Estimates of ED_{10} dose levels for biochemical and toxicological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin

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1. Introduction

Quantitative risk assessments for TCDD have focused primarily on cancer. However, cancer has often been considered a "high dose" effect. Studies by Mably et al[1] (1992) indicate that noncancer effects may be observed at much lower doses than cancer. One of the critical elements in the assessment of potential health risks associated with exposure to environmental chemicals is dose-response assessment. Recent approaches to dose-response assessment for noncancer endpoints employ the curve-fitting methodology of the benchmark dose. The present exercise applies this approach to data sets examining low dose biochemical and toxicological endpoints in animals exposed to TCDD. This approach was used to estimate the effective dose (ED_{10}) resulting in a 10% excess risk. In addition, the present analysis examines data sets from studies investigating noncancer effects of TCDD to determine if apparent thresholds exist for biochemical and/or toxicological responses.

2. Methods

A simple empirical modeling scheme was used to estimate the ED_{10} dose level for biochemical and toxicological endpoints. The Hill model of the form

$$R(d) = b + \frac{vd^n}{k^n + d^n}$$

was applied to the data, where R(d) is the response at dose d, and b,v,k and n are model parameters to be estimated from the data. The parameters each describe a different aspect of the dose-response curve: b is the background response, v is the maximum attainable response, k is the dose yielding half of v and n is the Hill coefficient describing the curvature of the dose-response. In risk assessments, the shape of the dose response curve plays a critical role, hence it is of interest to consider important classifications based on n. When n is near or below 1, risk is proportional to response or climbing more rapidly than proportional and has no apparent threshold. When n is much larger than 1 (n>1.5),the dose resposne curve is sigmoidal and has been described as appearing to have a threshold.

The 10% risk is defined as that dose satisfying the excess risk relationship

$$\frac{R(ED_{10}) - R(0)}{R(\infty) - R(0)} = 0.1$$

The initial data sets chosen are not meant to be all inclusive. Data sets with 5 or more dose levels and with the data reported in the original response units were chosen. In addition, not all endpoints were examined. The focus of this exercise is on the low-dose effects of TCDD. Hence experiments examining the lethal effects of TCDD were excluded from the present analysis. Furthermore, the exclusion of any data sets at this time does not preclude them from future studies. The data examined were analyzed using either group means weighted by the inverse of the standard error, unweighted means, or the individual animal data, depending on the availability.

3. Results

The data sets were divided into two categories based on dosing regimen. Estimates of the ED_{10} for biochemical and toxicological responses to TCDD following multiple sub chronic exposures are shown in Table 1. The biochemical responses examined include induction of hepatic mRNA, protein and activity of CYP1A1 and CYP1A2, induction of hepatic thyroxine glucuronidation, decreases in hepatic EGF receptor, decreases in hepatic and plasma retinol concentrations, and decreases in plasma thyroxine concentrations. The toxicological responses include alterations in serum enzymes, hepatic labeling index and increases in mean hepatic focal volumes. The studies examined include a 13 week study in female mice[2], a 13 week study in female Sprague Dawley rats[3] and a 30 week tumor promotion study in female Sprague-Dawley rats [4,5]. In the subchronic study in mice, the induction of CYP1A2 enzyme activity has a lower ED_{10} than induction of CYP1A1 although both responses had shape parameters equivalent to 1. In rats the two studies have similar ED_{10} and shape parameters for hepatic EROD induction[3] and CYP1A1 mRNA[5]. In contrast, induction of CYP1A2 enzyme activity [3] and mRNA[5] have similar ED_{10} but significantly different shape parameters (Table 1). Induction of CYP1A2 enzyme activity displays a threshold-like dose response while the induction of mRNA does not. Induction of hepatic thyroxine glucuronidation has a higher ED_{10} compared to CYP1A1 and CYP1A2 by a factor of approximately 2-3, although it has a shape parameter similar to CYP1A1 and CYP1A2. Of all the endpoints examined, decreases in hepatic retinol concentrations have the lowest ED_{10} at 0.28 ng/kg/d with n = 0.55. In contrast, changes in hepatic retinyl-palmitate have a relatively high ED_{10} at 237 ng/kg/d and a relatively high shape parameter (n=12.16) indicating a threshold-like dose response. Decreases in plasma retinol, and total and free thyroxine have higher ED_{10} values compared to hepatic enzyme induction.

