Comparative ability of TCDD to induce hepatic and skin cytochrome P-450 1A1 activity following 13 weeks of treatment.

DeVito, M.J.[a], Diliberto, J.[b], Birnbaum, L.S.[b]
[a]Center for Environmental Medicine, University of North Carolina, Chapel Hill, NC, USA.
[b]Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC, USA, 27711

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent toxicant which elicits a broad spectrum of toxic effects [1]. One of the best characterized effects of TCDD is the induction of cytochrome P-450 1A1 [2]. The induction of cytochrome P-450 1A1 is mediated through the Ah receptor. The Ah receptor is part of a complex containing several proteins, one of which is the 90-kDa heat shock protein [3]. Upon ligand binding, the Ah receptor dissociates from the complex and associates with a protein designated Aryl hydrocarbon Receptor Nuclear Transferase or Arnt [4]. The Arnt protein is required for the transformation and nuclear localization of the activated Ah receptor [4]. The activated receptor then binds as a heterodimer to response elements on the DNA upstream from the cytochrome P-450 1A1 gene [2]. Upon binding to these response elements, the Ah receptor elicits an increase in the transcription of the cytochrome P-450 1A1 gene[2]. The mechanism described above suggests that although the Ah receptor is necessary for the induction of cytochrome P-450 1A1, other factors are required which can alter the tissue response to TCDD. The present study compared the sensitivity of hepatic and skin tissue to the induction of cytochrome P-450 1A1 following 13 weeks of repeated exposure to low doses of TCDD. This dosing regimen should result in steady-state levels assuming a 12 day half-life for TCDD in mice[5].

Methods:

2,3,7,8-Tetrachlorodibenzo-p-dioxin was obtained from Ultra Scientific (purity > 98%). All other chemicals were obtained from Sigma Chemical Company. (Raleigh, NC) and allowed 7 days to acclimate to their new environment. Animals were randomly assigned to treatment groups (5 mice/ group), group housed, and allowed free access to food (Purina Rodent chow) and water. Animals were admin TCDD was initially dissolved in acetone, and this solution was added to corn oil. The acetone was subsequentially removed from the corn oil by evaporation.

Female B6C3F1 mice (60 days old) were obtained from Charles River Laboratories iver Laboratories 1.5, 4.5, 15, 45, or 150 ng of TCDD/kg 5 days/week for 13 weeks (65 treatments for total doses of 0, 98, 290, 980, 2,900 and 98,000 ng/kg). The chemicals were administered in corn oil by gavageat a volume of 10 ml/kg. Body weights were recorded and dosing volumes adjusted on a weekly basis. Three days after the last treatment, mice were anesthetized with CO_2, shaved with electric clippers, and sacrificed. Liver and skin were removed and homogenized in 4 and 10 volumes, respectively of ice-cold buffer containing 10% glycerol, 250 mM sucrose, 1 mM dithiothreitol, 0.5mM EDTA, 25 mM KCl, and 10 mM Hepes (pH 7.4). The homogenates were filtered through 2 layers of gauze and centrifuged for 25 minutes at 20,000 x g at 4°C. The resulting supernate was filtered through 2 layers of gauze, frozen in liquid nitrogen and stored at -70°C. Microsomes were prepared the day of the assay from the frozen supernates. The supernates were thawed and centrifuged at 105,000 x g for 60 minutes at 4°C. The resulting pellet was washed by resuspending in 1.15% KCL and 100 μM EDTA (pH 7.4) and centrifuged for 1 hour at 105,000 x g at 4°C. The final pellet (microsomal fraction) was resuspended in 0.1 mM phosphate buffer (pH 7.4). Ethoxyresorufin-O-deethylase (EROD) activity was determined spectrofluorimetrically using the dynamic assay method of Pohl and Fouts [6].

Intergroup comparisons of body weight, organ/body weight ratios, and EROD activity were analyzed using ANOVA followed by Scheffe's F-test with a preselected level of significance of $p < 0.05$.

