DOSE-RESPONSE MODELING OF RODENT NONCANCER ENDPOINTS FROM EXPOSURE TO TCDD USING A BENCHMARK DOSE APPROACH

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Introduction

Human health assessment of noncancer and cancer risks of toxic substances is predicated on two dichotomous methodologies. Noncancer risk has traditionally been estimated using a no-observed-adverse-effect-level (NOAEL) from experimental doses of a study, divided by uncertainty factors to determine a safe level of human exposure. Cancer risk often uses high-dose to low-dose linear extrapolation to establish an acceptable exposure level for carcinogenic risk. A benchmark dose approach, which may ultimately unite the different noncancer and cancer risk estimations for the same toxicant, is being evaluated as a replacement for the traditional NOAEL methodology currently being used to assess the noncancer effects of toxicants. As part of the ongoing reassessment of the potential health effects of TCDD and related compounds, a benchmark dose analysis was performed on the cancer and noncancer endpoints of TCDD. This paper presents the results of the analysis of the dose-response relationships for the noncancer endpoints of TCDD in experimental animals included in the Dose-Response Modeling chapter of the current U.S. EPA draft of exposure and health assessment of TCDD and related compounds.

Material and Methods

Empirical models were used to calculate effective doses and to assess dose-response curve shape for the noncancer endpoints induced by TCDD. The Hill model was primarily used for continuous dose-response studies described by the following equation: $R(d) = b + vd^n / [K^n + d^n]$, where $R(d)$ is the response at dose d, b is the background response, v is the maximum increase in response above background, k is the dose yielding half of v, and n is the Hill coefficient describing the curvature of the dose-response[1,2]. When n is near or below 1, risk is predicted to be approximately proportional to dose, or climbing more rapidly than proportional. When n is much larger than 1 ($n > 1.5$), the dose-response is non-linear and has been described as having a more threshold-like behavior. For these reasons, n will also be referred as the shape parameter.

Only data sets found in the published literature were examined in this analysis. Each of the included studies provided dose-response information on TCDD using at least three dose levels of TCDD and a control. The mean and an estimate of the variance of the data had to be presented in tabular form in the manuscript. Attempts to estimate the means and variances of data presented in

graphical forms proved unreliable and were not included in the analysis unless the original data were provided by the authors. Model fits, calculation of 1% effective doses (ED_{01}), and the 95% lower bound on the estimated ED_{01} (LED_{01}) were carried out using the U.S. EPA Benchmark Dose Software (BMDS) version 1.1b[3]. In addition, data sets were verified at random from independent model analysis to validate model fits derived from BMDS.

Data were divided into several categories based on exposure regimen and endpoint. Exposure categories were grouped as either single exposures or multiple exposures. For simplicity, effects were categorized as either *biochemical* (*e.g.*, alterations in mRNA, protein or enzyme activities), *hepatic* (*e.g.*, hepatotoxicity such as serum enzymes and histological effects), *immune* (*e.g.*, alterations in lymphocyte phenotypes and functions), *toxicity* (*e.g.*, body weight changes, developmental, reproductive and tissue toxicities), *tissue* (*e.g.*, alterations in tissue weight), *retinol* (*e.g.*, alterations in either serum or tissue retinoid concentrations), or *thyroid* (*e.g.*, alterations in serum thyroid hormone concentrations).

Results and Discussion

In the studies examining the effects of TCDD following multiple exposures in rodent models, the range of the EDs_{01} was highly variable within and across response categories (Figure 1). When examined by category, the median values for the ED_{01} for *biochemical* and *retinol* responses were lower than the median ED_{01} for other types of response. Of the 106 endpoints examined from studies using multiple exposures, 11 had ED_{01} values less than 0.1 ng/kg/day. Seven of the 11 endpoints with an ED_{01} below 0.1 ng/kg/day were markers of *immune* response. However, the ED_{01} for markers of *immune* function ranged over 6 orders of magnitude, decreasing the confidence of any particular ED_{01} value for this response. Under steady-state conditions, total body burden for TCDD corresponding to an ED_{01} of 0.1 ng/kg/day in rats and mice is 3.6 ng/kg and 1.6 ng/kg, respectively. By comparison, the current body burden for TCDD in humans is between 1-2 ng/kg body weight, assuming about 25% of body weight is lipid.

Of the endpoints for which an estimate was obtained, 43 had shape parameters less than 1.5, indicating linear dose-response relationships. Approximately half of the *biochemical* and half of the *tissue* responses indicated a linear dose-response relationship. The median shape parameter for the *tissue* responses was heavily influenced by the consistently linear shapes for alterations in thymic weight (10 of 11 dose-response curves for thymic changes had shape parameters less than 1.5). In contrast, only 18% of the *immune* function responses were linear. While there was some consistency of shape within certain categories of these endpoints, in general about half of the responses could be classed as either linear or non-linear. These observations do not strongly support linearity for TCDD dose-response, nor do they strongly support the existence of thresholds within the observable range.

