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

Russell E. Keenan,[1]
Richard J. Wenning,[1]
Alan H. Parsons,[1] and
Dennis J. Paustenbach [2]

[1] ChemRisk™ - A Division of McLaren, 1685 Congress Street, Portland, Maine 04102, U.S.A.

[2] ChemRisk™ - A Division of McLaren, 1135 Atlantic Avenue, Alameda, California 94501, U.S.A.

ABSTRACT

A better understanding of the mechanism of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), improvements in the histopathological interpretation of liver lesions, and improved risk assessment techniques warrant a re-analysis of the Kociba et al. (1978) bioassay. Because the histopathological criteria for liver lesions are different in 1990 than in 1978, a re-evaluation of the liver slides was conducted by an independent panel of pathologists. In contrast to previous findings, about 2/3 fewer tumors (using current NTP criteria) were observed in the livers of female Sprague-Dawley rats. The NOAEL for hepatocellular carcinomas was 0.01 μg/kg/day, rather than 0.001 μg/kg/day which had been reported in 1978. The weight-of-evidence suggests that 2,3,7,8-TCDD (not its metabolite) is the biologically active species and, as such, body weight rather than surface area is the most appropriate means to scale-up rodent data to predict the human response. Further, the nature of the lesions observed in the liver suggest that the dose-response analysis should be based on the incidence of hepatocellular carcinomas rather than the combined incidence of carcinomas and adenomas. Risk-specific-Doses (RsDs) were extrapolated on the basis of the revised, and survival-adjusted, tumor incidence data using the linearized multistage (LMS) model. Based on the survival-adjusted data, RsD estimates at a 1×10^{-6} risk level were 370 fg/kg/day for carcinomas only and 100 fg/kg/day for adenomas and carcinomas combined. The corresponding CPFs were 2,700 and 9,700 $(mg/kg\text{-}day)^{-1}$, respectively. These results suggest that the carcinogenic potency of 2,3,7,8-TCDD is at least 16-fold less than had been previously reported.

KEY WORDS

2,3,7,8-TCDD, cancer risk, dioxin, risk assessment, Kociba bioassay, linearized multistage model

INTRODUCTION

A number of regulatory agencies throughout the world have evaluated the potential hazards and, in particular, the carcinogenic risk posed by exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). In the absence of adequate dose-response data from epidemiologic studies, virtually all estimates of the risk to human health have been based on extrapolations from a 2-year chronic toxicity and oncogenicity study of Sprague-Dawley rats (Kociba et al., 1978). Numerous approaches have been used to evaluate the dose-response curve from this bioassay (Portier, 1984; Paustenbach et al., 1986; Sielken, 1987; Keenan et al., 1989; 1990a).

In the United States, Kociba et al. (1978) is cited by the U.S. Environmental Protection Agency (USEPA), the Centers for Disease Control (CDC), and the U.S. Food and Drug Administration (FDA) as the primary evidence supporting the carcinogenicity of 2,3,7,8-TCDD. Studies conducted by the National Toxicology Program (NTP) in 1982 have been cited by the USEPA as additional evidence supporting the findings of the Kociba et al. (1978) bioassay, confirming 2,3,7,8-TCDD's carcinogenic potential. Each of these agencies has derived cancer potency factors (CPF) and corresponding risk-specific dose (RsD) estimates on the basis of different interpretations of the tumor incidence rate observed in the bioassay using, as a matter of policy, the linearized multistage (LMS) model or another nonthreshold model for extrapolating low-doses.

Because the incidences of hepatocellular carcinomas and adenomas are the most sensitive adverse effect in the Kociba study, the appropriate histopathological classification of these lesions is critical. In 1990, a re-evaluation of the Kociba et al. (1978) histopathology was conducted by an independent Pathology Working Group (PWG) using the current NTP classification scheme for proliferative lesions in the rat liver (Maronpot et al., 1986; McConnell et al., 1988). This protocol or criterion is considerably different than the classification scheme of Squire and Leavitt (1975) used by Kociba in 1978 and Squire in 1980 in their diagnoses of pre-malignant "neoplastic nodules". Using the current NTP guidelines, pathologists now distinguish between hyperplasia, a non-neoplastic response to degenerative changes in the liver, and an adenoma, a benign condition involving clear differentiation of cells from the surrounding tissue (Maronpot et al., 1986).

