EVIDENCE ON THE CARCINOGENICITY OF

2,6-DIMETHYL-N-NITROSOMORPHOLINE

August 2012

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

The Office of Environmental Health Hazard Assessment's (OEHHA) Reproductive and Cancer Hazard Assessment Branch was responsible for the preparation of this document.

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PREFACE

Proposition 65 requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity.[1] It specifies that "a chemical is known to the state to cause cancer … 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 ..." The "state's qualified experts" regarding findings of carcinogenicity are the members of the Carcinogen Identification Committee (CIC) of the Science Advisory Board.[2]

The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. OEHHA selected 2,6-dimethyl-N-nitrosomorpholine for preparation of hazard identification materials. Upon selection, the public was given the opportunity to submit information relevant to the assessment of the evidence on the carcinogenicity of 2,6-dimethyl-N-nitrosomorpholine. OEHHA reviewed and considered those submissions in preparing this document.

OEHHA developed this document to provide the CIC with comprehensive information on the carcinogenicity of 2,6-dimethyl-N-nitrosomorpholine for use in its deliberations on whether or not the chemical should be listed under Proposition 65.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code 25249.5 *et seq*.)
[2] Title 27 Cal. Code of Regs. §25302

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1. EXECUTIVE SUMMARY

2,6-Dimethyl-N-nitrosomorpholine (DMNM) is a heterocyclic nitrosamine. Nitrosamines can form in certain industrial environments, such as rubber factories and machine workshops, where free secondary amines may come in contact with nitrosating agents. DMNM forms from the nitrosation of 2,6-dimethylmorpholine, a dehydration product of bis(2-hydroxypropyl)amine. Both of these compounds can be present in industrial oils, and are used in a variety of industrial processes. DMNM has also been used as a model compound in experimental laboratory investigations of carcinogenesis.

DMNM has been tested for carcinogenicity in rats, hamsters, guinea pigs, and trout. Significant increases in benign and malignant tumors have been observed at multiple sites in multiple species, often in multiple strains and both sexes, by various routes of exposure. More than ten tumor types induced by DMNM are considered rare, including nasal cavity and lung tumors in rats and hamsters; tracheal, tongue, esophageal, and forestomach tumors in rats; and gallbladder, pancreas, kidney, vaginal, and skin tumors in hamsters. Other DMNM treatment-related tumors observed in these studies are: liver and trachea tumors in the rat; liver, forestomach, larynx, trachea, and Harderian gland tumors in the hamster, and liver, glandular stomach and swimbladder tumors in trout.

DMNM has been shown to induce base-pair substitution mutations in *Salmonella*, primarily in the presence of metabolic activation, and X-linked recessive lethal mutations in *Drosophila*. *In vitro*, DMNM induces unscheduled DNA synthesis (UDS) in cultured primary rat hepatocytes and isolated hamster main pancreatic ducts. *In vivo*, DMNM induces single strand DNA breaks in pancreatic acinar cells of hamsters, but not rats. DMNM has been shown in *in vitro* studies to bind to hamster pancreas DNA, RNA, and protein. *In vivo*, DMNM has been shown to bind to hamster and rat liver DNA, RNA, and protein, and to form N-7 methylguanine in hamster and rat liver, and O^6-methylguanine in hamster liver.

DMNM, like many other nitrosamines, requires metabolic activation via cytochrome P450 for genotoxic and carcinogenic activity. Studies with α-deuterated DMNM and β-deuterated DMNM in hamsters indicate that metabolic activation can occur following oxidation of either the α- or the β-carbons of the molecule.

Metabolism of DMNM leads to formation of the mutagenic and carcinogenic metabolites N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine) (HPOP), N-nitrosobis(2-oxopropyl)amine (BOP), and N-nitrosobis(2-hydroxypropyl)amine (BHP).

Each of these metabolites shares species-specific target tumor sites with DMNM. For example, BHP, BOP and DMNM each induce liver, pancreas, and lung tumors in the hamster, and all four compounds (HPOP, BOP, BHP and DMNM) induce liver, lung, and nasal cavity tumors in the rat. These three DMNM metabolites also have similar genotoxicity profiles to DMNM. Specifically, all three DMNM metabolites induce mutations in *Salmonella* and Chinese hamster V79 lung cells, induce UDS *in vitro* in rat liver cells, and form DNA adducts (measured as methylated guanine) in hamster pancreatic ductal cells and rat and hamster liver cells. One metabolite, HPOP, also induces DNA single strand breaks in pancreatic acinar cells in rats and hamsters exposed *in vivo*.

DMNM is structurally similar to the carcinogenic and genotoxic heterocyclic nitrosamines nitrosomorpholine (NM) and nitrosopiperidine (NP). Like DMNM, NM and NP induce mutations in *Salmonella*, and mammalian cells *in vitro*, UDS in hepatocytes *in vitro*, and form DNA adducts *in vivo*. NM and NP share many species-specific target tumor sites with DMNM. For example, NM, NP and DMNM each induce nasal cavity, esophagus and liver tumors in the rat, and liver tumors in the hamster.

2. INTRODUCTION

2.1 Identity of 2,6-Dimethyl-N-nitrosomorpholine (DMNM)

2,6-Dimethyl-N-nitrosomorpholine (DMNM) is a heterocyclic nitrosamine. Stereoisomers and α- and β-deuterated forms of the compound have been studied for carcinogenicity, thus the structure is described in detail here. DMNM consists of a morpholine ring (a six-membered ring of four carbon atoms, one oxygen, and one nitrogen atom), a nitroso group (NO) bound to the ring nitrogen, and two methyl groups at the second and sixth positions (Figure 1). The carbons at positions 3 and 5 are referred to as α carbons (the first carbon connecting to the nitroso group), while those at positions 2 and 6 are β carbons (the second carbon connecting to the nitroso group). There are two stereoisomers (compounds with the same chemical formula and the only difference being the way the atoms are oriented in space) of DMNM. The c*is*-isomer of DMNM has both methyl groups extending either above or below the morpholine ring, and the *trans*-isomer has one methyl group extending above and the other extending below the ring.

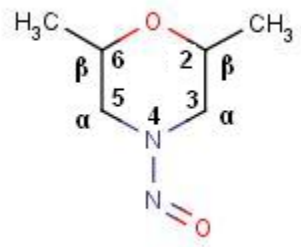


Figure 1. Chemical structure of DMNM

Molecular Formula:	$C_6H_{12}N_2O_2$
Molecular Weight:	144.17
CAS Registry Number:	1456-28-6
Synonyms:	2,6-Dimethyl-4-nitrosomorpholine; 2,6-Dimethylnitrosomorpholine; Dimethylnitrosomorpholine; Nitroso-2,6-dimethylmorpholine
Chemical Class:	Heterocyclic nitrososamines
Density:	1.22 g/cm^3
Flash Point:	93.9°C

Boiling point: 231.7°C (at 760 mmHg)

Water Solubility: 1.24×10^{5} mg/L (at 24°C)

Vapor pressure: 0.024 mmHg (at 25°C)

Henry's Law constant: 2.48×10^{-8} atm-m^3/mole (at 25°C)

Log P (Octanol-water coefficient): 0.32

2.2 Occurrence and Use

Nitrosamines can form in certain industrial environments, such as rubber factories and machine workshops, where free secondary amines may come in contact with nitrosating agents, such as nitric oxides present in the air or molten nitrate or nitrite salt baths (Spiegelhalder and Preussmann, 1983; Jönsson *et al.*, 2009). DMNM forms from the nitrosation of 2,6-dimethylmorpholine (Lijinsky and Reuber, 1982). 2,6-Dimethylmorpholine is a dehydration product of bis(2-hydroxypropyl)amine, and both of these compounds are used in variety of industrial processes and present in certain industrial oils (Yamamoto *et al.*, 1995). DMNM has been used as a model compound in experimental laboratory investigations of carcinogenesis.

3. DATA ON CARCINOGENICITY

3.1 Carcinogenicity Studies in Humans

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

3.2 Carcinogenicity Studies in Animals

The carcinogenicity of DMNM was studied in rats, hamsters, guinea pigs, and trout. These studies are listed in Table 1 by species and route of administration, and each is presented briefly below.

Table 1. **Overview of DMNM animal carcinogenicity studies**

Study No.	Species	Strain	Sex (M, F)	Route of Administration	Reference
1	Rat	Sprague Dawley	M	drinking water	Lijinsky and Taylor, 1975
2			F		
3	Rat	Sprague Dawley	M	drinking water	Lijinsky and Taylor, 1978
4	Rat	Fischer 344	F	drinking water	Lijinsky and Reuber, 1980; 1982
5	Rat	Wistar	NR*	drinking water	Vollrath *et al*., 1986
6	Rat	Fischer 344	M	gavage	Lijinsky *et al*., 1982b
7			F		
8	Rat	Sprague Dawley	M	*s.c*. injection	Ovelar *et al*., 1981
9			F		
10	Rat	Wistar	M	*i.p*. injection	Konishi *et al*., 1987
11	Rat	Fischer 344	F	Intravesicular injection	Lijinsky *et al*., 1991
12	Hamster	Syrian golden	M	gavage	Mohr *et al*., 1977; Reznik *et al*., 1978
13			F		
14	Hamster	Syrian golden	M	gavage	Rao *et al*., 1981
15	Hamster	Syrian golden	M	gavage	Lijinsky *et al*., 1982b
16	Hamster	European	M	gavage	Althoff *et al*., 1985
17			F		
18	Hamster	Syrian golden	M & F	*s.c.* injection	Althoff *et al*., 1978
19	Hamster	European	M	*s.c*. injection	Althoff *et al*., 1985
20			F		
21	Hamster	Syrian golden	M	*s.c*. infusion	Kokkinakis and Scarpelli, 1989
22	Guinea Pig	Strain 2	M	gavage	Cardy and Lijinsky, 1980
23	Guinea Pig	Random-bred	M	gavage	Rao *et al*., 1980
24	Trout	Shasta	M & F	diet	Hendricks *et al*., 1995

*NR: Not reported

3.2.1 Studies in Rats

The carcinogenicity of DMNM has been investigated in three strains of rats, Sprague Dawley, Fischer 344, and Wistar. The compound was administered via drinking water, gavage, subcutaneous (*s.c*.) injection, intraperitoneal (*i.p*.) injection, and intravesicular bladder injection. Specifically, studies of DMNM consist of three drinking water studies

in Sprague Dawley rats (two in males, one in females) (Lijinsky and Taylor, 1975; 1978), one drinking water study in female Fischer 344 rats (Lijinsky and Reuber, 1980; 1982), one drinking water study in Wistar rats (Vollrath *et al*., 1986), two gavage studies in Fischer 344 rats (one in males, one in females) (Lijinsky *et al*., 1982b), two *s.c*. injection studies in Sprague Dawley rats (one in males, one in females) (Ovelar *et al.*, 1981), one single *i.p*. injection study in male Wistar rats (Konishi *et al.*, 1987), and one intravesicular bladder injection study in female Fischer 344 rats (Lijinsky *et al.*, 1991).

3.2.1.1 Drinking water studies

- **Lijinsky and Taylor (1975)**

Fifteen male and 15 female Sprague Dawley rats were given DMNM in drinking water five days per week (equivalent to 5 mg/week), and tap water *ad libitum* for the remaining two days. The treatment duration was 30 weeks, after which the animals received tap water *ad libitum* until natural death occurred. No concurrent controls were included in either study. DMNM-treated male rats were dead by week 31 (Lijinsky and Taylor, 1978). DMNM-treated female rats were dead by week 34.

Among male rats, DMNM induced tumors at multiple sites (Table 2). Adenocarcinoma of the nasal turbinates, a rare tumor in rats (Schwartz *et al*., 1994; Boorman *et al*., 1990), occurred in all treated animals. A majority of the animals also had esophageal tumors, which were mostly benign. Forestomach tumors, which are rare in rats (Brown and Hardisty, 1990; Haseman *et al*., 1990), occurred in 4/15 male rats. Benign tumors of the trachea were also observed in three male rats.

Table 2. Tumor incidence in male Sprague Dawley rats given DMNM 5 days/week for 30 weeks in drinking water (Lijinsky and Taylor, 1975)

Tumor Site & Type		DMNM (5 mg/week)
Nasal turbinates	Adenocarcinoma	15/15
Esophagus	Squamous cell papilloma[1]	11/15
	Squamous cell carcinoma[1]	2/15
	Combined squamous cell papilloma and carcinoma	13/15
Forestomach	Combined squamous cell papilloma and carcinoma	4/15
Trachea	Squamous cell papilloma	3/15

[1]reported in Lijinsky and Taylor (1978)

DMNM also induced tumors at multiple sites in female rats (Table 3). Rare adenocarcinomas of the nasal turbinates were observed in 13/15 treated females. All (15/15) females had esophageal tumors, which were mostly benign. One rare forestomach tumor (Brown and Hardisty, 1990; Haseman *et al*., 1990), one hepatocellular carcinoma, and two benign tumors of the trachea were also observed in female rats.

Table 3. Tumor incidence in female Sprague Dawley rats given DMNM 5 days/week for 30 weeks in drinking water (Lijinsky and Taylor, 1975)

Tumor Site & Type		DMNM (5 mg/week)
Nasal turbinates	Adenocarcinoma	13/15
Esophagus	Combined squamous cell papilloma and carcinoma	15/15
Forestomach	Squamous cell, not otherwise specified	1/15
Liver	Hepatocellular carcinoma	1/15
Trachea	Squamous cell papilloma	2/15

- **Lijinsky and Taylor (1978)**

Fifteen six– to eight-week old male Sprague Dawley rats were given DMNM in drinking water five days per week (equivalent to 1 mg/week) and tap water *ad libitum* for the remaining two days. The treatment duration was 30 weeks, after which the animals received tap water *ad libitum* until natural death occurred. No concurrent controls were included in this study. All DMNM-treated animals were dead by approximately week 56.

Four rats were observed with malignant nasal turbinate tumors, one with a benign esophageal tumor and one with a benign forestomach tumor (Table 4).

Table 4. Tumor incidence in male Sprague Dawley rats given DMNM 5 days/week for 30 weeks in drinking water (Lijinsky and Taylor, 1978)

Tumor Site & Type		DMNM (1 mg/week)
Nasal turbinates	Squamous cell carcinoma and adenocarcinoma	4/15
Esophagus	Papilloma	1/15
Forestomach	Papilloma	1/15

- **Lijinsky and Reuber (1980; 1982)**

Groups of female Fischer 344 rats (20/group) were exposed to *cis*-DMNM, *trans*-DMNM, or an isomeric mixture of DMNM[3] (2 parts *cis*: 1 part *trans*) in drinking water for five days per week. The remaining two days of the week tap water was provided *ad libitum. cis*-DMNM was administered at concentrations of 12.5 mg/L for 30 weeks (total dose: 38 mg) and 31 mg/L for 27 weeks (total dose: 84 mg); *trans*-DMNM was administered at 6.8 mg/L for 30 weeks (total dose: 20 mg) and 17 mg/L for 22 weeks (total dose: 37 mg) (Lijinsky and Reuber, 1982). The isomeric mixture of DMNM was administered in drinking water at concentrations of 20 mg/L for 30 weeks (total dose: 60 mg) and 50 mg/L for 22 weeks (total dose: 110 mg) (Lijinsky and Reuber, 1980; 1982). At the conclusion of the treatment period animals received tap water *ad libitum* until natural death occurred.

Rats receiving the isomeric mixture and the high concentration of *cis*-DMNM did not survive beyond 35 weeks. Animals receiving the high dose of *trans*-DMNM did not survive past 30 weeks. Animals receiving the low dose of *cis*– and *trans*-DMNM respectively had two survivors each by week 45. No specific group of untreated animals was designated as a concurrent control group for this experiment; however, a continuous series of untreated control animals from the same animal colony was maintained at the same facility. The authors noted that lifespan and spontaneous tumor incidence in these untreated animals were no different than that already reported for controls in Lijinsky *et al*. (1981), a study conducted in the same laboratory during the same timeframe. Also, the authors noted that esophageal tumors were never observed in untreated Fischer 344 rats from this colony, and that control animals rarely died earlier than 18 months of age (the reported survival of untreated Fischer rats from this laboratory is 130 weeks).

