

**RISK ASSESSMENT OF 2,3,7,8-TCDD  
USING A BIOLOGICALLY-BASED CANCER MODEL:  
A RE-EVALUATION OF THE KOCIBA ET AL. (1978) BIOASSAY**

Maxwell W. Layard,<sup>1</sup>  
Dennis J. Paustenbach,<sup>2</sup>  
Richard J. Wenning,<sup>3</sup> and  
Russell E. Keenan<sup>3</sup>

<sup>1</sup> Layard Associates, 2242 San Antonio Avenue, Alameda, California 94501, U.S.A.

<sup>2</sup> ChemRisk™ - A Division of McLaren, 1135 Atlantic Avenue, Alameda, California 94501, U.S.A.

<sup>3</sup> ChemRisk™ - A Division of McLaren, 1685 Congress Street, Portland, Maine 04102, U.S.A.

**ABSTRACT**

The Moolgavkar-Knudson-Venzon (M-K-V) two-stage model for carcinogenesis was used to predict the risk-specific dose (RsD) based on the incidence of tumors reported by Kociba et al. (1978) for Sprague-Dawley rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). In addition, the results from the recently completed histopathology re-evaluation of the same study by an independent Pathology Working Group (PWG, 1990), using current National Toxicology Program (NTP) pathology criteria, were also evaluated using the M-K-V model. Preliminary estimates of the RsD at a  $1 \times 10^{-6}$  risk level based on the 1978 histopathology results were 10 fg/kg/day if carcinomas and hyperplastic nodules were combined and 150 fg/kg/day if only carcinomas were considered. In contrast, RsDs based on the histopathology re-examination using current pathology criteria were 80 fg/kg/day when adenomas and carcinomas were combined and 400 fg/kg/day if only hepatic carcinomas were considered. Since the M-K-V model is intended only to be used for malignant tumors, the most appropriate RsD is 400 fg/kg/day ( $10^{-6}$  risk). This value is approximately 60-fold greater than USEPA's RsD ( $10^{-6}$  risk) of 6.4 fg/kg/day. In light of the more biologically relevant basis of the M-K-V model, these results can be expected to be more valid than those derived from statistically based models.

**KEY WORDS**

2,3,7,8-TCDD, dioxin, M-K-V model, cancer risk, biologically-based model

## INTRODUCTION

There has been considerable recent interest in the use of stochastic two-stage biologically-based models of carcinogenesis such as that developed by Moolgavkar, Knudson, and Venzon, (M-K-V model) (Moolgavkar and Knudson, 1981; Moolgavkar, 1986; Moolgavkar et al., 1988) for estimating the cancer risk posed by low level exposure to environmental chemicals. The advantages of using biologically-based models over more conventional statistically-based models include the incorporation of important concepts in the cancer process and the use of model parameters that are biologically relevant. Although the linearized multistage (LMS) model, which has traditionally been used in cancer risk assessments for regulatory purposes, is conceptually based on a multi-step theory of carcinogenesis, the M-K-V model has the advantage that its form is not dependent on the dose-response data.

The U.S. Environmental Protection Agency (USEPA), as well its Science Advisory Board (SAB), have expressed interest in the development of the M-K-V model to describe the carcinogenic behavior of 2,3,7,8-TCDD in experimental animals and in humans (Loehr, 1989). Although the M-K-V model was applied to 2,3,7,8-TCDD by Thorlund (1988), the analysis contained several shortcomings and received only limited critical review. This paper incorporates information that was not available in 1987 and is markedly different from the previous effort.

The recent re-evaluation of the histopathology slides of the hepatic lesions from the Kociba et al. (1978) bioassay by an independent group of pathologists (PWG, 1990) prompted a re-examination of the M-K-V model to describe these data. The PWG's analysis was based on the National Toxicology Program's current criteria for evaluating hepatic lesions in rodents which are appreciably different than the criteria used in the 1970's (Keenan et al., 1990a).

In this paper, we report the results of an analysis of 2,3,7,8-TCDD-induced hepatocellular lesions in female Sprague-Dawley rats using the M-K-V model and the tumor incidence data from Kociba et al. (1978) and from the results of the histopathology reanalysis. Risk estimates were compared to estimates obtained using the conventional linearized multistage model (Keenan et al., 1990b).