In studies examining the effects of dioxin in adult rats and mice following a single exposure, the median ED_{01} was above 10 ng/kg for all endpoints examined (Figure 2). *Biochemical* and *immune* responses had the lowest median ED_{01} estimates, 180 and 65 ng/kg, respectively. *Hepatic* and *toxic* responses gave median EDs_{01} greater than 10,000 ng/kg. Once again there was large variability in the EDs_{01} for a given category and, in general, varied approximately three orders of magnitude within each category. The ED_{01} estimates were below the lowest dose tested for 23 of the 75 endpoints examined. Of these 23 estimates, the ED_{01} was less than one order of magnitude lower than the lowest dose tested for approximately half (10) of the values. Following a single exposure to TCDD, 33 of the 77 (43%) endpoints examined had shape parameters less than 1.5, indicating linear dose-response relationships. There was no consistent pattern in the shape of the

dose-response relationships for the *biochemical*, *immune*, and *tissue* response categories. In these categories both linear and threshold-like dose-response relationships were observed. All endpoints in the *toxicity* category exhibited threshold-like dose-response relationships.

Following a single exposure, a number of developmental effects have been examined (Figure 3). These effects have been categorized as *biochemical*, *tissue*, or *toxicity*. The majority of the effects examined were considered *tissue* responses. The range of $ED_{s_{01}}$ was more than five orders of magnitude, and the median values for all response categories were greater than 100 ng/kg, with an overall median of 140 ng/kg. One recent finding on the effects of TCDD on developmental reproductive effects in rodents is that the $ED_{s_{01}}$ for the developmental reproductive effects in mice are 10 to 1,000 times higher than those in the rats. The $ED_{s_{01}}$ for the developmental effects were within the dose range tested in 26 out of 58 endpoints for which an estimate was obtained Of the 32 estimates that were below the experimental range, approximately half (17) were less than an order of magnitude below the lowest dose tested. The shape parameter for the developmental effects was less than 1.5 for only 18 of the 60 endpoints analyzed.

The activation of the aryl hydrocarbon receptor by TCDD initiates a cascade of events beginning with altered gene expression, and many of these biochemical changes, particularly the alterations in growth factors and their receptors, may mediate the toxic effects of TCDD. The role of other biochemical changes, *e.g.*, induction of aldehyde dehydrogenase, is less certain. When considering the biochemical and toxicological effects of TCDD as a continuum, one can consider the biochemical changes as initiators of cellular processes that lead to the toxicological effects. Hence, understanding the shape of the dose-response relationships for the biochemical effects may provide insight into the shape of the dose-response relationship for toxic responses, particularly in the low dose region.

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This abstract does not necessarily reflect U.S. EPA policy.

References

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Figure 1. ED 01 Values for Multiple-Dose Studies by Endpoint.

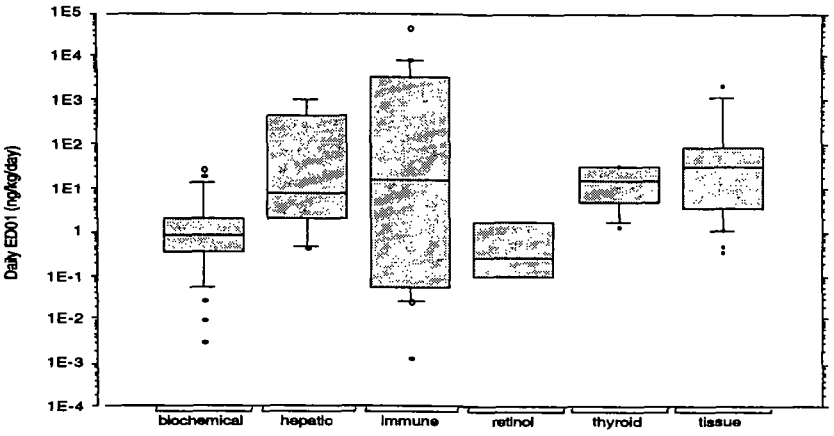

Figure 2. ED_{01} Values for Single-Dose Adult Studies by Endpoint.

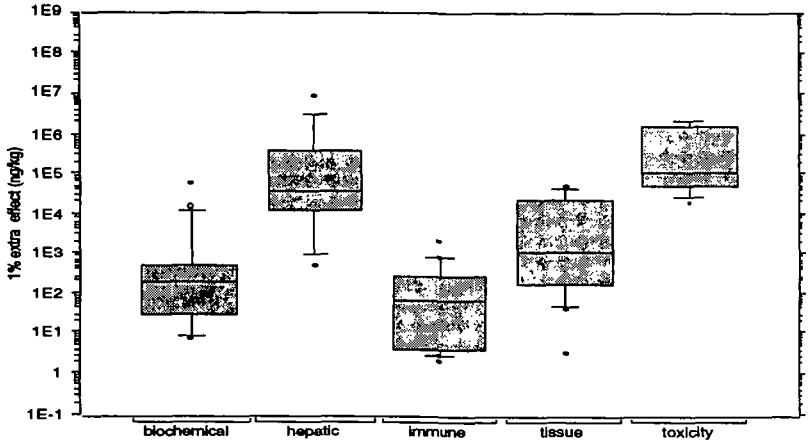

Figure 3. ED_{01} Values for Single-Dose Developmental Studies by Endpoint.