IMPACT OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING ON BENCHMARK DOSE CALCULATIONS FOR TCDD-INDUCED NONCANCER ENDPOINTS

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Introduction

In any health risk assessment based on experimental animal data, inclusion of mechanistic data is ideal when available. The tremendous increase in the use of mechanistic data in both cancer and noncancer risk assessments was recently summarized by Haber *et al.*[1]. The use of mechanistic data serves two purposes: to increase biological plausibility and reduce uncertainty in the extrapolation of animal data to human exposure. To assess noncancer health effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a benchmark dose (BMD) analysis was previously conducted[2].

A benchmark dose is a statistically derived dose that results in a prespecified increase in effect, and the 1% effect level was chosen in the BMD analysis. One of the major advantages in performing a BMD analysis is that all the dose-response data is used, not just the single dose determined as having no or little adverse effect. Currently, many regulatory agencies rely on a no/lowest observed adverse effect level (N/LOAEL) approach combined with uncertainty factors to estimate a virtually "safe" human dose.

In the previous BMD analysis of noncancer effects following TCDD exposure, a body burden resulting in a 1% maximum increase over background (BB_{01}) was calculated based on a daily ED_{01} using simple kinetic assumptions such as steady-state conditions, appropriate half-life of TCDD, and absorption of TCDD[2]. The purpose of the current analysis presented in this paper was to evaluate the impact of increased biological realism through use of a mechanistic physiologically-based pharmacokinetic (PBPK) model in estimating the BB_{01} values for noncancer endpoints following exposure to TCDD in female Sprague-Dawley rats. The PBPK model[3] predicts absorption and distribution of TCDD within experimental observations, as well as TCDD-induced gene expression of mRNA and protein levels following exposure to TCDD.

Material and Methods

An initiation-promotion study was conducted in female Sprague-Dawley rats as described in detail by Tritscher et al.[4]. Data from this TCDD study were used to conduct the BMD analysis. The dose metric of average daily dose (0, 3.5, 10.7, 35.7, and 125 ng/kg/day TCDD) used in the BMD analysis was approximated from biweekly gavage doses of TCDD administered in the study. A mechanistic PBPK model[3] was used to obtain the dose metric of body burden from female

Sprague-Dawley rats treated with TCDD via biweekly gavage. TCDD-induced CYP mRNA gene expression was quantitated by Walker *et al.*[5] by competitive RT-PCR analysis. CYP1A1 and 1A2 proteins were quantitated by Tristcher *et al.* from hepatic microsomes isolated from frozen liver tissue by a double antibody radioimmunoassay procedure using purified CYP isozymes as standards[4]. Total and nonspecific binding of epidermal growth factor (EGF) to the [^{125}I]-EGF receptor was measured in isolated hepatic plasma membranes by Sewall *et al.*[6]. Scatchard analysis was used to determine the apparent maximum binding capacity (B_{max}). Total cholesterol was measured in serum collected at time of necropsy and analyzed on a Monarch 2000 using commercially available reagents[7].

Based on the mechanism of action of TCDD[8], the first measurable observation following TCDD exposure is increased transcription of TCDD-inducible genes. In this analysis, the first measurable effect was an alteration in TCDD-induced mRNA and proteins following TCDD exposure. These biochemical effects were termed as "proximal" effects. A noncancer effect subsequent to increased transcription of TCDD-inducible genes is alteration of growth factors (*e.g.*, alteration in EGFR). These effects were termed "distal" in this analysis. Finally, changes in tissue response to TCDD exposure were considered most "distal". Alternations of hepatic enzymes measured in serum were indicative of these distal effects.

An empirical modeling scheme was employed to estimate ED_{01} for the biological and toxicological effects induced by TCDD. The Hill model was used for the dose-response of noncancer endpoints 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[9, 10]. ED_{01} estimates were converted to body burden resulting in a 1% maximum increase over background and termed BB_{01K}. Assuming steady-state conditions, BB_{01K}(ng/kg) for TCDD was calculated from the ED_{01} by the equation: BB_{01K}(ng/kg) = ED_{01}(ng/kg/day) * half-life(days) / ln(2) * f, where f is the fraction of dose absorbed and assumed to be 100% for TCDD administered via gavage and half-life was 25 days in the female Sprague-Dawley rat. The PBPK-modeled body burdens were used as the dose metric to estimate BB_{01} values (BB_{01PBPK}) for the same TCDD-induced noncancer effects using the Hill model described above. These BB_{01PBPK} values were compared directly to the BB_{01K} values obtained based on the EDs_{01}. The U.S. Environmental Protection Agency's Benchmark Dose Software (BMDS), version 1.2.1[11] was used to model all ED_{01} and BB_{01PBPK} estimates.

Results and Discussion

The impact of mechanistic PBPK modeling was evaluated for benchmark dose analysis of biochemical and toxicological effects resulting from a 30-week TCDD initiation-promotion study in female Sprague-Dawley rats[4, 7]. The BMD analysis included estimation of an ED_{01}, an equivalent BB_{01} based on simple conversion of the ED_{01} (BB_{01K}), and a BB_{01} based on body burdens modeled from a PBPK model[3] (BB_{01PBPK}). The biochemical effects were categorized based on the proximity of the specific effects and TCDD binding to and activation of the aryl hydrocarbon receptor (AhR)[8].

Based on the mode of action of TCDD[8], increased transcription of TCDD-induced genes such as cytochrome P450 (CYP) 1A1 is considered the most proximal response immediately following exposure to TCDD. The ED_{01} for CYP1A1 mRNA gene expression was 1.6 ng/kg/day, the BB_{01K} was 59.0 ng/kg, and the BB_{01PBPK} was 17.8 ng/kg (see Table 1). The PBPK-model body burden at the 1% effect level was approximately three-fold lower than the body burden based on a daily ED_{01}. TCDD-inducible CYP proteins follows transcription of mRNA, and these CYP proteins were previously characterized by Tristcher *et al.*[4]. The ED_{01} value for CYP1A1 was 0.4 ng/kg/day, which is equivalent to BB_{01K} of 14.9 ng/kg. The PBPK-modeled BB_{01PBPK} was 7.0 ng/kg, about two-fold lower than the BB_{01K}.

The next category of TCDD-induced responses included those effects considered more distal than gene induction based on TCDD-AhR binding to DREs on DNA. Dose-dependent decreases in EGFR following TCDD exposure in female Sprague-Dawley rats were demonstrated by Sewall *et al.*[6]. The ED_{01} for decreased maximum binding of EGFR was 1.7 ng/kg/day. The equivalent BB_{01K} for this effect was 60.9 ng/kg. The BB_{01PBPK} was 31.7 ng/kg, about two-fold less than the equivalent body burden based on the ED_{01}.

The last group of noncancer effects included in the BMD analysis was serum chemistry endpoints that indicated hepatotoxicity of TCDD. Changes in liver enzymes and total cholesterol measured in serum that were statistically different from values reported in control animals were included in the analysis. The serum clinical chemistry parameters resulted in a wide range of ED_{01} estimates. An ED_{01} of 0.4 ng/kg/day for total cholesterol represented the lowest ED_{01}. The BB_{01K} value equivalent to this ED_{01} was 15.0 ng/kg. Comparatively, the BB_{01PBPK} for total cholesterol was nearly two-fold lower and estimated to be 9.2 ng/kg.

In summary, mechanistic data was used to evaluate the previous BMD analysis of noncancer effects following TCDD exposure in Sprague-Dawley rats. Inclusion of a revised PBPK model reduced uncertainty in the use of default methodologies. Evidence to support a reduction of uncertainty exists in the basis of the PBPK model[3]. The model reliably predicts TCDD distribution throughout the body and incorporates pharmacokinetic and pharmacodynamic events subsequent to the absorption and distribution of TCDD throughout the rat. The pharmacodynamic events predicted by the model include the induction and proteolysis of AhR protein following exposure to TCDD, as well as biochemical events following the binding of TCDD to the AhR such as TCDD-induced gene expression on the mRNA and protein levels. These model predictions are similar to experimental observations. Furthermore, the model's robustness was tested on data sets not used in model revision, and the model predicted TCDD-induced responses within the range of experimental observations.

Increased biological realism through use of a PBPK model in estimating the BB_{01} values for noncancer endpoints following exposure to TCDD in female Sprague-Dawley rats was achieved in this analysis. The use of a simple kinetic method may lead to an underestimation of risks associated with a 1% maximum response over background for the endpoints presented in this analysis.

References

1. Haber, L. T., Maier, A., Zhao, Q., Dollarhide, J.S., Savage R.E, and Dourson, M.L. (2001) Applications of mechanistic data in risk assessment: The Past, present, and future. Toxicological Sciences. 61, 32-39.

2. Kim, A., Devito, M., Walker, N., Parham, F., Ye, F., Cogliano, J., and Portier, C. (2000) Dose-response modeling of rodent noncancer endpoints from exposure to TCDD using a benchmark dose approach. *In:* Organohalogen Compounds, pp. 288-291.

3. Kohn, M. C., Walker, N.J., Kim, A.H., and Portier, C.J. (2001) Physiological modeling of a proposed mechanism of enzyme induction by TCDD. Toxicology. 162, 193-208.

4. Tritscher, A. M., Goldstein, J. A., Portier, C. J., McCoy, Z., Clark, G. C., and Lucier, G. W. (1992) Dose-response relationships for chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a rat-tumor promotion model: Quantification and immunolocalization of CYP1A1 and CYP1A2 in the liver. Cancer Research. 52(12), 3436-3442.

5. Walker, N. J., Portier, C. J., Lax, S. F., Crofts, F. G., Li, Y., Lucier, G. W., and Sutter, T. R. (1999) Characterization of the dose-response of CYP1B1, CYP1A1, and CYP1A2 in the liver of female Sprague-Dawley rats following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicology and Applied Pharmacology. 154(3), 279-286.

6. Sewall, C. H., Lucier, G. W., Tritscher, A. M., and Clark, G. C. (1993) TCDD-mediated changes in hepatic epidermal growth factor receptor may be a critical event in the hepatocarcinogenic action of TCDD. Carcinogenesis. 14(9), 1885-93.

7. Maronpot, R. R., Foley, J. F., Takahashi, K., Goldsworthy, T., Clark, G., Tritscher, A., Portier, C., and Lucier, G. (1993) Dose response for TCDD promotion of hepatocarcinogenesis in rats initiated with DEN: Histologic, biochemical, and cell proliferation endpoints. Environ. Health Perspect. 101, 634–642.

8. Poland, A., Kimbrough, R. D., and Cold Spring Harbor Laboratory. (1984) Biological mechanisms of dioxin action, p. xiv, 500. [Cold Spring Harbor, N.Y.]: Cold Spring Harbor Laboratory.

9. McGrath, L. F., Cooper, K. R., Georgopoulos, P., and Gallo, M. A. (1995) Alternative models for low dose-response analysis of biochemical and immunological endpoints for tetrachlorodibenzo-*p*-dioxin. Regul Toxicol Pharmacol. 21(3), 382-96.

10. Murrell, J. A., Portier, C. J., and Morris, R. W. (1998) Characterizing dose-response: I: Critical assessment of the benchmark dose concept. Risk Anal. 18(1), 13-26.

11. U.S. Environmental Protection Agency. (2001) Benchmark Dose Software, Version 1.2.1. www.epa.gov/ncea/bmds.htm.

Table 1. TCDD-Induced ED_{01} Values, Equivalent BB_{01} Values Based on Average Daily Dose as the Dose Metric, and BB_{01} Values Based on PBPK-Modeled Body Burdens as the Dose Metric.

Response Category	Effect	ED_{01} (ng/kg/day)	BB_{01K} (ng/kg)	BB_{01PBPK} (ng/kg)	BB_{01K}: BB_{01PBPK}
Biochemical	CYP1A1 mRNA	1.6 (1.1)	59.0 (38.3)	17.8 (9.8)	3.3
Biochemical	CYP1A1 Protein	0.4 (0.2)	14.9 (7.0)	7.0 (3.6)	2.1
Growth Factor	EGFR Max Binding (B_{max})	1.7 (0.4)	60.9 (14.7)	31.7 (7.5)	1.9
Hepatic	Total Cholesterol	0.4 (0.1)	15.0 (2.0)	9.2 (1.1)	1.6

Lower 95% confidence limits on ED_{01}, BB_{01K}, and BB_{01PBPK} values are shown in parentheses.
BB_{01K} = Equivalent BB_{01} based on simple kinetic conversion of the daily ED_{01}.
BB_{01PBPK} = BB_{01} based on PBPK-modeled body burdens.