THE TOXIC EQUIVALENCE FACTOR FOR 2,3,7,8-TETRACHLORODIBENZOFURAN (TCDF): INCORPORATION OF TOXICOKINETIC AND TOXICODYNAMIC CONSIDERATIONS

Simon T[1], Budinsky RA[2], Rowlands JC[2]
[1] Ted Simon LLC, Winston, GA, USA 30187; [2]The Dow Chemical Company, Midland MI, USA 48674

Introduction

2,3,7,8-Tetrachlorodibenzofuran (TCDF) is unique among the dioxin and furan congeners in that it is: a) The furan analogue of 2,3,7,8-tetrachlorodibenzo-p-dioxin - TCDD, b) largely undetected in human biomonitoring or food studies, and c) rapidly metabolized via 4-hydroxylation and glucuronidation. Notwithstanding, TCDF has been assigned a relatively high potency with a TEF value of 0.1. TCDF is prevalent in soil from historic chlor-alkali sites but it occurs in small amounts or not at all in blood and food. The presence in soil has made TCDF an an important congener risk assessment and remediation activities because of the high TEF.[1-5]

The rapid metabolism, short half-life and consequent lack of bioaccumulation of TCDF relative to TCDD raise doubts about the validity of its relatively high TEF value.[6-7] For example, in mink, TCDF does not bioaccumulate as expected, and the metabolic clearance is 4-8 hours depending on the degree of CYP1A1 induction.[8-9] A comparison of relative potencies based on administered doses versus those based on tissue concentrations indicate the need to consider toxicokinetics in evaluating TCDF.[10] This enhanced clearance of TCDF is CYP1A1-dependent and impacts estimates of its relative oral bioavailability, a concern about the 2005 WHO TEFs when evaluating dioxin-like chemicals (DLCs) in soil.[11] Although TCDF is structurally analogous to TCDD, the two congeners have different gene transcription responses.[12-13] The relative potency database for TCDF is based on enzyme induction, occurrence of cleft palate, and effects in sheep red blood cell results.[14-19] Relative potency values for TCDF range from 0.006 to 0.63 based on both in-vivo and in-vitro data with a 50th percentile value of 0.08.[20] When limited to in-vivo data, the range is 0.006 to 0.5 with a 50th percentile value of 0.03.[20] This suggests the TCDF differs from TCDD with regard to both toxicokinetics, toxicodynamics, or both.

Since additivity of TEF-adjusted doses rests on the assumption of the same mechanism, it should be expected that the same extent of toxicity will be seen between TCDF and TCDD following TEF adjustment of the dosages. However, as noted, TCDF and TCDD have different gene expression profiles.[12,13] Finally, the experimental data upon which the TEFs are based is almost entirely from rodents. In-vitro studies comparing TCDD and TCDF in human and rodent cell culture allows a means to adjust for extrapolation issues related to the toxicodynamic aspects of TEFs for human health risk assessment.[15,21] Comparisons with non-rodent species may inform the TEFs for ecological risk assessment.[8]

Overall, there is significant uncertainty surrounding the TCDF TEF value of 0.1. However, there is also the possibility of addressing this uncertainty by analyzing currently available data or conducting future studies. Here, we have attempted to mine the current data to assess potential adjustment factors for the TEF value for TCDF and develop species-specific TEFs for this congener. The following analysis of five different species uses an approach for examining variation in relative potency using both toxicokinetic and toxicodynamic data to develop species-specific relative potency estimates for TCDF.

Materials and methods

Studies on bioavailability or toxicokinetics (TK) and studies on toxicodynamics (TD) of TCDF and TCDD were assembled from the scientific literature using PUBMED. For each species considered, Monte Carlo simulation was used to obtain distributions of separate relative potency estimates (REPs) for TK and TD.[22] Bioavavailability was estimated as the sum of the amounts in liver and adipose tissue at the conclusion of dosing divided by the total dosage amount. For observational studies in which animals were not administered known dosages but exposed to environmental concentrations, bioavailability could be estimated using an empirical equation based on the liver/adipose concentration ratio. The ratio between the bioavailability of TCDF and TCDD was the bioavailability equivalence factor (BEF).