Using the current NTP guidelines, the number and distribution of hepatic lesions observed by the PWG (1990) were markedly different from that originally reported (Table 1). They found that, due to a change in the classification criteria, there were substantially fewer cancerous tumors (about 2/3 less) observed in the study than previously believed. The lesions previously referred to as "hyperplastic nodules" or "neoplastic nodules" were predominantly benign and classified as lesions due to overt hepatic toxicity. Unlike previous reviews of the slides, they were read by the PWG pathologists without their prior knowledge of the dose groups from which the animals were drawn.

In this paper, we compare and contrast risk estimates derived from the application of the LMS model to the liver tumor incidences reported by Kociba et al. (1978) and by the PWG (1990). To evaluate the differences in the approach to selecting risk estimates, the following underlying assumptions were examined: a) significance of early mortality in the bioassay; b) interspecies extrapolation procedures; c) appropriateness of combining tumors at multiple organ sites; and d) classification of rat proliferative hepatocellular lesions.

METHODS

In order to compare with prior regulatory values, RsDs and CPFs were determined using the Global'86 LMS Model (Howe et al., 1986) which assumes no-threshold and low-dose linearity. Data were fitted to a conventional 3-stage model (one less than the number of dose groups in the bioassay). It should be noted, however, that both the USEPA Dioxin Task Force and the USEPA Science Advisory Board have acknowledged that this model is probably not appropriate for estimating the risks of low level exposure to 2,3,7,8-TCDD. RsDs and CPFs were developed by: 1) extrapolating from rats to humans using a body weight correction factor; 2) presenting the 95% lower confidence limit (LCL) and the maximum likelihood estimate (MLE) from the LMS model; 3) considering the combined incidences of hepatocellular carcinomas and adenomas or the incidence of carcinomas alone; and, 4) adjusting for early mortality.

RESULTS AND DISCUSSION

Risk estimates for 2,3,7,8-TCDD based on the overall tumor incidence rates reported by Kociba et al. (1978) and by the PWG (1990) are presented in Table 2. Based on the overall incidence of hepatocellular carcinomas reported by Kociba et al. (1978) and scaling up to humans by body weight rather than by surface area, the RsD (1×10^{-6} risk) was 250 fg/kg/day at a 95% LCL. The MLE of risk (10^{-6}) was 400 fg/kg/day and the corresponding CPF was 3,900 $(mg/kg\text{-}day)^{-1}$. Risk estimates based on the combined tumor incidence data do not appear appropriate because of ambiguity regarding lesions diagnosed as neoplastic nodules under the Squire and Leavitt (1975) tumor classification scheme (Maronpot et al., 1986). Risk estimates derived from the Kociba et al. (1978) bioassay should be based only on the incidence of hepatocellular carcinomas.

Using the PWG (1990) tumor incidence data, the RsD (10^{-6}) was 670 fg/kg/day (based on the overall incidence of hepatocellular carcinomas, a 95% LCL, and a body weight scaling factor) which represents about a 3-fold reduction in risk than using the original Kociba results. The MLE of risk (10^{-6}) was 480,000 fg/kg/day and the corresponding CPF was 1,500 $(mg/kg\text{-}day)^{-1}$. When the combined incidence of hepatocellular carcinomas and adenomas was considered in the LMS model, the comparable RsD (10^{-6}) was 120 fg/kg/day, the MLE of risk (10^{-6}) was 180 fg/kg/day, and the corresponding CPF was 8,200 $(mg/kg\text{-}day)^{-1}$.

Survival-adjusted tumor incidence rates among treated and untreated animals were considered by the USEPA (1985) to be the appropriate basis for calculating risk estimates. They corrected for the high mortality observed in the high and mid-dose groups by censuring all animals that died prior to the appearance of the first "neoplastic nodule". Similar corrections, therefore, were used in this analysis. Risk estimates based on the survival-adjusted data sets are presented in Table 3.

