The Use of Tissue Burden as a Dose Metric for TCDD-Inducible Responses in Rat Liver is Endpoint-Specific

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) is the most widely studied and toxic congener of a family of dioxin-like compounds that are ubiquitous throughout the environment. Their lipophilicity and biological persistence result in long-term exposure to human populations. TCDD exposure results in a broad spectrum of biological responses including altered gene expression, altered metabolism, endocrine disruption, growth dysregulation, and immunosuppression. TCDD is a multi-site, trans-species carcinogen in both sexes (1).

TCDD is not a direct acting-genotoxic agent. All effects, including carcinogenicity, of TCDD are thought to be mediated via the aromatic hydrocarbon receptor (AHR). TCDD is a carcinogen in multiple rodent models, and several studies have shown that TCDD acts as a tumor promoter in a chronic two-stage initiation/promotion models (2-4). In a chronic two-stage initiation/promotion study, a significant increase in cell proliferation was observed in the livers of female Sprague-Dawley rats treated biweekly with an averaged daily dose of 125 ng TCDD/kg/day for 30 weeks (5).

Enzyme induction of cytochrome P450 1A1 (CYP1A1) and 1A2 (CYP1A2) has been widely studied as a sensitive biochemical response mediated by the AHR (6). Dose-responses for induction of CYP1A1 and CYP1A2 have been demonstrated in chronic tumor promotion studies (4). The appropriateness of using enzyme induction data as a surrogate for cancer risk estimates has also been evaluated by other investigators (4,7,8).

In this study, we compared the use of tissue burden as a dose metric for a "simple" TCDD-inducible response directly related to AHR activation (*e.g.*, CYP1A1 induction) with that of a more "complex" inducible response that has multiple levels of regulation and is linked to the mechanism of hepatocarcinogenesis (*e.g.*, induction of cell proliferation).

Material and Methods

Female Sprague-Dawley rats were randomly divided into groups containing 6-10 animals per group. The animals were housed three to a cage under conditions of controlled temperature (70 ± 0.5°F), humidity (50 ± 5%), and lighting (12 hour light/12 hour dark), and received food and water *ad libitum*.

Female Sprague-Dawley rats, initiated with 175 mg DEN/kg bodyweight at 10 weeks of age, were treated with TCDD by either a discontinuous dosing regimen with the same daily averaged dose of 125 ng/kg/day TCDD for varying exposure durations, or continuous treatment with different doses of TCDD (two distinct studies using high and low dose ranges) for 30 weeks. Control animals in all three studies received biweekly gavage of corn oil. In the tumor promotion study in which rats received the same daily dose of 125 ng/kg/day TCDD, but had different dosing regimens, three groups were treated with TCDD: (1) 30 weeks with TCDD via biweekly gavage, followed by 30 weeks of corn oil gavage; (2) corn oil for 16 weeks, TCDD for 30 weeks, followed by 14 weeks of corn oil gavage; and (3) 60 weeks of TCDD. In the low-dose continuous study, rats were treated with TCDD via biweekly gavage at daily averaged doses of 0.1, 0.3, 1.0, 3.5, and 125 ng/kg/day. In the high-dose continuous study, rats were treated with biweekly gavage at daily averaged doses of 3.5, 10.7, 35.7, and 125 ng/kg/day TCDD (5). Osmotic pumps containing 30 mg/mL 5-bromo-2'-deoxyuridine (BrdU) were implanted subcutaneously seven days prior to necropsy. All animals were killed one week after the last treatment with either corn oil or TCDD, by asphyxiation with CO_2. Sections of liver were removed and minced, and aliquots frozen immediately in liquid nitrogen.

Quantitative RT-PCR was carried out using a competitive titration assay using a heterologous recombinant internal standard RNA as previously described (9). Cell proliferation was measured by immunohistochemical detection of BrdU incorporation into the nuclei of hepatocytes undergoing replicative DNA synthesis and expressed as the labeling index (LI) (percentage of hepatocytes positively labeled). TCDD analysis was performed by Triangle Laboratories Inc. (Durham, NC) by GC/MS as previously described (3). Concentrations of TCDD were determined relative to tissue wet weight and expressed as parts per trillion (ppt).

Results and Discussion

Dose-dependent increases in TCDD-inducible mRNA levels of CYP1A1 were observed with increasing liver burden of TCDD (Figure 1). In the absence of administered TCDD, there was a detectable level of each TCDD-inducible enzyme mRNA present at very low levels, as well as detectable concentrations of TCDD in liver, and is attributed to polychlorinated dibenzodioxins and polychlorinated dibenzofurans present in standard laboratory animal feed (10). Levels of CYP1A1 mRNA ranged from $7.0 \pm 7.2 \times 10^{4}$ copies/μg RNA in control animals to $7.3 \pm 5.5 \times 10^{8}$ copies/μg total RNA in 60-week TCDD treated rats in the discontinuous study.

The dose-response relationship between liver TCDD burden and CYP1A1 levels was the same, regardless of whether the dosing regimen was discontinuous or continuous. Induction of CYP1A1 from the low-dose continuous study was quantitated using different internal standards and primers than previously employed by Vanden Heuval et al. (11), and the current results were comparable to levels of CYP1A1 previously published (12).

The relationship between TCDD-induction of cell proliferation and liver TCDD burden is shown in Figure 2. By comparison with CYP1A1, liver burden was not a suitable dose metric for TCDD-induced changes in cell proliferation. These data indicate that the use of liver burden as a dose metric for TCDD-inducible response is endpoint-specific and that time of exposure is essential for predicting changes in cell proliferation and should be incorporated into a suitable dose metric for TCDD. This finding may have important implications in the risk assessment for human exposure to TCDD.

