Tissue CYP1A1 activity reflects tissue 2,3,7,8-tetrachlorodibenzo-*p*-dioxin concentration

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent compound of the dioxin-family, including polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs). Tissue disposition of TCDD has been shown to be dose- and time-dependent in rats and mice[1,2]. To describe this phenomenon, several physiologically based pharmacokinetic (PB PK) models for TCDD in rats including pharmacodynamic responses, such as hepatic cytochrome P4501A (CYP1A) induction, have been developed[3]. In mice, a PB PK model has been presented after TCDD exposure[4].

Studies from our laboratory indicate that hepatic CYP1A induction is a sensitive TCDD-inducible phenomenon which can be detected hours after acute exposure[5,6]. This response has shown to be reversible in rodents and is associated with the toxicokinetics of the administered compound[2,7]. Review of the data suggest that tissue CYP1A activities reflect tissue TCDD concentrations, regardless of dose or dosing regimen. To test this hypothesis, we studied the dependency of CYP1A induction in liver, lung, and skin on TCDD tissue concentration in female B6C3F1 mice after either acute or subchronic exposure.

Methods

Chemicals: TCDD was purchased from Radian corporation (acute studies and subchronic study 1) or Ultra Scientific (subchronic study 2) (purities >98%). [1,6-^{3}H]TCDD was synthesized by Chemsyn Science Laboratories (Lenexa, TX) (purity >98%). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and treatment: Female B6C3F1 mice (60 days old) were obtained from Charles River Breeding Laboratories, Raleigh, NC. Water and food were given *ad libitum*. The animals were held under controlled conditions of temperature (22°C ± 1) and lighting (12/12 light/dark cycle). Mice were randomly assigned to treatment groups (5 per group), and group housed. Animals were dosed by gavage with corn oil solutions of the test chemical 5 days a week for 13 weeks in the subchronic studies (0, 0.15, 0.45, 1.5, 4.5, 15, 45, 150, and 450 ng [^{3}H]TCDD/kg/day in study 1, and 0, 1.5, 4.5, 15, 45, and 150 ng TCDD/kg/day in study 2) or a single oral dose (0, 0.1, 1, or 10 µg [^{3}H]TCDD/kg) in the acute studies. After 13 weeks of exposure in the subchronic studies, the mice were killed. In the acute studies, mice were killed after 7, 14, 21, or 35 days posttreatment. Liver, lung, and skin were removed and homogenized in S9-buffer as described and stored at -70°C until analyses[8].

Cytochrome P450 activities: Ethoxyresorufin *O*-deethylase activity (EROD), a marker for CYP1A1, was determined in liver, lung, and skin as described[2,8].

Tissue analyses: TCDD-derived radioactivity in tissues was determined as described[2]. TCDD analyses in non-radioactive samples was analyzed by GC-MS as reported[9].

Results

Liver: Figure 1 presents the hepatic TCDD concentration at multiple time points after acute exposure to 1 and 10 µg TCDD/kg. TCDD concentration decreased time-dependently with a half-life of about 7 days at all dose levels. Figure 2A shows hepatic CYP1A1 activity versus hepatic TCDD concentration after acute and subchronic TCDD exposure. The increase in EROD activity was dependent upon hepatic TCDD concentration, regardless of dose or dosing regimen.
Lung: Figure 2B shows pulmonary CYP1A1 activity versus pulmonary TCDD concentration after acute and subchronic TCDD exposure. EROD activity reflected pulmonary TCDD concentration, regardless of dose or dosing regimen.
Skin: Figure 2C gives CYP1A1 activity in skin versus TCDD concentration in skin after acute and subchronic TCDD exposure. Again, the increase in EROD activity was dependent upon the TCDD concentration in skin, regardless of dose or dosing regimen.

Discussion

Acute and subchronic exposure to TCDD induced CYP1A1 activity in liver, lung, and skin. Enzymatic CYP1A activities reflected TCDD tissue concentrations, regardless of time period of/after dosing and how much TCDD was administered. This is in contrast to the area under the curve in Figure 1: e.g., the hepatic TCDD concentration after 7 days of 1 µg TCDD/kg exposure is about the same as after 36 days of 10 µg TCDD/kg exposure. However, the area under the curve in figure 1 is much higher at the latter point. This indicates that the area under the curve is not the appropriate dose metric for rapidly reversible effects. Instead, rapidly reversible effects such as TCDD-induced CYP1A induction reflect tissue concentration.

[This abstract does not necessarily represent USEPA policy.]