TD differences were estimated using ethyoxyresorufin-O-dethylase (EROD) as a measure of CYP1A1 induction via AHR activation. Iterative Monte Carlo fitting was used to develop the IEF distributions.[22] For TD REPs or

induction equivalence factors (IEFs), values at the EC20, EC50 and EC80 were calculated.[23] For each species, a proposed "adjusted" total toxic equivalence factor (TEF_{adj}) was calculated as the product of the BEF and the three IEFs. The average of the central values and confidence limits represent the potential range of TEF_{adj} values.[22,23] The median values, confidence limits and distributions for the TEF_{adj} derived here for rat, mouse, mink, seal and humans are shown in Table 1 below. Distributions representing REPs for toxicokinetics and toxicodynamics are also shown. Because metabolism of TCDF would tend to affect both these aspects, a correlation coefficient of -0.25 was used in the TEF calculation. This choice of this value is arbitrary, but it is an attempt to avoid double-counting the effect of metabolism on the TEF.

Results and Discussion

Rats

Bioavailability was measured from a mixture of congeners including TCDD and TCDF administered in both soil mixed with food and by corn oil gavage.[11] Values of the BEF from soil was greater than that from oil. The IEFs were obtained from dose response in rat primary hepatocytes.[21] A random choice between these two BEF distributions was multiplied by the distributions for the IEFs to obtain the TEF_{adj}.[21]

Mice

Bioavailability and toxicodynamic data were obtained from a 13-week bioassay.[10,24] TCDD and TCDF were administered by corn oil gavage. There were five dose groups. The arithmetic means and standard deviations of liver and adipose amounts were used as the parameters of a lognormal distributions used to estimate the BEF. IEFs were calculated as described.

Mink

Bioavailability for TCDF was calculated from a feeding study.[9] To obtain bioavailability for TCDD, an empirical model of bioavailability (Fig. 1) based on the liver/adipose concentration ratio was developed from 87 data points in mice, rats swine and mink; the model is applicable to all dioxin congeners.[9,11,24,25] During model development, the relationship of dose to bioavailability was examined and the influence of dose was found to be essentially non-existent; the effect of dose is likely to be captured in the liver/adipose ratio, which has also been used as a measure of hepatic sequestration in swine.[26]

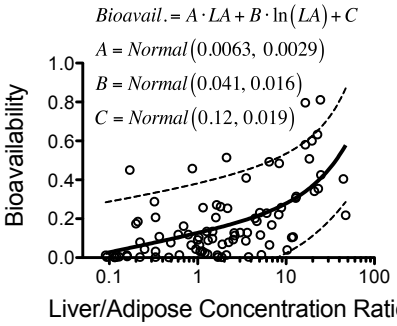

Figure 1. Empirical Model to predict Bioavailability of DLCs from Liver/Adispose Concentration Ratio. Dashed lines show the 90% prediction interval.

To estimate bioavailability of TCDD in mink, 16 paired data for TCDD in liver and adipose tissue were used as input to the empirical model.[9] The ratio of the distributions of TCDF bioavailability and TCDD bioavailability was used as the BEF distribution.

In mink, data were available on EROD induction for TCDF and 4-PeCDF, but not for TCDD.[27] To simulate the TCDD dose response model, ECx values obtained from the 4-PeCDF dose response model were adjusted downwards with using a REP distribution developed from 20 in-vitro measurements of effects known to be associated with AHR activation.[20] This distribution was used to adjust the EC50 value used in the dose response model for PeCDF. This adjustment assumes that 4-PeCDF and TCDD have parallel dose response curves.

In contrast to the other species, the difference between the IEF20 and IEF50 values for the mink was about two orders of magnitude. This indicates the dose response curves are not parallel and that IEF values developed at response levels less than the EC50 may not be reliable.