[3] DMNM typically exists as an isomeric mixture of *cis*- and *trans*-DMNM.

As shown in Table 5, tumors of the nasal cavity, tongue, esophagus and forestomach which are considered to be rare in Fischer rats (Goodman *et al.*, 1979), were observed in female rats administered the isomeric mixture of DMNM, or either of the isomers, while no tumors were reported at these sites in untreated Fischer rats from this laboratory. Among the four tumor sites, the strongest responses were seen in the esophagus and the weakest in the forestomach for all three forms of DMNM. The authors described the tumors observed at these sites as mostly basal cell papillomas and carcinomas, with occasional squamous cell tumors occurring as well.

The isomeric mixture of DMNM induced statistically significant increases in:

- nasal cavity carcinomas and combined papillomas and carcinomas
- tongue carcinomas, and combined papillomas and carcinomas
- esophagus carcinomas, papillomas, and combined papillomas and carcinomas

and increases that did not achieve statistical significance in forestomach papillomas and carcinomas.

cis-DMNM induced statistically significant increases in:

- nasal cavity carcinomas and combined papillomas and carcinomas
- tongue combined papillomas and carcinomas
- esophagus carcinomas and combined papillomas and carcinomas

and borderline statistically significant increases in tongue and forestomach combined papillomas and carcinomas (p=0.053).

trans-DMNM induced statistically significant increases in:

- nasal cavity carcinomas and combined papillomas and carcinomas
- tongue combined papillomas and carcinomas
- esophagus carcinomas and combined papillomas and carcinomas

and non-significant increases in forestomach combined papillomas and carcinomas.

Table 5. Tumor incidence in female Fischer 344 rats administered an isomeric mixture of DMNM, *cis*-DMNM, or *trans*-DMNM in drinking water (Lijinsky and Reuber, 1980; 1982)

Tumor Site &Type		DMNM concentration in drinking water (mg/L) *[Total dose (mg)]*						
		isomeric DMNM *cis:trans = 2:1*		*cis*-DMNM		*trans*-DMNM		Control[1]
		20 *[60]*	50 *[110]*	12.5 *[38]*	31 *[84]*	6.8 *[20]*	17 *[37]*	0 *[0]*
Nasal cavity	Papilloma	NR[2]	NR	NR	NR	NR	0/20	0/20
	Carcinoma	14/20*	0/20	6/20*	2/20	10/20*	0/20	0/20
	Combined papilloma and carcinoma	15/20*	0/20	6/20*	2/20	11/20*	0/20	0/20
Tongue	Papilloma	3/20	0/20	NR	1/20	NR	0/20	0/20
	Carcinoma	8/20*	0/20	4/20[#]	0/20	3/20	0/20	0/20
	Combined papilloma and carcinoma	11/20*	0/20	12/20*	1/20	7/20*	0/20	0/20
Esophagus	Papilloma	3/20	8/20*	NR	NR	NR	NR	0/20
	Carcinoma	16/20*	12/20*	11/20*	14/20*	11/20*	9/20*	0/20
	Combined papilloma and carcinoma	19/20*	20/20*	17/20*	20/20*	18/20*	20/20*	0/20
Forestomach	Papilloma	3/20	0/20	NR	NR	NR	1/20	0/20
	Carcinoma	0/20	3/20	2/20	1/20	1/20	0/20	0/20
	Combined papilloma and carcinoma	3/20	3/20	3/20	4/20[#]	2/20	1/20	0/20

[1]These studies did not include a specific concurrent control group; however, a continuous series of unexposed rats from the same animal colony was maintained in the same facility. The authors state that esophageal tumors are never observed in these untreated controls, and cite Lijinsky *et al*. (1981) for published tumor incidence data for 20 unexposed female Fischer 344 rats studied in the same laboratory during the same timeframe. These data for untreated controls are reported here.

[2]NR- not reported

[#]p = 0.053, *p < 0.05, pairwise comparison to untreated females using Fisher exact test (performed by OEHHA)

- **Vollrath *et al*. (1986)**

Fifty white Wistar rats received 5 mg DMNM per rat per week in drinking water until moribund or natural death (DMNM Group I). Histopathology assessment was limited to the nasal tissues and brain of the animals. No concurrent control group was included in the study design. A serious irregularity in the conduct of the study involved the deaths of 20 rats early in the treatment period (before the occurrence of nasal tumors) from pneumonia. The investigators replaced the animals that died of pneumonia with a second group of rats (DMNM Group II). Occurrence of the first nasal tumor differed between the groups, occurring at week 28 in Group I and at week 22 in Group II. The authors suggested this may have been due to changes in the stability of the DMNM administered to the rats. Specifically, the investigators observed a single spectrophotometric peak at 240 nm in the freshly synthesized and freshly prepared aqueous solution of DMNM. When the same DMNM was used to prepare aqueous solutions three and four months later, a second peak was observed at 210 nm.

The authors observed the nasal tumor incidence for DMNM Groups I and II combined as 45/50 and characterized the tumors as originating in the olfactory epithelium. Nasal tumors are rare in rats (Boorman *et al*., 1990; Haseman *et al*., 1990).

3.2.1.2 Gavage studies

- **Lijinsky *et al*. (1982b)**

Groups of seven-week old male and female Fischer rats (20/sex) were administered 10 mg DMNM by gavage twice a week for 12 weeks, resulting in total doses of 240 mg per rat. No concurrent controls were included in either study. Treated male rats did not survive past 30 weeks and treated female rats had 2 survivors at 30 weeks and none at 40 weeks.

In male rats, DMNM induced benign and malignant tumors of the esophagus, malignant tumors of the nasal cavity and lung, and one benign liver tumor (Table 6). Esophageal, lung, and nasal tumors are rare in male Fischer 344 rats.

Table 6. Tumor incidence in male Fischer 344 rats following gavage treatment with DMNM twice per week for 12 weeks (Lijinsky *et al*., 1982b)

Tumor Site & Type		DMNM (20 mg/week)
Esophagus	Squamous and basal cell carcinoma	6/20
	Combined benign & malignant tumor	16/20
Lung	Adenocarcinoma	1/20
Nasal cavity	Squamous cell carcinoma	3/20
Liver	Benign tumor	1/20

In female rats, DMNM induced benign and malignant tumors of the esophagus and forestomach, malignant tumors of the nasal cavity and lung, and one tongue tumor (Table 7). Esophageal, lung, tongue, forestomach, and nasal tumors are rare in female Fischer rats.

Table 7. Tumor incidence in female Fischer 344 rats following gavage treatment with DMNM twice per week for 12 weeks (Lijinsky *et al*., 1982b)

Tumor Site & Type		DMNM (20 mg/week)
Esophagus	Squamous and basal cell carcinoma	10/20
	Combined benign and malignant tumor	20/20
Nasal cavity	Squamous cell carcinoma	7/20
Forestomach	Carcinoma	1/20
	Combined benign & malignant tumor	4/20
Lung	Adenocarcinoma	4/20
Tongue	type not specified	1/20

3.2.1.3 S.C. injection studies

- **Ovelar *et al.* (1981)**

Male and female eight-week old Sprague Dawley rats (15 per sex) were injected subcutaneously with one of three dose levels of DMNM or olive oil (vehicle control) once weekly for life. The DMNM doses injected were 93.6, 46.8, and 23.4 mg/kg body weight (bw) for male rats; and 77.3, 38.6, and 19.3 mg/kg bw for female rats, representing 1/5, 1/10, and 1/20 of the LD_{50} for DMNM for each sex respectively. All DMNM-treated animals were observed until natural death occurred and control animals were killed after the last treated animal had died. Mean body weight of male rats in the high-dose group was 240 g, compared to 530 g for the male controls. Mean body weight of female rats in the high-dose group was 171 g, compared to 336 g for females in the controls. Survival was lower among treated rats at all doses, as compared to controls. At the high dose, male rats survived an average of 18 weeks, as compared to 43 weeks in the controls. Female rats survived an average of 20 weeks at the high dose compared to 44 weeks in the controls.

Among male rats, treatment-related increases in tumors of the esophagus, lung, and liver were observed (Table 8). Specifically, a significant increase in combined squamous cell papillomas and carcinomas of the esophagus was observed in all dose groups ($p<0.001$ by pairwise comparison with the control group), with 100 percent incidence in the low- and mid-dose groups. A significant increase in combined lung adenomas and squamous cell carcinomas occurred in all dose groups ($p< 0.001$ by pairwise comparison with the controls at the low and high dose and $p< 0.05$ at the mid-dose). Lung tumors increased with a significant positive trend across all doses ($p< 0.001$). An increase in liver hemangiosarcomas was also observed in the mid- and high- dose groups ($p< 0.05$ by pairwise comparison with the control group) and there was a significant positive trend with dose ($p< 0.01$).

Table 8. Tumor incidence in male Sprague Dawley rats receiving weekly *s.c.* injections of DMNM for life (Ovelar *et al*., 1981)

Tumor Site & Type		Dose (mg/kg bw/week)				Trend test[1]
		0	23.4	46.8	93.6	
Esophagus	Combined squamous cell papilloma & carcinoma	0/15	14/14**	14/14**	9/14**	0.004
Lung	Combined adenoma & squamous cell carcinoma	0/15	9/14**	5/14*	10/14**	<0.001
Liver	Hemangiosarcoma	0/15	0/14	5/14*	4/14*	<0.01

[1] Exact trend test (by OEHHA)

* p< 0.05; ** p<0.001, pairwise comparison with controls by Fisher exact test (performed by OEHHA)

Among female rats, treatment-related increases in tumors of the esophagus, lung, and liver were observed (Table 9). A significant increase in combined squamous cell papillomas and carcinomas of the esophagus was observed in all dose groups (p< 0.001 by pairwise comparison with the control group), with 100 percent tumor incidence in the low- and mid-dose groups. A significant increase in combined lung adenomas and squamous cell carcinomas occurred in all dose groups (p< 0.001 by pairwise comparison with controls). Lung tumors increased with positive trend across all doses (p< 0.001). An increase in liver hemangiosarcomas was also observed in the high-dose group (p< 0.05 by pairwise comparison with the controls) and there was a significant positive trend across all doses (p< 0.01).

Table 9. Tumor incidence in female Sprague Dawley rats receiving weekly *s.c.* injections of DMNM for life (Ovelar *et al*., 1981)

Tumor Site & Type		Dose (mg/kg bw/week)				Trend test[1]
		0	19.3	38.6	77.3	
Esophagus	Combined squamous cell papilloma & carcinoma	0/15	14/14**	15/15**	14/15**	<0.001
Lung	Combined adenoma & squamous cell carcinoma	0/15	8/14**	8/15**	8/15**	<0.001
Liver	Hemangiosarcoma	0/15	0/14	2/15	5/15*	<0.01

[1] Exact trend test (by OEHHA)

* p< 0.05; ** p<0.001, pairwise comparison with controls by Fisher exact test (performed by OEHHA).

3.2.1.4 Single i.p. injection study

- **Konishi *et al.* (1987)**

Groups of six-week old male Wistar rats were injected once intraperitoneally with one of three dose levels of DMNM or saline solution (vehicle control). Twelve rats received a DMNM dose of 110 mg/kg bw; 11 rats received 221 mg/kg bw, and nine rats received 442 mg/kg bw. Ten control rats were injected with saline solution. Rats were killed at 55 weeks and histology was performed. No data on body weights or survival were reported.

As shown in Table 10, a single injection of DMNM increased the incidence of total bronchiolo-alveolar tumors (combined adenoma, adenocarcinoma and adenosquamous carcinoma) of the lung in the high-dose group ($p<0.05$), with a positive dose-related trend across all doses ($p<0.005$). A dose-related increase in the incidence of bronchiolo-alveolar adenomas ($p<0.05$) was also observed.

Table 10. Lung tumor incidence in male Wistar rats treated with a single *i.p.* injection of DMNM and observed for 55 weeks (Konishi *et al*., 1987)

Lung Region & Tumor Type		Dose (mg/kg bw)				Trend test[1]
		0	110	221	442	
Bronchial	Papilloma	0/10	2/12	1/11	1/9	N.S.[2]
Bronchiolo-alveolar	Adenoma	0/10	0/12	1/11	2/9	$p < 0.05$
	Adenocarcinoma	0/10	0/12	0/11	1/9	N.S.
	Adenosquamous carcinoma	0/10	0/12	0/11	1/9	N.S.
	Total bronchiolo-alveolar tumors	0/10	0/12	1/11	4/9*	$p < 0.005$

[1] Exact trend test (performed by OEHHA).
[2] N.S. Not significant;
*$p<0.05$, pairwise comparison with controls by Fisher exact test (performed by OEHHA)

3.2.1.5 Intravesicular injection study

- **Lijinsky *et al.* (1991)**

Either DMNM or a 25% ethanol solution (vehicle control) was administered by intravesicular injection into the bladders of female ten-week old Fischer 344 rats (12/group) twice per week under light metophane anesthesia for 18 weeks. Treated rats received 12 mg DMNM per week. Controls were given injections of 25% ethanol

twice weekly for up to 30 weeks. The animals were maintained until death. The median survival was 21 weeks for the treated rats, and the controls survived on average 102 weeks. All treated rats developed tumors of the esophagus. No tumors were observed in the controls.

3.2.2 Studies in Hamsters

DMNM was studied in two strains of hamsters by gavage (Mohr *et al.*, 1977; Reznik *et al.*, 1978; Rao *et al.*, 1981; Lijinsky *et al*., 1982b; Althoff *et al.*, 1985) and *s.c.* injection (Althoff *et al.*, 1978; 1985; Kokkinakis and Scarpelli, 1989). Syrian golden hamsters were used in all studies, except the gavage and *s.c.* injection studies of Althoff *et al.* (1985), in which European hamsters were studied.

3.2.2.1 Gavage studies

- **Mohr *et al.* (1977); Reznik *et al.* (1978)**

Male and female eight-week old Syrian golden hamsters (15/sex/group) received DMNM dissolved in olive oil once a week by gavage for life (Mohr *et al.*, 1977; Reznik *et al.*, 1978). Weekly doses of DMNM were either 0 (vehicle control), 9.19, 18.37, 36.74 or 73.48 mg/kg bw, corresponding to 0, 1/40, 1/20, 1/10, and 1/5 of the LD_{50} (367 mg/kg bw). Dosed animals were observed until natural death. Control animals in each experiment were killed after the last DMNM-treated animal had died.

In the female hamster study, survival was decreased in a dose-dependent manner in treated animals, ranging between 24 and 65 weeks. The control animals survived an average of 60 weeks. No tumors were observed in the control group. Treated females often developed tumors in several organs simultaneously, and treatment-related increases in tumors of the nasal cavity, trachea, lung, forestomach, liver, gallbladder, and pancreas were observed (Table 11). Tumor incidences were significantly increased by both trend tests and pairwise comparisons with control for the following sites: the posterior region of the nasal cavity (combined benign and malignant, rare tumor), trachea (benign only), forestomach (combined benign and malignant), liver (combined benign and malignant), gallbladder (combined benign and malignant; rare tumor), and pancreas (benign, and combined benign and malignant). Tumors of the anterior region of the nasal cavity showed a marginally significant dose-dependent increase by trend ($p = 0.066$), and were significantly increased in the second highest dose group by pairwise comparison. A significant increase in combined benign and malignant lung tumors was observed in the two middle dose groups. Lung tumors in the Syrian golden hamster are considered rare, with a spontaneous incidence of less than 0.1 - 0.5% (IARC, 1996).