Acknowledgments

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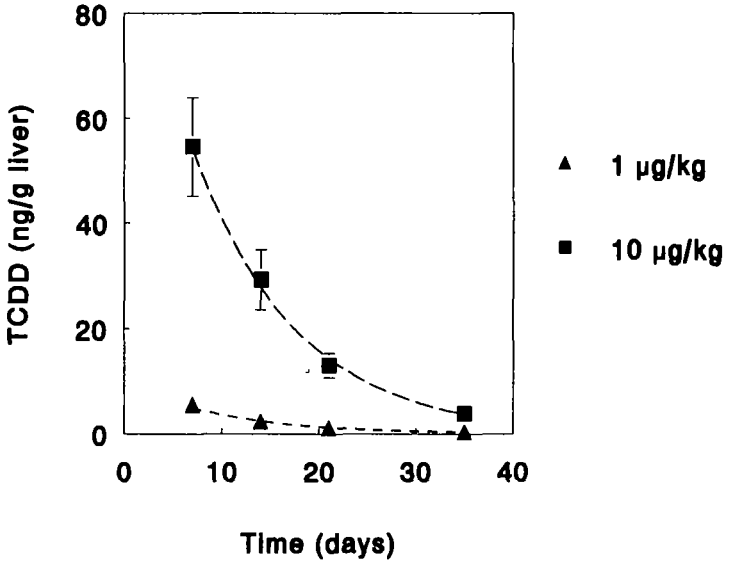

Figure 1. Hepatic TCDD concentration after acute exposure to 1 or 10 µg TCDD/kg during time in female B6C3F1 mice (mean ± SE).

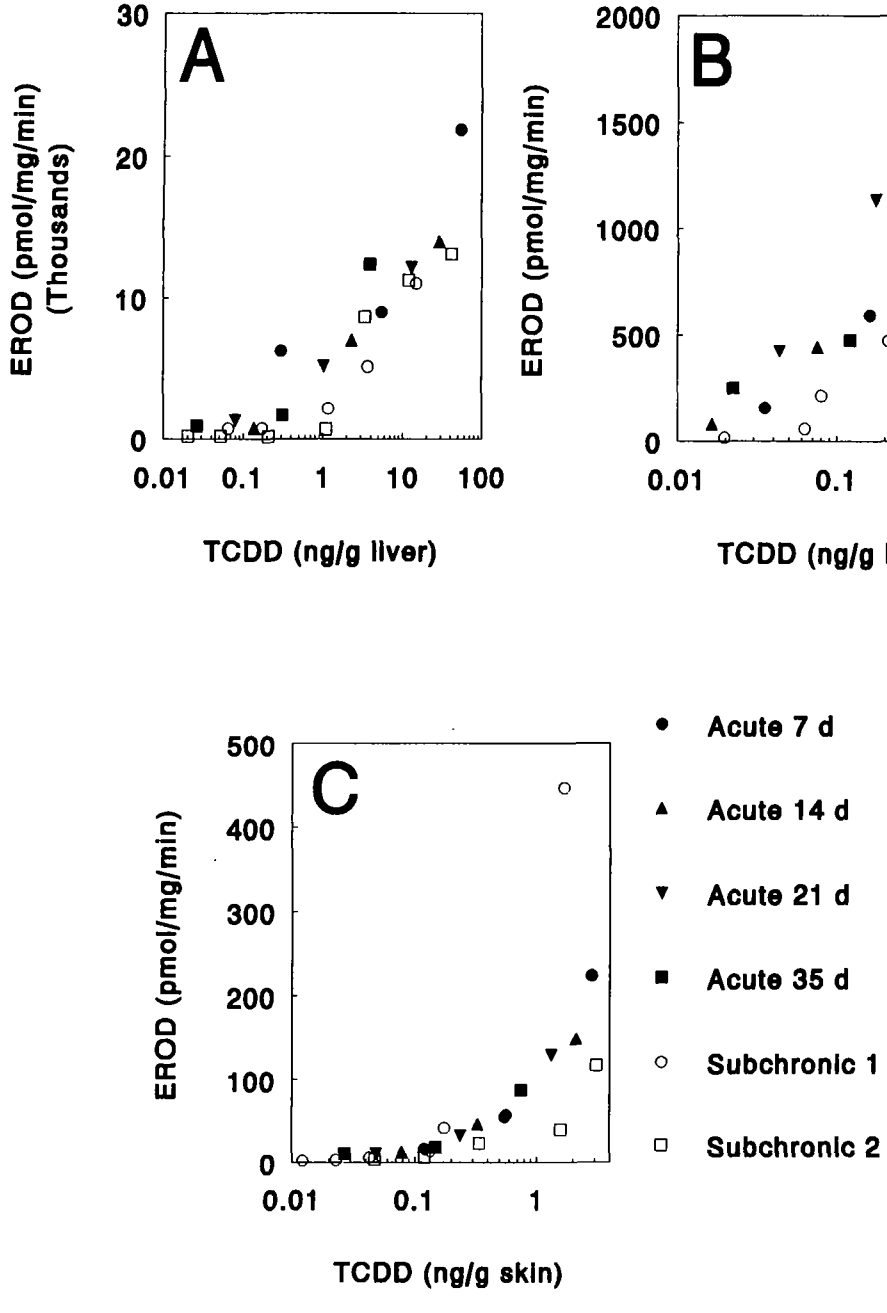

Figure 2A-C. CYP1A1 activities versus TCDD tissue concentration levels after acute and subchronic TCDD exposure in liver (A), lung (B), and skin (C). Data points represent the mean of 5 animals.