

**NO SIGNIFICANT RISK LEVEL (NSRL)
FOR THE PROPOSITION 65 CARCINOGEN
MX
(3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE)**

May 2001

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency

SUMMARY OF FINDINGS

The cancer potency of MX ("mutagen X"; 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) was estimated from dose-response data of multiple MX-responding tumor sites observed among male and female rats exposed orally (Komulainen *et al.*, 1997; 2000). These sites were liver, adrenal and thyroid in both sexes and mammary gland in the female. Other sites were associated with treatment, but were judged likely to contribute only minimally to the overall potency estimate, and thus were not included in the analyses. For each of the tumor sites listed above, a probability distribution of cancer potency estimates was derived using likelihood theory. The linear term (q_1) of the multistage model fit to dose response data for a given site represents the cancer potency for that site. A combined distribution representing cancer potency for all selected sites affected by MX was derived through Monte Carlo analysis. The upper 95 percent confidence bound indicated by the combined distribution for these MX-related tumor sites was taken as the cancer potency for MX. The potency derivation takes into account body size differences between humans and experimental animals. The Proposition 65 "no significant risk level" (NSRL) is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. The cancer potency estimate and corresponding NSRL are given in Table 1.

Table 1. Cancer Potency and NSRL for MX

Chemical	Cancer Potency (mg/kg-day) ⁻¹	NSRL (µg/day)
<i>MX</i>	6.37	0.11

INTRODUCTION

This report describes the derivation of a cancer potency value and NSRL for MX ("Mutagen X", CAS number 77439-76-0, molecular weight 217.4). "MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone)" was listed on December 22, 2000 as known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq.*). MX is a chlorination disinfection byproduct, which forms from the reaction of chlorine with humic acids in raw water. MX has been measured in drinking water samples in the United States and several other countries. Levels detected in drinking water were low, ranging from 2 to 67 ng/L (reviewed in OEHHA, 2000).

This document discusses the studies available for cancer dose response assessment, and summarizes the deviations of the cancer potency estimates and NSRLs. A description of the methodology used is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

The carcinogenicity of MX was investigated in one series of studies (Komulainen *et al.*, 1997; 2000). Male and female Wistar rats (50 animals per dose group, five weeks of age) were administered MX in their drinking water at mean concentrations of 0, 5.9, 18.7 or 70.0 µg/mL for 104 weeks. The authors reported that the water concentrations and consumption rates resulted in average daily doses of 0, 0.4, 1.3 or 5.0 mg/kg body weight for male rats and 0, 0.6, 1.9 or 6.6 mg/kg body weight for female rats.

MX induced cancer at multiple sites in male and female rats (Komulainen *et al.*, 1997; 2000). Summaries of the tumor incidences are presented in Table 2 for male rats and Table 3 for female rats. In addition to the tumor findings presented in Tables 2 and 3, Komulainen *et al.* (1997) reported increases in tumor formation, significant by trend test, but not by pairwise comparisons between treated and control groups, for the skin basal cell tumors, alveolar and bronchiolar adenoma, pancreas Langerhans' cell adenoma and carcinoma (combined) and lymphoma and leukemia (combined) among male rats and lymphoma and leukemia (combined) among female rats.

Table 2. Tumors in male Wistar rats receiving MX in drinking water for 104 weeks (Komulainen *et al.*, 1997; 2000)

Tissue	MX, mg/kg-day				p-value (trend) ^d
	Control	0.4	1.3	5.0	
Liver					
carcinoma	0/50	0/50	2/50	1/50	0.2015
adenoma	0/50	1/50	2/50	4/50	0.0196
adenoma or carcinoma	0/50	1/50	3/50	5/50 ^a	0.0107
cholangioma	0/50	0/50	1/50	4/50	0.0054
Adrenal gland					
cortical adenoma	5/50	2/50	7/50	14/50 ^a	0.0006
Thyroid gland					
follicular carcinoma	0/49	1/50	9/50 ^b	27/49 ^c	<0.0001
follicular adenoma	2/49	20/50 ^c	34/50 ^c	21/49 ^c	0.0173
follicular adenoma or carcinoma	2/49	20/50 ^c	38/50 ^c	44/49 ^c	<0.0001

Bolding indicates datasets selected for potency estimation.