The authors described the tumors observed in the anterior regions of the nasal cavity as mainly papillary polyps and papillomas, whereas the tumors observed in the posterior region of the nasal cavity were primarily adenocarcinomas. The squamous cell papillomas of the larynx were mostly small and occurred near the first tracheal ring. Tracheal papillary polyps occurred in all parts of the trachea; these are considered benign neoplasms (IARC, 1982). The pulmonary tumors were described as adenomas, adenocarcinomas, and squamous cell tumors. Liver cholangiomas and cholangiocarcinomas often occurred together within the same animal. Gallbladder tumors were described as either papillary polyps or papillary adenocarcinomas that often invaded the liver parenchyma. Forestomach tumors were reported to be mostly squamous cell papillomas, with multiple tumors occurring in the same animal's forestomach. Metastasis of ductal pancreatic carcinomas to the lungs was observed in one animal per treatment group for all but the lowest dose group.

With regard to non-neoplastic findings, the authors reported frequent pronounced hyperplasia of the pancreatic duct epithelia in treated animals, and occasional goblet cell metaplasia. Pronounced atrophy of pancreatic acinar cells was also commonly observed in treated animals.

Table 11. Tumor incidence in female Syrian golden hamsters administered DMNM by weekly gavage (Mohr *et al.*, 1977; Reznik *et al.*, 1978)

Tumor Site and Type		Control	DMNM (mg/kg/week)				Trend Test[1]
			9.19	18.37	36.74	73.48	
Nasal cavity ***Anterior: nasoturbinal, maxilloturbinal, & maxillary sinuses***	Combined papilloma & adenocarcinoma	0/13	0/15	0/13	5/14*	1/12	0.066
Nasal cavity ***Posterior: endo- and exoturbinates***	Combined papilloma & adenocarcinoma	0/13	0/15	1/13	7/14**	4/12*	p≤0.05
Trachea	Papillary polyp[2]	0/13	2/15	3/13	3/14	8/12***	p≤0.001
Lung	Combined adenoma & adenocarcinoma	0/13	3/15	5/13*	5/14*	2/12	N.S.
Larynx	Squamous cell papilloma	0/13	3/15	2/13	0/14	1/12	N.S.
Forestomach	Combined squamous cell papilloma & carcinoma	0/13	1/15	3/13*	5/14*	3/12	p≤0.05
Liver	Combined cholangioma & cholangiocarcinoma	0/13	0/15	1/13	3/14	6/12**	p≤0.001
Gallbladder	Combined papillary polyps & adenocarcinoma	0/13	0/15	5/13*	0/14	4/12*	p≤0.05
Pancreas	Ductal adenoma	0/13	2/15	6/13**	5/14*	5/12*	p≤0.05
	Ductal adenocarcinoma	0/13	1/15	2/13	1/14	2/12	N.S.
	Combined adenoma & carcinoma [Metastases to lungs]	0/13 [0]	3/15 [0]	7/13** [1]	6/14** [1]	6/12** [1]	p≤0.01

* p≤0.05; ** p≤0.01; *** p≤0.001; pairwise comparison with controls by Fisher exact test (performed by OEHHA).

N.S.: not significant

[1]Exact trend test (performed by OEHHA).

[2]Benign tumors (IARC, 1982)

In the male hamster study, animals in the highest dose group died with visible pancreatic tumors as early as week 30. Survival was decreased in a dose-dependent pattern in treated males, ranging between 32 and 60 weeks. The average survival for the control males was 65 weeks. No tumors were seen in the controls. Treated males often developed tumors in several organs simultaneously. Treatment-related increases in tumors were observed in the nasal cavity, trachea, lung, forestomach, liver, gallbladder, pancreas and kidney and possibly also in the larynx (Table 12). Tumor incidences were significantly increased by both trend tests and pairwise comparisons with controls for the following sites: the posterior region of the nasal cavity (combined benign and malignant), trachea (benign), forestomach (combined benign and malignant), liver (combined benign and malignant), and pancreas (malignant and combined benign and malignant). Significant increases by pairwise comparisons with controls were also observed for tumors of the anterior region of the nasal cavity (combined benign and malignant, in the low- and second-highest dose groups; rare), lung (combined benign and malignant, in all dose groups; rare), gallbladder (combined benign and malignant, in the third-highest dose group; rare), and pancreas (benign, in the third-highest and highest dose groups; rare/uncommon). The increase in benign kidney tumors, rare in hamsters, was marginally significant in the high dose group as compared to controls.

The authors described the tumors observed in the anterior regions of the nasal cavity as mainly papillary polyps and papillomas, whereas the tumors observed in the posterior region of the nasal cavity were primarily adenocarcinomas. The squamous cell papillomas of the larynx were mostly small and occurred near the first tracheal ring. Tracheal papillary polyps occurred in all parts of the trachea; these are considered benign neoplasms (IARC, 1982). The pulmonary tumors were described as adenomas, adenocarcinomas, and squamous cell tumors. As noted previously, lung tumors are considered rare in hamsters (IARC, 1996). Liver cholangiomas and cholangiocarcinomas often occurred together within the same animal. Gallbladder tumors, rare, were described as either papillary polyps or papillary adenocarcinomas that often invaded the liver parenchyma. Forestomach tumors were reported to be mostly squamous cell papillomas, with multiple tumors occurring in the same animal's forestomach. The renal tumors observed were adenomas, rare in Syrian hamsters (IARC, 1996). Metastasis of ductal pancreatic carcinomas to the lungs occurred in one animal in the third-highest dose group, five animals in the second-highest dose group, and three animals in the highest dose group.

With regard to non-neoplastic findings, the authors reported frequent pronounced hyperplasia of the pancreatic duct epithelia in treated animals, and occasional goblet

cell metaplasia. Pronounced atrophy of pancreatic acinar cells was also commonly observed in treated animals.

Table 12. Tumor incidence in male Syrian golden hamsters administered DMNM by weekly gavage (Mohr *et al.*, 1977; Reznik *et al.*, 1978)

Tumor Site and Type		Control	DMNM (mg/kg/week)				Trend Test[1]
			9.19	18.37	36.74	73.48	
Nasal cavity ***Anterior: nasoturbinal, maxilloturbinal, & maxillary sinuses***	Combined papilloma & adenocarcinoma	0/15	4/14*	3/14	4/12*	3/13	N.S.
Nasal cavity ***Posterior: endo- and exoturbinates***	Combined papilloma & adenocarcinoma	0/15	1/14	2/14	1/12	5/13*	p≤0.01
Trachea	Papillary polyp[2]	0/15	4/14*	3/14	3/12[&]	6/13**	p≤0.01
Lung	Combined adenoma & adenocarcinoma	0/15	7/14**	9/14***	5/12**	5/13*	N.S.
Larynx	Squamous cell papilloma	0/15	2/14	1/14	3/12[&]	2/13	N.S.
Forestomach	Combined squamous cell papilloma & carcinoma	0/15	1/14	7/14**	3/12[&]	4/13*	p≤0.05
Liver	Combined cholangioma & cholangiocarcinoma	0/15	0/14	3/14	4/12*	5/13*	p≤0.01
Gallbladder	Combined papillary polyps & adenocarcinoma	0/15	2/14	4/14*	2/12	3/13[&]	N.S.
Pancreas	Ductal adenoma	0/15	2/14	7/14**	1/12	4/13*	N.S.
	Ductal adenocarcinoma	0/15	0/14	5/14**	6/12**	6/13**	p≤0.001
	Combined adenoma & carcinoma [Metastases to lungs]	0/15 [0]	2/14 [0]	10/14*** [1]	6/12** [5]	9/13*** [3]	p≤0.001
Kidney	Adenoma	0/15	0/14	0/14	0/12	3/13[&]	p≤0.01

[&] 0.05<p<0.1 (marginally significant); * p≤0.05; ** p≤0.01; *** p≤0.001; pairwise comparison with controls by Fisher exact test (performed by OEHHA).

N.S.: not significant

[1]Exact trend test (performed by OEHHA).

[2]Benign tumors (IARC, 1982)

- **Rao *et al*. (1981)**

Male Syrian golden hamsters, 20 animals per treatment group, were administered by gavage once per week for 30 weeks either an isomeric mixture of DMNM (2 parts *cis*: 1 part *trans*) at doses of 18 or 50 mg/kg bw/wk, *cis*-DMNM at doses of 11.5 or 31 mg/kg bw/wk, *trans*-DMNM at doses of 6.5 or 17 mg/kg bw/wk, α-deuterated DMNM (nitroso-2,6-dimethylmorpholine-3,3,5,5-d_4) at doses of 18 or 50 mg/kg bw/wk, or β-deuterated DMNM (N-nitroso-2,6-dimethyl-2,6-d_2-morpholine) at a dose of 50 mg/kg bw/wk. The control group of 20 animals received olive oil (the vehicle) by gavage once per week for 30 weeks. Animals were observed until death or termination of the experiment at the end of 66 weeks.

Survival was decreased in all treatment groups, as compared to the control animals, which all survived to the end of the study. Treatment-related effects on survival, from most to least affected, were as follows: α-deuterated DMNM, isomeric mixture of DMNM and *cis*-DMNM (equivalent effects on survival), β-deuterated DMNM, and *trans*-DMNM. All animals in the high-dose α-deuterated DMNM group died by week 28, and average survival in the low-dose group was 36-44 weeks. Average survival in the high-dose isomeric mixture of DMNM and *cis*-DMNM groups was somewhat less than 44 weeks, and 52 weeks in the low-dose groups. Average survival in the β-deuterated DMNM group was 52 weeks, and greater than 66 weeks in both the high- and low-dose *trans*-DMNM groups.

As shown in Table 13, tumors of the trachea, lung, liver, pancreas, and kidney were observed in each of the treatment groups. Tumors developed earlier in the α-deuterated 50 mg/kg/week treatment group (19-28 weeks) than in the β-deuterated 50 mg/kg/week treatment group (32-66 weeks). The authors reported that five control animals had benign tumors (tumor site and type not stated); no malignant tumors were reported in controls. Because of the lack of information on the types of benign tumors observed in the controls, interpretation of the effects of the various treatments on benign tumor incidence is uncertain, and in some cases not possible. Hence, the discussion that follows focuses on the malignant tumor findings.

The isomeric mixture of DMNM induced statistically significant increases in:

- Liver angiosarcoma
- Pancreatic carcinoma (with a positive dose-related trend)

cis-DMNM induced statistically significant increases in:

- Liver angiosarcoma

- Pancreatic carcinoma
- Kidney carcinoma (with a positive dose-related trend; rare)

α-Deuterated DMNM induced statistically significant increases in:

- Liver angiosarcoma
- Pancreatic carcinoma (with a positive dose-related trend)
- Kidney carcinoma (rare)

β-Deuterated DMNM induced statistically significant increases in:

- Kidney carcinoma (rare).

Tumors observed in the *trans*-DMNM-treated groups were primarily benign, and the increases reported in malignant tumors did not reach statistical significance at p = 0.05. A consistent pattern of higher cholangioma incidence occurring in the low- *versus* the high-dose groups was observed for all DMNM forms tested at two doses (e.g., isomeric DMNM, *cis*-DMNM, t*rans*-DMNM, and α-deuterated DMNM).

Epithelial hyperplasia of large- and medium-sized ducts or atrophy of acinar tissues was often found in animals with adenocarcinomas of the pancreas. Both focal and/or diffuse endothelial hyperplasia were frequently observed in animals with angiosarcomas of the liver. Hyperplastic hepatic nodules were present in 20-30% of the animals in both DMNM-treated groups. Focal dysplasia of renal tubular epithelium was found in all animals treated with DMNM, regardless of doses administered or the presence of renal cell carcinoma.

Because of the different dose levels administered to the *cis*- and *trans*-DMNM treatment groups and the small group sizes, it is difficult to compare the tumor incidence data across sites and determine with confidence whether one isomeric form is more carcinogenic than the other. However, when comparing the site-specific tumor incidence data observed in the isomeric DMNM treatment groups with those observed in the *cis*- and *trans*-DMNM treatment groups, it appears that *cis*-DMNN is more potent than the isomeric mixture of DMNM, which is more potent than *trans*-DMNM.

Potency comparisons between the α- and β-deuterated forms of DMNM and with non-deuterated DMNM are complicated by the use of just one high dose level for β-deuterated DMNM, poor survival in the α-deuterated DMNM groups, and small group sizes. Tumors occurred much earlier in the α-deuterated DMNM groups (19 - 24 weeks) than in the β-deuterated DMNM group (32 - 66 weeks). No consistent patterns

in site-specific cancer potencies were found between the α-deuterated and β-deuterated DMNM treatment groups. β-Deuterated DMNM-treated animals had a lower incidence of angiosarcoma of the liver (3 *versus* 8) and lung adenoma (10 *versus* 13), but a higher incidence of kidney carcinoma (7 *versus* 2) and trachea papilloma (4 *versus* 2) as compared to α-deuterated DMNM-treated animals receiving the same dose (50 mg/kg/week). The number of tumor-bearing animals did not differ significantly among the animals receiving DMNM or α-deuterated DMNM at a dose of 18 mg/kg/week, or among the animals receiving either isomeric DMNM, α-deuterated DMNM or β-deuterated DMNM at a dose of 50 mg/kg/week. Because the cleavage of carbon-deuterium bonds occurs at a slower rate than cleavage of carbon-hydrogen bonds, these results suggest that in the hamster conditions that favor oxidation of the β carbons of DMNM lead to greater carcinogenic activity in the lung and liver, while conditions that favor oxidation of the α carbons lead to greater activity in the kidney and possibly the trachea.

Table 13. Tumor incidence in male Syrian golden hamsters administered DMNM by weekly gavage for 30 weeks (Rao *et al*., 1981)

Tumor Type and Site		Control (olive oil)	Isomeric DMNM *cis*: *trans* = 2:1 (mg/kg/week)		*cis*-DMNM (mg/kg/week)		*trans*-DMNM (mg/kg/week)		α-deuterated DMNM (mg/kg/week)		β-deuterated DMNM (mg/kg/week)
			18	50	11.5	31	6.5	17	18	50	50
Trachea	Papilloma	NR	3/20	2/20	1/20	7/20	0/20	0/20	3/20	2/20	4/20
Lung	Adenoma	NR	8/20	11/20	6/20	16/20	2/20	1/20	16/20	13/20	10/20
	Carcinoma	0/20	1/20	0/20	2/20	0/20	0/20	0/20	0/20	0/20	0/20
Liver	Benign vascular tumor	NR	0/20	3/20	0/20	2/20	0/20	0/20	0/20	0/20	0/20
	Cholangioma	NR	11/20	0/20	8/20	4/20	6/20	3/20	7/20	0/20	4/20
	Cholangiocarcinoma	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20
	Hepatocellular carcinoma	0/20	1/20	0/20	1/20	0/20	0/20	2/20	1/20	0/20	0/20
	Angiosarcoma	0/20	0/20	6/20** trend[1]:***	0/20	8/20** trend:***	0/20	0/20	0/20	8/20** trend:***	3/20
Pancreas	Cystadenoma	NR	14/20	14/20	11/20	17/20	2/20	4/20	10/20	15/20	16/20
	Carcinoma	0/20	1/20	5/20* trend:**	0/20	6/20** trend:***	0/20	0/20	2/20	4/20* trend:**	2/20
Kidney	Carcinoma	0/20	3/20	2/20	2/20	4/20* trend:*	0/20	1/20	5/20*	2/20	7/20**

NR: not reported.

*p≤0.05; **p≤0.01; ***p≤0.001, pairwise comparison with controls by Fisher exact test (performed only for malignant tumors by OEHHA).

[1]Exact trend test (performed only for malignant tumors by OEHHA).

- **Lijinsky *et al*. (1982b)**

Twenty male Syrian golden hamsters, seven-weeks old, were administered 6 mg of *cis*-DMNM dissolved in a 50% ethanol/water solution by weekly gavage for 30 weeks, for a total dose of 180 mg (Lijinsky *et al*., 1982b). No concurrent control animals were included in this study. Treated animals were allowed to die naturally or were killed when moribund. Fifteen animals survived to week 30, only two survived to week 40 and none survived at week 50. All treated animals developed tumors, often at multiple sites. Tumors of the pancreas, lung and liver were most frequently observed in treated animals, followed by nasal cavity and forestomach tumors (Table 14). About 90% of the animals with pancreatic tumors had ductal adenocarcinomas. Tumors of the nasal cavity and lung were considered rare in Syrian hamsters, and the spontaneous incidence of pancreatic tumors in Syrian hamsters is less than 2% (IARC, 1996).