The toxicological responses have higher ED_{10} values compared to hepatic enzyme induction with the exception of alterations in serum ALT. Furthermore, with the exception of serum ALT and S. DeH in animals pretreated with DEN, all the toxicological responses have values of $n > 1.5$ indicating threshold-like dose response curves (Table 1). Increases in hepatic mean focal volume and labeling index have similar ED_{10}'s between 31 and 32 and both exhibit threshold like dose response curves. Down-regulation of hepatic EGF receptor has been proposed to play a role in the TCDD-induced hepatic proliferation and in the induction of foci and tumors. While the ED_{10} for down regulation of hepatic EGF receptor is lower than either the labeling or focal ED_{10}, it also has a marginal threshold-like dose-response curve, which, when corrected for tissue concentration the nonlinearity disappears[13].

Estimates of the ED_{10} for the induction of hepatic enzymes in rats treated with a single dose of TCDD ranged from approximately 70-157 ng/kg and all had n equivalent or less than 1. In mice, induction of hepatic enzymes had ED_{10} values of 108 - 650 ng/kg with n equivalent to 1 for all endpoints except induction of hepatic CYP1A2 mRNA and increased Ah receptor binding which exhibit threshold like dose response curves. In animals subchronically exposed to TCDD, enzyme induction had the lowest ED_{10} values. In contrast several of the toxicological endpoints in animals acutely exposued to TCDD have much lower ED_{10} values than hepatic enzyme induction. For example, the ED_{10} value for inhibition of the plaque forming cells in response to the sheep red blood cells is approximatley 22 times lower than the ED10 for hepatic EROD induction, although the decreased immune response displays a threshold-like dose response curve. The lowest ED_{10} is for decreased cauda epididymal sperm count in rats prenatally exposed to TCDD[1].
The ED_{10} for this response is between 4 and 10 ng/kg and has values of n equivalent to 1, suggesting a lack of a threshold for this response. Other toxicological response such as cleft palate induction and thymic atrophy have ED_{10}'s equivalent or higher to hepatic enzyme induction.

4. Discussion

In general, most of the functional measures of noncancer toxicity following TCDD exposure exhibit dose-response relationships which have no apparent threshold. However, many of the few endpoints for which shape could be analyzed which represent host morbidity (thymus cellularity, sperm morphology, fertility and cleft palate) exhibited threshold-like dose-response relationships. The exception to this is the decreased cauda epididymal sperm counts from rats prenatally exposed to TCDD. This response does not appear to have a threshold-like dose response and it also has the lowest ED_{10} for any study using a single administration.

In the studies using an acute exposure, the endpoints with the lowest ED_{10} are the decreased immune response to sheep red blood cells and the decreased cauda epididymal sperm counts in rats exposed prenatally. In the subchronic studies, hepatic enzyme induction have the lowest ED_{10}. Comparable studies on immune competence and developmental toxicity have not been reported following subchronic exposures. Hence, enzyme induction may have the lowest ED_{10} in subchronic studies because more sensitive endpoints have not been examined.

There is ample evidence that noncancer endpoints are extremely sensitive to the toxic effects of TCDD. The available data do not generally provide enough information to develop biologically based mechanistic models for all noncancer endpoints. Few if any of the molecular events beyond ligand binding to the Ah receptor are understood for these effects. The only information we have to develop mechanistic models is dose-response relationships. Future studies that better characterize target tissues and the molecular mechanisms underlying these events are indicated. Because of the importance of generating reliable estimates of the risk for noncancer effects, the development of biologically based dose-response models for these effects is an urgent research need.

This abstract does not necessarily represent USEPA policy.

5.References

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Table 1. Excess Risk ED_{10} for Biochemical and toxicological effects of TCDD in Studies with Multiple Dose Administration[1].