Results:

The administration of TCDD over a 90 day period did not result in any mortality or morbidity by the lack of change in body weight gains or organ/body weight ratios compared to controls. Hepatic EROD activity was significantly increased at all doses tested. The lowest dose tested (1.5 ng/kg/day) resulted in a 2.2 fold induction while the highest dose (150 ng/kg/day) produced a 40 fold induction. Hepatic EROD activity is maximally induced to 45-50 fold by the single administration of 10,000 ng of TCDD/kg. Thus, the administration of 9,800 ng/kg over a 90 day period (150 ng/kg/day) produced near maximal induction of hepatic EROD activity. The estimated ED50 for induction of hepatic EROD activity is 65 ng/kg/day.

Skin EROD activity in control mice was 140 times lower than hepatic EROD activity (0.89 nmol/min./mg protein vs 126 nmol/mg protein/min respectively). All doses tested significantly induced skin EROD activity ($p < 0.05$). Doses as low as 1.5 ng/kg/day produced a 1.7 fold induction of skin EROD activity. Skin EROD activity was induced 53 fold over control animals at the highest dose tested. The estimated ED50 for induction of skin EROD activity is 72 ng/kg/day. There was a significant correlation between induction of skin EROD activity and hepatic EROD activity ($r^2 = 0.826$; $p < 0.0001$). The similarity of the ED50s and the significant correlation between skin and hepatic EROD activity indicates that the relative responses in these tissues were

equally sensitive to the actions of TCDD based on the administered dose.

Discussion:

The induction of cytochrome P-450 1A1 by TCDD is mediated through the Ah receptor[2]. The unoccupied receptor is found in the cytosolic fraction and its nuclear accumulation is dependent upon ligand binding [7]. Nuclear accumulation of the Ah receptor is significantly correlated with increased cytochrome P-450 1A1 mRNA levels in hepatic tissue from mice[8]. The present data do not show evidence of a threshold for the induction of EROD activity in hepatic or extrahepatic tissue. The lack of threshold for induction of hepatic cytochrome P-450 1A1 protein levels and EROD activity by TCDD has also been demonstrated in rats [9]. Following chronic administration, rats are slightly more sensitive than mice for hepatic EROD induction. The ED50 for hepatic EROD induction in rats is 10 ng/kg/day[9] compared to 65 ng/kg/day in mice.

Based on relative responses and administered dose, hepatic and skin tissue are equally sensitive to the effects of TCDD. If we were to compare the sensitivity of hepatic and skin tissue on a per cell basis, the sensitivities may be different. For example, hepatic concentration of TCDD is approximately 15 times greater than that of skin in B6 mice[5]. The relative response in hepatic tissue and skin are the same but on a per cell basis the skin has much less TCDD suggesting that it may be the more sensitive of the two tissues. Future experiments incorporating tissue dosimetry are indicated to better characterize these responses.

In summary, at steady-state levels, the relative responses in hepatic and skin tissue are equivalent when compared based on administered dose. Furthermore, the present study indicates that there is no apparent threshold for the induction of hepatic and extrahepatic EROD activity by TCDD.

This abstract does not necessarily reflect USEPA policy.

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TABLE 1

THE EFFECTS OF TCDD ON HEPATIC AND SKIN EROD ACTIVITY

	HEPATIC		SKIN	
TCDD (ng/kg/day)	EROD[a] ACTIVITY	FOLD[c] INDUCTION	EROD ACTIVITY	FOLD INDUCTION
0.0	126 ± 16	1.0 ± 0.1	0.89 ± 0.12	1 ± 0.1
1.5	271 ± 39*	2.2 ± 0.3*	1.54 ± 0.15*	1.7 ± 0.2*
4.5	323 ± 38*	2.6 ± 0.3*	2.50 ± 0.29*	2.8 ± 0.3*
15	764 ± 67*	6.1 ± 0.5*	9.29 ± 2.30*	10.4 ± 2.7*
45	2245 ± 212*	17.8 ± 1.7*	15.65 ± 2.25*	17.6 ± 2.5*
150	4996 ± 407*	39.7 ± 3.2*	47.01 ± 4.55*	52.8 ± 5.1*

a - EROD activity is expressed as nmol/mg protein/min and is presented as mean ± SD for five animals/group.
b - Fold induction is derived by dividing the group average by the average for the control values.
* - significantly greater than controls ($p < 0.05$).