Table 14. Tumor incidence in male Syrian golden hamsters receiving *cis*-DMNM by weekly gavage for 30 weeks (Lijinsky *et al.*, 1982b)

Tumor Site and Type		*cis*-DMNM (6 mg/week)
Nasal cavity	Olfactory adenocarcinoma	12/20
Lung	Adenoma	19/20
Forestomach	Benign tumors, not otherwise specified	4/20
Liver	Hemangiosarcoma	16/20
	Cholangiocarcinoma	2/20
Pancreas	Ductal adenocarcinoma	17/20
	Combined benign & malignant tumors	19/20

- **Althoff *et al.* (1985)**

Male and female six- to eight-month-old European hamsters were administered weekly doses of DMNM by gavage at 0, 37, and 112 mg/kg bw for life (Althoff *et al*., 1985). In both the male and female studies the control and high-dose groups consisted of 15 animals each. In the male study, the low-dose group consisted of seven animals. In the female study, the low-dose group consisted of 13 animals. Control animals in each study received solvent only.

In the male study, survival was decreased in a dose-dependent pattern, with average survival of 75 weeks, 29 weeks, and 14 weeks in the control, low-, and high-dose groups, respectively. The first tumor was seen at 12 weeks in the high-dose group, at 23 weeks in the low-dose group, and at 68 weeks in the control. DMNM induced respiratory epithelial tumors of the nasal cavity and lungs, and hemangioendotheliomas in the liver in males (Table 15). No tumors were observed at these sites in the controls.

In the female study, a dose-dependent decrease in survival was also observed, with average survival of 79 weeks, 37 weeks, and 23 weeks in the control, low-, and high-dose groups, respectively. The first tumor was observed at 17 weeks in the high-dose group, at 30 weeks in the low-dose group, and at 68 weeks in the control. Tumors of

Table 15. Tumor incidence in male and female European hamsters administered DMNM by weekly gavage for life (Althoff *et al.*, 1985)

Tumor Site and Type		DMNM (mg/kg/week)			Trend Test[1]
		0	37	112	
Male					
Nasal cavity	Combined benign and malignant	0/15	5/7***	5/15*	0.07
Larynx	Combined benign and malignant	0/15	0/7	1/15	N.S.
Trachea	Combined benign and malignant	0/15	1/7	0/15	N.S.
Lung	Combined benign and malignant	0/15	0/7	5/15*	p≤0.01
Liver	Hemangioendothelioma	0/15	6/7***	0/15	N.S.
Female					
Nasal cavity	Combined benign and malignant	0/15	10/13***	10/15***	p≤0.001
Larynx	Combined benign and malignant	0/15	1/13	1/15	N.S.
Trachea	Combined benign and malignant	0/15	2/13	5/15*	p≤0.05
Lung	Combined benign and malignant	0/15	1/13	7/15**	p≤0.001
Liver	Hemangioendothelioma	0/15	8/13**	2/15	N.S.
	Cholangiocellular adenoma	0/15	0/13	1/15	N.S.
	Cholangiocellular carcinoma	0/15	0/13	1/15	N.S.

* p≤0.05; ** p≤0.01; *** p≤0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA).

N.S.: not significant at p=0.05

[1]Exact trend test (performed by OEHHA)

the nasal cavity, lung and trachea showed significant treatment-related increases by both pairwise comparison and trend test in females, and liver hemangioendotheliomas were also significantly increased in the low-dose group (Table 15). No tumors were observed at these sites in the controls. Non-neoplastic effects of DMNM included hyperplasia, metaplasia and dysplasia of the respiratory epithelium.

3.2.2.2 S.C. injection studies

- **Althoff *et al*. (1978)**

DMNM was administered to eight-week old Syrian golden hamsters by weekly *s.c.* injection in olive oil at doses of 0, 8, 16 and 32 mg/kg bw for life (Althoff *et al.*, 1978). The control group received weekly injections of olive oil. Each dose group consisted of 30 animals (15 males and 15 females). Tumor findings were reported by dose group; gender-specific tumor incidence data were not reported. The last experimental animal died at 56 weeks, at which time all surviving control animals were sacrificed.

A dose-dependent decrease in survival was observed; average survival was 32 weeks for the high-dose group, 47 weeks for the low-dose group, and 50 weeks for controls. The respiratory tract was the main target of DMNM carcinogenicity following *s.c.* injection in Syrian golden hamsters, with the first tumor observed at week 15. Significant increases in tumors of the nasal cavity, larynx, trachea, lung, forestomach, pancreas, gallbladder, liver/bile duct/gallbladder, vagina, and Harderian gland were observed (Table 16). Nasal cavity tumors were the most common type of respiratory tract tumor, and these included papillomas (seen in the anterior nasal cavity) and adenocarcinomas (seen in the middle and posterior nasal cavity). Nearly 100% (29/30) of the animals in the high dose group developed nasal cavity tumors. These lesions frequently invaded the olfactory bulb and the brain, as well as surrounding tissues such as the palate and Harderian gland. One nasal cavity tumor metastasized to the lungs.

Regarding non-neoplastic effects, multifocal hyperplastic, metaplastic, and dysplastic areas of the respiratory epithelium were observed in all DMNM-treated animals, and nodular proliferation of the terminal bronchial epithelium was commonly seen in treated, but not control animals. Treated animals had a higher incidence of multifocal proliferative or adenomatous lesions of the epithelia of the biliary (50-53% in treated animals *versus* 20% in controls) and pancreatic ducts (77-90% in treated animals *versus* 30% in controls) than the controls.

Table 16. Tumor incidence in Syrian golden hamsters administered weekly *s.c.* injections of DMNM for life (Althoff *et al.*, 1978)

Tumor Site and Type		DMNM (mg/kg bw/week)				
		0	8	16	32	Trend Test[1]
Nasal cavity	Combined papilloma and adenocarcinoma	0/30	24/30***	25/30***	29/30***	p≤0.001
Larynx	Combined papillary polyp and carcinoma	0/30	4/30[&]	6/30*	8/30**	p≤0.01
Trachea	Papillary polyp	0/30	9/30***	9/30***	12/30***	p≤0.001
Lung	Combined adenoma and adenocarcinoma	0/30	7/30**	7/30**	12/30***	p≤0.001
Forestomach	Combined papilloma and carcinoma	1/30	11/30***	11/30***	9/30**	p≤0.05
Pancreas	Combined adenoma and adenocarcinoma	0/30	2/30	5/30*	9/30***	p≤0.001
Gallbladder	Polypoid adenoma	0/30	3/30	2/30	5/30*	p≤0.05
Liver, bile duct and gallbladder	Combined adenoma, carcinoma, hemangioma, cholangioma, and cholangiocarcinoma	1/30	4/30	8/30*	7/30*	p≤0.05
Kidney	Adenoma	0/30	0/30	2/30	3/30	p≤0.05
Harderian gland	Adenoma	0/30	5/30*	5/30*	5/30*	0.065
Skin	Combined trichoepithelioma and squamous cell papilloma	0/30	1/30	1/30	3/30	p≤0.05
Vagina	Papilloma	0/15	13/15***	12/15***	8/15***	p≤0.01

* p≤0.05; ** p≤0.01; *** p≤0.001, [&] marginally significant with 0.05<p<0.1, pairwise comparison with control by Fisher exact test (performed by OEHHA).
NS: Not significant at p=0.05
[1]Exact trend test (performed by OEHHA).

- **Althoff *et al.* (1985)**

DMNM was administered to groups of 15 six- to eight-month old male and female European hamsters by *s.c.* injection at doses of 0 (solvent only) or 85 mg/kg bw once per week, for life (Althoff *et al*., 1985). In both the male and the female studies, the effect of hibernation on the carcinogenicity of DMNM was also investigated in separate groups of controls (n = 10) and treated animals (n = 24) kept at 4°C and 90% humidity for 12 weeks to simulate hibernation conditions from December to March.

In the male study, DMNM treatment reduced survival as compared to controls, in animals that did not undergo hibernation (20 *versus* 71 weeks), and in animals that did (26 *versus* 105 weeks). The first tumor was seen at week 17 in the DMNM-treated non-hibernation group, and at week 20 in the DMNM-treated hibernation group. The first tumor in the non-hibernation controls was seen at 63 weeks, while no tumors were observed in the hibernation controls.

DMNM induced statistically significant increases in combined benign and malignant tumors of the nasal cavity and the lungs in both the hibernation and non-hibernation males (Table 17). Non-significant increases in tumors of the trachea and larynx were observed in both the DMNM-treated hibernation and non-hibernation groups, compared to no tumors at these sites in the respective controls. Other tumors observed in non-hibernation animals included hemangioendotheliomas of the liver in two DMNM-treated males, compared to none in the controls, and transitional cell carcinoma of the renal pelvis or urinary bladder in two DMNM-treated males, compared to one transitional cell papilloma in the control group. Other tumors in the hibernation animals included cholangiocellular carcinomas of the liver in two DMNM-treated males, compared to none in the controls, and transitional cell carcinoma of the renal pelvis or urinary bladder in two DMNM-treated males, compared to none in the controls. Non-neoplastic effects of DMNM included hyperplasia, metaplasia and dysplasia of the respiratory epithelium.

In the female study, DMNM treatment reduced survival as compared to controls, in animals that did not undergo hibernation (25 *versus* 81 weeks), and in animals that did (27 *versus* 102 weeks). The first tumor was seen at week 13 in the DMNM-treated non-hibernation group, and at week 22 in the DMNM-treated hibernation group. The first tumor in the non-hibernation controls was seen at 63 weeks, while no tumors were observed in the hibernation controls.

DMNM induced statistically significant increases in combined benign and malignant tumors of the nasal cavity in both the hibernation and non-hibernation females (Table 17). Increases in combined benign and malignant lung tumors were statistically significant in the non-hibernating females, but did not reach statistical significance in hibernating females. Non-significant increases in tumors of the trachea and larynx were observed in both the DMNM-treated hibernation and non-hibernation groups, compared to no tumors at these sites in the respective controls. Other tumors observed in non-hibernation animals included hemangioendotheliomas of the liver in two DMNM-treated females, compared to none in the controls, and transitional cell carcinoma of the renal pelvis or urinary bladder in three DMNM-treated females, compared to none in controls. Other tumors in the hibernation animals included a liver cholangiocellular adenoma in one DMNM-treated female and liver cholangiocellular carcinomas in two DMNM-treated females, compared to no cholangiocellular tumors in the controls, and transitional cell carcinomas of the renal pelvis or urinary bladder in two DMNM-treated females, compared to none in the controls. Non-neoplastic effects of DMNM included hyperplasia, metaplasia and dysplasia of the respiratory epithelium.

Table 17. Tumor incidence in male and female European hamsters administered DMNM by weekly *s.c.* injection for life (Althoff *et al.*, 1985)

Tumor Site and Type		DMNM (mg/kg/week)		DMNM + Hibernation (mg/kg/week)	
		0	85	0	85
Male					
Nasal cavity	Combined benign and malignant	2/15	12/15**	0/10	17/24**
Larynx	Benign	0/15	2/15	0/10	2/24
Trachea	Combined benign and malignant	0/15	3/15	0/10	5/24
Lung	Combined benign and malignant	0/15	8/15**	0/10	10/24*
Liver	Hemangioendothelioma	0/15	2/15	0/10	0/24
	Cholangiocellular carcinoma	0/15	0/15	0/10	2/24
Renal pelvis/urinary bladder	Transitional cell papilloma	1/15	0/15	0/10	0/24
	Transitional cell carcinoma	0/15	2/15	0/10	2/24
Female					
Nasal cavity	Combined benign and malignant	0/15	12/15**	0/10	23/24**
Larynx	Benign	0/15	2/15	0/10	3/24
Trachea	Combined benign and malignant	0/15	3/15	0/10	3/24
Lung	Combined benign and malignant	0/15	9/15**	0/10	6/24
Liver	Hemangioendothelioma	0/15	2/15	0/10	0/24
	Cholangiocellular adenoma	0/15	0/15	0/10	1/24
	Cholangiocellular carcinoma	0/15	0/15	0/10	3/24
Renal pelvis/urinary bladder	Transitional cell papilloma	0/15	0/15	0/10	0/24
	Transitional cell carcinoma	0/15	3/15	0/10	2/24

* p≤0.05; ** p≤0.001, pairwise comparison with controls by Fisher exact test (performed by OEHHA)

- **Kokkinakis and Scarpelli (1989)**

cis-DMNM (> 98% purity) was administered to male Syrian golden hamsters by continuous *s.c*. infusion via an osmotic pump for a week (Kokkinakis and Scarpelli, 1989). Fifteen animals received a total dose of 750 mg/kg bw and 10 animals received

a total dose of 950 mg/kg bw. *cis*-DMNM was dissolved in 30% ethanol. Ten control animals were infused with saline for seven days. Animals were observed for 25 weeks after treatment.
No toxic effects or deaths were observed in either dose group six weeks after treatment. Twenty-five weeks after treatment, 5 high-dose animals and 12 low-dose animals survived. As shown in Table 18, a low incidence of pancreatic and liver tumors was observed in *cis*-treated animals in this model system. No survival or tumor data were reported for control animals.

Table 18. Tumor incidence in male Syrian golden hamsters administered *cis*–DMNM via continuous *s.c.* infusion for 7 days (Kokkinakis and Scarpelli, 1989)

Tumor Site and Type	*cis*-DMNM (mg/kg bw)	
	750	950
Pancreas		
Carcinomas *in situ*	0/12	2/5
Ductal adenocarcinoma	0/12	1/5
Liver		
Cholangioma	0/12	2/5
Cholangiocarcinoma	0/12	1/5
Hepatocellular carcinoma	1/12	0/5

3.2.3 Studies in Guinea Pigs

3.2.3.1 Gavage Studies

Two sets of gavage studies of DMNM have been conducted in two different types of male guinea pigs in two laboratories (Cardy and Lijinsky, 1980; Rao *et al.*, 1980).

- **Cardy and Lijinsky (1980)**

DMNM was administered by gavage in olive oil to groups of 20 eight week old male "strain 2" guinea pigs twice a week at doses of 80 mg/kg/wk for 12 weeks (total dose 960 mg/kg) or 32 mg/kg/wk for 35 weeks (total dose 1120 mg/kg) (Cardy and Lijinsky, 1980). The control group, 20 animals, received olive oil at 2 ml/kg/wk for 90 weeks. Animals were allowed to live until moribund or natural death. In the 80 mg/kg/wk dose group, two animals survived for 90 weeks and none for 100 weeks. In the 32 mg/kg/wk dose group, 18 animals survived for 40 weeks and none for 50 weeks. In the control

group, 20 animals survived for 50 weeks, five were sacrificed for examination at week 76, six survived for 110 weeks, and none for 120 weeks.

The liver was the primary target organ for the carcinogenicity of DMNM administered by gavage in the guinea pig. The most common tumor observed in the liver was of vascular endothelial origin, *i.e*., hemangioendothelial sarcoma (also known as hemangiosarcoma). DMNM induced statistically significant increases in hemangiosarcoma of the liver in both treatment groups (Table 19). Six additional malignant liver tumors of various types occurred in the 80 mg/kg/wk group, including two bile duct carcinomas (Table 19).

The authors observed that the vascular endothelial tumors induced by DMNM frequently metastasized to other organs. Metastases were mainly to the lungs, but were also observed in the spleen, pancreatic lymph node, kidney, and urinary bladder. Information on the incidence of tumor metastasis was not provided in the paper.

Many liver sections showed massive cystic biliary proliferation and fibrosis which was referred to as “cystic biliary fibroadenosis.” This non-reversible proliferative lesion of the bile duct occurred in all 16 animals in the 80 mg/kg/wk dose group, as compared with none in the control or 32 mg/kg/wk groups, and often destroyed large areas of liver parenchyma. The authors speculated that this progressive lesion was a precursor to the development of the bile duct carcinomas seen in the 80 mg/kg/wk dose group.