Seals: Liver and blubber concentrations of TCDF and TCDD were available from 7 male Baikal Seals.[28] These were used with the empirical model in Fig. 1 to estimate the BEF. A heterologous expression system, COS-7 cells from African Green Monkey kidney, with low endogenous AHR activity were transfected with Baikal Seal AHR coupled to a luciferase reporter gene.[29] This system was used to obtain dose response data for DLCs in Baikal Seals. These dose response data were used to estimate IEF distributions. Because of the low endogenous

AHR activity in COS-7 cells, it was not necessary to use the negative correlation to avoid double-counting the effect of metabolism.

Humans

The REP for bioavailability was determined from autopsy data from eight subjects.[30] Liver/adipose concentration ratios were used to estimate bioavailability measures for both TCDD and TCDF with the empirical model. The IEF distributions were estimated from Hill model parameters developed from dose response in primary hepatocytes.[21]

Table 1. Species-specific BEF, IEF and TEF distributions [LN (GM, GSD) indicates a lognormal distribution with a geometric mean (GM) and geometric standard deviation given by the two numerical values]

Species	Toxicokinetic REP /Bioavailability Equivalence Factor (BEF)	Toxicodynamic REP/ Induction Equivalence Factor (IEF)	Total Toxic Equivalence Factor (TEF) Median (90% CI)
Rat	Soil: LN (0.647, 1.14) Oil: LN (0.30, 1.23)	REP20: LN (0.111,1.63) REP50: LN (0.0216,1.55) REP80: LN (0.0047, 1.65)	0.02 (0.008 – 0.06)
Mouse	LN (0.164, 1.50)	REP20: LN (0.102, 1.81) REP50: LN (0.242, 1.65) REP80: LN (0.563, 1.79)	0.05 (0.03 – 0.14)
Mink	LN (0.053, 1.76)	REP20: LN (0.001, 5.89) REP50: LN (0.03, 4.25) REP80: LN (0.07, 4.18)	0.002 (0.0002 – 0.02)
Baikal Seals	LN (1.09, 1.22)	REP20: LN (0.16, 4.08) REP50: LN (0.096, 2.19) REP80: LN ((0.054, 3.20)	0.1 (0.02 – 0.9)
Humans	LN (1.10, 1.41)	REP20: LN (0.013, 1.60) REP50: LN (0.012, 1.56) REP80: LN (0.11, 1.62)	0.13 (0.06 – 0.3)

Figure 2 shows the TEFs graphically. What is striking about this figure is the mink is about 1-2 orders of magnitude less than any other species. The mink used of this TEF derivation were investigated as part of an ecological risk assessment of the Tittibawassee River and no adverse effects were seen in these animals.[8,9,27] The human TEF distribution is likely as high as it is because it was estimated from individuals exposed at low background levels. Because humans are much less sensitive than rodents to activation of the AHR by exogenous ligands, it is likely that relatively little CYP1A1 enzyme induction had occurred from background exposure to produce higher metabolism. Hence, TCDF in the liver would be metabolized more slowly than with higher exposure. Similarly for Baikal Seals, higher levels of TCDF occur in the liver than in adipose tissue, suggesting that TCDF metabolism may occur to a lesser extent than in terrestrial mammals.[31] One important caveat is that although data on CYP1A1 induction by DLCs are available in many species, enzyme induction *per se* is not a measure of toxicity; hence, TEFs based on enzyme induction should be viewed with caution.

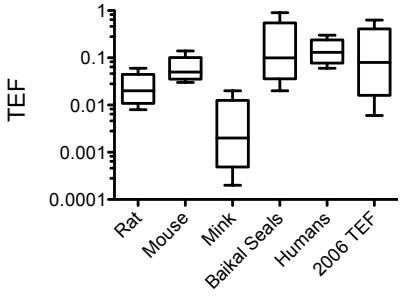

Figure 2. Boxplots of TEF distributions in the five species compared with those from the TEF database.[20]

Development of TEF_{adj} with consideration of both toxicokinetics and toxicodynamics is consistent with current ecological risk assessment policies.[32] Therefore, because of the large differences in the BEF and IEF values, and the resulting TEF_{adj} for the five species considered here, we are proposing that species-specific TEF_{adj} be used in risk assessment and that future TEF evaluations consider species differences in both toxicokinetics and toxicodynamics.

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