^a Significantly different from control animals by pairwise Fisher Exact Test, $p \leq 0.05$

^b Significantly different from control animals by pairwise Fisher Exact Test, $p \leq 0.01$

^c Significantly different from control animals by pairwise Fisher Exact Test, $p \leq 0.001$

^d Results of exact trend test (Cox, 1958)

Table 3. Tumors in female Wistar rats receiving MX in drinking water for 104 weeks (Komulainen *et al.*, 1997; 2000)

Tissue	MX, mg/kg-day				p-value (trend) ^d
	Control	0.6	1.9	6.6	
Mammary gland					
adenocarcinoma	3/50	2/50	5/50	11/50 ^a	0.0014
fibroadenoma	23/50	25/50	32/50	34/50 ^a	0.0118
adenoma	0/50	0/50	3/50	1/50	0.2758
adenoma or adenocarcinoma	3/50	2/50	7/50	12/50 ^a	0.0009
atypic hyperplasia	0/50	0/50	3/50	2/50	0.1103
atypic hyperplasia, adenoma, or adenocarcinoma	3/50	2/50	9/50	13/50 ^b	0.0006
Liver					
carcinoma	1/50	1/50	3/50	0/50	0.8054
adenoma	1/50	1/50	1/50	10/50 ^b	<0.0001
adenoma or carcinoma	2/50	2/50	4/50	10/50 ^a	0.0013
cholangiocarcinoma	1/50	0/50	0/50	2/50	0.1548
cholangioma	0/50	4/50	10/50 ^c	33/50 ^c	<0.0001
cholangioma or cholangiocarcinoma	1/50	4/50	10/50 ^b	34/50 ^c	<0.0001
Adrenal glands					
cortical adenoma	5/50	10/50	12/50	16/50 ^b	0.0091
Thyroid gland					
follicular carcinoma	1/50	3/49	6/50	22/50 ^c	<0.0001
follicular adenoma	4/50	16/49 ^b	36/50 ^c	36/50 ^c	<0.0001
follicular adenoma or carcinoma	5/50	18/49 ^b	38/50 ^c	47/50 ^c	<0.0001
C-cell carcinoma	0/50	0/49	0/50	1/50	0.2513
C-cell adenoma	11/50	11/49	10/50	16/50	0.0871

Bolding indicates datasets selected for potency estimation.

^a Significantly different from control animals by pairwise Fisher Exact Test, $p \leq 0.05$

^b Significantly different from control animals by pairwise Fisher Exact Test, $p \leq 0.01$

^c Significantly different from control animals by pairwise Fisher Exact Test, $p \leq 0.001$

^d Results of exact trend test (Cox, 1958)

APPROACH TO DOSE RESPONSE ANALYSIS

MX is a direct acting mutagen and clastogen (reviewed in OEHHA, 2000). MX caused mutations in numerous strains of bacteria. MX induced mutations, chromosomal aberrations, sister chromatid exchanges (SCEs), strand breaks, and unscheduled DNA synthesis in human and other mammalian cells *in vitro*. MX exhibited mixed results in *in vivo* genotoxicity studies,

following oral or intraperitoneal (i.p.) exposure of rodents to MX. Significant increases in strand breaks or alkali-labile sites, micronuclei or SCEs were observed in blood lymphocytes, kidney, stomach, jejunum, ileum, colon, duodenum, liver, lung, brain, spleen and bladder following oral or i.p. administration of MX to rodents. Several MX-derived DNA adducts have been characterized. Available evidence suggests that MX may cause mutations through DNA adduction and misrepair and through an unusual, thermodynamic mechanism in which MX ionizes DNA bases.

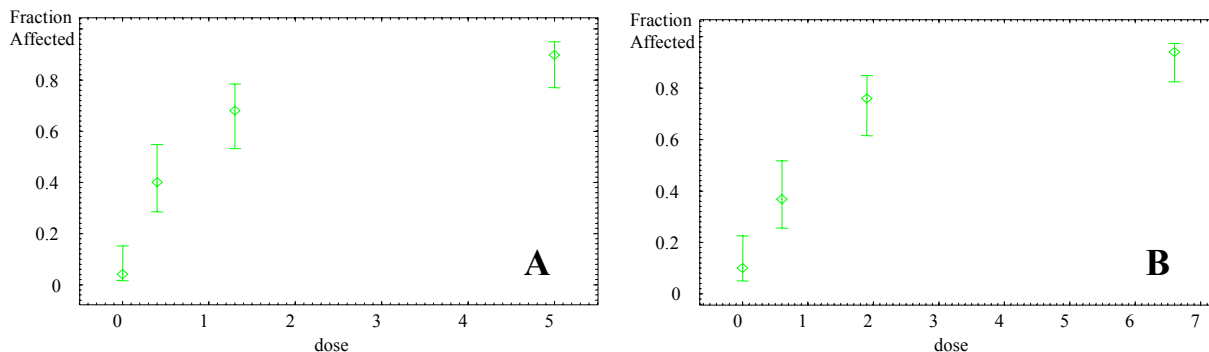
The available data suggest that MX causes cancer primarily through a genotoxic mode of action, although the precise mechanism of carcinogenesis is not known. The available evidence suggests that a proliferative mechanism involving thyroid hormone disruption is not operative in MX-induced carcinogenesis in rats (reviewed in OEHHA, 2000). MX was reported to induce cellular proliferation in the stomach of Wistar rats, a site at which tumors were not observed in carcinogenicity studies in the same rat strain. MX was also reported to act as a tumor promoter in an initiation/promotion study of the glandular stomach in Wistar rats (reviewed in OEHHA, 2000). However, since MX did not appear to increase the incidence of tumors at this site, the significance of this finding for dose response analysis and cancer potency estimation is unclear. There are also insufficient data to support dose adjustments based on pharmacokinetic models. Therefore, the default approach (i.e., a linearized multistage model and interspecies scaling) has been applied. The approach used is described in detail in the Appendix. Additionally, since MX induced tumors at multiple sites in both male and female rats, a combined cancer potency estimate was derived for the more sensitive MX-treatment related cancer sites judged likely to contribute to the overall cancer potency using Monte Carlo analysis (see below).

DOSE-RESPONSE ASSESSMENT

Cancer potency estimates were derived from sensitive tumor responses from the studies, as evident by pair-wise and trend tests (Tables 2 and 3). These include thyroid gland follicular cell adenoma and carcinoma (combined), adrenal gland adenoma, and liver adenoma and carcinoma (combined) among both male and female rats, as well as mammary gland atypic hyperplasia, adenoma and carcinoma (combined) and liver cholangioma and cholangiocarcinoma (combined) among female rats.

The shape of the dose-response curves for MX-induced thyroid tumors in males and females is supralinear, i.e., the trend in tumor incidence is less than linear with increasing doses (Figure 1). This may reflect non-linear pharmacokinetics, competing causes of death or other non-linear biological processes. Confidence intervals are sufficiently small that the data are not consistent with a linear relationship (Figure 1) and removal of the top dose group from the analysis significantly improved the chi square goodness of fit of the multistage model. Since a linear curvefit through all the data points would underestimate risks at low doses, cancer potencies were estimated from the thyroid tumor data for males and females, following removal of the top dose group from each dataset. The resultant cancer potency estimates are summarized in Table 4. Dose-response curves for sites other than the thyroid gland did not display any indications of supralinearity; thus, analyses for non-thyroid tumors included data from all dose groups. Time-dependent (survival) analysis, e.g., application of the multistage-Weibull model, was not conducted since most tumors were observed towards the end of the study or at autopsy.

Figure 1. Thyroid follicular cell adenoma and carcinoma (combined) among (A) male and (B) female Wistar rats (Komulainen *et al.*, 2000)



Since MX induced tumors at multiple sites in both male and female rats, a combined potency estimate for all treatment-related tumor sites was derived for each sex, using Monte Carlo analysis. For each tumor site, a distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term (q_1) of the multistage model was generated with the MSTAGE 2.01 computer program (created by Edmund Crouch), which had been modified to tabulate percentile values. A combined distribution (Figure 2) was created by adding q_1 for each tumor site, according to its distribution, through one million Monte Carlo trial simulations (Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound of the combined distribution was taken as the basis of the cancer potency estimate for the combined tumor sites (Table 4).

For males, distributions of the cancer potency estimates were combined for the following tumor sites: liver adenoma and carcinoma (combined), adrenal gland cortical adenoma, and thyroid follicular cell adenoma and carcinoma (combined, top dose group removed). For female rats, distributions of the cancer potency estimates were combined for the following tumor sites: mammary gland atypic hyperplasia, adenoma and adenocarcinoma (combined), liver adenoma and carcinoma (combined), liver cholangioma and cholangiocarcinoma (combined), adrenal gland cortical adenoma, and thyroid follicular cell adenoma and carcinoma (combined, top dose group removed) (Figure 2).

Figure 2. Combined distribution of potency estimates for all MX-related tumor sites among male rats (A) and female rats (B)

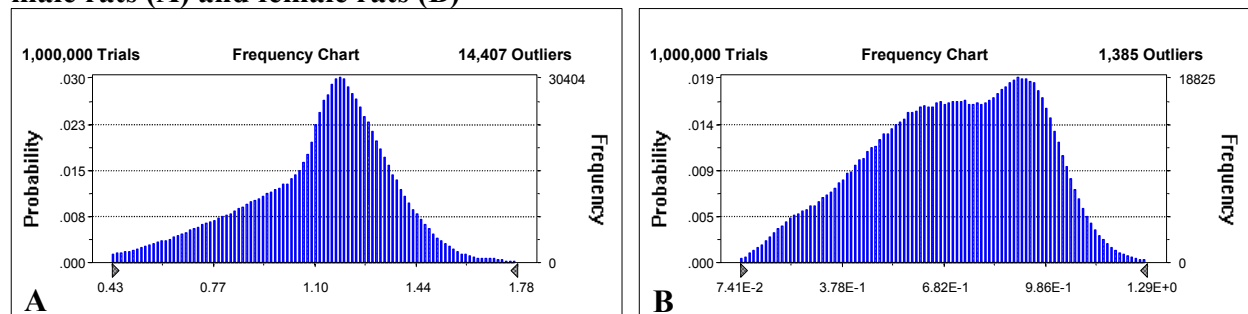


Table 4. Human cancer potency estimates for selected MX-induced tumors

Tumor site	Cancer Potency Estimate (mg/kg-day) ⁻¹	
	Males ¹	Females ¹
Thyroid follicular cell adenoma or carcinoma ^{2,3,4}	7.25	5.01
Adrenal gland cortical adenoma ^{3,4}	0.412	0.421
Mammary gland atypic hyperplasia, adenoma or adenocarcinoma ⁴	---	0.391
Liver adenoma or carcinoma ^{3,4} Liver cholangioma or cholangiocarcinoma ⁴	0.241 ---	0.273 1.01
All MX-related tumor sites	7.57	5.17

Bolding indicates values selected as the basis of the NSRL.

¹The authors reported average terminal (104 wk) body weights of the rats for each dose group, but did not provide average body weights for the duration of the experiment. Default values of 0.5 kg for male rats and 0.35 kg for female rats (Gold and Zeiger, 1997) were used for the inter-species extrapolation to human-equivalent potencies, since they appeared consistent with the 104 wk body weights (which averaged 0.59 kg for males and 0.37 kg for females across dose groups) (Komulainen *et al.* 1997).

²Top dose group removed from the analysis (see text).

³Distributions of q_1 combined using Monte Carlo analysis for males that were used in deriving the potency for "all MX-related tumor sites"

⁴Distributions of q_1 combined using Monte Carlo analysis for females that were used in deriving the potency for "all MX-related tumor sites"

Cancer potency estimates of 7.57 (mg/kg-day)⁻¹ for male rats and 5.17 (mg/kg-day)⁻¹ for females rats were derived from the combined distribution of cancer potency estimates for all MX-related

tumor sites (Komulainen *et al.*, 1997; 2000). Since both studies were of equal quality, and since the two potency estimates were not statistically different from one another, the potency estimates from the male and female rats were averaged to form a combined estimate of 6.37 (mg/kg-day)⁻¹.

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10⁻⁵. The combined cancer potency estimate for all MX-related tumor sites, 6.37 (mg/kg-day)⁻¹, derived above was used to calculate the NSRL for MX (0.11 µg/day). It should be noted that basing the NSRL estimate on the combined (averaged) cancer potency estimate for the most sensitive sex and site - thyroid follicular cell tumors in both sexes (6.13 [mg/kg-day]⁻¹) - yields the same value (0.11 µg/day).

REFERENCES

Cox DR (1958). The regression analysis of binary sequences. *J R Stat Soc B* **20**:215-242.

Gold LS, Zeiger E (1997). *Handbook of Carcinogenic Potency and Genotoxicity Databases*. CRC Press, Inc., Boca Raton.

Komulainen H, Kosma VM, Vaittinen SL, Vartiainen T, Kaliste-Korhonen E, Lotjonen S, Tuominen RK, Tuomisto J. (1997). Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone in the rat. *J Natl Cancer Inst* **89**(12):848-856.

Komulainen H, Kosma VM, Vaittinen SL, Vartiainen T, Kaliste-Korhonen E, Lotjonen S, Tuominen RK, Tuomisto J (2000). Carcinogenicity of 3-chloro-4-(dichloromethyl)-5-hydroxy(5H)-furanone (MX) in Wistar rats. Combined frequencies of indicated tumours. Personal communication from Dr. Hannu Komulainen, Laboratory of Toxicology, National Public Health Institute, Kuopio, Finland to Dr. Thomas McDonald, California Environmental Protection Agency, February 8, 2000.

Office of Environmental Health Hazard Assessment (OEHHA, 2000). *Evidence on the Carcinogenicity of MX (mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone)*. OEHHA, California Environmental Protection Agency, Oakland, California, December 2000.

APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE AN NSRL FOR MX

Procedures for the development of Proposition 65 NSRLs are described in regulation (California Code of Regulations, Title 22, Sections 12701 and 12703). Consistent with these procedures, the specific methods used to derive the NSRL for MX are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

"Multistage" polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (CDHS, 1985; U.S. EPA, 1996; Anderson *et al.*, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1d + q_2d^2 + \dots + q_id^i)] \quad (1)$$

with constraints,

$$q_i \geq 0 \text{ for all } i.$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the data. The parameter q_0 represents the background lifetime incidence of the tumor. The parameter q_1 , or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as q_1^* , the upper 95% confidence bound on q_1 (CDHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units of mg/kg-day, the parameters q_1 and q_1^* are given in units of $(\text{mg/kg-day})^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977). To estimate potency in animals (q_{animal}) from experiments of duration T_e , rather than the natural life span of the animals (T), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$q_{\text{animal}} = q_1^* \cdot (T/T_e)^3 \quad (2)$$

Following Gold and Zeiger (1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1988), the natural life span of mice and rats is assumed to be two years, so that for experiments lasting T_e weeks in these rodents:

$$q_{\text{animal}} = q_1^* \cdot (104/T_e)^3 \quad (3)$$

To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by the U.S. EPA (Anderson *et al.*, 1983) as "extra risk", and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

For MX, cancer potency is taken as the upper 95 percent confidence bound for the multiple sites affected by MX. The methods to estimate the combined distribution are described in the main text (pp. 5-8).

Calculation of the lifetime average dose

The study authors reported the lifetime average dose of MX for each of the relevant dose groups (Komulainen *et al.*, 1997). The authors reported that the drinking water concentrations of MX and consumption rates resulted in average daily doses of 0, 0.4, 1.3 or 5.0 mg/kg body weight for male rats and 0, 0.6, 1.9 or 6.6 mg/kg body weight for female rats.

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. As described in the California risk assessment guidelines (CDHS, 1985), a dose in units of milligram per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-third power when animal potency is expressed in units $(\text{mg}/\text{kg}\cdot\text{day})^{-1}$:

$$q_{\text{human}} = q_{\text{animal}} \cdot (bw_h / bw_a)^{1/3} \quad (4)$$

A.3 Risk-Specific Intake Level Calculation

The intake level (I , in mg/day) associated with a cancer risk R , from exposure is:

$$I = \frac{R \cdot bw_h}{q_{\text{human}}} \quad (5)$$

where bw_h is the body weight, and q_{human} the theoretical cancer potency estimate for humans.

Daily intake levels associated with lifetime cancer risks above 10^{-5} exceed the no significant risk level for cancer under Proposition 65 (Title 22 California Code of Regulations, Section 12703). Thus for a 70 kg person, the NSRL is given by:

$$\text{NSRL} = \frac{10^{-5} \cdot 70\text{kg}}{q_{\text{human}}} \quad (6)$$

APPENDIX REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265-267.

Anderson EL and the U.S. Environmental Protection Agency Carcinogen Assessment Group (1983). Quantitative approaches in use to assess cancer risk. *Risk Analysis* 3:277-295.

California Department of Health Services (CDHS, 1985). Guidelines for Chemical Carcinogen Risk Assessment and Their Scientific Rationale. California Department of Health Services, Health and Welfare Agency, Sacramento, CA.

Crump KS (1981). An improved procedure for low-dose carcinogenic risk assessment from animal data. *J Environ Path Toxicol* **52**:675-684.

Crump KS, Guess HA, Deal LL (1977). Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogenicity data. *Biometrics* **33**:437-451.

Gold LS, Zeiger E (1997). *Handbook of Carcinogenic Potency and Genotoxicity Databases*. CRC Press, Inc., Boca Raton.

U.S. Environmental Protection Agency (U.S. EPA, 1996). Proposed guidelines for carcinogen risk assessment. Federal Register 61(79):17960-18011 (April 23, 1996).

U.S. Environmental Protection Agency (U.S. EPA, 1988). Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Office of Health and Environmental Assessment, Washington D.C. EPA/600/6-87/008.