

# NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN N-CARBOXYMETHYL-N-NITROSOUREA

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

## SUMMARY OF FINDINGS

The cancer potency of N-carboxymethyl-N-nitrosoourea (CMNU) was estimated from dose-response data of multiple CMNU-responding tumor sites observed among female rats exposed orally (Maekawa *et al.*, 1983). These sites were the large and small intestines, oral cavity and Zymbal's gland. 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. The cancer potencies for the affected sites were summed probabilistically, according to their distributions, to obtain a combined distribution. This combined distribution representing cancer potency for multiple sites affected by CMNU was derived through Monte Carlo analysis. The upper 95 percent confidence bound, indicated by the combined distribution for these CMNU-related tumor sites, was taken as the cancer potency for CMNU. 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 N-Carboxymethyl-N-nitrosoourea.**

Chemical	Cancer Potency (mg/kg-day) <sup>-1</sup>	NSRL (µg/day)
N-carboxymethyl-N-nitrosoourea	1.0	0.70

## INTRODUCTION

This report describes the derivation of a cancer potency value and NSRL for N-carboxymethyl-N-nitrosoourea (CMNU, CAS number 60391-92-6, molecular weight 147.1). "N-Carboxymethyl-N-nitrosoourea" was listed on January 25, 2002 as known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq.*). CMNU is a naturally occurring N-nitrosoourea compound with no known commercial uses. CMNU is formed from reaction of glycoyamine and nitrite (reviewed in OEHHA, 2002). Glycoyamine (also called

guanidinoacetate) is the direct metabolic precursor of creatine and is present in a variety of mammalian muscle samples and other foods (OEHHA, 2002).

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

## **STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT**

The carcinogenicity of CMNU has been investigated in two drinking water studies, one in male MRC Wistar rats receiving CMNU in the drinking water five days per week for 74 weeks and followed until death (Bulay *et al.*, 1979), and one in female Donryu rats receiving CMNU in drinking water on a daily basis for 68 weeks and then sacrificed (Maekawa *et al.*, 1983). The study in female rats by Maekawa *et al.* (1983) is the best study for cancer potency estimation for several reasons. First, Bulay *et al.* (1979) used only one treated group plus controls, whereas Maekawa *et al.* (1983) employed three dosed groups plus controls. Secondly, a stronger carcinogenic response was observed among the female rats compared to the male rats. Dose levels and study duration were similar in both studies.

Maekawa *et al.* (1983) administered CMNU in drinking water continuously to four groups of female Donryu rats (40 animals per dose group) at concentrations of 0, 100, 200 or 400 ppm. Dosing was continued for 68 weeks, at which time all survivors were sacrificed. The appearance of the first tumor occurred at 35 weeks. The tumor incidence data reported in Table 2 are based on the number of rats surviving past 35 weeks of age. There was a slight dose-related reduction in the mean survival of CMNU-treated rats compared to controls. The mean survival times for the 0, 100, 200 and 400 ppm dose groups were 66, 65, 64, and 59 weeks, respectively. All animals were “autopsied completely and examined macroscopically for tumors in various organs and tissues.” All atypical lesions, tumors and tissues were examined microscopically.

Increased incidences of intestinal hyperplasia, adenoma and adenocarcinoma were observed in the two highest dose groups compared to controls. Strong dose-related trends ( $p < 0.0001$ , Mantel-Haenszel trend test) were observed for each of these three effects (Table 2). All of the intestinal adenomas, adenocarcinomas and hyperplasias were found in the jejunum or ileum, except for one adenoma of the duodenum and one adenoma of the large intestine observed in the high dose group. Fibromas, fibrosarcomas and myosarcomas of the intestines were also observed in a few animals in the two highest dose groups, with a significant trend ( $p < 0.05$ ).

Increases in squamous cell papilloma or carcinoma of the oral cavity and Zymbal's gland were observed among the CMNU-treated female rats. The incidences for both sites were low and not statistically significant by pair-wise tests, but were significant by trend tests ( $p < 0.05$ ) (Table 2).

The incidences of mammary fibroadenoma and total mammary tumors (i.e., fibroma, fibroadenoma, adenoma, and adenocarcinoma) among the CMNU-treated rats were increased ( $p < 0.05$ ) in the low- and mid-dose groups, but not in the high-dose group, relative to controls (Table 2). The number of mammary tumors per tumor-bearing rat was elevated above controls for all treatment groups. The mammary tumors were not statistically significant by trend tests (Table 2). Incidences of leukemia were slightly increased in the high-dose group relative to controls. The increases in leukemia were not statistically significant by pair-wise or trend tests.

It is uncertain whether the mammary tumors and leukemias were treatment-related; thus, they were not included in the cancer potency estimates.

**Table 2. Tumors and Preneoplastic Effects in Female Donryu rats Receiving CMNU Via Drinking Water for 68 Weeks (Maekawa *et al.*, 1983).**

Tumor Site and Type		Average Daily Dose (mg/kg-d)				Trend <sup>c</sup>
		0	5.7	11.4	22.9	
Intestines (large and small)	Hyperplasia	0/36	4/40	12/38 <sup>b</sup>	24/34 <sup>b</sup>	p<0.0001
	Adenoma	0/36	4/40	15/38 <sup>b</sup>	23/34 <sup>b</sup>	p<0.0001
	Adenocarcinoma	0/36	1/40	9/38 <sup>a</sup>	19/34 <sup>b</sup>	p<0.0001
	Fibroma	0/36	0/40	1/38	0/34	p=0.42
	Fibro-/myo-sarcoma	0/36	0/40	0/38	3/34	p<0.05
Oral cavity	Squamous-cell papilloma or carcinoma	0/36	1/40	1/38	3/34	p=0.02
Mammary gland	Fibroma	0/36	1/40	2/38	0/34	p=0.52
	Fibroadenoma	9/36	27/40 <sup>b</sup>	27/38 <sup>b</sup>	10/34	p=0.65
	Adenoma	0/36	0/40	2/38	1/34	p=0.12
	Adenocarcinoma	0/36	1/40	0/38	0/34	p=0.69
	Total mammary tumors	9/36	28/40 <sup>b</sup>	30/38 <sup>b</sup>	11/34	p=0.54
	No. of mammary tumors per tumor-bearing rat	1.0	2.0	2.7	2.2	
Zymbal's gland	Squamous-cell papilloma or carcinoma	0/36	0/40	1/38	3/34	p=0.006
Leukemia	Acute myelogenous	1/36	1/40	1/38	3/34	p=0.08

<sup>a</sup> Significantly increased relative to the control group, p<0.01 (Fisher Exact Test).

<sup>b</sup> Significantly increased relative to the control group, p<0.001 (Fisher Exact Test).

<sup>c</sup> Mantel-Haenszel trend test.

## APPROACH TO DOSE-RESPONSE ANALYSIS

Like other N-alkyl-N-nitrosourea compounds, CMNU is a direct acting mutagen and clastogen. CMNU induced mutations in several strains of bacteria, and caused a wide array of chromosomal aberrations in mammalian cells *in vitro*. CMNU bears strong structural resemblance to other N-alkyl-N-nitrosourea compounds (e.g., ENU), which are carcinogenic in rodents, pigs and primates. Other compounds that, like CMNU, are carboxymethylating agents also have been shown to cause cancer in rodent studies (reviewed in OEHHA, 2002).

The available data suggest that CMNU causes cancer primarily through a genotoxic mode of action, although the precise mechanism of carcinogenesis is not known. There are insufficient

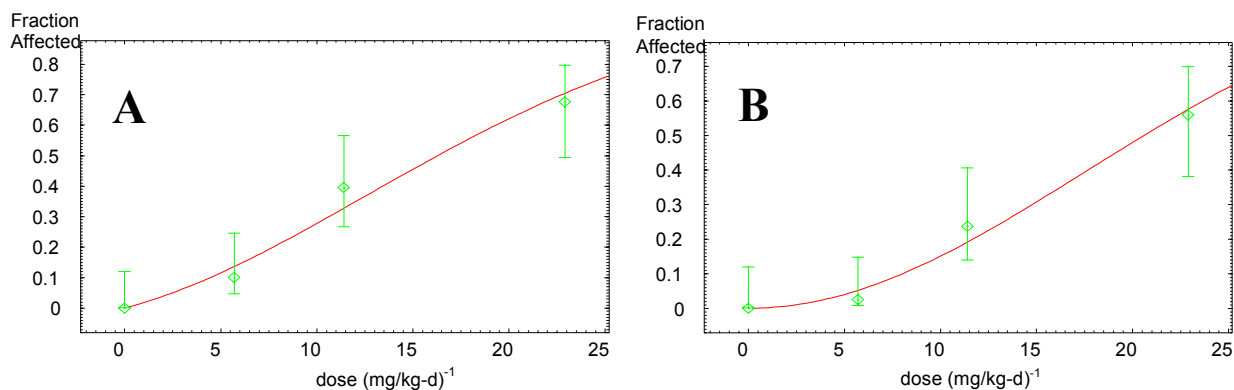
data to support dose adjustments based on pharmacokinetic models. Therefore, the default approach (i.e., a linearized multistage model and interspecies scaling) has been applied. The approach used is described in detail in the Appendix. Additionally, since CMNU induced tumors at multiple sites in female rats, a combined cancer potency estimate using Monte Carlo analysis was derived for cancer sites judged to be clearly associated with CMNU exposure (see below).

## DOSE RESPONSE ASSESSMENT

Cancer potency estimates were derived for tumor responses in CMNU-treated female rats (Maekawa *et al.*, 1983) that were judged to be clearly associated with treatment (Table 2). These sites were adenoma of the large and small intestines (combined), adenocarcinoma of the large and small intestines (combined), fibrosarcoma and myosarcomas of the large and small intestines (combined), squamous-cell papilloma and carcinoma of the oral cavity (combined), and squamous-cell papilloma and carcinoma of the Zymbal's gland (combined).

Intestinal tumors were the most responsive tissue judged to be clearly associated with exposure to CMNU. The dose-response curve for CMNU-induced intestinal adenomas is shown in Figure 1A. The dose-response curve for intestinal adenocarcinomas is shown in Figure 1B. The study authors did not report combined incidence of benign and malignant intestinal tumors. Thus, cancer potency estimates were derived separately for intestinal adenomas and intestinal adenocarcinomas (Table 3).

**Figure 1. CMNU-Induced Adenomas (A) or Adenocarcinomas (B) of the Intestines in Female Rats (Maekawa *et al.*, 1983).**

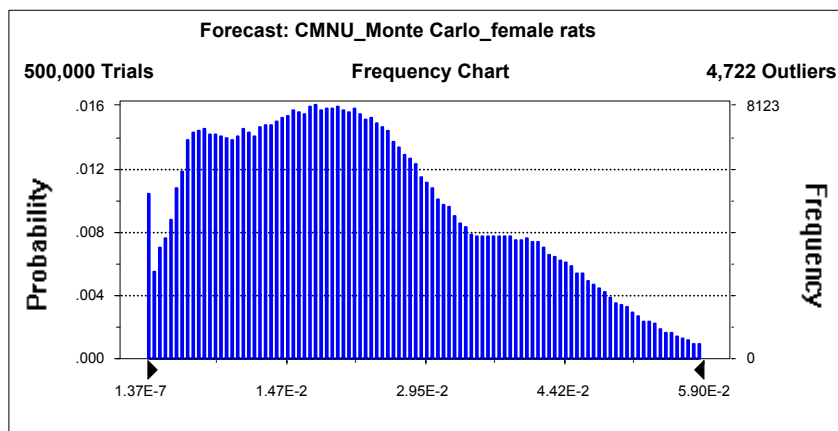


Since CMNU induced tumors at multiple sites in female rats, a combined potency estimate was derived using Monte Carlo analysis for those tumor sites judged to be clearly associated with exposure to CMNU. Normally for tumors arising from the same cell type and site, such as the intestinal adenomas and adenocarcinomas, incidence derived from the number of animals with either or both tumors is used. However, the study authors did not report the combined incidence of intestinal adenoma and adenocarcinomas for CMNU-treated female rats (Maekawa *et al.*, 1983). Because these tumors do not occur independently (adenocarcinoma generally progresses from the adenoma) the potencies for the two sites could not be summed. The incidences of adenoma exceeded that for adenocarcinoma for each dose group. The incidences for intestinal adenoma would more closely represent that for the two tumor types combined. Therefore, this

tumor was chosen over the adenocarcinoma to quantitate risk for either type of tumor in the intestine.

Figure 2 gives the combined multiple site potency distribution. 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 computer program (Crouch, 1998), 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 500,000 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 3).

**Figure 2. Combined Distribution of Potency Estimates for CMNU-Related Tumor Sites in Female Rats<sup>1</sup>.**



<sup>1</sup> The 95 percent upper confidence bound of this distribution,  $0.0472 \text{ (mg/kg-d)}^{-1}$ , represents the 68-week animal cancer potency in female rats for treatment-related tumors judged to be clearly associated with CMNU. This corresponds to a lifetime animal potency of  $0.167 \text{ (mg/kg-d)}^{-1}$  and a human potency estimate of  $1.0 \text{ (mg/kg-d)}^{-1}$  (see Appendix).

Distributions of the cancer potency estimates were combined for the following sites: adenoma of the large and small intestines (combined), fibrosarcoma and myosarcomas of the large and small intestines (combined), squamous-cell papilloma and carcinoma of the oral cavity (combined), and squamous-cell papilloma and carcinoma of the Zymbal's gland (combined) in female rats (Figure 2).

Using methods described in the Appendix, animal potencies derived from the 68-week study (Maekawa *et al.*, 1983) are projected to estimates of potencies associated with lifetime exposures. These lifetime values are then used to predict human cancer potency. Table 3 provides human potency estimates derived from each of the individual treatment-related sites, as well as the combined estimate. Because the values given in Table 3 are derived from the 95 percent upper confidence bounds, the sum of the values for the individual sites does not correspond to the combined estimate. The estimate for combined treatment-related cancers corresponds to the upper 95 percent confidence bound on the distribution obtained through the Monte Carlo simulation (Figure 2).

**Table 3. Human Cancer Potency Estimates for Selected CMNU-Induced Tumors Among Female Rats.**

<b>Tumor site</b>	<b>Cancer Potency (mg/kg-day)<sup>-1</sup></b>
Intestines (small and large) adenoma <sup>1</sup>	0.91
Intestines (small and large) adenocarcinoma	0.39
Intestines (small and large) fibrosarcoma or myosarcoma <sup>1</sup>	0.052
Oral cavity squamous cell papilloma or carcinoma <sup>1</sup>	0.14
Zymbal's gland squamous cell papilloma or carcinoma <sup>1</sup>	0.10
Multiple CMNU-related tumor sites	<b>1.0</b>

<sup>1</sup> Distributions of q<sub>1</sub> combined using Monte Carlo analysis that were used in deriving the potency for “multiple CMNU-related tumor sites.”

Bolding indicates value selected as the basis of the NSRL.

A cancer potency estimate of 1.0 (mg/kg-day)<sup>-1</sup> was derived from the combined distribution of cancer potency estimates for multiple CMNU-related tumor sites among female rats (Maekawa *et al.*, 1983) (Figure 2).

### **NO SIGNIFICANT RISK LEVEL**

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10<sup>-5</sup>. The combined cancer potency estimate for multiple CMNU-related tumor sites, 1.0 (mg/kg-day)<sup>-1</sup>, derived above was used to calculate the NSRL for CMNU (0.70 µg/day).

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## APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR N-CARBOXYMETHYL-N-NITROSOUREA

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 N-Carboxymethyl-N-nitrosourea (CMNU) are outlined in this Appendix.

### 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 (mg/kg-day)<sup>-1</sup>. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977). To estimate potency in animals ( $q_{\text{animal}}$ ) 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:

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

Potencies from the 68 week rat bioassays of CMNU (Maekawa *et al.*, 1983) were adjusted to reflect the projected response from lifetime exposure (for the rat, assumed to be two years), *i.e.*,  $q_1^* \cdot (104/68)^3$ .

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 lifetime average dose

Maekawa *et al.* (1983) treated female rats with CMNU via drinking water at concentrations of 0, 100, 200, or 400 ppm for 68 weeks. The average daily dose of CMNU during the study duration

was estimated by assuming a default water consumption rate of 0.02 liters per day and a default body weight of 0.35 kg for female rats (Gold and Zeiger, 1997). The average lifetime daily dose was estimated to be 0, 5.7, 11.4 and 22.9 mg/kg-d for the 0, 100, 200 and 400 ppm dose groups, respectively.

## 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 ( $q_{\text{human}}$ ) can be achieved by multiplying the animal potency ( $q_{\text{animal}}$ ) 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  $(\text{mg}/\text{kg}\text{-day})^{-1}$ :

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

A default body weight of 70 kg for humans and 0.35 kg for female rats was assumed (Gold and Zeiger, 1997).

## 3. Risk-Specific Intake Level Calculation

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

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

where  $bw_h$  is the body weight, and  $q_{\text{human}}$  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:

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



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