Study Description	Dose[2] Regime	Endpoint	Shape[3] Parameter	10% extra effect	Data[4]
DeVito, et al., 1994[2]	5x/week	liver EROD (CYP1A1 activity)	1.35	17.35	UW
female B6C3F1 mice	13 weeks	liver 4OH-AA (CYP1A2 activity)	0.72	0.76	UW
van Birgelen, et al. 1995[3]	daily	EROD (CYP1A1 activity)	1.23	7.31	W
female S-D rats	13 weeks	4OH-AA (CYP1A2 activity)	2.18	6.86	W
		hepatic retinol	0.55	0.28	W
		hepatic retinyl-palmitate	12.16	236.90	W
		plasma retinol	3.33	27.56	W
		plasma TT4	0.54	317.17	W
		plasma FT4	0.88	51.96	W
van Birgelen, et al. 1995[4] female S-D rats	daily 13 weeks	liver T4UGT	0.93	19.55	W
Maronpot, et al., 1993[5]	1x/2wks	Alk. Phos. (DEN)	2.70	22.77	RAW
female S-D rats	30 weeks	Alk. Phos. (SAL)	9.31	30.56	RAW
		ALT	0.50	0.41	RAW
		Triglycerides	1.49	14.38	RAW
		S. DeH. (DEN)	0.57	15.43	RAW
		S. DeH. (SAL)	15.63	29.31	RAW
		5-prime Nuc	0.35	-	RAW
		Liver labeling index	12.63	32.39	RAW
Tritscher, et al., 1992[6]	1x/2wks	Max. EGF receptor	1.58	6.77	RAW
female S-D rats	30 weeks	Avg. Conc 1A1 (protein)	1.21	3.06	RAW
		Avg. Conc 1A2 (protein)	0.66	9.06	RAW
		Mean focal volume	11.98	31.16	RAW

[1] Doses are expressed as ng/kg (single dose)
[2] Dose regime: single dose indicated length of time until termination
[3] Shape of the dose response curve expressed as exponent in Hill equation or power law fit to the data
[4] Data types used in the analyses: W-group means weighted by inverse of standard error, UW-unweighted means, RAW-raw data, B - raw binomial data.

Table 2. Excess Risk ED_{10} for Biochemical and toxicological effects of TCDD in Studies with Single Dose Administration[1].

Study Description	Dose[2] Regime	Endpoint	Shape[3] Parameter	10% extra effect	Data[4]
Kitchin & Woods, 1979[7]	Single,	liver cytochrome p-450	0.53	68.72	W
female S-D rats	3 days	liver benzopyrene hydroxylase	1.37	77.33	W
Abraham, et al., 1988[8]	Single,	liver cytochrome p-450	0.72	86.77	W
female Wistar rats	7 days	liver EROD (CYP1A1 activity)	0.97	157.27	W
Narasimhan, et al., 1993[9]	Single,	liver EROD (CYP1A1 activity)	0.97	645.77	W
female B6C3F1 mice	24 hrs.	liver Cyp1A1 (mRNA)	1.00	108.52	W
		liver Cyp1A2 (mRNA)	3.88	391.23	W
		Total Ah receptor binding	3.85	648.71	UW
	S, 4 days	Spleen PFC/10^6cells	2.60	29.08	UW
Davis & Safe 1988[10] C57BL/6N mice	S, 4 days	Spleen PFC/10^6cells	3.96	359.95	UW
Olson, et al., 1980[11]	Single,	Thymus weight	1.37	57007.0	W
male golden syrian hamsters	50 days	Spleen weight	5.75	346037.	W
Mably, et al., 1992[1]	Single	Sperm morph. - day 120	4.20	145.60	W
preg. female,		Fertility index	23.06	360.50	W
male offspring,		Cauda sperm count day 63	0.86	4.27	W
Holtzman S-D rats		Cauda sperm count - day 120	0.97	6.83	W
		Cauda sperm count/g - day 120	1.50	10.10	W
		DSP/g - day 49	1.48	21.41	W
		DSP/g - day 63	0.39	0.87	W
		DSP/g - day 120	1.72	10.14	W
Birnbaum, et al. 1989[12]	S	Cleft palate 6D-10	6.96	10791.0	B
preg. female	S	Cleft palate 6D-12	5.21	7797.4	B

[1] Doses are expressed as ng/kg (single dose)
[2] Dose regime: single dose indicated length of time until termination
[3] Shape of the dose response curve expressed as exponent in Hill equation or power law fit to the data
[4] Data types used in the analyses: W-group means weighted by inverse of standard error, UW-unweighted means, RAW-raw data, B - raw binomial data.