Using the PWG (1990) survival-adjusted tumor incidence data, the RsD (10^{-6}) was 370 fg/kg/day (based on hepatocellular carcinomas, a 95% LCL, and a body weight scaling factor). The MLE of risk (10^{-6}) was 370,000 fg/kg/day and the corresponding CPF was 2,700 $(mg/kg\text{-}day)^{-1}$. Risk estimates based on the combined tumor incidence rate were less affected by the survival-adjustment procedure than those based on the incidence of carcinomas only. Since the first adenomas appeared earlier in the study (at week 55) than did the first carcinomas (at week 81), fewer animals were censured from the combined tumor analysis. The comparable RsD (10^{-6}) considering the combined

incidence of hepatocellular carcinomas and adenomas was 100 fg/kg/day. The MLE of risk (10^{-6}) was 150 fg/kg/day and the corresponding CPF was 9,700 $(ng/kg\text{-}day)^{-1}$. These results indicate at least a 16-fold difference between risk estimates based on the survival-adjusted, combined incidences of adenomas and carcinomas reported by the PWG (1990) and those calculated by the USEPA (1985) (6.4 fg/kg/day at 1 x 10^{-6} risk).

Until the USEPA adopts the results of a biologically-based cancer model, it is likely that the agency will continue to rely on the LMS model to assess the carcinogenicity of 2,3,7,8-TCDD. In light of that constraint, regulatory policy may necessitate that the dose-response behavior of 2,3,7,8-TCDD in rodents be extrapolated to humans based on the combined incidence of hepatocellular carcinomas and adenomas (as described by the PWG in their recent report). From this, it would appear that using current EPA policy guidelines, the most appropriate RsD (10^{-6}), for TCDD is 100 fg/kg/day. While this figure is greater than the RsD (10^{-6}) of 86 fg/kg/day for combined liver lesions in the male B6C3F1 mouse (Kimbrough et al., 1984), a number of issues must be considered, including the high background rate of liver tumors in mice and the relevance of this species as a model to accurately predict the human cancer response, before the NTP study should be considered a more appropriate data set than Kociba et al (1978).

REFERENCES

Howe, R.B., Crump, K.S., and Landingham, C.V. (1986). *Global'86. A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses*. K.S. Crump and Company, Inc., Ruston, LA.

Keenan, R.E., Wenning, R.J., and Parsons, A.H. (1989). *Sensitivity Analysis for Dioxin Risk Assessment and Implications for Determining Acceptable Levels of Daily Exposure*. Submitted to State of Maine Department of Health Services, Scientific Advisory Panel, Augusta, Maine. November 28.

Keenan, R.E., Wenning, R.J., Parsons, A.H., and Paustenbach, D.J. (1990a). A re-evaluation of the cancer potency of 2,3,7,8-TCDD and suggested exposure levels. *Fund. Appl. Toxicol.* (submitted).

Kimbrough, R.D., Falk, H., and Stehr, P. (1984). Health implications of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) contamination of residential soil. *J. Toxicol. Environ. Health 14*:47-93.

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.

Layard, M., Paustenbach, D.J., Wenning, R.J., and Keenan, R.E. (1990b). Risk assessment of 2,3,7,8-TCDD using a biologically-based cancer model: A re-evaluation of the Kociba et al. (1978) bioassay (abstract submitted to Dioxin'90 Conference).

Maronpot, R.R., Montgomery, C.A., Boorman, G.A., and McConnell, E.E. (1986). National Toxicology Program nomenclature for hepatoproliferative lesions of rats. *Toxicol. Pathol. 14*:263-273.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1988). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. In: *Carcinogenicity, The Design, Analysis, and Interpretation of Long-term Animal Studies*. (H.C. Grice and J.L. Cimineri, Eds.) pp. 183-196. ILSI Monographs. Springer-Verlag, New York, NY.

National Toxicology Program (NTP) (1982). *Carcinogenesis Bioassay of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (CAS No. 1746-01-6) in Osborne-Mendel Rats and B6C3F1 Mice (Gavage Study)*. Technical Report Series No. 209. NIEHS, NC.

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.

Paustenbach, D.J., Shu, H.P., and Murray, F.J. (1986). A critical examination of assumptions used in risk assessments of dioxin contaminated soil. *Reg. Toxicol. Pharmacol. 6*:284-307.

Portier, C.J., Hoel, D.G., and Ryzin, J.V. (1984). Statistical analysis of the carcinogenesis bioassay data relating to the risks from exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. In: *Public Health Risks of the Dioxins.* (W.W. Lowrance, Ed.) pp. 99-120. William Kaufmann, Los Altos, CA.

Sielken, R.L. (1987). Quantitative cancer risk assessments for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Fd. Chem. Toxicol. 25*:257-267.

Squire, R.A. and Levitt, M.H. (1975). Report of a workshop on classification of specific hepatocellular lesions in rats. *Cancer Res. 35*:3,214-3,223.

U.S. Environmental Protection Agency (USEPA) (1985). *Health Assessment Document for Polychlorinated Dibenzo-p-Dioxins.* EPA/600/8-84/014F. Office of Health and Environmental Assessment, Washington, D.C.

Table I. Histopathological Interpretation by PWG (1990) and Kociba et al. (1978) of Hepatic Lesions in Female Sprague-Dawley Rats Exposed to 2,3,7,8-TCDD in the Kociba et al. (1978) Bioassay.

Treatment Dose (μg/kg/day)	0	0.001	0.01	0.1
Kociba et al. (1978)				
Hepatocellular hyperplastic nodules	8/86 (9%)[a]	3/50 (6%)	18/50 (36%)*	23/48 (48%)*
Hepatocellular carcinoma	1/86 (1%)	0/50 (0%)	2/50 (4%)	11/48 (23%)*
Total combined incidence	9/86 (10%)	3/50 (6%)	18/50 (36%)*	34/48 (23%)*
PWG (1990)				
Hepatocellular carcinoma	0/86 (0%)	0/50 (0%)	0/50 (0%)	4/45 (9%)*
Hepatocellular adenoma	2/86 (2%)	1/50 (2%)	9/50 (18%)*	14/45 (31%)*
Total combined incidence	2/86 (2%)	1/50 (2%)	9/50 (18%)*	18/45 (40%)*

Source: Kociba et al. (1978) and PWG (1990).
* Denotes a statistically significant difference ($p<0.05$) from control by the Fischer Exact Test.
a. Number of responses/number of animals examined (percent response).

Table 2. Plausible Alternative Risk-specific Doses (RsDs) and Cancer Potency Factors (CPF) for 2,3,7,8-TCDD Based on the Overall Incidences of Hepatic Lesions Observed by PWG (1990) and Kociba et al. (1978).

	Kociba et al. (1978)		PWG (1990)	
Set of Assumptions	RsD at 1 x 10^{-6} Incremental Carcinogenic Risk (fg/kg-day)	CPF $(mg/kg\text{-}day)^{-1}$	RsD at 1 x 10^{-6} Incremental Carcinogenic Risk (fg/kg-day)	CPF $(mg/kg\text{-}day)^{-1}$
Body Weight + 95% LCL[a] (MLE)[b] + Total Hepatic Lesions	57 (77)	17,500	120 (180)	8,200
Body Weight + 95% LCL (MLE) + Hepatocellular Carcinomas	250 (400)	3,900	670 (480,000)	1,500

a. The 95% lower confidence limit (LCL) is that dose for which there is a 95% statistical confidence that the true RsD is no lower than this value.
b. Maximum likelihood estimate.

Table 3. Plausible Alternative Risk-specific Doses (RsDs) and Cancer Potency Factors (CPF) for 2,3,7,8-TCDD Based on the Survival-Adjusted Incidences of Hepatic Lesions Observed by PWG (1990) and Kociba et al. (1978).

	Kociba et al. (1978)		PWG (1990)	
Set of Assumptions	RsD at 1 x 10^{-6} Incremental Carcinogenic Risk (fg/kg-day)	CPF $(mg/kg\text{-}day)^{-1}$	RsD at 1 x 10^{-6} Incremental Carcinogenic Risk (fg/kg-day)	CPF $(mg/kg\text{-}day)^{-1}$
Body Weight + 95% LCL[a] (MLE)[b] + Total Hepatic Lesions	35 (48)	28,200	100 (150)	9,700
Body Weight + 95% LCL (MLE) + Hepatocellular Carcinoma	130 (270)	7,700	370 (370,000)	2,700

a. The 95% lower confidence limit (LCL) is that dose for which there is a 95% statistical confidence that the true RsD is no lower than this value.
b. Maximum likelihood estimate.