disposition of TCDD following repeated low-level oral exposures. Ideally, TCDD dosimetry would be evaluated in an animal model under steady-state conditions. For that reason, studies were conducted to examine the disposition of TCDD following subchronic oral dosing in the mouse.

The present study describes the time-course (Phase I) and dose-response (Phase II) effects of subchronic dosing by oral gavage of [^{3}H]TCDD/day (Mon-Fri) in mice. Time-points used in Phase I were 30, 60, 90, and 120 days with dosing of either 1.5 or 150 ng [^{3}H]TCDD/kg/day. Doses used in Phase II were 0.15, 0.45, 1.5, 4.5, 15, 45, 150, or 450 ng [^{3}H]TCDD/kg/day with a time point of 90 days. Endpoints examined were dosimetry in liver and adipose tissue; and CYP1A1 and CYP1A2 activities in liver.

Materials and Methods

Chemicals: TCDD was purchased from Cambridge (purity ≥98%). [1,6-^{3}H]TCDD (radiopurity ≥98%; specific activity ~29.5 Ci/mmole at the time of use) was purchased from Cambridge Isotope Laboratories (Woburn, MA).

Animals and treatment: Female B6C3F1 mice (60 days old, ~20 g) were obtained from Charles River Breeding Lab. (Raleigh, NC). Animals (maintained on a 12-hr light/dark cycle at 22±1° C and 55±5% relative humidity), were acclimatized 1 week prior to dosing. Mice, randomly assigned to treatment groups of 5 animals/group, were group housed in polycarbonate cages containing heat-treated hardwood shavings (Beta Chips, Northeastern Products Inc., Warrensburg, NY) and given feed (Purina 5001 Rodent Chow, Ralston Purina Co., St. Louis, MO) and water *ad libitum*. Animals were dosed by gavage with corn oil solutions at a dosing volume of 10 ml/kg of graded doses of [^{3}H]TCDD 5 days a week for either 30, 60, 90, or 120 days. Three days after the last dose, animals were killed. For determination of tissue dosimetry and CYP1A1 and CYP1A2 activities, adipose tissue and liver were removed. Hepatic S-9 fractions were prepared[5] and stored at −70° C until analysis. Microsomal fractions were prepared [5] on the day of CYP1A activity analyses.

Cytochrome P450 activity: Ethoxyresorufin *O*-deethylase (EROD), marker for CYP1A1, and acetanilide-4-hydroxylase (ACOH), marker for CYP1A2, activities were measured in hepatic microsomes [5].

Sample analysis: Radioactivity in tissues was determined by combustion (Packard 306B Biological Oxidizer, Downers Grove, IL) followed by liquid scintillation spectrometry (Beckman Scintillation Counter, Beckman Instruments, Fullerton, CA). Form of radioactivity localized in hepatic and adipose tissue has been demonstrated to be predominantly (if not all) unmetabolized TCDD[6].

Statistics: All data are presented as mean ± standard deviation. Intergroup comparisons of the log transformation of enzyme activities were performed by ANOVA followed by Protected Fisher's Least Significiant Difference test.

Results and Discussion

Time-Course: Hepatic and fat distributions (Fig. 1 and 2) of [^{3}H]TCDD were dose-dependent. At the low dose (1.5 ng/kg/day), concentration of TCDD was greater in fat than in liver. In contrast at the high dose (150 ng/kg/day), greater concentrations of

TCDD were found in liver than in fat. At time-points >60 days, concentrations in fat (low dose) and liver (high dose) increased only slightly. TCDD-induced hepatic CYP1A1 and CYP1A2 as measured by EROD (Fig. 3) and ACOH activities were dose- but not time-dependent. Fold induction of ACOH activity was <1.5 at the low dose and >5 at the high dose. At the high dose, increased hepatic sequestration of TCDD was reflected by increase in hepatic EROD and ACOH activities.

Dose-Response: Fig. 4 demonstrates dose-dependency of [^{3}H]TCDD in liver and fat. Fold induction of ACOH activity ranged from 1.1-1.8. for doses 0.15 to 1.5 ng/kg/day; 1.8 ($p<0.04$) at 4.5 ng/kg/day; 4.8 ($p<0.0001$) at 15 ng/kg/day; and 11 ($p<0.0001$) 45-450 ng/kg/day. Hepatic EROD activity was significantly increased ($p<0.02$) starting at the lowest dose of 0.15 ng/kg/day. The nearly half-maximal induction response of CYP1A1 (Fig. 5) occurred at the 15 ng/kg/day dose. At this dose, liver and fat concentrations were equal and the cross-over phenomenon occurred. At high doses (i.e., induced animals), greater TCDD concentrations were found in liver than in fat--indicating hepatic sequestration due to the inducible binding protein, CYP1A2[7].

This study provides direct evidence of low dose effects at steady-state conditions on TCDD disposition and CYP1A1 and CYP1A2 induction. Futhermore, this study demonstrates the importance of CYP1A2 as a crucial determinant of hepatic sequestration.

Implications

The present study emphasizes the importance of high to low dose extrapolation of results from animals studies at steady-state conditions for purposes of human risk assessment of TCDD and related compounds.

(This does not necessarily reflect EPA policy.)

References

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Fig. 1

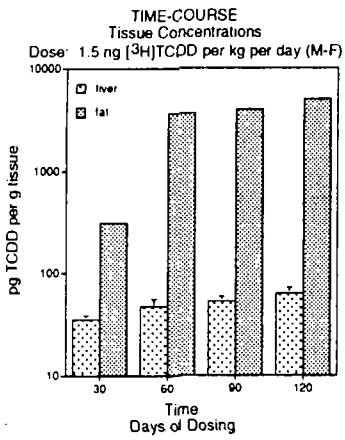

Fig. 2

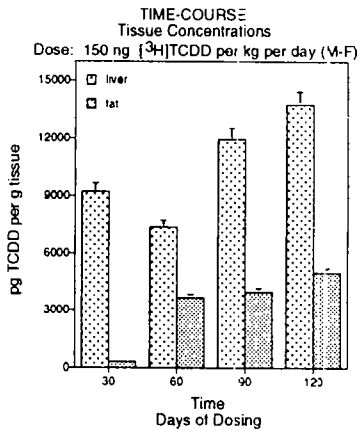

Fig. 3

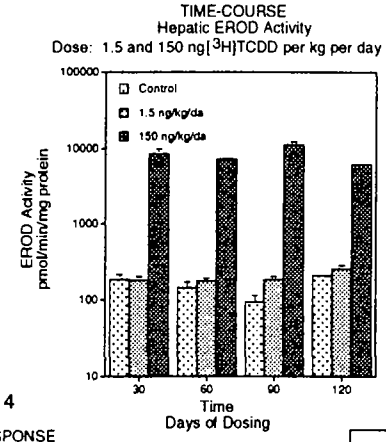

Fig. 4

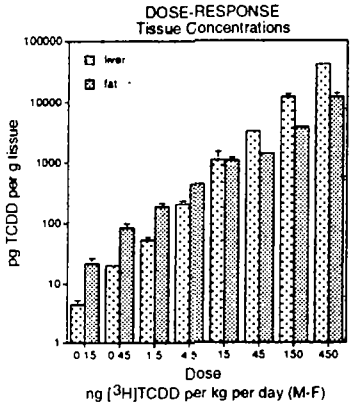

Fig. 5