Table 19. Liver tumor incidence in male strain 2 guinea pigs administered DMNM by gavage twice a week (Cardy and Lijinsky, 1980)

Liver Tumor Type	Control (olive oil)	DMNM (mg/kg/week) [Total dose (mg/kg)]	
		80 for 12 weeks[1] [960]	32 for 35 weeks [1120]
Hemangioendothelial sarcoma (Hemangiosarcoma)	0/20	6/16*	19/20**
Hepatocellular carcinoma	0/20	1/16	0/20
Bile duct carcinoma (Cholangiocarcinoma)	0/20	2/16	0/20
Undifferentiated carcinoma	0/20	1/16	0/20
Undifferentiated sarcoma	0/20	2/16[2]	0/20

* p≤0.01; ** p≤0.001, pairwise comparison with controls by Fisher exact test (performed by OEHHA).

[1] Four animals died on the 12th week and were not necropsied.

[2] One tumor was considered by the authors to be an osteosarcoma.

- **Rao *et al.* (1980)**

DMNM was administered weekly for 23 weeks to groups of 18 male random-bred guinea pigs by gavage in olive oil at two dose levels: 14 and 28 mg/kg/wk. A control group of 12 animals received olive oil by gavage for 23 weeks. Animals in the control and high dose groups were sacrificed at 37 weeks, and animals in the low dose group were sacrificed at 54 weeks.

Similar to the findings of Cardy and Lijinsky (1980), the liver was the target organ in random-bred guinea pigs, with statistically significant increases in hemangiosarcomas and cholangiomas (Table 20). In the low dose group, two-thirds of the animals developed hemangiosarcomas of the liver, with the first tumor appearing at week 36. In the high dose group, 82% (14/17) of the animals developed hemangiosarcomas of the liver, with the first tumor appearing at week 26. The metastases of hemangiosarcomas to other organs were observed. Metastases to the lungs occurred in 3 low-dose animals and one high-dose animal. Metastases to the mesenteries occurred in two high-dose animals, and to the lymph nodes in one low-dose animal. Cholangiomas occurred in 60% (9/15) of the low dose group and 47% (8/17) of the high dose group. No tumors occurred in the control group.

Focal and diffuse hyperplasia of the hepatic sinusoidal endothelial cells was reported in the vicinity of the hemangiosarcomas, and occasionally at distant sites. Extramedullary hematopoiesis was commonly found in livers adjacent to hemangiosarcomas, and occasionally in the tumors. Other pathological findings in the liver included fatty infiltration, oval cell proliferation, and peliosis hepatis in both the low- and high-dose groups. Peliosis hepatis (also called angiectasis) appeared as small dark blood-filled spaces in the liver as a result of dialated sinusoids containing red blood cells. This lesion was induced by nitrosamines in rats and can be a precursor of hemangiomas and hemangiosarcomas (Eustis *et al*., 1990). In addition, the authors reported that the gall bladder showed marked focal epithelial hyperplasia in two high-dose animals.

Other tumors observed in the DMNM-treated animals included one malignant mesenchymal tumor in the low dose group; one malignant lymphoma, one hepatocellular carcinoma, and one bronchioalveolar adenoma in the high dose group.

Table 20. Liver tumor incidence in male random-bred guinea pigs administered DMNM by weekly gavage (Rao *et al.*, 1980)

Liver Tumor Type	Control[1] (olive oil)	DMNM (mg/kg/week)[1]	
		14	28
Hemangiosarcoma	0/12	10/15**	14/17**
Cholangioma (Bile duct adenoma)	0/12	9/15**	8/17*

* p≤0.01; ** p≤0.001, pairwise comparison with controls by Fisher exact test (performed by OEHHA).
[1]Administered for 23 weeks

3.2.4 Diet study in Trout

- **Hendricks *et al*. (1995)**

Two groups of 100 eight-week old Shasta strain rainbow trout were fed a diet containing 1556 mg DMNM per kg dry weight of diet for up to 18 months, and two groups of 100 control trout were fed a control diet for up to 18 months. Approximately 40% of the fish in each treatment and control group were sacrificed at 9 months; the remaining fish were sacrificed at 18 months. The two treated groups and the control groups were

combined for reporting purposes. Fish were fed to satiety three times daily up to six months of age, and twice a day thereafter.

The average total dietary dose for the 18-month DMNM treatment group was 781 mg DMNM. Internal organs were examined grossly and histology was performed on liver, portions of head kidney[4], kidney, spleen, gill, gonads, thymus, thyroid, heart, stomach, pyloric ceca[5], duodenum, rectum, pancreas, and swimbladder. Growth and survival of trout was similar for treated and control groups. No differences in liver weights were observed for treated compared to untreated trout.

DMNM induced tumors of the liver in trout after 9 months of dietary exposure, and tumors of the liver, glandular stomach, and swimbladder after 18 months (Table 21). Specifically, after 9 months dietary exposure, statistically significant ($p<0.001$) increases in combined hepatocellular carcinoma and hepatocholangiocellular carcinoma, and combined hepatocellular adenoma, carcinoma, and hepatocholangiocellular carcinoma were observed in DMNM-treated trout, compared to controls (Table 21).

After 18 months of dietary exposure, statistically significant increases in both benign and malignant liver tumors were observed, with 69% (78/113) of DMNM-treated trout developing either benign or malignant liver tumors compared to none in controls; also after 18 months exposure 47% (53/113) of DMNM-treated trout developed papilloma of the glandular stomach, compared to none in the controls; and 4% (4/113) of DMNM-treated trout developed swimbladder papilloma compared to none in the controls. Hyperplasia of the glandular stomach was observed in 9/64 DMNM-treated trout after 9 months of dietary exposure, compared to none in the controls ($p<0.001$); hyperplasia was not observed in treated or control trout after 18 months.

While fish such as trout lack complete organ homology to humans, trout are sensitive to many classes of chemical carcinogens, have well described tumor pathology; and show responsiveness to tumor promoters and inhibitors (Baily *et al*., 1996).

[4] Fish kidneys are narrow, straplike, and extend the entire length of the abdominal cavity. The kidney is greatly expanded at the anterior end and is called the head kidney. The expansion is due to blood sinuses.

[5] Pyloric ceca secrete the digestive enzymes required to digest some food. Fish without the pyloric ceca have digestive enzyme production in the liver and pancreas.

Table 21. Tumor incidence in Rainbow trout administered DMNM in the diet for 9 or 18 months (Hendricks *et al*., 1995)

Tumor site*	Tumor type	Duration of dietary exposure to DMNM[1]			
		9 months		18 months	
		control	DMNM	control	DMNM[2]
Liver	Hepatocellular adenoma	0/66	2/64	0/113	12/113**
	Combined hepatocellular carcinoma & hepato-cholangiocellular carcinoma	0/66	9/64**	0/113	66/113**
	Combined hepatocellular adenoma & carcinoma & hepatocholangio-cellular carcinoma	0/66	11/64**	0/113	78/113**
Glandular stomach	Papilloma	0/66	0/64	0/113	53/113**
Swim-bladder	Papilloma	NR[3]	NR	0/113	4/113*

[1] 1556 mg of DMNM given per kg dry weight of diet.
[2] Average total dose per fish is equal to 781 mg DMNM.
[3] NR- Not reported
*marginally statistically significant (p=0.06); pairwise comparison with controls by Fisher exact test (performed by OEHHA)
** p<0.001, pairwise comparison with controls by Fisher exact test (performed by OEHHA)

3.3 Other Relevant Data

3.3.1 Genotoxicity

The genotoxicity of DMNM was tested in the *Salmonella typhimurium* reverse mutation assay, the *Drosophila* X-linked recessive lethal mutation assay, *in vitro* in isolated hamster main pancreatic ducts and rat hepatocyte primary culture unscheduled DNA synthesis (UDS) assays, and *in vivo* for induction of single strand DNA breaks in pancreatic acinar cells of rats and hamsters. *In vitro* binding to hamster pancreas DNA, RNA, and protein, and *in vivo* binding to hamster and rat liver DNA has also been

investigated. DMNM is positive in multiple test systems, inducing mutations, UDS, and DNA single strand breaks, and binds covalently with DNA, RNA, and protein *in vitro* and *in vivo*.

Several studies using the *Salmonella* reverse mutation (Ames) test indicate that DMNM is mutagenic in several *Salmonella* tester strains, including TA 100, TA 1530, TA 1535, and TA 98 (see Table 22). Reverse mutations in strains TA 100, TA 1535, and TA 1530 are indicative of basepair substitution, whereas they are due to frameshift mutations in TA 98. Specifically, DMNM is mutagenic in TA 100 with metabolic activation by S9 from the liver (but not pancreas or lung) from the hamster, rat, mouse, rabbit, and monkey. DMNM was not mutagenic in TA 100 in the presence of S9 from human liver, pancreas or lung. The lack of activity with S9 from human tissue correlates with the low cytochrome P-450 concentration in human biopsied liver as compared to animal liver tissue (Yamazaki *et al*., 1986). Similarly, cytochrome P-450 content in S9 preparations from human lung tissue was not detectable (Mori *et al*., 1986b). Induction of rat liver enzymes with phenobarbital (PB) and hamster liver enzymes with polychlorinated biphenyls (PCB) led to an increase in mutagenicity compared to uninduced tissue in TA 100 (See Table 22). DMNM was also positive in tester strain TA 1530 both with and without metabolic activation by S9 from hamster liver, although mutagenicity was less in the absence of S9. In strain TA 1535, DMNM was positive with liver and pancreas S9 from rat, hamster, and mouse (induced and un-induced). DMNM was primarily negative in tester strain TA 98 in the presence or absence of rat liver S9 induced with PB, or 3-methylcholanthrene (3-MC). It was slightly positive in one study with rat liver S9 induced with PCB.

Mutagenicity assays which compared the isomeric mixture of DMNM with its purified *cis*- and/or *trans*-diastereomers yielded the following results. *Cis*-, *trans*-, and the isomeric mixture of DMNM were slightly mutagenic when tested in TA 1530 in the absence of S9 (Wislocki and Gingell, 1980). Mutagenicity was greatly enhanced with metabolic activation with PB-induced hamster liver S9, and *cis*-DMNM was more mutagenic compared to *trans*-DMNM and the isomeric mixture with this metabolic activation system (Wislocki and Gingell, 1980). In a study using TA 1535, metabolic activation with rat liver S9 (Aroclor induced) significantly increased the mutagenicity of the isomeric mixture and *trans* isomer but not the *cis* isomer (Andrews and Lijinsky, 1980). In a different study using TA 1535, both the *cis* and *trans* isomers were equally mutagenic in the presence of hamster pancreas S9 (Rao *et al*., 1980).

DMNM was positive in the *Drosophila melanogaster* X-linked recessive lethal mutation assay (Nix *et al.*, 1980; see Table 22). The F_2 generation was scored for X-linked lethal mutations. DMNM induced recessive lethal mutations in mature sperm and spermatids in *Drosophila* at the highest concentration (20 mM) tested.

DMNM induced UDS *in vitro* in a study utilizing the rat hepatocyte primary culture (HPC) repair test (Yamazaki *et al.*, 1985; see Table 23). The test uses freshly isolated, non-replicating adult hepatocytes and measures DNA damage as autoradiographic UDS. DMNM was also positive in another *in vitro* UDS study using isolated main pancreatic ducts from hamsters (Scarpelli *et al*., 1982).

The potential of DMNM to cause single strand breaks in pancreatic acinar cell DNA from rats and hamsters exposed *in vivo* was examined by Curphey *et al*. (1987) (Table 23). Rats and hamsters were injected intraperitoneally with DMNM, exposed for one hour, sacrificed, and the nuclei were harvested from the pancreatic cells. DNA damage was assessed by determining single strand DNA breaks. DMNM produced single strand DNA breaks in hamster, but not rat pancreatic acinar cells.

Incubation of regenerating hamster pancreas slices for 90 minutes with ^{3}H-labeled DMNM, *cis*-, or *trans*-DMNM resulted in covalent binding to DNA, RNA, and protein (Rao *et al*., 1981). No significant differences were observed in these *in vitro* studies between the *cis*-, *trans*-, and isomeric mixture of DMNM and the levels of covalent binding to the hamster pancreas slices.

The covalent binding of ^{3}H-labeled DMNM in the liver of Syrian golden hamsters and F344 rats was investigated six hours after gavage administration (Lijinsky, 1985). A similar percentage of the administered dose of ^{3}H-labeled DMNM was measured as label bound to cellular macromolecules in hamster and rat liver (5.5% for hamsters and 4.1% for rats). Levels of two specific alkylated nucleic acids (N-7 methylguanine and O^6-methylguanine) were also assessed in the livers of these animals. DMNM treatment resulted in the formation of N-7 methylguanine and O^6-methylguanine in hamster liver. In the rat, DMNM treatment resulted in the formation in the liver of a much lower level of N-7 methylguanine than in the hamster, and no detectable formation of O^6-methylguanine (Lijinsky, 1985).

Table 22. Non-mammalian mutagenicity tests

Endpoint	Strain	Conc. tested	Results		Activation System	References
			+ S9	- S9		
Reverse mutations *Salmonella typhimurium*	TA 100	0-20 mg/plate	+	-	Hamster liver S9	Mori *et al*., 1987
			+	-	Rat liver S9	
			+	-	Mouse liver S9	
			+	-	Rabbit liver S9	
			+	-	Monkey liver S9	
			-	-	Hamster pancreas S9	
			-	-	Rat pancreas S9	
			-	-	Mouse pancreas S9	
			-	-	Rabbit pancreas S9	
			-	-	Monkey pancreas S9	
	TA 100	0-20 mg/plate	-	-	Hamster pancreas S9 +/- PCB	Mori *et al*., 1986b
			-	-	Rat pancreas S9 +/- PCB	
			-	-	Mouse pancreas S9 +/- PCB	
			-	-	Rabbit pancreas S9	
			-	-	Monkey pancreas S9	
			-	-	Human pancreas S9	
			-	-	Hamster lung S9 +/- PCB	
			-	-	Rat lung S9 +/- PCB	
			-	-	Mouse lung S9 +/- PCB	
			-	-	Rabbit lung S9	
			-	-	Monkey lung S9	
			-	-	Human lung S9	
	TA 100	0-20 mg/plate; 10 mg/plate highest activity	(+)	-	Rat liver S9	Mori *et al.,* 1985
			+	-	Rat liver S9 PB induced	
			+	-	Rat liver S9 PCB induced	
			+	-	Rat liver S9 3-MC induced	
	TA 100	10 mg/plate	+	NR	Rat liver S9	Yamazaki *et al.,* 1986
			+	NR	Hamster liver S9	
			+	NR	Mouse liver S9	
			+	NR	Rabbit liver S9	
			+	NR	Monkey liver S9	
			-	-	Human liver S9	
	TA 100	10 mg/plate	+	-	Rat liver S9 PCB induced	Mori *et al*., 1983
			-	-	Rat lung S9 PCB induced	
	TA 100	10 mg/plate	+	-	Rat liver S9	Mori *et al.* 1986a; Mori & Konishi 1991
			+	-	Rat liver S9 PB induced	
			++	-	Rat liver S9 PCB induced	
			+	-	Rat liver S9 3-MC induced	
			+	-	Hamster liver S9	
			++	-	Hamster liver S9 PB induced	
			++	-	Hamster liver S9 PCB induced	
			+	-	Hamster liver S9 3-MC induced	
			+	-	Mouse liver S9	
			+	-	Mouse liver S9 PB induced	

Endpoint	Strain	Conc. tested	Results		Activation System	References
			+ S9	- S9		
			+	-	Mouse liver S9 PCB induced	
			+	-	Mouse liver S9 3-MC induced	
	TA 100	0-2 µmol/ plate	+	-	Hamster liver S9 Aroclor 1254 induced	Gingell *et al.*, 1980
	TA 1530*****	0.06-2.0 µmol/plate	+	(+)	Hamster liver S9 PB induced; plus S9 (supernatant added to S9 mix); *cis*, *trans*, and mix DMNM were tested	Wislocki & Gingell, 1980
	TA 1530	0-2 µmol/ plate	+	-	Hamster liver S9 Aroclor 1254 induced	Gingell *et al.*, 1980
	TA 1535	0-2 µmol/plate	+	-	Hamster liver S9 Aroclor 1254 induced	Gingell *et al*., 1980
	TA1535**	0-1000 µg/plate	(+)	(+)	Rat liver S9 Aroclor 1254 induced	Andrews & Lijinsky, 1980
	TA 1535 (assumed)	0-1000 µg/plate	(+)	-	Rat liver S9 Aroclor 1254 induced	Andrews & Lijinsky, 1984
			+	-	Hamster liver S9 Aroclor 1254 induced	
	TA 1535	0-10 µmol/plate	+	NR	Mouse liver S9	Zeiger & Sheldon, 1978
			+	NR	Rat liver S9	
	TA 1535	10 mg/plate	+	+	Hamster pancreas S9	Scarpelli *et al.*, 1980
			+	+	Hamster pancreas S9 TCDD induced	
			+	+	Hamster pancreas S9 BNF induced	
			+	+	Hamster pancreas S9 3-MC induced	
			+	+	Hamster pancreas S9 indole-3-carbinol induced	
			+	+	Hamster pancreas S9 chlorpromazine induced	
			+	+	Hamster pancreas S9 Aroclor 1254 induced	
			+	+	Hamster pancreas S9 PB induced	
	TA 1535	1-1000 µg	+	NR	Rat liver S9; PB induced	Andrews *et al.,* 1978
	TA1535***	NR	+	-	Hamster pancreas S9	Rao *et al.*, 1981
			+	+	Hamster liver S9	
	TA 98	10 mg/plate	-	-	Rat liver S9 PB induced	Mori *et al*., 1985
			-	-	Rat liver S9 PCB induced	
			-	-	Rat liver S9 3-MC induced	
	TA 98	10 mg/plate	(+)	-	Rat liver S9 PCB induced	Mori *et al.*, 1983
			-	-	Rat lung S9 PCB induced	
X-linked recessive	*Drosophila melano-*	5-20 mM	+		NA	Nix *et al*., 1980

Endpoint	Strain	Conc. tested	Results		Activation System	References
			+ S9	- S9		
lethal mutation	*gaster*					

*_cis_-DMNM is more mutagenic than _trans_-DMNM; **_trans_-DMNM is more mutagenic than _cis_- DMNM;
***_cis_- and _trans_- DMNM equally mutagenic in the presence of hamster pancreas S9
- negative; (+) slightly positive; + positive; ++ strongly positive
NR: Not Reported; NA: Not Applicable; PB: Phenobarbital; PCB: Polychlorinated Biphenyls; TCDD: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; 3-MC: 3-Methylcholanthrene; BNF: β-Naphthoflavone

Table 23. Mammalian genotoxicity tests

Endpoint	Assay System	*In vitrolin vivo*	Results	Reference
Unscheduled DNA synthesis	Rat hepatocyte primary culture	*In vitro*	+	Yamazaki *et al.*, 1985
	Hamster isolated main pancreatic ducts	*In vitro*	+	Scarpelli *et al*., 1982
Single strand DNA breaks	Rat pancreatic acinar cells	*in vivo*	-	Curphey *et al.*, 1987
	Hamster pancreatic acinar cells	*in vivo*	+	

In summary, DMNM has been shown to induce base-pair substitution mutations in *Salmonella*, primarily in the presence of metabolic activation, and X-linked recessive lethal mutations in *Drosophila*. *In vitro*, DMNM induces UDS in cultured primary rat hepatocytes and isolated hamster main pancreatic ducts. *In vivo*, DMNM induces single strand DNA breaks in pancreatic acinar cells of hamsters, but not rats. DMNM has been shown in *in vitro* studies to bind to hamster pancreas DNA, RNA, and protein. *In vivo*, DMNM has been shown to bind to hamster and rat liver DNA, RNA, and protein, and to form N-7 methylguanine in hamster and rat liver, and O^6-methylguanine in hamster liver.

3.3.2. Pharmacokinetics and metabolism

The pharmacokinetics of DMNM has been studied in rats, hamsters, and guinea pigs (Gingell *et al*., 1976a; 1978; Reznik-Schuller *et al.*, 1980; Underwood and Lijinsky, 1982; Kokkinakis *et al.*, 1983; Lijinsky, 1985), and *in vitro* metabolism studies of DMNM have been conducted with liver and pancreas microsomes and other subcellular fractions from rats, hamsters and rabbits (Scarpelli *et al.,* 1982; Kokkinakis *et al.*, 1983; 1984; 1985).

Absorption and distribution

DMNM is rapidly absorbed and distributed following a single gavage dose of ^{3}H-DMNM (2 mg) to female Sprague-Dawley rats and Syrian golden hamsters (Underwood and Lijinsky, 1982). Within an hour, radioactivity was detected in all tissues examined in rats and hamsters (*i.e.*, blood, liver, kidney, lung, spleen, esophagus, pancreas, urine), without apparent accumulation in any tissue. No significant species differences in the distribution of radiolabel across tissues was observed. Five, 24, and 48 hours after ^{3}H-DMNM administration, urine and blood had the highest levels of radioactivity, followed by the liver (Underwood and Lijinsky, 1982).

Metabolism

DMNM metabolites have been detected in several *in vivo* studies, including in the blood and urine of male Syrian golden hamsters following *i.p.* injection (Gingell *et al.*, 1976a; 1978), in the blood, liver and pancreas in hamsters following *i.v.* injection (Kokkinakis *et al*., 1983), and in the urine of female Sprague-Dawley rats, Syrian golden hamsters, and guinea pigs following gavage administration (Underwood and Lijinsky, 1982). Up to eleven metabolites of DMNM have been detected in the urine of rats, hamsters and guinea pigs in these studies, two of which were identified as N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP) and N-nitrosobis(2-hydroxypropyl)amine (BHP). Table 24 presents data from the rat, hamster and guinea pig gavage studies on the levels of DMNM, HPOP and BHP in urine 24 hours after dosing (Underwood and Lijinsky, 1982). These data indicate that the metabolism of DMNM is qualitatively similar across these three species.

Table 24. Urinary metabolites* 24 hours after one-time gavage of DMNM (Underwood and Lijinsky, 1982)

Chemical	Hamster	Rat	Guinea pig
DMNM (Parent compound)	0.8%	1.7%	0.5%
HPOP	2.8%	5.5%	2.4%
BHP	9.5%	4.4%	3.6%

***Percentage of administered dose

In vitro metabolism studies in hamster liver postmitochondrial supernatants (S9) and microsomes (Kokkinakis *et al*., 1983; 1984), hamster pancreas S9 and microsomes (Scarpelli *et al*., 1982; Kokkinakis *et al*., 1983), rat liver microsomes (Kokkinakis *et al*., 1984) and rabbit liver microsomes (Kokkinakis *et al*., 1985) have detected multiple metabolites of DMNM, including HPOP, BHP, N-nitroso-bis(2-oxopropyl)amine (BOP), N-nitrosomethyl(2-oxopropyl)amine (MOP), N-nitrosomethyl(2-hydroxypropyl)amine, (MHP) and six unidentified peaks. HPOP was identified in the rat and rabbit liver microsomal studies (Kokkinakis *et al*., 1984; 1985), while BHP, BOP, MOP, and MHP were identified in the hamster liver microsomal and S9 studies (Kokkinakis *et al*., 1983), and BHP and BOP in the hamster pancreas S9 studies (Scarpelli *et al*., 1982).

The *in vivo* studies indicate that DMNM is rapidly metabolized. In the hamster, high blood levels of DMNM observed immediately following *i.p*. injection declined rapidly within the first 30 minutes, and approached baseline levels after three hours (Gingell *et al.*, 1976a). In these same animals, blood levels of the DMNM metabolite HPOP gradually increased after dosing, and peaked between 90 – 120 minutes. Additional studies by these investigators confirmed this time course for blood levels of DMNM and HPOP following *i.p*. injection of DMNM in the hamster, and also reported the appearance of BHP in the blood at two hours (Gingell *et al*., 1978). Rapid metabolism of DMNM to HPOP was also observed by Kokkinakis *et a*l. (1983) in the hamster, following *i.v.* injection of DMNM directly into the portal vein. Ten minutes after injection, HPOP was detected in the liver and pancreas, and trace levels of HPOP were detected in the blood (Kokkinakis *et al*., 1983). Additional evidence for the rapid metabolism of DMNM comes from the gavage dosing studies of Underwood and Lijinsky (1982) in rats, hamsters and guinea pigs. Multiple DMNM metabolites were detected in each species' urine within the first 8 hours of a single gavage dose of [^{3}H]DMNM. In addition, at 24

hours, only 8% of the administered dose was present in the blood of rats and hamsters (Underwood and Lijinsky, 1982).

DMNM is metabolized by multiple pathways and enzyme systems, as shown in Figure 2. Studies in hamster liver microsomes have shown that the β-hydroxylation pathway is catalyzed by mixed-function oxidases and requires NADPH and oxygen to form HPOP, which is a stable compound (Kokkinakis *et al.,* 1983). HPOP is further metabolized to BOP, MOP, MHP (Gingell *et al*., 1976b; Pour *et al.*, 1980), and BHP (Gingell *et al.*, 1976b; 1978; Kokkinakis *et al.,* 1983). The α-hydroxylation pathway metabolizes DMNM to form reactive nitrosamides, which may undergo non-enzymatic degradation to yield diazonium or carbonium ions, which may react with cellular nucleophiles, such as DNA, RNA, and proteins (Kokkinakis *et al.,* 1984). While α-hydroxylation is thought to be the major metabolic pathway for N-nitrosomorpholine and other cyclic nitrosamines (Hecht and Young, 1981), β-hydroxylation appears to be an equally important pathway for the metabolism and bioactivation of DMNM, based on metabolism studies of α- and β-deuterated DMNM (Kokkinakis *et al*., 1984). Metabolism studies of DMNM using subcellular fractions reported that S9 fractions prepared from hamster liver and pancreas catalyzed DMNM metabolism to a lesser extent than microsomal fractions, while no activity was observed in the cytosolic fractions (Kokkinakis *et al.,* 1983). These studies suggest that the enzymes involved in activation of DMNM are associated with the endoplasmic reticulum.

HPOP exists as a tautomeric mixture of 2 open-chain stereoisomers (isomers A and B; A dominates) and 2 cyclic stereoisomers (isomers C and D; D dominates), which are readily interconvertible and exist in equilibrium (Kokkinakis *et al.*, 1987) (Figure 2). The cyclic form of HPOP resembles glucopyranose (a cyclic form of glucose containing a pyranose ring). It has been hypothesized that its similarity to glucopyranose may facilitate its uptake by pancreatic islet cells, which are the progenitor cells of the pancreatic tumors induced by DMNM in the hamster (Pour *et al*., 1979).

As shown in Figure 2, HPOP is a common metabolite of DMNM (Underwood and Lijinsky, 1982; Kokkinakis *et al.*, 1983), BOP (Gingell *et al.*, 1976b; Pour *et al*., 1979; Kokkinakis *et al.*, 1987), and BHP (Gingell *et al.*, 1976b). *In vivo* and *in vitro* studies have shown that HPOP, BOP and BHP are metabolites of DMNM (Underwood and Lijinsky, 1982; Kokkinakis *et al.*, 1983). Studies in the hamster have shown that HPOP and BHP are metabolites of BOP (Gingell *et al.*, 1976b; Pour *et al*., 1980), BHP is a metabolite of HPOP, and HPOP and BOP are metabolites of BHP (Gingell *et al.*, 1976b). The metabolism of DMNM to MOP has been shown in hamster liver

microsomes *in vitro* (Kokkinakis *et al.*, 1983), and the metabolism of MOP to MHP has been demonstrated in hamsters *in vivo* (Pour *et al*., 1980).

In vivo and *in vitro* studies of DMNM indicate that multiple forms of cytochromes P450 are involved in the hydroxylation of DMNM to HPOP. *In vivo*, increases in DMNM metabolism were observed in rabbits pretreated with phenobarbitol (an inducer of CYP2B4 and CYP2E1) and ethanol (an inducer of CYP2E1) (Kokkinakis *et al.*, 1985), and in hamsters pretreated with TCDD (2,3,7,8-tetrachlorodibenzodioxin, an inducer of CYP1A1 and CYP1B1) (Scarpelli *et al.,* 1982). The metabolism of DMNM in hamster liver microsomes (Kokkinakis *et al.,* 1983) and rabbit liver microsomes (Kokkinakis *et al.,* 1985) was inhibited by cytochrome P450 inhibitors, such as SKF-525A (a CYP3A4/5 inhibitor; Franklin and Hathaway, 2008), and α-benzoflavone (a CYP1A2 inhibitor; Hollenberg, 2002). In addition, Kokkinakis *et al.* (1985) demonstrated using antibodies to specific cytochromes P450 in studies with rabbit liver microsomes and incubations with reconstituted isozymes that CYP2B4, CYP2E1, CYP2C3 and CYP3A6 each can metabolize DMNM to HPOP.

In vivo studies of HPOP in the hamster suggest that cytochromes P450 are involved in the metabolism of HPOP to DNA reactive and carcinogenic species (Kokkinakis and Scarpelli, 1989). In these studies, hamsters fed a diet containing 8% protein and administered HPOP had lower levels of hepatic cytochrome P450, lower levels of 7-methylguanine in hepatic DNA, increased glucuronidation of HPOP, and developed fewer pancreatic ductal adenocarcinomas than hamsters exposed to the same dose of HPOP but fed a diet containing 20% protein (Kokkinakis and Scarpelli, 1989).

In vitro studies suggest some species and organ differences in the rate of β-hydroxylation of DMNM. Regarding species differences, studies conducted with liver microsomes found that DMNM was metabolized to HPOP seven times faster in hamster liver microsomes than in rat liver microsomes (Kokkinakis *et al*., 1984). Regarding organ differences, the metabolite profiles were different between hamster liver and pancreas S9 fractions, suggesting different enzymes may be involved in the metabolism of DMNM in these organs (Scarpelli *et al*., 1982). Within the pancreas, differences were observed between hamster pancreas cell types, with S9 prepared from acinar and islet cells displaying a similar rate of metabolism of DMNM, but different proportions of individual metabolites (Scarpelli *et al*., 1982). In the case of S9 prepared from pancreatic acinar cells, the relative amounts of HPOP, BHP, and BOP formed were roughly equivalent, while for S9 prepared from pancreatic islet cells the relative amounts were as follows: [BHP] > [HPOP] >> [BOP] (Scarpelli *et al*., 1982).

Excretion

In vivo studies indicate that DMNM is not excreted to any significant extent in urine (as the parent compound) or expired air (Lijinsky, 1985) or feces (as parent or metabolites). Specifically, no DMNM was detected in hamster urine following *i.p*. administration (Gingell *et al*., 1976a; 1978), and as shown above in Table 24, only small amounts of DMNM were excreted in urine 24 hours following a single gavage dose to hamsters (0.8% of the administered dose), rats (2%), and guinea pigs (0.5%) (Underwood and Lijnsky, 1982). In the gavage studies, only a very small amount of radioactivity was detected at 48 hours in hamster feces (0.4% of the administered dose); radiolabel was not detected in rat or guinea pig feces. As discussed above, rapid metabolism of DMNM results in the excretion of DMNM metabolites in the urine. For example, a significant amount of the total dose of ^{3}H-DMNM is excreted in the urine eight hours after gavage administration in the hamster (54%), rat (39%), and guinea pig (30%) (Underwood and Lijinsky, 1982).

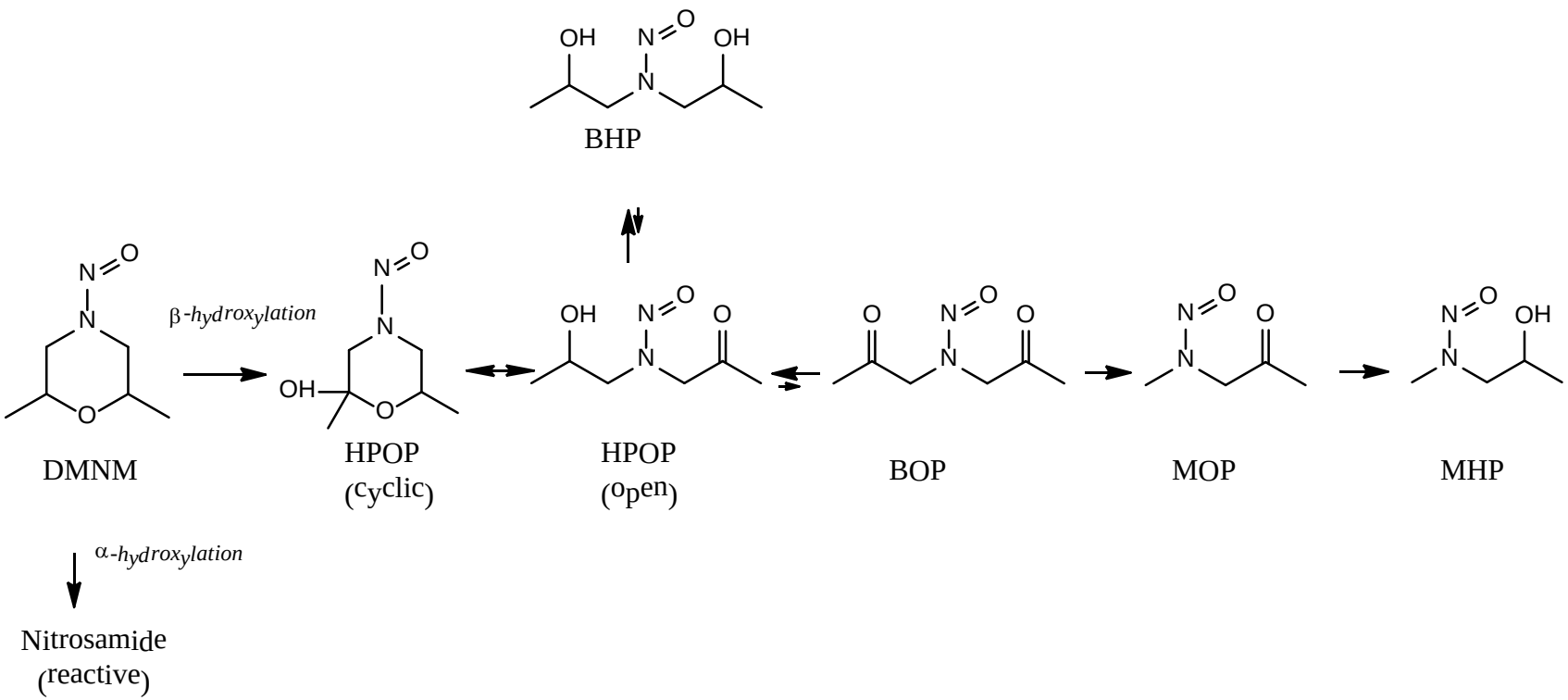


Figure 2. Metabolic pathways of DMNM

3.3.3. Animal Tumor Pathology

Rat

In rats treated with DMNM, tumors of the nasal cavity, trachea, lung, tongue, esophagus, forestomach, and liver (hemangiosarcomas) were observed (Ovelar *et al*., 1981; Lijinsky *et al*., 1991; Lijinsky and Taylor 1975; Konishi *et al*., 1987; Lijinsky and Reuber, 1980; 1982).

DMNM induced nasal tumors arising from the respiratory epithelium and the olfactory epithelium (Lijinsky & Taylor, 1975; Lijinsky and Reuber, 1980; 1982; Vollrath *et al*., 1986). All types of nasal tumors are rare in rats (Boorman *et al*., 1990; Schwartz *et al*., 1994). The tumors observed include adenocarcinomas, squamous cell carcinomas, and neuroblastomas (estesioneuroblastomas). Adenocarcinomas may arise from respiratory epithelium of the anterior naso- or maxillary turbinates, the septal glands, Bowman's glands, or Steno's gland, and the respiratory epithelium of the olfactory region (Schwartz, 1994). The adenocarcinomas observed (Lijinsky and Taylor, 1975) arose in the olfactory region and invaded caudally into the olfactory region of the brain. Squamous cell carcinomas can arise from the squamous epithelium of the anterior nose or from metaplastic squamous epithelium in the respiratory or olfactory portions of the nasal cavity. An immuno-cytochemistry study by Vollrath *et al*. (1986) described squamous cell carcinomas primarily originating from the olfactory epithelium, with some tumors also originating from the respiratory epithelium. The olfactory-derived tumors may be of neuroendocrine origin (esthesioneuroblastoma), based on the presence of neuroendocrine granules in rat nasal tumors, along with elevated levels of adrenocorticotropic hormone and calcitonin. Because esthesioneuroblastomas arise from the olfactory epithelium, they should be evaluated separately from tumors arising from the respiratory epithelium (McConnell *et al*., 1986).

Benign squamous papillomas of the trachea were observed in rats treated with DMNM (Lijinsky and Taylor, 1975). Tracheal neoplasms are rare in rats (Schwartz *et al*., 1994).

DMNM induced benign and malignant lung tumors in rats (Ovelar *et al*., 1981; Konishi *et al*., 1987). Bronchiolo-alveolar tumors seldom occur spontaneously in rats (Boorman and Eustis, 1990; Schwartz *et al*., 1994). The tumors observed by investigators include adenomas, adenocarcinomas, adenosquamous carcinomas, and squamous cell carcinomas. Bronchiolo-alveolar adenomas are considered to have the potential to progress from benign to malignant phenotypes (McConnell *et al*., 1986). Squamous cell carcinomas of the lung are also rare in rats (Boorman and Eustis, 1990; Schwartz *et al*.,

1994) and the origin of this tumor is uncertain. It may arise from bronchi, bronchioles, or alveoli, but may also arise from keratinizing epidermal cysts originating in the alveoli (Boorman and Eustis, 1990).

DMNM induced tongue basal cell papillomas and carcinomas, and squamous cell carcinomas (Lijinsky and Reuber, 1980; 1982). Neoplasms of the tongue are rare in rats (Whiteley *et al*., 1996). Tumors of the tongue arise principally from the stratified squamous epithelium but can also arise from the connective tissue. Squamous cell-papilloma and carcinoma are considered to have the potential to progress from benign to malignant phenotypes (Whiteley *et al*. 1996; McConnell *et al*., 1986).

Esophageal tumors are rare in rats (Whiteley *et al*., 1996; Brown and Hardisty, 1990; Haseman and Arnold, 1990). The esophageal tumors induced by DMNM include papillomas and verrucous carcinomas (a rare form of squamous cell carcinoma) (Lijinsky and Taylor, 1975; 1978; Lijinsky and Reuber, 1980; 1982; Lijinsky *et al*., 1982b). Proliferative lesions of the esophagus typically arise from the stratified squamous epithelium, and carcinomas may arise from epithelium or within papillomas (Whiteley *et al*., 1996). Squamous cell papilloma and carcinoma are considered to have the potential to progress from benign to malignant phenotypes (McConnell *et al*., 1986).

DMNM induced squamous cell papillomas and carcinomas of the forestomach (Lijinsky and Taylor, 1975; Lijinsky and Reuber, 1982). Squamous cell carcinomas arise through proliferation of the non-glandular epithelium, and are locally invasive. They are considered rare in rats (Frantz *et al*., 1991; Haseman and Arnold, 1990). Squamous cell papillomas and carcinomas are considered to have the potential to progress from benign to malignant phenotypes (McConnell *et al*., 1986; Frantz *et al*., 1991).

Significant increases in liver hemangiosarcomas in male and female rats were observed (Ovelar *at al*., 1981). The lesions appeared as hemorrhagic nodules. Hemangiosarcomas consist of irregular, poorly formed vascular channels; they are locally invasive and can metastasize to other organs (Mitsumori, 1990).

Hamster

In hamsters, DMNM administered by gavage or *s.c.* injection induced significant increases in tumors of the nasal cavity (combined benign and malignant), trachea (combined benign and malignant), larynx (combined benign and malignant), lung (benign and malignant), forestomach (combined benign and malignant), liver (benign and malignant), gallbladder (combined benign and malignant), pancreas (benign and

malignant), and kidney (malignant) (Mohr *et al.*, 1977; Reznik *et al.*, 1978; Althoff *et al.*, 1978; Rao *et al.*, 1981; Lijinsky *et al*., 1982b; Althoff *et al.*, 1985; Kokkinakis and Scarpelli, 1989; Section 3.2.2). In addition to tumors at these sites, tumors of the skin (benign), Harderian gland (benign), and vagina (benign) were also seen in *s.c.* injection studies (Section 3.2.2.2).

Nasal cavity tumors were seen both in the anterior and posterior regions, including papillary polyps/papillomas and adenocarcinomas. Most of the posterior region tumors were adenocarcinomas, and tumors found in the anterior region were mainly papillary polyps and papillomas. Metastases to the lungs were observed. Nasal cavity tumors are rare in untreated Syrian golden hamsters, with a spontaneous incidence of less than 0.1% (IARC, 1996). IARC (1996) states that "even low incidences of tumors in this organ can be regarded as evidence for a potential carcinogenic risk posed by the agent under study."

Larynx tumors were squamous cell papillomas, papillary polyps and carcinomas. Squamous cell carcinomas may arise from benign papillomas (IARC, 1996). Larynx tumors have been reported occasionally in untreated hamsters (IARC, 1996).

Trachea tumors were benign papillary polys, papillomas, and carcinomas that occurred in all parts of the trachea. Benign papillomas may progress to carcinomas (IARC, 1996). Tumors of the trachea have been reported occasionally in untreated hamsters (IARC, 1996).

Lung tumors included squamous cell tumors, adenomas, adenocarcinomas, and carcinomas, and were seen in over 50% of some DMNM-treated groups. Lung tumors are rare in untreated Syrian golden hamsters, with a spontaneous incidence of no more than 0.1-0.5% (IARC, 1996). Squamous cell carcinomas may arise from benign papillomas (IARC, 1996).

Forestomach tumors were squamous cell papillomas and carcinomas. According to IARC (1996), spontaneous neoplasms of the forestomach are infrequent in the hamster. The incidences of spontaneous squamous cell papilloma of the forestomach were 4.1% in female hamsters and 6.1% in males; and the incidence of squamous cell carcinoma in males was 0.1%. Squamous cell carcinomas may either develop from benign papillomas or evolve directly from atypical epithelium (IARC, 1996).

Liver tumors included vascular tumors (hemangiomas or hemangioendotheliomas, hemangiosarcoma), cholangiocellular/biliary tumors (cholangiomas and cholangiocarcinomas), and hepatocellular carcinomas. Cholangiomas and cholangiocarcinomas were often seen within the same animal. The incidence of spontaneous epithelial (combined hepatocellular and cholangiocellular) carcinomas in untreated Syrian hamsters is less than 1%, as is the incidence of spontaneous hemangiomas and hemangioendotheliomas (IARC, 1996). Progression of cholangioma to cholangiocarcinoma is likely (IARC, 1996).

Gallbladder tumors were papillary polyps, polypoid adenomas, and adenocarcinomas. According to IARC (1996), gallbladder tumors are very rare in domestic and laboratory animals.

Pancreas tumors were cystadenomas, ductal adenomas and adenocarcinomas. Some of the pancreatic carcinomas metastasized to the lungs. Epithelial hyperplasia of ducts or atrophy of acinar tissues was often seen in DMNM-treated animals with pancreatic adenocarcinomas. Adenocarcinomas often appear to develop simultaneously with adenomas. These tumors are mostly of the ductal and less frequently of the acinar cell type (IARC, 1982). The spontaneous incidence of ductal/ductular adenomas and adenocarcinomas in the Syrian hamster is 0.3-2% (IARC, 1996). Adenomas may contain foci of malignant cells, indicating that progression to malignancy can occur (IARC, 1996).

Kidney tumors were adenomas and carcinomas of the renal tubular epithelium. In addition, renal dysplasia of the renal tubular epithelium was very common in DMNM-treated animals. Spontaneous renal tumors are extremely rare in the Syrian hamster, occurring in less than 0.1% of untreated animals (IARC, 1996).

Skin tumors were benign trichoepitheliomas (arising from the epithelium or hair follicle) and squamous cell papillomas. These are rare in the hamster (IARC, 1996). Squamous papillomas can progress to carcinomas.

The tumors of the vagina seen in DMNM-treated animals were benign squamous cell papillomas. The incidence of spontaneous tumors of the vagina in the hamster is exceedingly low (IARC, 1996).

The Harderian gland tumors were benign adenomas. It is possible that Harderian gland adenomas may progress to carcinomas in the hamster, since such progression is seen in mice (IARC, 1994).

Guinea pig

In guinea pigs, DMNM induced malignant vascular tumors (hemangiosarcomas), and benign bile duct adenomas (cholangiomas) (Cardy and Lijinsky 1980; Rao *et al.*, 1980; Section 3.2.3). Hemangiosarcomas often metastasized to other organs such as lungs and spleen. Other types of liver tumors observed included cholangiocarcinoma, undifferentiated carcinoma and sarcoma, and hepatocellular carcinoma.

Trout

DMNM induced liver and stomach tumors in rainbow trout (Hendricks *et al*., 1995). Swimbladder tumors were also observed in DMNM-treated trout.

The liver tumors were adenomas and carcinomas, and the carcinomas included hepatocellular carcinomas and mixed hepato-cholangiocellular carcinomas. As is true in mammals, liver adenomas in trout are expected to have the potential to progress from benign to malignant phenotypes (Hendricks *et al.*, 1995; Sinnhuber *et al*., 1978). Stomach tumors were benign papillary adenomas of the glandular stomach. Swimbladder tumors were benign papillomas.

3.3.4 Structure-Activity Comparisons

DMNM is a heterocyclic nitrosamine that shares structural similarities with other carcinogenic cyclic nitrosamines. DMNM's metabolites include N-nitrosopropylamines which present concerns for carcinogenicity. The structures of two structurally similar compounds and three DMNM metabolites are shown in Table 25; tumor target sites are shown in Table 26 and genotoxicity findings for these compounds are shown in Table 27.

Heterocyclic nitrosamines

NM (nitrosomorpholine) is structurally similar to DMNM, the difference being that it has no methyl groups on the morpholine ring. NM is on the Proposition 65 list of chemicals known to cause cancer. NM is an IARC Group 2B carcinogen (IARC, 1987) and is listed as "reasonably anticipated to be a human carcinogen" in the NTP Report on Carcinogens (NTP, 2011). NM induces tumors at multiple sites in multiple species, and shares several common target tumor sites with DMNM, including nasal cavity (rat,

hamster), trachea (hamster), esophagus (rat), liver (rat, mouse, hamster, trout), lung (mouse), and kidney (rat) (Table 26). NM is mutagenic in *Salmonella* and mammalian cell assays, induces UDS in hepatocytes *in vitro*, and chromosomal aberrations *in vitro* in hamster and human cell lines (Table 27). NM has also been shown to form DNA adducts *in vivo* (Loeppky *et al.,* 2002).

NP (nitrosopiperidine) is similar to DMNM and NM, in that it is also a six-membered heterocyclic nitrosamine, although it is an unsubstituted piperidine ring, rather than a morpholine ring. NP is on the Proposition 65 list of chemicals known to cause cancer. NP is an IARC Group 2B carcinogen (IARC, 1987) and is listed as "reasonably anticipated to be a human carcinogen" in the NTP Report on Carcinogens (NTP, 2011). NP induces tumors at multiple sites in multiple species, and shares several common target tumor sites with DMNM, including nasal cavity (rat, hamster), larynx tumors (hamster), lung (mouse, hamster), esophagus (rat, hamster), forestomach (mouse, hamster), and liver (rat, mouse, hamster, monkey) (Table 26). NP is mutagenic in *Salmonella* and mammalian cell assays, and induces UDS in hepatocytes *in vitro* (Table 27).

N-Nitrosopropylamine metabolites

HPOP (N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine) is a common metabolite of DMNM, BHP, and BOP and occurs in open and cyclic forms (Gingell *et al*., 1976a & 1980; Kokkinakis *et al*., 1983 & 1993). HPOP is formed from DMNM via β-hydroxylation (Kokkinakis *et al*., 1983). HPOP induces tumors in the rat at several of the same sites as does DMNM, including the nasal cavity, lung, esophagus, and liver (Table 26). HPOP is mutagenic in *Salmonella* and mammalian cell assays and causes DNA single strand breaks (Table 27) (Curphey *et al*., 1987). HPOP has been shown to induce DNA adducts *in vitro* and *in vivo* (Kokkinakis *et al*., 1993; Lijinsky, 1985).

BHP (N-nitrosobis(2-hydroxyproply)amine) is formed by reduction of HPOP. BHP induces tumors at multiple sites in multiple species, and shares several common target tumor sites with DMNM, including nasal cavity (rat, hamster), larynx (hamster), trachea (hamster), lung (rat, mouse, hamster), liver (rat, mouse, hamster), and pancreas (hamster) (Table 26). BHP is mutagenic in *Salmonella* and mammalian cell assays (Table 27). BHP has been shown to induce DNA adducts *in vitro* and *in vivo* (Kokkinakis *et al*., 1993; Lijinsky, 1985).

BOP (N-nitrosobis(2-oxopropyl)amine) is formed by oxidation of HPOP. BOP induces tumors at multiple sites in multiple species, and shares several common target tumor sites with DMNM, including nasal cavity (rat, mouse), lung (rat, mouse, hamster), liver

(rat, mouse, hamster, guinea pig), pancreas (hamster), and kidney (rat) (Table 26). BOP is mutagenic in *Salmonella* and mammalian cell assays (Table 27). BOP has been shown to induce DNA adducts *in vitro* and *in vivo* (Kokkinakis *et al*., 1993; Lijinsky, 1985).

Table 25. DMNM, two related heterocyclic nitrosamines, and three metabolites

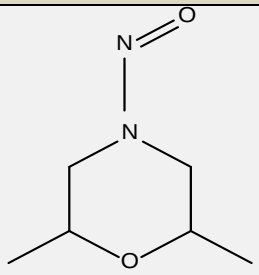
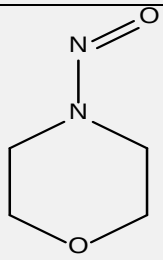
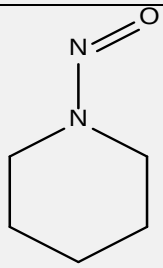
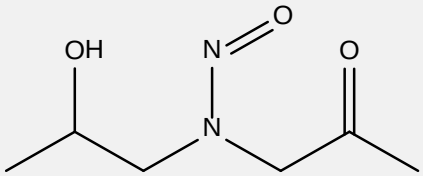
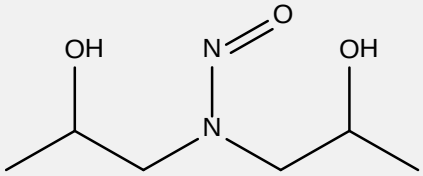
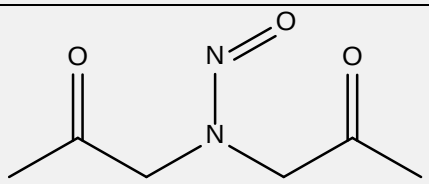
DMNM & related heterocyclic nitrosamines	
2,6-Dimethylnitrosomorpholine (DMNM)	
Nitrosomorpholine (NM) Proposition 65 Carcinogen	
Nitrosopiperidine (NP) Proposition 65 Carcinogen	
DMNM metabolites	
N-Nitroso(2-hydroxypropyl)(2-oxopropyl)amine (open) **(**HPOP)	
N-Nitrosobis(2-hydroxyproply)amine (BHP)	
N-Nitrosobis(2-oxopropyl)amine (BOP)	

Table 26. Comparison of target tumor sites across species[1] for DMNM and some metabolites and structurally related cyclic nitrosamines[2]

Chemical	Liver				Pancreas			Lung			Esophagus			Fore-stomach			Nasal cavity			Larynx and/or trachea			Kidney			Other
	R	M	H	G	R	M	H	R	M	H	R	M	H	R	M	H	R	M	H	R	M	H	R	M	H	
DMNM and structurally related heterocyclic nitrosamines																										
DMNM*[3,4,5,6]	+		+	+			+	+		+	+			+		+	+		+	+		+			+	Gall bladder (H) Vagina (H) Tongue (R) Harderian Gland (H) Skin (H) Renal Pelvis/urinary bladder (H) Glandular stomach (T) Swimbladder (T)
NM*[4]	+	+	+						+		+						+		+			+	+			Thyroid (R)
NP**[4]	+	+	+						+	+	+		+		+	+	+		+			+				Pharynx (R)
DMNM metabolites																										
HPOP [4,5,6]	+							+			+						+									Bladder (R)
BHP[4]	+	+	+				+	+	+	+							+		+			+				
BOP	+	+	+	+			+	+	+	+							+	+					+			Thyroid (R) Gall Bladder (H) Bladder (R) Kidney (R) Ureter (R) Testis (R)

[1] Rat (R), Mouse (M), Hamster (H), and Guinea Pig (G), Trout (T), [2] Source of data: CCRIS

[3]DMNM-not tested in the mouse; [4]NM, NP, HPOP, BHP not tested in guinea pig; [5]HPOP not tested in the mouse; [6]HPOP not tested in the hamster

*Also liver tumors in trout; **Also liver tumors in monkey

Table 27. Comparison of DMNM and some metabolites and structurally related cyclic nitrosamines: Genotoxicity[1] and DNA adduct formation

DNMN and structurally related compounds					
Test system / Chemical	**Mutagenicity in *Salmonella typhimurium***	**Mutagenicity in mammalian cells**	**Positive for UDS (*in vitro*)**	**Positive in other assays**	**DNA adduct formation**
DMNM	+	NT	+	X-linked recessive-lethal mutation assay in *Drosophila melanogaster;(*Nix *et al.,* 1980*)* DNA single strand breaks (Curphey *et al*.,1987)	+ *in vivo* (Lijinsky, 1985)
NM	+	+ (Chinese Hamster V 79 lung cells)	+	*In vitro* chromosomal aberrations: HEPG2 Hepatoma (human); V 79 cells	+ *in vivo* *(Loeppky et al., 2002)*
NP	+	+ (mouse lymphoma cells)	+	NT	NT
DNMN metabolites					
HPOP	+	+ (Chinese Hamster V 79 lung cells)	NT	DNA single strand breaks (Curphey *et al*., 1987)	+ *in vitro* and *in vivo* (Kokkinakis *et al*., 1993; Lijinsky, 1985)
BHP	+	+ (Chinese Hamster V 79 lung cells)	NT	NT	+ *in vitro* and *in vivo* (Kokkinakis *et al*., 1993; Lijinsky 1985)
BOP	+	+ (Chinese Hamster V 79 lung cells)	NT	NT	+ *in vitro* and *in vivo* (Kokkinakis *et al*., 1993; Lijinsky, 1985)

[1]Source of data: CCRIS or as cited

NT: Not tested

In summary, DMNM and its metabolites induce tumors, including rare tumors, in multiple species and at multiple sites, and many tumor sites are shared amongst species. All compounds are mutagenic in *Salmonella* and mammalian systems. DMNM, NM, and NP test positive for UDS (*in vitro*). DMNM and its metabolite, HPOP, also cause single strand DNA breaks. The DMNM metabolites HPOP, BHP, and BOP, and the structural analogue NM, all induce DNA adducts.

4. MECHANISMS

DMNM induces tumors at multiple sites in multiple species (Table 26). A body of evidence suggests that DMNM induced the observed tumors via a genotoxic mechanism or mechanisms. As discussed under Section 3.3.1 *Genotoxicity,* DMNM induces mutations in *Salmonella* and *Drosophila*, UDS *in vitro* in rat liver and hamster pancreas cells, and DNA single strand breaks *in vivo* in rat and hamster pancreas. DMNM has been shown to bind to DNA in *in vitro* and *in vivo* in the hamster and the rat, forming N-7 methyl guanine and O^6-methylguanine adducts.

DMNM, like many other nitrosamines, requires metabolic activation via cytochrome P450 for genotoxic and carcinogenic activity. Studies with α-deuterated DMNM and β-deuterated DMNM in hamsters indicate that metabolic activation can occur following oxidation of either the α- or the β-carbons of the molecule (Rao *et al*., 1981). Metabolism of DMNM leads to formation of the mutagenic and carcinogenic metabolites HPOP, BOP, and BHP (See Section 3.3.2 Metabolism and Pharmacokinetics and Section 3.3.4 Structure-Activity Comparisons). Each of these three metabolites share species-specific target tumor sites with DMNM (Table 26). For example, BHP, BOP and DMNM each induce liver, pancreas, and lung tumors in the hamster, and all four compounds (HPOP, BOP, BHP and DMNM) induce liver, lung, and nasal cavity tumors in the rat. These three DMNM metabolites also have a similar genotoxicity profile as DMNM. Specifically, all three DMNM metabolites induce mutations in *Salmonella* and mammalian cells, induce UDS *in vitro* in rat liver cells (Yamazaki *et al*., 1985), and form DNA adducts (measured as methylated guanine) in hamster pancreatic ductal cells and rat and hamster liver cells (Lijinsky, 1985; Kokkinakis *et al*., 1993). One metabolite, HPOP, also induces DNA single strand breaks in pancreatic acinar cells in rats and hamsters exposed *in vivo* (Curphey *et al*., 1987).

Additional mechanistic information comes from studies of DMNM in specialized experimental rat models by Yamamoto *et al.* (1995) and Pera *et al.* (2001). Yamamoto *et al.* (1995) examined the activity of several nitroso compounds, including DMNM and the DMNM metabolite BHP, to induce gamma glutamyl transferase (GGT) positive liver foci in a short-term assay in the Wistar rat. The appearance of GGT-liver foci is considered indicative of an initiation step toward full neoplastic transformation, *i.e*. it is an early indicator of carcinogenicity (Williams, 1989; Hanigan, 1998). In these studies male Wistar rats (11 per group) were fed the test substance (e.g., DMNM, BHP), or control diet for a total of three weeks. Two weeks into the three-week treatment period the rats underwent a two-thirds hepatectomy. Five weeks after the beginning of the experiment the rats were given 0.02% acetylaminofluorene, in the diet for 2 weeks, and at six weeks the rats received a single dose of carbon tetrachloride. At eight weeks the rats were sacrificed, the liver removed, and examined histologically for GGT-positive foci. DMNM proved to be the most potent in inducing GGT-positive liver foci of the nitroso compounds tested in this study. BHP also increased the number of GGT-positive foci, as compared to controls. The authors concluded that DMNM and BHP were tumor initiators in this assay system.

Pera *et al.* (2001) examined differences in the expression of three cellular proteins, cyclin D1, p53, and Ki-67, in normal, hyperplastic, dysplastic, and neoplastic esophageal tissues, in Sprague Dawley rats treated with DMNM after undergoing an esophago-jejunostomy to produce a model of chronic duodenal content reflux esophagitis. p53 and cyclin D1 are cell cycle-related genes, and increased expression of cyclin D1 has been associated with the development of esophageal carcinomas in humans (Arber *et al*., 1999; Naitoh *et al*., 1995). Ki-67 is a gene whose expression is indicative of rapid cell proliferation and which has been used in cancer diagnostics (Bullwinkel *et al*., 2006). DMNM-treatment in this rat model produced a very high incidence (20/24) of esophageal tumors, including adenosquamous cell carcinomas, adenocarcinomas and squamous cell carcinomas. Expression of cyclin D1, p53 and Ki-67 proteins in esophageal tissues was assessed by means of immunohistochemical analysis. Increased expression of cyclin D1 coincided with increased expression of Ki-67, and appeared to be an early event that increased as the cells progressed along the continuum from normal to hyperplastic, dysplastic, and neoplastic. The pattern of increased p53 protein expression paralleled that of cyclin D1, but with a somewhat later onset, starting in the dysplastic stage. The authors concluded that cyclin D1 expression may be an early event in DMNM-induced rat esophageal tumorigenesis, and that both cyclin D1 and p53 are likely to play a role in the development and progression of esophageal lesions to malignancy.

In summary, the tumorigenicity of DMNM is likely mediated by oxidation via cytochrome P450, resulting in the formation of multiple genotoxic and carcinogenic metabolites (*e.g.*, HPOP, BHP, BOP). Consistent with this, DMNM has been shown to have liver tumor initiating activity in a short-term *in vivo* assay in the rat. In rat esophageal tissue, DMNM-induced tumorigenic progression was associated with an increase in expression of proteins involved in cell cycle control (cyclin D1, p53) and cell proliferation (Ki-67). The fact that DMNM induces tumors in a number of different tissues in multiple species suggests that it acts by a general genotoxic mechanism(s), such as mutagenicity. However, it should also be considered that DMNM may cause tumors by more than one mechanism and that different mechanisms may operate in different tissues.

5. REVIEWS BY OTHER AGENCIES

DMNM has not been classified as to its potential carcinogenicity by the U.S. EPA, the U.S. Food and Drug Administration, the National Toxicology Program, the National Institute for Occupational Safety and Health, or the International Agency for Research on Cancer.

6. SUMMARY AND CONCLUSIONS

6.1 Summary of Evidence

Evidence for carcinogenicity of DMNM comes from multiple animal studies in which significant increases in benign and/or malignant tumors (including several rare tumor types) have been observed at multiple sites in multiple species (rats, hamsters, guinea pigs, and trout), often in multiple strains and both sexes, by various routes of exposure.

The following significant increases in tumor incidences were observed following DMNM treatment:

Nasal cavity tumors (rare in rats and hamsters)

- Adenocarcinomas or squamous cell carcinomas
    - Male Sprague Dawley rats (drinking water: 2 studies)
    - Female Sprague Dawley rats (drinking water)
    - Male Fischer 344 rats (gavage)
    - Female Fischer 344 rats (drinking water: 2 studies; gavage)

    - Male Syrian golden hamsters (gavage)
- Combined papillomas and carcinomas or adenocarcinomas, or combined benign and malignant tumors (not otherwise specified)
    - Female Fischer 344 rats (drinking water: 2 studies)
    - Male Syrian golden hamsters (gavage)
    - Female Syrian golden hamsters (gavage; *s.c*. injection)
    - Male European hamsters (gavage; *s.c*. injection)
    - Female European hamsters (gavage; *s.c*. injection)
- Olfactory epithelial tumors (neuroblastomas, estesioneuroblastomas)
    - Wistar rats (drinking water)

Laryngeal tumors

- Combined benign and malignant tumors
    - Syrian golden hamsters (*s.c*. injection)

Tracheal tumors (rare in rats)

- Squamous cell papillomas, or benign tumors (not otherwise specified)
    - Male Syrian golden hamsters (gavage: 2 studies)
    - Female Syrian golden hamsters (gavage)
    - Syrian golden hamsters (*s.c*. injection)
    - While not statistically significant, these types of tumors were also seen in male Sprague Dawley rats (drinking water), female Sprague Dawley rats (drinking water), and male Syrian golden hamsters (gavage: 3 studies)

- Combined benign and malignant tumors (not otherwise specified)
    - Female European hamsters (gavage)
    - While not statistically significant, these types of tumors were also seen in male European hamsters (*s.c*. injection: 2 studies), and female European hamsters (*s.c*. injection: 2 studies)

Lung tumors (rare in rats and hamsters)

- Adenomas
    - