LINEAR VS. NONLINEAR EXTRAPOLATION FOR DOSE-RESPONSE ASSESSMENT OF TCDD

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Introduction

As early as 1993, scientists recognized that the hallmark of receptor-mediated toxicity was a sigmoidal dose response curve and that there would exist a safe exposure level below which no effects would occur.[1,2] Both first order and higher order Hill models have been successfully used to fit dose-response data for TCDD.[3,4] Recently, it was demonstrated that receptor-mediated effects producing any number of first order steps of arbitrary complexity will telescope into a first order Hill function for the apical effect.[5] The early bioassays of TCDD in rats administered initiators before TCDD because of the prevailing assumption that TCDD acted as a tumor promoter.[6-8]

Given the plethora of dose-response data and models for TCDD effects, including cancer, that appear sigmoidal in shape, the clear recognition of the role of TCDD as tumor promoter, and the knowledge of the mode of action of TCDD acting via aryl hydrocarbon receptor activation, EPA's choice of linear extrapolation for cancer dose response is questionable, particularly given the recommendation of the National Research Council to use nonlinear extrapolation for the carcinogenic effects of TCDD.[9,10] Here, dose response modeling is used to examine the possibility that a threshold does indeed exist for the TCDD-mediated animal carcinogenesis and to develop a reference dose based on a key event that is protective of cancer.

Materials and Methods

Dose response studies of TCDD in female rats chosen as key data sets in EPA's Reanalysis document for cancer dose-response assessment were refit with the dichotomous Hill model to determine if the goodness of fit (GOF) was better or worse than that for the multistage model preferred by EPA. A better GOF for a sigmoidal model such as the dichotomous Hill would suggest a threshold did indeed exist.

Given the scientific consensus of the sigmoidal nature of dose-response curves for receptor-mediated effects, it is notable that EPA confined the dose response modeling in the Reanalysis document to the multistage model. The reason may be that EPA's BMDS software proved impossible to use for fitting the dichotomous Hill model for almost all of the datasets. Here, the maximum likelihood fitting procedures from BMDS source code files were implemented in MS-Excel in double precision.

Results

Dose response analyses of tumor responses in a variety of tissues in female rats using the dichotomous Hill model were compared to those presented in Appendix F of EPA's Reanalysis document using the identical data.[9,11-13] The table below shows Akaike's information criteria (AIC) and the Chi-square p-values for both fits. For the dichotomous Hill model, lower AIC values and higher p-values than the multistage model are in bold.

Study	Endpoint	EPA's Preferred Multistage Model		Dichotomous Hill Model	
		AIC	p-value	AIC	p-value
Kociba et al. (1978)[11]	Hepatocellular adenomas or carcinomas	143.261	0.2449	**142.528**	**0.9995**
NTP (1982)[12]	Neoplastic nodules or hepatocellular carcinoma	135.19	0.2175	135.42	**0.7093**
NTP (2006)[13]	Cholangiocarcinoma	113.508	0.9933	116.8498	0.9674
NTP (2006)	Hepatocellular adenoma	72.7815	0.9330	74.9751	**0.9929**
NTP (2006)	Oral squamous cell carcinoma	126.963	0.2700	**124.5606**	**0.4996**
NTP (2006)	Pancreatic adenoma or carcinoma	29.373	0.6403	**28.8193**	**0.9999**
NTP (2006)	Lung cystic keratinizing epithelioma	56.9427	0.5067	**53.9151**	**0.9999**

In 6/7 datasets, the dichotomous Hill model fit the data better based on the Chi-square p-value. In 4/7 datasets, the dichotomous Hill model showed a combination of greater parsimony and better fit based on the AIC.

Discussion

In the public summary of their report, the NRC concluded "that EPA's decision to rely solely on a default linear model lacked adequate scientific support."[10] The animal dose-response evaluations in Table 1 suggest that the dose response for hepatocarcinogenesis in animals is indeed nonlinear.

Recently, a nonlinear evaluation of hepatocarcinogenesis in rats and development of a reference dose (RfD) for cancer followed EPA's methodology.[14] Regarding the likely MOA for TCDD-induced hepatocarcinogenesis, the underlying key events consist of AHR activation, the early clonal growth of altered hepatic foci, inhibition of intrafocal apoptosis and increased proliferation of altered cells.[15-21] Increased cell division within foci is likely also fostered by toxic hepatopathy.[22,23] Toxic hepatopathy appeared to be necessary for tumor occurrence, and it is likely that sustained liver injury is necessary for hepatocarcinogenesis.[14,24,25]

TCDD is not a direct-acting mutagen or tumor initiator. Therefore, it is appropriate to apply nonlinear extrapolation to cancer risk assessment of TCDD and develop a threshold toxicity RfD for cancer. One could also use a key event in the MOA to develop a nonlinear toxicity criterion.

For example, toxic hepatopathy is a more sensitive response than liver cancer and necessary for tumor formation.[14] Evidence of elevated liver enzymes in humans was observed in both the Seveso and NIOSH cohorts.[26,27] While a rigorous analysis of human relevance of these clinical findings of liver injury, using the key events framework has not yet been conducted for TCDD, the possible concordance of affected organ provides some confidence that toxic hepatopathy is an appropriate key event upon which to base an RfD.[28-33]

A body burden of 48 ng/kg corresponds to the rat $BMDL_{01}$ for toxic hepatopathy.[14] In humans, a body burden of 48 ng/kg corresponds to an intake of 0.022 ng/kg/d based on the Response Surface for the Emond model.[9] If one applies an extrapolation factor of 3 for interspecies toxicodynamic differences and another factor of 3 for intraspecies toxicokinetic differences, the resulting RfD will be 2.2 pg/kg/d, virtually identical to the WHO-TDI of 2.3 pg/kg/d. [34]

For all chemicals, not just TCDD, the use of linear low dose extrapolation for all chemicals is inconsistent with the body of biological knowledge about the maintenance of homeostasis, the principles of physical chemistry of reactions of xenobiotics with biological molecules, and the growing body of dose response data using newer high throughput data and a systems biology conceptual approach. Indeed, the majority of biological data on dose-response suggests that most chemicals, including genotoxic carcinogens, exhibit a dose threshold.[35]

Continued existence for any organism is a matter of maintaining homeostasis in the face of the stress of life. Organisms have the capacities and redundant systems to deal with a variety of stressors, but these capacities and redundancies are finite. This is the basis of biological thresholds.

It remains unclear why the Reanalysis document did not include a development of nonlinear toxicity criteria from animal data consistent with the recommendations of the NRC? The scientific consensus regarding the MOA of TCDD carcinogenesis in animals suggests that a nonlinear toxicity criterion is indeed appropriate.

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