EVIDENCE ON THE CARCINOGENICITY OF

N-CARBOXYMETHYL-N-NITROSOUREA

DRAFT

October 2001

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

AUTHORS AND REVIEWERS

The Office of Environmental Health Hazard Assessment's Reproductive and Cancer Hazard Assessment Section was responsible for the preparation of this document. Members of other technical sections within the Office of Environmental Health Hazard Assessment were drawn from to conduct internal peer review.

Primary Author

Thomas A. McDonald, M.P.H., Ph.D.
Staff Toxicologist
Reproductive and Cancer Hazard Assessment Section

Internal OEHHA Reviewers

George V. Alexeeff, Ph.D, D.A.B.T.
Deputy Director for Scientific Affairs

Lauren Zeise, Ph.D.
Chief, Reproductive and Cancer Hazard Assessment Section

Martha S. Sandy, Ph.D.
Chief, Cancer Toxicology and Epidemiology Unit
Reproductive and Cancer Hazard Assessment Section

Andrew G. Salmon, M.A., D.Phil.
Chief, Air Toxicology and Risk Assessment Unit
Air Toxicology and Epidemiology Section

Susan Klasing, Ph.D.
Staff Toxicologist
Pesticide and Environmental Toxicology Section

PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq*.) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of carcinogenicity are identified as the members of the Carcinogen Identification Committee of the OEHHA Science Advisory Board (22 CCR 12301).

N-Carboxymethyl-N-nitrosourea (CMNU) was assigned a final priority of 'high' carcinogenicity concern and placed on the Final Candidate list of chemicals for Committee review on August 6, 1999. A public request for information relevant to the assessment on the evidence on the carcinogenicity of this chemical was announced in the *California Regulatory Notice Register* on August 6, 1999. No information was received as a result of this request.

This draft document *Evidence on the Carcinogenicity of N-Carboxymethyl-N-Nitrosourea* was developed to provide the Committee with relevant information for use in its deliberations. It reviews the available scientific evidence on the carcinogenic potential of CMNU. A public meeting of the Committee to discuss this evidence is scheduled for December 18, 2001. At this meeting it is expected that the Committee will render an opinion on whether CMNU has been clearly shown to cause cancer. Written public comment on the document should be submitted to OEHHA by December 4, 2001, in order to be considered by the Committee in advance of the meeting. During the December 2001 meeting, the public will have an opportunity to present verbal comments to the Committee.

TABLE OF CONTENTS

LIST OF TABLES

1 EXECUTIVE SUMMARY

N-Carboxymethyl-N-nitrosourea (CMNU) is a naturally occurring compound that is formed primarily from the reaction of glycocyamine and nitrite, which are present in the diets of most individuals. The typical daily dose of CMNU received by humans is unknown, but is expected to vary widely and depend primarily on nitrite and meat intake.

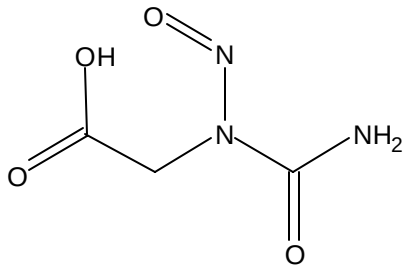
CMNU administered in the drinking water induced adenocarcinomas of the large and small intestine in two independent studies, one in male MRC Wistar rats and another in female Donryu rats. Treatment-related increases in squamous cell carcinoma of the skin were observed in male rats, and increases in squamous cell oral cavity and Zymbal's gland tumors were significant by trend test in female rats. CMNU has not been tested for carcinogenicity in mice.

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., N-ethyl-N-nitrosourea), which are carcinogenic in rodents, pigs and primates.

2 INTRODUCTION

2.1 Identity of N-Carboxymethyl-N-Nitrosourea (CMNU)

Figure 1. Structure of CMNU



Molecular Formula: $C_3H_5N_3O_4$
Molecular Weight = 147.1
CAS Registry No. 60391-92-6
Chemical Class: Nitrosourea; N-alkyl-N-nitrosourea

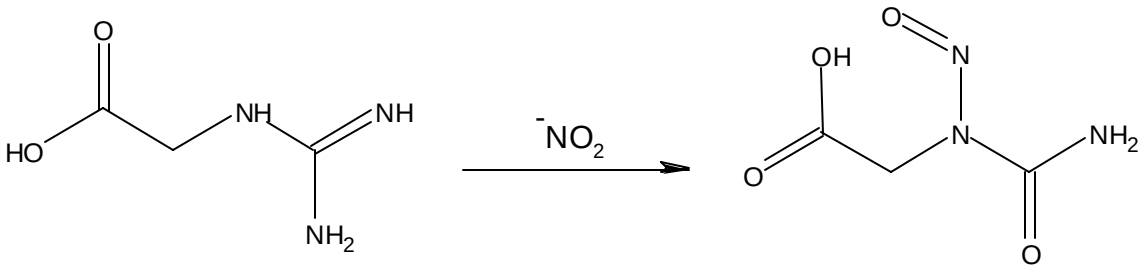
Synonyms: CMNU; carboxymethylnitrosourea; N-carbamoyl-N-nitrosoglycine; N-(aminocarbonyl)-N-nitroglycine; N-(aminocarbonyl)-N-nitroglycine; nitroso hydantoic acid; 1-(carboxymethyl)-1-nitrosourea (RTECS, 2000).

CMNU is stable at acidic pH, but degrades in alkaline conditions (Yamamoto *et al.*, 1976; Buley *et al.*, 1979). Buley *et al.* (1979) examined the stability of CMNU in aqueous solutions at different pH. The half-life of CMNU was greater than 50 h in acidic conditions (pH < 6), 14 h at neutral pH, 2.7 h at pH 8, and less than one hour at a pH higher than 8. CMNU, like other alkylnitrosourea-compounds, is sensitive to degradation by light (Yamamoto *et al.*, 1976; Maewaka *et al.*, 1983). CMNU dissolved in water and placed in clear glass tubes degraded after 50 percent after 97 h; however, the same aqueous solution was stable when protected from light and maintained at room temperature (Yamamoto *et al.*, 1976).

2.2 Occurrence and Use

CMNU is a naturally occurring N-nitrosourea compound with no known commercial uses. CMNU is formed from reaction of glycocyamine and nitrite (Figure 2) (Yamada *et al.*, 1976; Yoshikawa *et al.*, 1979). Glycocyamine (also called guanidinoacetate) is the direct metabolic precursor of creatine (Devlin, 1982) and is present in a variety of mammalian muscle samples and other foods (Yoshikawa *et al.*, 1979). For example, the average concentration of glycocyamine in the muscle of untreated rabbits ranged from 7.5 to 17.0 mg per 100 g of tissue (e.g., ~ 100 ppm) (Melville and Hummel, 1951). Nitrite is a compound produced endogenously, is added to cured meats as a preservative and color enhancer, and is a common drinking water contaminant (e.g., from fertilizer runoff to well water sources) (OEHHA, 2000). Nitrite is also derived from dietary sources of nitrate, since the body readily converts nitrates to nitrites (OEHHA, 2000). Acidic reaction conditions, such as those present in the stomach, are favorable to formation of CMNU from glycocyamine and nitrite (Yamamoto *et al.*, 1976). The rates of formation of CMNU under gastric conditions were proportional to the concentration of glycocyamine and nitrite (Yamada *et al.*, 1976). However, direct measurements of the amount of CMNU formed endogenously in animals or humans have not been made, and such measurements would be complicated by the fact that CMNU is likely to be a reactive, short-lived species.

Figure 2. Formation of CMNU from glycocyamine and nitrite



CMNU can also form from the reaction of hydantoic acid (carbamoylglycine) and nitrite (Yamada *et al.*, 1976). Hydantoic acid has been detected in some plants (Mirvish, 1972).

3 DATA ON CMNU CARCINOGENICITY

Two carcinogenicity studies have been reported for CMNU, one in male rats (Buley *et al.*, 1979) and the second in female rats (Maekawa *et al.*, 1983). In both cases, CMNU was administered in the drinking water. No cancer studies of CMNU in mice have been reported. CMNU has been tested for genotoxicity in multiple strains of bacteria and in a mammalian cell line *in vitro*. Additional relevant data include comparisons with structurally similar compounds.

3.1 Carcinogenicity Studies in Humans.

No data on long-term effects of human exposure to CMNU were found in a recent search by OEHHA.

3.2 Carcinogenicity Studies in Animals.

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).

Rat Drinking Water Study: Buley et al., 1979

As part of an investigation into the carcinogenic potential of six nitrosamide-like compounds, Buley *et al.* (1979) administered CMNU to 40 eight-week old male MRC Wistar rats via the drinking water, five days per week for 74 weeks. CMNU was dissolved in a citrate buffer (pH 4, 2 g/L) and then administered in drinking water at a concentration of 260 mg/L (ppm). The authors estimated that the average rat received a total dose of four grams of CMNU per kg body weight over the course of the experiment, which corresponds to 10.8 mg/kg body weight per day on the days dosed. The daily dose of CMNU was approximately 19-fold lower than the acute lethal-dose-50 percent (LD_{50}), 210 mg/kg body weight, estimated from a study of male rats given a single intraperitoneal injection of CMNU (Buley *et al.*, 1979). Control groups of male rats were also studied. One control group of 50 male rats received plain drinking water (untreated controls). Two additional groups of 22 and 24 male rats received drinking water containing sodium citrate buffer at the same concentration as that received by the CMNU-treated group (vehicle controls). The only difference in the two vehicle control groups was that they were run at different times (i.e., sequentially). The second vehicle control group was run to confirm the observations of tumors of the parathyroid and nervous system in the first vehicle control group. No parathyroid tumors were observed in the second vehicle control group. Tumor incidence data for these two vehicle control groups were presented separately by the study authors, but were combined here for the analysis presented in Table 1. Animals were observed until natural death or were sacrificed when moribund. Of the 40 CMNU-treated animals, 35 were alive at 80 weeks of age, 14 at 100 weeks, two at 120 weeks and all rats were dead by 140 weeks. Survival among the vehicle control rats was similar to the CMNU-treated rats. All animals were autopsied.

As shown in Table 1, an increased incidence of adenocarcinoma of the intestines (large and small) was observed among CMNU-treated rats relative to vehicle or untreated controls

($p<0.05$). The study authors also reported a combined category of total gastrointestinal tract (GIT) tumors, in which CMNU-treated rats exhibited an increased incidence of tumors relative to vehicle or untreated controls ($p\leq0.03$). This combined category of tumors included squamous cell carcinomas of the tongue, squamous cell papilloma of the forestomach, and adenocarcinomas of the small and large intestines. Neither the increased incidence of tumors of the tongue nor of the forestomach was large enough to reach statistical significance as evaluated

Table 1. Tumors in male MRC Wistar rats receiving 260 ppm CMNU via drinking water, five days per week for 74 weeks (Buley *et al.*, 1979)

Tumor Site and Type		Treatment Group		
		untreated control	vehicle control	CMNU
Gastrointestinal tract (GIT)	Squamous cell (SC) carcinoma of tongue	0/47	0/46	2/40
	SC papillomas of forestomach	2/47	2/46	3/40
	SC papillomas of the tongue or forestomach	2/47	2/46	5/40[a]
	Adenocarcinoma of large and small intestines	0/47	0/46	4/40[b]
Skin	SC papillomas	Not reported	0/46	3/40[c]
	SC carcinomas	Not reported	0/46	3/40[c]
	SC papillomas or carcinomas	1/47	0/46	6/40[b]

[a] p=0.16 for pairwise comparison between treated and vehicle (citrate-buffer) controls (Fisher Exact Test).

[b] Significantly increased incidence relative to vehicle (citrate-buffer) controls (Fisher Exact Test, $p<0.05$).

[c] p=0.097 for pairwise comparison between treated and vehicle (citrate-buffer) controls (Fisher Exact Test).

by pairwise comparison with the Fisher exact test. It is unclear whether each of the tumor types included by Buley *et al.* (1979) in the GIT tumor category are sufficiently related neoplasms to justify their analysis in combination (see Section 3.3.4 Pathology for discussion).

Buley *et al.* (1979) reported in the text of the report that the incidence of adrenal gland tumors among CMNU treated male rats was significantly increased when compared to vehicle controls: "Eight rats had adrenal tumors (6 pheochromocytomas and 2 cortical adenomas), 106 wk, P<0.03)." However, the specific incidence data of adrenal gland tumors for the vehicle controls were not reported. The finding of increased adrenal gland tumors among CMNU-treated animals relative to vehicle controls may be limited by the observation of adrenal gland tumors in the untreated control group. The authors reported observing seven adrenal gland tumors (type not specified) in the untreated controls, but they did not report whether these tumors occurred in male or female rats. Groups of female rats had been utilized as controls for experiments of compounds other than CMNU. Thus, the incidence of adrenal gland tumors in the untreated control male rats cannot be determined.

CMNU-treated rats exhibited significantly increased incidence of squamous cell papillomas and carcinomas (combined) of the skin compared to untreated (p=0.034) or vehicle controls (p=0.008). The incidences of papillomas or carcinomas, separately, were marginally significant (p=0.097) in pair-wise comparisons with the vehicle controls. In addition, Buley *et al.* (1979) indicates that a seventh CMNU-treated rat had a skin tumor, although the text describes three animals with skin papillomas, three with skin carcinomas, and does not identify the type of skin tumor in the seventh animal. Buley *et al.* (1979) concluded that CMNU was a weak carcinogen in MRC Wistar rats.

Rat Drinking Water Study: Maekawa et al., 1983

Maekawa *et al.* (1983) conducted a carcinogenicity study of female rats given CMNU via drinking water. Four groups of female Donryu rats (40 animals per dose group) were administered CMNU in their drinking water continuously 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 all three endpoints (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.

Table 2. Tumors and preneoplastic effects in female Donryu rats receiving 0, 100, 200 or 400 ppm CMNU via drinking water for 68 weeks (Maekawa *et al.*, 1983)

Tumor Site and Type		Dose, ppm				Trend[c]
		0	100	200	400	
Intestines (large and small)	Hyperplasia	0/36	4/40	12/38[b]	24/34[b]	p<0.0001
	Adenoma	0/36	4/40	15/38[b]	23/34[b]	p<0.0001
	Adenocarcinoma	0/36	1/40	9/38[a]	19/34[b]	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[b]	27/38[b]	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[b]	30/38[b]	11/34	p=0.54
	No. of mammary tumors per tumor-bearing rat	1.0	2.0	2.7	2.2	
Zymbal 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

[a] Significantly increased relative to the control group, p<0.01 (Fisher Exact Test)

[b] Significantly increased relative to the control group, p<0.001 (Fisher Exact Test)

[c] Mantel-Haenszel trend test

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.

For female rats treated with CMNU, tumors of the oral cavity and Zymbal gland were significantly increased with dose ($p\leq0.02$ by Mantel-Haenszel trend test). Marginally significant increases were observed by pairwise comparisons between high dose and control animals in the incidences of squamous-cell papillomas and carcinomas of the oral cavity ($p=0.11$) and Zymbal's gland ($p=0.11$).

3.3 Other Relevant Data

In addition to the reported animal bioassays, other relevant data related to the possible carcinogenicity of CMNU are available. This information includes studies of genetic toxicity and structure-activity comparisons.

3.3.1 Genetic Toxicology

CMNU is a direct acting mutagen and clastogen. Ishidate *et al.* (1981) reported that CMNU was positive for mutagenicity in the *Salmonella* reverse mutation assay without metabolic activation. Although several strains of *Salmonella* (i.e., TA98, TA100 and TA1537) were tested, Ishidate *et al.* (1981) reported results only as positive or negative in the *Salmonella* reverse mutation assay, and did not provide information on results obtained with individual testing strains. CMNU was found not to be mutagenic in the *Salmonella* strain TA1535 in the absence of metabolic activation (Lee *et al.*, 1977), nor was a reaction mixture of glycocyamine and nitrite (precursors of CMNU) mutagenic in *Salmonella* strain TA1535 (Endo *et al.*, 1974).

Yoshikawa *et al.* (1979) reported that CMNU was mutagenic without metabolic activation in three *Escherichia coli* tester strains, namely a wild type strain (H/r30R), a strain deficient in excision repair (Hs30R *uvrA*-), and a strain defective in DNA polymerase I (R15 *polA*-). The strain deficient in excision repair were more sensitive to CMNU-induced mutations than the wild type and polymerase-defective strains. In a related experiment, Kohda *et al.* (1987) studied the mutagenicity of CMNU and other N-nitrosourea compounds (N-alkyl-, N-hydroxyalkyl-, N-haloalkyl-N-nitrosoureas). Two *E. coli* tester strains were used, a wild type strain and a mutant strain deficient in excision–repair capacity (i.e., *uvr*A mutant). CMNU increased the mutation frequency proportionally with dose in both tester strains; however, CMNU was far more efficient in inducing mutations in the repair-deficient bacteria as compared with the wild type bacteria. The general conclusion of the study was that nitrosourea compounds containing bulky or electronegative side chains (e.g., isobutyl-, 2-hydroxypropyl-, carboxymethyl-, carboxypropyl-) produced alkylation products that were more easily repaired compared to those produced by the short-chained alkyl-N-nitrosoureas.

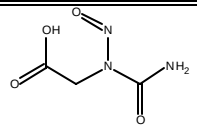
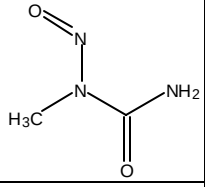
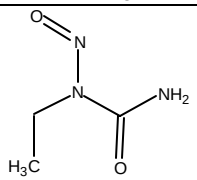
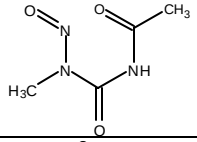
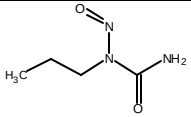
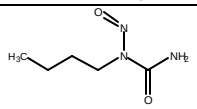
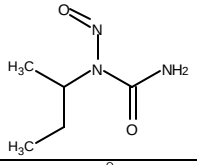
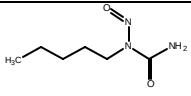
In mammalian cells, CMNU[1] (12.5 µM in saline) was reported to elicit a strong response in inducing chromosomal aberrations in Chinese hamster lung fibroblast cells (Ishidate and Odashima, 1977). The types of aberrations induced included chromatid gaps, chromatid or chromosomal breaks, translocations, ring formations, and fragmentations. Relative to the other 134 compounds tested, CMNU was placed in the highest response category (i.e., greater than 50 percent of cells contained aberrations) (Ishidate and Odashima, 1977). Ishidate *et al.* (1981) also found that CMNU induced increases in chromosomal aberrations in Chinese hamster lung fibroblast cells, using the same doses and protocols as described in the earlier publication by Ishidate and Odashima (1977).

3.3.2 Structure-Activity Comparisons

CMNU bears strong structural resemblance to other N-alkyl-N-nitrosourea compounds (Table 3), which are carcinogenic in rodents, pigs and primates (OEHHA, 1988; CancerChem, 2000). N-methyl-N-nitrosourea (MNU) and N-ethyl-N-nitrosourea (ENU) are listed as causing cancer under Proposition 65. As shown in Table 3, individual N-alkyl-N-nitrosourea compounds administered to rats via the drinking water (studies conducted in Maekawa's laboratory) induced tumors in different target organs. The tissue specificity of the carcinogenic action of the various N-alkyl-N-nitrosoureas is likely due to a combination of factors, including lipophilicity, the types of DNA adducts formed, and the DNA-repair capacity of the target organ (Maekawa and Mitsumori, 1990). CMNU was not observed to induce tumors of the central nervous system as has been observed for some other nitrosourea compounds. One of the sites most sensitive to the action of CMNU in the rat is the intestine; this is also a common target of other nitrosourea compounds such as ENU, N-isobutyl-N-nitrosourea and N-propyl-N-nitrosourea. Other sites such as the oral cavity and forestomach are also common targets for tumor induction by N-alkyl-N-nitrosoureas.

[1] Results were reported using the CMNU synonym, "nitroso hydantoic acid."

Table 3. Target tumor sites in rats administered N-alkyl-N-nitrosoureas in drinking water (adapted from Maekawa and Mitsumori, 1990)

Chemical	Structure	Rat Strain	Dose in Water (ppm)	Target Tumor Sites
CMNU		Donryu	100 – 400	intestine, oral cavity, skin
MNU*		Donryu F344 ACI	100 – 400 100 – 200 200	CNS, forestomach CNS, tongue, stomach CNS, stomach
ENU*		Donryu F344 F344	100 – 400 100 – 400 0.3 – 10	marrow intestine CNS, intestine
Ac-MNU		ACI	12 – 66	CNS, stomach
PNU		Donryu F344	150 – 600 100 – 200	marrow thymus, intestine
BNU		Donryu F344	100 – 400 400	marrow upper digestive tract
i-BNU		Donryu	100 – 400	intestine
AMU		Donryu	100 – 400	forestomach, marrow

Abbreviations are: ANU, N-amyl-N-nitrosourea; Ac-MNU, N-methyl-N-nitroso-N'-acetylurea; BNU, N-butyl-N-nitrosourea; CMNU, N-carboxymethyl-N-nitrosourea; CNS, central nervous system; ENU, N-ethyl-N-nitrosourea; i-BNU, N-isobutyl-N-nitrosourea; MNU, N-methyl-N-nitrosourea; PNU, N-propyl-N-nitrosourea

* On the Proposition 65 list of chemicals known to cause cancer.

3.3.3 Pharmacokinetics and Metabolism

No studies of the pharmacokinetics or metabolism of CMNU were located in the literature; however, one might expect similar behavior to structurally similar compounds such as MNU and ENU. MNU and ENU are widely distributed in rats shortly after administration, as measured by radiolabel distribution and DNA adduct formation in multiple tissues (IARC, 1978). N-alkyl-nitrosoureas are reactive with short half-lives (e.g., the half-life of ENU follow i.v. administration was five to six minutes) (IARC, 1978).

3.3.4 Pathology

In two separate drinking water studies, one in male rats (Buley *et al.*, 1979) and one in female rats (Maekawa *et al.*, 1983), the same tumor type was observed, namely adenocarcinoma of the intestines. Additionally, squamous cell tumors of the skin were increased in male rats treated with CMNU, and squamous cell tumors of the oral cavity and Zymbal's gland were increased in female rats treated with CMNU. In addition, observations of marginal increases in squamous cell tumors of the oral cavity (i.e., tongue) and forestomach (combined) in treated male rats are consistent with the increases in squamous cell tumors of the oral cavity observed in treated female rats.

Buley *et al.* (1979) state that "After a complete necropsy, the organs were fixed in 10 % buffered formalin, and tissues were prepared for histology by conventional methods and stained with hematoxylin and eosin." Buley *et al.* (1979) reported the tumor types observed but did not describe any additional microscopic observations regarding the tumors. There was a statistically significant increased incidence of total tumors of the gastrointestinal tract (GIT), as categorized by the authors, in nine of 40 CMNU-treated rats, relative to two of 46 vehicle controls or two of 47 untreated controls ($p \leq 0.02$, calculated by OEHHA). These tumors included squamous cell carcinomas of the tongue, squamous cell papilloma of the forestomach, and adenocarcinomas of the small and large intestines. Tumors originating from cells of the same embryonic origin are combined for analyzing the significance of findings in treated animals. Thus, according to guidelines for combining neoplasms used by the National Toxicology Program (McConnell *et al.*, 1986), squamous cell papillomas and carcinomas of the tongue, esophagus and forestomach (non-glandular) are often combined for statistical analyses. Likewise epithelium-derived neoplasms from various sites along the small and large intestines are combined to evaluate the intestinal tract as a whole (McConnell *et al.*, 1986). There was no discussion in the guidelines of combining squamous cell tumors of the oral cavity and forestomach with adenocarcinomas of the intestines (McConnell *et al.*, 1986), and combining these types of tumors for analyzing the significance of findings appears questionable.

Maekawa *et al.* (1983) described the pathology of the CMNU-induced tumors among female rats. The majority of gastrointestinal tumors "were located in the small intestines (jejunum and ileum) except for a few tumors in the duodenum and large intestine. Tumors in the small intestine were pedicular or sessile and differed in size." "Adenomas were composed of cylindrical cells with many mitotic figures, but cellular and structural atypism was rare and no invasion of tumor cells into the submucosa was observed . . . Most adenocarcinomas were of the

papillo-tubular type. Tumor cells were cylindrical or cuboidal with large, polymorphic nuclei and some were goblet cells. They often infiltrated into the submucosa, muscle layer, and serosa. . . . Of four mesencymal tumors, one was identified as a fibroma and three as fibro- or leiomyosarcomas. All tumors of the large intestine were identified as adenomas with thin pedicules. In many cases, intestinal tumors were multiple" (see Table 2). "In four rats of the 400 ppm group, intestinal invagination due to induction of tumors was observed. In addition, mucosal hyperplasia or preneoplastic changes of the small intestine were also found in many rats in the experimental groups . . . and showed dose-effect relationships. . . " The authors did not observe neoplastic or histological changes in other regions of the digestive tract, but did identify a few squamous-cell papillomas and carcinomas of the lip and oral cavity. In addition to the tumors reported in Table 2, Maekawa *et al.* (1983) also reported a combined category of total intestinal tumors, in which CMNU-treated rats exhibited an increased incidence of tumors in all dose groups relative to controls in pair-wise comparisons ($p<0.05$), and a strong dose-response trend ($p<0.0001$) was observed. The combined category was comprised of both epithelial- and mesenchymal-derived intestinal neoplasms (e.g., adenoma, adenocarcinomas, fibroma), tumor types that are not generally combined using current criteria (McConnell *et al.*, 1986).

Maekawa *et al.* (1983) reported that tumors of the mammary gland differed in size and consistency. Most tumors were classified as fibroadenomas; some were identified as adenomas or adenocarcinomas. There were no differences in the distribution of tumor histologic type between control and CMNU-treated rats. As shown in Table 2 above, mammary tumor multiplicity was greater in tumor-bearing CMNU-treated rats relative to controls.

3.4 Mechanism

CMNU is a direct acting mutagen and clastogen, based on results from *in vitro* tests in bacteria and mammalian cells (see Section 3.3.1 Genetic Toxicity). CMNU is a carboxymethylating agent, which likely gives rise to carboxymethyl DNA adducts (Harrison *et al.*, 1997). Harrison *et al.* (1997) noted that carboxymethylating agents, in addition to CMNU, are mutagenic and cause cancer in animal test systems. For example, azaserine [diazoacetate (ester) L-serine] and N-nitrosoglycocholic acid are compounds known to form carboxymethyl adducts with DNA *in vivo* (reviewed in Harrison *et al.*, 1997). Azaserine has been the subject of more than 50 publications demonstrating its potential to induce pancreatic cancer in animals (CancerChem, 2000). N-Nitrosoglycocholic acid administered orally to rats resulted in increases in stomach and liver cancer (CancerChem, 2000). N-Nitrosated peptides that contain glycine on the C-terminus, such as N-(N'-acetyl-L-prolyl)-N-nitrosoglycine, are expected to be carboxymethylating agents (Harrison *et al.*, 1997) and have been observed to be carcinogenic in rodents (Anderson and Blowers, 1994). Thus, a genotoxic mode of action is likely responsible for the observed carcinogenic effects.

4 OTHER REVIEWS

To date, the carcinogenic activity of CMNU does not appear to have been evaluated by the International Agency for Research on Cancer, the National Toxicology Program, the U.S.

Environmental Protection Agency, the National Institutes of Occupational Safety and Health, or the U.S. Food and Drug Administration.

5 SUMMARY AND CONCLUSIONS

5.1 Summary of Evidence

CMNU administered in the drinking water induced adenocarcinomas of the large and small intestines in two independent studies, one in male MRC Wistar rats and another in female Donryu rats. Treatment-related increases in squamous cell carcinoma of the skin were observed in male rats, and increases in squamous cell oral cavity and Zymbal's gland tumors were significant by trend test in female rats. CMNU has not been tested for carcinogenicity in mice.

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.

5.2 Conclusion

There is evidence for the carcinogenicity of CMNU, based on the development of adenocarcinoma of the intestines in male and female rats of different strains, squamous cell skin tumors in male rats, and squamous cell oral cavity and Zymbal's gland tumors in female rats. Further evidence includes observations of mutagenicity in bacteria, clastogenicity in mammalian cells *in vitro*, and close structural analogies to well-recognized carcinogens.

6 REFERENCES

Anderson D, Blowers SD (1994). Limited cancer bioassay to test a potential food chemical. *Lancet* **344**(8918):343-344.

Bulay O, Mirvish SS, Garcia H, Pelfrene AF, Gold B, Eagen M (1979). Carcinogenicity test of six nitrosamides and a nitrosocyanamide administered orally to rats. *J Natl Cancer Inst* **62**(6):1523-1528.

CancerChem (2000). Version 3.0. Electronic database of U.S. Public Health Service Publication No. 149, *Survey of Compounds Which Have Been Tested for Carcinogenic Activity*, GMA Industries, Inc.

Devlin TM (ed.) (1982). *Textbook of Biochemistry with Clinical Correlations.* John Wiley and Sons, New York, pp. 785.

Endo H, Takahashi K, Aoyagi H (1974). Screening of compounds structurally and functionally related to N-methyl-N'-nitro-N-nitrosoguanidine, a gastric carcinogen *Gann* **65**(1):45-54.

Harrison KL, Fairhurst N, Challis BC, Shuker D Eg (1997). Synthesis, characterization, and immunochemical detection of O6-(carboxymethyl)-2'-deoxyguanosine: A DNA adduct formed by nitrosated glycine derivatives. *Chem Res Toxicol* **10**(6):652-659.

International Agency for Research on Cancer (IARC, 1978). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some N-Nitroso Compounds.* IARC, World Health Organization, Lyon, France, Volume 17, pp. 204, 241-242.

Ishidate M, Odashima S (1977). Chromosome tests with 134 compounds on Chinese hamster cells in vitro - a screening for chemical carcinogens. *Mutat Res* **48**(3-4):337-353.

Ishidate M, Sofuni T, Yoshikawa K (1981). Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and/or carcinogens. *Gann Monograph on Cancer Res* **27**:95-108.

Kohda KH, Ninomiya S, Washizu K, Shiraki K, Ebie M, Kawazoe Y (1987). Studies on chemical carcinogens and mutagens. 42. Mutagenicity of a series of N-alkyl-N-nitrosoureas, N-hydroxyalkyl-N-nitrosoureas, N-haloalkyl-N-nitrosoureas and N-carboxyalkyl-N-nitrosoureas in *Escherichia coli* tester strains - dependence on the *Uvra* DNA-repair system. *Mutat Res* **177**(2):219-28.

Lee K, Gold B, Mirvish SS (1977). Mutagenicity of 22 N-nitrosamides and related compounds for *Salmonella typhimurium* TA1535. *Mutat Res* **48**(2):131-138.

Maekawa A, Mitsumori K (1990). Spontaneous occurrence and chemical induction of neurogenic tumors in rats - influence of host factors and specificity of chemical-structure. *Crit Rev Toxicol* **20**(4):287-310.

Maekawa A, Ogiu T, Matsuoka C, Onodera H, Furuta K, Tanigawa H, Odashima S (1983). Induction of tumors in the small intestine and mammary gland of female Donryu rats by continuous oral administration of N-carboxymethyl-N-nitrosourea. *J Cancer Res Clin Oncol* **106**(1):12-16.

McConnell EE, Solleveld HA, Swenberg JA, Boorman GA (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *J Natl Cancer Inst* **76**:283-289.

Melville RS, Hummel JP (1951). Creatine and glycocyamine metabolism in rabbits in vitamin E deficiency. *J Biol Chem* **191**:383-389.

Mirvish (1972). Studies on N-nitrosation reactions: kinetics of nitrosation, correlation with mouse feeding experiments, and natural occurrence of nitrosatable compounds (ureides and guanidines). In Nakahara W, Takayama S, Sugimura T (eds.) *Topics in Chemical Carcinogenesis.* Tokyo University Press, Tokyo, pp. 279-295.

Office of Environmental Health Hazard Assessment (OEHHA, 1988). *Risk-specific Intake Levels for the Proposition 65 Carcinogen N-nitroso-N-ethylurea.* OEHHA, California Department of Health Services, California, October 1, 1988.

Office of Environmental Health Hazard Assessment (OEHHA, 2000). *Evidence on Developmental and Reproductive Toxicity of Sodium Nitrite.* OEHHA, California Environmental Protection Agency, Sacramento, California, March, 2000 draft, located at http://www.oehha.org/prop65/hazard_ident/sodnitn.html.

Registry of Toxic Effects of Chemical Substrates (RTECS, 2000). National Institute for Occupational Safety and Health.

Yamada T, Yamamoto M, Tanimura A (1976). Studies on the formation of nitrosamines (IV) kinetical studies on the carboxymethylnitrosourea formation from glycocyamine and sodium nitrite. *J Food Hyg Soc* **17**(2):182-186.

Yamamoto M, Yamada T, Tanimura A (1976). The formation of nitrosamines. III. The reaction products of glycocyamine and sodium nitrite. *Shokuhin Eiseigaku Zasshi* **17**(2):176-181.

Yoshikawa K, Uchino H, Yamamoto M, Yamada T, Tanimura A, Kondo S (1979). Effect of N-carboxymethyl-N-nitrosourea on viability and mutagenic response of repair-deficient strains of *Escherichia coli*. *Gann* **70**(5):705-708.