A Preliminary Evaluation of the Toxic Equivalency Factor (TEF) for 2,3,4,7,8-PCDF (4-PCDF) Using Data from the Recent NTP Dioxin Bioassays

Robert Budinsky[1], Thomas, B Starr[2], Dennis Paustenbach[3], Bryce Landenberger[1], Donald Fontaine[1]

[1]The Dow Chemical Company
[2]TBS Associates, Raleigh, NC USA
[3]ChemRisk

Introduction

PCDD/PCDF toxic equivalency factors (TEFs)[i] are being routinely applied now in human cancer risk assessments for dioxin-like compounds despite the fact that until very recently only two congeners (1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin) other than 2,3,7,8-TCDD had been evaluated for carcinogenicity in a standard cancer bioassay[ii,iii]. However, in December 2003, draft reports from the National Toxicology Program (NTP) cancer bioassays were made available for 2,3,7,8-TCDD, 4-PCDF, PCB 126, and a (presumably) equipotent mixture of these three compounds[iv,v,vi,vii,viii]. These data provide important new information for evaluating the accuracy of TEFs in predicting the potential human cancer hazard for 4-PCDF and PCB126 alone and in combination with TCDD. We present herein results from a series of simple statistical tests demonstratting that the current TEF of 0.5 for 4-PCDF, when combined appropriately with the new TCDD bioassay data, fails to predict accurately the results from the new 4-PCDF bioassay.

Methods

We modeled TCDD dose-response relationships for liver tumors (adenomas and bile duct tumors combined) and combined liver and lung tumors as a function of: 1) administered dose [ng/kg/day], 2) liver concentration at terminal sacrifice [nM], and, 3) area under the liver concentration curve [AUC, pg/g x weeks], also at terminal sacrifice. Values for these dose metrics are presented in Table 1 for each dose group in the TCDD and 4-PCDF bioassays. The AUC values were generated from measured liver concentrations using the standard trapezoidal rule for integration.

Animals with multiple tumors were counted at most once in incidence rate numerators, and the corresponding denominators were adjusted for the competing risks of intercurrent mortality with the poly-3 method[ix]. The liver tumors consisted of adenomas and cholangiolar carcinomas. Lung tumors were comprised predominantly of cystic keratinizing epitheliomas, with a few additional alveolar/bronchiolar adenomas.

Weibull models were fit to the TCDD dose-response data using USEPA's Benchmark Dose software, version 1.3.2 (AUC doses were divided by 10,000 prior to model parameter estimation). The predicted numbers of tumor-bearing animals for each 4-PCDF bioassay dose group were then generated by 1) applying the current TEF of 0.5 to the 4-PCDF doses to obtain TCDD-equivalent doses; 2) using the fitted dose-response model for TCDD to estimate the probability of being tumor-bearing at each 4-PCDF dose; and 3) multiplying each such probability by the poly-3 adjusted number of animals at risk in the corresponding 4-PCDF dose group. Under the null hypothesis that the true value of the 4-PCDF TEF equals 0.5 (the current WHO estimate[1]), the sum of [(Observed – Predicted)^2 / Predicted] over the 4-PCDF dose groups should be approximately distributed as a chi-squared random variate with degrees of freedom equal to the number of 4-PCDF dose groups.

Results

Tables 2, 3 and 4 show the results obtained from applications of this simple goodness-of-fit test of the null hypothesis that the 4-PCDF TEF is equal to 0.5 based on administered dose, liver concentration, and area under the liver concentration curve (AUC), respectively.

The last cells for "# Observed" in each of these tables has two entries, one for liver tumors alone and the other for combined liver or lung tumors, so each table provides results for two distinct tests of the null hypothesis, one based on liver tumors only, the other on the combination of liver and lung tumors.

Thus, a total of six tests of the 4-PCDF TEF null hypothesis were undertaken, and each was rejected, i.e., there was no indication that a 0.5 TEF value fit the data. The highest p-value obtained, namely, 0.01851, arose from predictions based upon the combination of liver and lung tumors as a function of administered dose. Liver tumors alone produced a far smaller and highly significant p-value of 0.00084 using administered dose, and the remaining p-values (see Table 3 and Table 4) were extremely small ($p < 1x10^{-25}$), indicating that once the prominent pharmacokinetic differences between TCDD and 4-PCDF were accounted for by use of a more appropriate target tissue dose metric (e.g. AUC), the discrepancies between observed and 0.5 TEF-predicted 4-PCDF tumor incidences are so extreme as to be unsupportive of the current TEF value. Our results demonstrate clearly that the current TEF of 0.5 for 4-PCDF is much too high to be consistent with the most recent carcinogenicity data for TCDD and 4-PCDF.

Discussion

The new NTP bioassay data for TCDD and 4-PCDF provide an ideal opportunity for testing the accuracy of the current TEF for 4-PCDF. Virtually identical experimental designs and protocols were utilized in these studies avoiding many of the factors such as protocol, species, strain, endpoint, and investigator differences that might otherwise compromise the validity of a quantitative comparison of findings and a determination of a relative potency factor(s). Our analyses of these new data demonstrate highly significant discrepancies between observed and predicted tumor incidence rates when the current TEF for 4-PCDF is used to predict the 4-PCDF tumor incidence rates.

One potential explanation of these discrepancies is that the pharmacokinetic differences between TCDD and 4-PCDF in the mammalian studies upon which 4-PCDF's current TEF is based were not adequately considered when the TEF was established[1,x,xi]. It is interesting to note that Waern et al.'s (1991) liver tumor promotion study indicated that 4-PCDF was only 0.007 times as active as TCDD based on liver concentration comparisons[10]. However, the discrepancies we have found between the observed and predicted tumor incidence rates for 4-PCDF are far greater, not smaller, when either liver concentration or AUC, both of which implicitly incorporate pharmacokinetic differences, are utilized as dose metric alternatives to administered dose.

Another possible explanation for the failure of the current 4-PCDF TEF to accurately predict 4-PCDF tumor incidence rates is that it was derived from data for non-cancer and non-chronic endpoints, and there is little evidence that such endpoints are useful quantitative predictors of carcinogenicity either in general, or, more specifically, for dioxin-like compounds[xii] (c.f., Starr et al. (1999) for a discussion of the numerous difficulties with TEFs). In any event, we find that the current 0.5 TEF for 4-PCDF simply does not work with any of the three dose metrics we explored. Thus, a reduced TEF is warranted for 4-PCDF.

Our conclusion that a reduced TEF for 4-PCDF is warranted is also supported by the recent analyses of CYP1A1 and CYP1A2 induction data from the same NTP studies. Toyoshiba et al. (2004)[xiii] recently reported a range of relative potency factors for CYP1A1 and CYP1A2 based on administered dose which were all lower than the current TEF for 4-PCDF. Although Toyoshiba et al. (2004) stated they had also conducted analyses using tissue concentrations, results from these analyses were not included in their report.

A simple, yet rigorous assessment of the current TEF for 4-PCDF, has been made possible by the availability of the new NTP cancer bioassay data. While the current TEF for this congener has been derived with data from a number of studies, none were the pivotal cancer bioassay data needed to support the use of TEFs for cancer risk assessment. Furthermore, the current TEF for 4-PCDF does not take into consideration pharmacokinetic differences with respect to tissue concentrations and target organ distribution patterns.

Consistent with the USEPA's draft dioxin reassessment, it is reasonable to continue to refine TEF estimates for dioxin and furan congeners as better data become available, and to base risk assessment and management decisions on these refined estimates that implicitly take into account pharmacokinetic and pharmacodynamic difference in response to exposure. DeVito et al. (1997)[xiv] discussed the need to be cognizant of differences in tissue disposition when evaluating relative potencies and they have recommended that TEFs be derived in terms of tissue equivalent doses. Our analysis indicates clearly that the current TEF for 4-PCDF is substantially higher than is indicated by the newly available bioassay data.

Table 1. Dose Metric Data

Dose (TEQ) ng/kg/d	TCDD		4-PCDF	
	Liver Conc nM	Liver AUC pg/g x wks*	Liver Conc nmol	Liver AUC pg/g - wks*
0	0.1	8,176.	5	36,401
3	2.0	55,320.	52	1,339,162
10	7.0	203,099.	183	4,589,442
22	14.0	434,791.	368	10,136,372
46	20.0	786,704.	774	21,445,272
100	29.0	1,507,044.	1,468	41,065,441

*: As determined at 104 weeks

Table 2. Observed Vs Predicted Numbers of Tumor-Bearing Animals Using Administered Dose

4-PCDF Dose, ng/kg/d	# Observed with Liver or Liver or Lung Tumors	# Predicted with Liver Tumors	# Predicted with Liver or Lung Tumors	$(Obs-Pre)^2/Pre$ for Liver Tumors	$(Obs-Pre)^2/Pre$ for Liver or Lung Tumors
0	1 / 41.97 *	0.	0.3838	**	0.9891
6	0 / 38.07	0.0054	0.3504	0.0054	0.3504
20	1 / 36.67	0.1093	0.3984	7.2618	0.9084
44	1 / 37.91	0.8183	0.9423	0.0404	0.0035
92	3 / 36.13	4.7310	4.6151	0.6333	0.5625
200	6 / 37.18 8 / 38.09	23.4457	25.9522	12.9811	12.4183
Chi-square goodness-of-fit test with 5 or 6 degrees of freedom			**Sum**	20.9221	15.2349
			p-value	0.00084	0.01851

*: Number observed / poly-3 adjusted number at risk.

**: Number predicted is exactly zero, so control group was excluded from Sum.

Table 3. Observed Vs Predicted Numbers of Tumor-Bearing Animals Using Liver Concentration

4-PCDF Liver, nM	# Observed with Liver or Liver or Lung Tumors	# Predicted with Liver Tumors	# Predicted with Liver or Lung Tumors	$(Obs-Pre)^2/Pre$ for Liver Tumors	$(Obs-Pre)^2/Pre$ for Liver or Lung Tumors
5	1 / 41.97 *	0.000133	0.3807	7,518.3 **	1.0077
52	0 / 38.07	16.4075	17.3762	16.4075	17.3762
183	1 / 36.67	36.67	36.67	34.6973	34.6973
368	1 / 37.91	37.91	37.91	35.9364	35.9364
774	3 / 36.13	36.13	36.13	30.3791	30.3791
1468	6 / 37.18 8 / 38.09	37.18	38.09	26.1483	23.7702
Chi-square goodness-of-fit test with 5 or 6 degrees of freedom			**Sum**	143.5685	142.1592
			p-value	3.12E-29	2.1E-28

*: Number observed / poly-3 adjusted number at risk.
**: Number predicted is small and unstable, so control group was excluded from Sum.

Table 4. Observed Vs Predicted Numbers of Tumor-Bearing Animals Using Area under the Liver Concentration Curve (AUC)

4-PCDF Liver AUC, pg/g –wk	# Observed with Liver or Liver or Lung Tumors	# Predicted with Liver Tumors	# Predicted with Liver or Lung Tumors	$(Obs-Pre)^2/Pre$ for Liver Tumors	$(Obs-Pre)^2/Pre$ for Liver or Lung Tumors
36,401	1 / 41.97 *	0.000068	0.3798	14,705.0 **	1.0128
1,229,162	0 / 38.07	3.1250	3.0243	3.1250	3.0243
4,589,442	1 / 36.67	35.5866	36.3181	33.6147	34.3456
10,136,372	1 / 37.91	37.91	37.91	35.9364	35.9364
21,445,272	3 / 36.13	36.13	36.13	30.3791	30.3791
41,065,441	6 / 37.18 8 / 38.09	37.18	38.09	26.1483	23.7702
Chi-square goodness-of-fit test with 5 or 6 degrees of freedom			**Sum**	129.2035	128.4684
			p-value	3.51E-26	2.46E-25

*: Number observed / poly-3 adjusted number at risk.
**: Number predicted is small and unstable, so control group was excluded from Sum.

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