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References

(1) Huff J, Lucier G, and Tritscher A; *Annu Rev Pharmacol Toxicol* **1994**, 34, 343.
(2) Clark G, Tritscher A, Maronpot R, Foley J, and Lucier G, p. 389, In *Banbury Report 35: Biological basis for risk assessment of dioxins and related compounds.*, Cold Spring Harbor Laboratory Press, **1991**
(3) Lucier G W, Tritscher A, Goldsworthy T, Foley J, Clark G, Goldstein J, and Maronpot R; *Cancer Res* **1991**, 51, 1391.
(4) Tritscher A M, Goldstein J A, Portier C J, McCoy Z, Clark G C, and Lucier G W; *Cancer Res* **1992**, 52, 3436.
(5) Maronpot R R, Foley J F, Takahashi K, Goldsworthy T, Clark G, Tritscher A, Portier C, and Lucier G; *Environ. Health. Perspect.* **1993**, 101, 634.
(6) Whitlock J P, Jr.; *Annu Rev Pharmacol Toxicol* **1990**, 30, 251.
(7) Leung H W, Paustenbach D J, Murray F J, and Andersen M E; *Toxicol Appl Pharmacol* **1990**, 103, 399.
(8) Birnbaum L S; *Environ Health Perspect* **1994**, 9, 157.
(9) Vanden Heuvel J P, Clark G C, Kohn M C, Tritscher A M, Greenlee W F, Lucier G W, and Bell D A; *Cancer Res* **1994**, 54, 62.
(10) Vanden Heuvel J P, Clark G C, Tritscher A, and Lucier G W; *Fundam Appl Toxicol* **1994**, 23, 465.
(11) Vanden Heuvel J P, Clark G C, Thompson C L, McCoy Z, Miller C R, Lucier G W, and Bell D A; *Carcinogenesis* **1993**, 14, 2003.
(12) Sewall C H, Flagler N, Vanden Heuvel J P, Clark G C, Tritscher A M, Maronpot R M, and Lucier G W; *Toxicol Appl Pharmacol* **1995**, 132, 237.

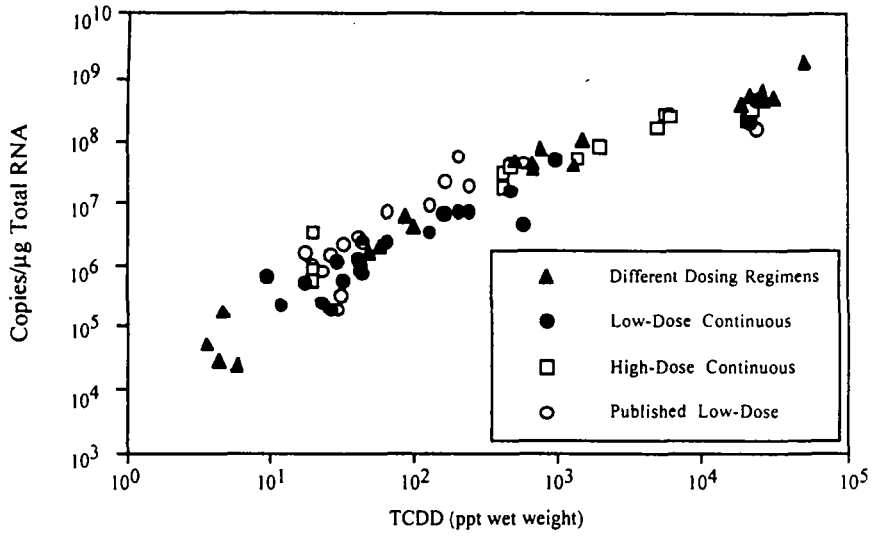

Figure 1. Relationship between liver burden of TCDD and CYP1A1 RNA levels. Each point represents one animal.

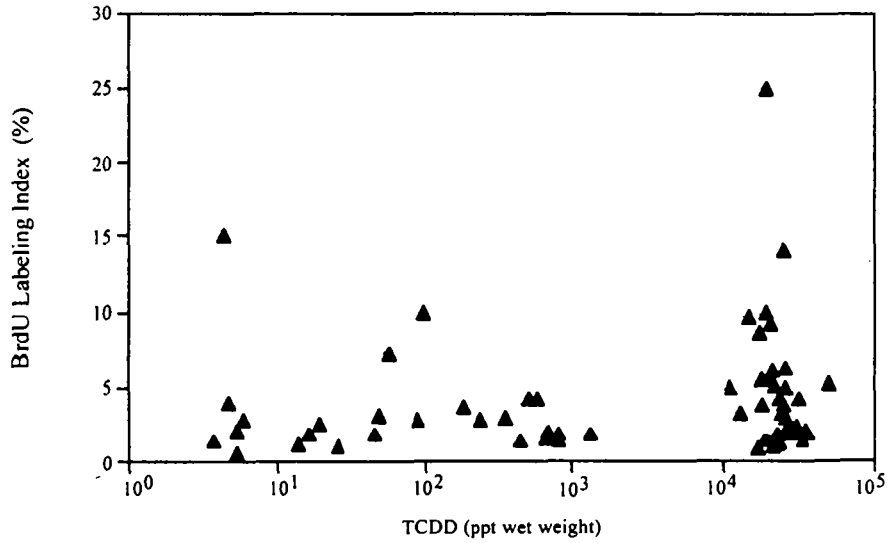

Figure 2. Relationship between liver burden of TCDD and BrdU labeling indices in the discontinuous study. Each point represents one animal.