## METHODS

The biological basis of the two-stage M-K-V model has been extensively discussed elsewhere (Moolgavkar and Knudson, 1981; Moolgavkar, 1986; Moolgavkar et al., 1988). Briefly, the crucial features of the model are that it can accommodate: a) the transition of target stem cells into cancer cells via an intermediate stage in two rate-limiting, irreversible, and hereditary (at the level of the cell) steps; and, b) growth and differentiation of normal target and intermediate cells.

The M-K-V model was used to describe the liver tumorigenicity data from the two-year chronic toxicity and oncogenicity bioassay conducted by Kociba et al. (1978). In the Kociba study, 50 Sprague-Dawley (Spartan substrain) rats of each sex were maintained for up to 24 months on diets

containing 1,000, 10,000, or 100,000 µg/kg/day of 2,3,7,8-TCDD; an additional 86 animals of each sex were maintained as study controls. The following information from Kociba et al. (1978) and PWG (1990) were available for each animal: the exact date of death; the presence or absence of hepatocellular carcinoma, hyperplastic nodule (Kociba et al., 1978), or adenoma (PWG, 1990); and, the age at death or sacrifice. Both malignant tumors (hepatocellular carcinomas) and benign lesions (hyperplastic nodules or adenomas) were considered in these analyses.

In the context of the M-K-V model, the hazard, or incidence, function at time  $t$ , denoted by  $h(t)$ , is the instantaneous rate of appearance of malignant tumors. The incidence function predicted by the model is

$$h(t) = \mu(t)E[Y(t)|Z(t)=0] \quad (1),$$

where  $Y(t)$  and  $Z(t)$  represent the number of intermediate (pre-malignant) and malignant cells, respectively, at time  $t$ ,  $\mu(t)$  represents the second event (mutation) rate, and  $E$  is the conditional expectation of  $Y(t)$  given  $Z(t) = 0$ . In fitting the model to bioassay data, several parameters of the incidence function are estimated, including parameters reflecting the dependence of transition and growth rates on the dose of carcinogen (Moolgavkar et al., 1988).

The probability that a malignant cell is generated by time  $t$  is

$$P(t) = 1 - \exp(-\int_0^t h(s)ds) \quad (2).$$

Since the liver tumors observed by Kociba et al. (1978) and the PWG (1990) were considered incidental (non-fatal), the contribution to the likelihood function, used in maximum likelihood estimation of the model parameters, for an animal that died at time  $t$  is  $P(t)$  if the animal had a tumor, or  $(1-P(t))$  if it was free of tumors. The maximum likelihood estimates of the parameters were computed using the computer program GENSTAT 5 (Release 1.3, 1990; Numerical Algorithm Group, Downers Grove, IL).

## RESULTS AND DISCUSSION

Preliminary estimates of the RsD ( $1 \times 10^{-6}$  risk) based on the tumor incidence data reported by Kociba et al. (1978) and by the PWG (1990) in female Sprague-Dawley rats are presented in Table 1. Using the data reported in 1978, the RsD ( $10^{-6}$ ) based on hepatocellular carcinomas was estimated to be 150 fg/kg/day. When the combined incidence of hepatocellular carcinomas and hyperplastic nodules was considered, the RsD ( $10^{-6}$ ) was approximately 10 fg/kg/day. Higher RsDs were calculated using the tumor incidence data from the recent re-evaluation of the histopathology slides. The RsD ( $10^{-6}$ ) was estimated to be 80 fg/kg/day when adenomas and hepatocellular carcinomas were combined and 400 fg/kg/day when only hepatocellular carcinomas were considered.

Since the M-K-V model is intended only to be used for malignant tumors (it quantitatively allows for pre-cancerous lesions), the most appropriate R<sub>sD</sub> (10<sup>-6</sup>) for 2,3,7,8-TCDD is 400 fg/kg/day. This value is approximately 60-fold greater than the USEPA's current R<sub>sD</sub> (10<sup>-6</sup>) of 6.4 fg/kg/day based on the LMS model and survival-adjusted tumor incidence data from Kociba et al (1978). However, this value is well below the range of allowable daily intakes (ADIs) established by a number of regulatory agencies in Western Europe and North America. Because 2,3,7,8-TCDD is not genotoxic, ADIs ranging from 1,000 to 10,000 fg/kg/day have been developed based on the application of a safety factor to either the NOAEL (no-observable-adverse-effect-level) or the LOAEL (lowest-observable-adverse-effect-level) of exposure in rodents (Keenan et al., 1990a).

Parameter estimates obtained from fitting the M-K-V model to these data suggest that 2,3,7,8-TCDD may have little effect on the intermediate cell net growth rate, and that the effect of 2,3,7,8-TCDD on the first and second stage transition rates may be dictated by just one of those rates. Since it is not possible from the Kociba lifetime feeding experiment to identify which of the two transition rates of the model is most affected by the presence of the chemical, a careful analysis of promotion studies involving TCDD is warranted. Further, the proliferation of pre-malignant lesions observed in the bioassay suggests that the first transition rate is dependent on the dose of 2,3,7,8-TCDD. In the absence of better data on dose-related effects on cell transition and growth rates, we plan to conduct a sensitivity analysis to approximate the upper and lower bounds of each model parameter and to examine the impact on the risk estimates. The range of plausible results, however, is constrained by the fit of the dose-response curve to the bioassay data.

## REFERENCES

- Keenan, R.E., Wenning, R.J., Parsons, A.H., and Paustenbach, D.J. (1990a). A reevaluation of the cancer potency of 2,3,7,8-TCDD and suggested exposure levels. *Fund. Appl. Toxicol.* (submitted).
- Keenan, R.E., Wenning, R.J., Parsons, A.H., and Paustenbach, D.J. (1990b). A re-evaluation of the tumor histopathology of Kociba et al. (1978) using 1990 criteria: Implications for the risk assessment of 2,3,7,8-TCDD using the linearized multistage model (abstract submitted to Dioxin'90 Conference)
- Kociba, R.J., Keyes, D.G., Beyer, J.E., Carreon, R.M., Wade, C.E., Dittenber, D.A., Kalnins, R.P., Frauson, L.E., Park, C.N., Barnard, S.D., Hummel, R.A., and Humiston, C.G. (1978). Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in rats. *Toxicol. Appl. Pharmacol.* 46:279-303.
- Loehr, R.C. (1989). Letter to William Reilly, Administrator, USEPA from Raymond C. Loehr, Chair, Executive Committee, USEPA Science Advisory Board. November 28.
- Moolgavkar, S.H. and Knudson, A.G. (1981). Mutation and cancer: a model for human carcinogenesis. *J. Natl. Cancer Inst.* 66:1,037-1,052.
- Moolgavkar, S.H. (1986). Carcinogenesis modeling: from molecular biology to epidemiology. *Ann. Rev. Public Health* 7:151-169.

Moolgavkar, S.H., Dewanji, A, and Venzon, D.J. (1988). A stochastic two-stage model for cancer risk assessment. I. The hazard function and the probability of tumor. *Risk Anal.* 8:383-392.

Pathology Working Group (PWG) (1990). *Pathology Working Group Report on 2,3,7,8-Tetrachlorodibenzo-p-dioxin Chronic Toxicity / Carcinogenicity Study in Sprague-Dawley Rats*. Prepared by R.M. Sauer, PWG Chairperson, PATHCO, INC. and submitted to R.A. Michaels, Chairperson, Maine Scientific Advisory Panel. March 13.

Thorslund, T.W. (1988). Quantitative dose-response model for the tumor promoting activity of TCDD. In: *A Cancer Risk-Specific Dose Estimate for 2,3,7,8-TCDD. Review Draft*. Appendix B. EPA/600/6-88/007 Aa and Ab. Office of Health and Environmental Assessment, Washington, D.C.

**Table 1. Hepatic Tumor Incidence in Female Sprague-Dawley Rats Observed by Kociba et al. (1978) and PWG (1990) and Preliminary Risk-specific Dose (RsD) Estimates from the M-K-V Model.**

Treatment Dose (ug/kg/day)	Hepatic Tumor Incidence			
	Kociba et al. (1978)		PWG (1990)	
	Hepatocellular carcinoma	Hepatocellular carcinoma & hyperplastic nodules	Hepatocellular carcinoma	Hepatocellular carcinoma & adenomas
0	1/86 (1%)	9/86 (10%)	0/86 (0%)	2/86 (2%)
0.001	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
0.01	2/50 (4%)	18/50 (36%)	0/50 (0%)	9/50 (18%)
0.1	11/48 (23%)	34/48 (71%)	4/45 (9%)	18/45 (40%)
R <sub>sD</sub> at 1 x 10 <sup>-6</sup> risk level (fg/kg-day)	150	10	400	80