NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN 5-(MORPHOLINOMETHYL)-3-[(5-NITROFURFURYLIDENE) AMINO]-2-OXAZOLIDINONE

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SUMMARY OF FINDINGS

The cancer potency of 5-(morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone (MNAO) was estimated from dose-response data of mammary tumors among female rats who were exposed orally to MNAO hydrochloride (Cohen *et al*., 1973). The cancer potency estimate corresponds to the upper 95 percent confidence bound on the linear term of the multistage model fit to cancer dose-response data in experimental animals. 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 for MNAO was obtained from studies on MNAO hydrochloride in which the dose was adjusted for differences in molecular weight. The cancer potency estimate and corresponding NSRL are given in Table 1.

Table 1. Cancer Potency and NSRL for MNAO

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (μg/day)
MNAO	3.9	0.18

INTRODUCTION

This report describes the derivation of the cancer potency value and no significant risk level (NSRL) for MNAO (CAS number 139-91-3, commonly called furaltadone, molecular weight 360.8). MNAO was listed on April 1, 1988 as a chemical known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.). MNAO belongs to a class of nitrofuran antibiotics. Its use in humans was stopped because of toxicity (IARC, 1974). MNAO had been approved for use in veterinary medicine, but the U.S. Food and Drug Administration has since withdrawn approval as a result of the evidence of carcinogenicity (FDA, 1996).

This document discusses the studies available for cancer dose-response assessment, and summarizes the derivation of the cancer potency estimate and NSRL. A description of the methodology is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

One long-term study has investigated the carcinogenic potential of MNAO when administered by the oral route, a feeding study in female Sprague-Dawley rats (Cohen *et al*., 1973). The animals received a cumulative dose of five g/rat of MNAO hydrochloride in the diet over 46 weeks, after which they received control diet for the following 20 weeks. This agent was observed to be strongly carcinogenic, inducing high incidences of mammary adenocarcinomas and lymphoblastic lymphomas. Among the mammary tumors in treated rats (see Table 2), 6/32 were fibroadenomas (1/25 in controls, p=0.1) and 25/32 were adenocarcinomas (0/25 in controls, p<<0.001). Mammary tumors appeared earlier in the treated group than in controls (week 30 versus week 50), and treated rats frequently had multiple tumor masses. A statistically significant increase in the incidence of lymphoblastic lymphoma, a rare tumor in this rat strain, was also observed among the treated animals (7/32 compared to 0/25 for controls, p=0.013). Rare transitional cell carcinomas of the renal pelvis (2/32 compared to 0/25 among controls, p=0.3) and several transitional cell hyperplasias (4/32 compared to 0/25 among controls, p=0.09) were also observed.

Table 2. Incidence of Tumors in Female Sprague-Dawley Rats Treated With MNAO Hydrochloride By Dietary Administration (Cohen *et al*., 1973)

Cumulative Dose[1] (g/rat)	Average Dose[2] (mg/kg-day)	Mammary Tumor Incidence[3]		Lymphoblastic Lymphoma Incidence
		Total	Adenocarcinoma	
0	0	1/25	0/25	0/25
5	36	31/32[4]	25/32[4]	7/32[5]

1 The authors reported a cumulative dose of 5 gm MNAO hydrochloride per rat.

2 Average dose is calculated by dividing the cumulative dose by 0.270 kg (body weight estimated from the reported growth curve) and 462 days (length of experiment), and multiplying by the adjustment factor 324.3/360.8 (ratio of the molecular weights of MNAO and MNAO hydrochloride).

3 Tumor incidence reported here is the number of tumor-bearing animals/total number of animals alive at 10 weeks, as reported by the authors. The authors define total mammary tumors, as adenocarcinomas and fibroadenomas, where the latter includes fibroadenomas, adenofibromas and adenomas

4 Significantly different from controls by Fisher exact test, p<0.001.

5 Significantly different from controls by Fisher exact test, p=0.01.

APPROACH TO DOSE-RESPONSE ANALYSIS

MNAO induced mutations in bacterial and nonhuman test systems (GENE-TOX, 2000). These findings are suggestive that a genotoxic mode of action is plausible. There is insufficient information on the precise mechanism of carcinogenicity to permit the development of a biologically based model for cancer potency estimation. 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 is described in detail in the Appendix.

DOSE-RESPONSE ASSESSMENT

A cancer potency estimate was derived for MNAO from the study of Cohen *et al*. (1973). Table 3 summarizes potency estimates associated with each of the tumor sites and types that are reported in Table 2. The most sensitive tumor site observed in female rats was the mammary gland, and the most sensitive tumor classification was "total mammary tumors." Incidence data for total mammary tumors was therefore selected as the basis of the human potency and NSRL calculations. The human cancer potency of MNAO, which includes adjustments for the shortened study duration and rodent-human differences in body size, is estimated to be 3.9 $(mg/kg\text{-}day)^{-1}$.

Table 3. Human Cancer Potency Estimates for MNAO by Female Rat Tumor Site and Type

Tumor[1]	Cancer Potency Estimate $(mg/kg\text{-}day)^{-1}$
Mammary, total	**3.9**
Mammary adenocarcinoma	1.5
Lymphoblastic lymphoma	0.30

[1]Incidence data reported by Cohen *et al*. (1973).

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The cancer potency estimate for total mammary tumors, the most sensitive tumor site and type, was used to calculate the NSRL for MNAO (0.18 µg/day).

REFERENCES

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR MNAO

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 NSRLs for MNAO 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):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \cdots + q_j d^j)] \qquad (1)$$

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:

$$q_{animal} = q_1^* \bullet (T/T_e)^3 \qquad (2)$$

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:

$$q_{animal} = q_1^* \bullet (104/T_e)^3 \qquad (3)$$

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.

Calculation of the average daily dose

The average daily dose in units of mg/kg-day was calculated for each of the relevant dose groups, based on the dose level, duration and regimen described in the experiments above. In this case 0.270 kg was estimated as the average body weight for female rats from the growth curve provided by the study authors.

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 $(mg/kg\text{-}day)^{-1}$:

$$q_{human} = q_{animal} \bullet (bw_h / bw_a)^{1/3} \qquad (4)$$

A.3 Risk-Specific Intake Level Calculation

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

$$I = \frac{R \bullet bw_h}{q_{human}} \qquad (5)$$

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:

$$NSRL = \frac{10^{-5} \bullet 70kg}{q_{human}} \qquad (6)$$

APPENDIX REFERENCES

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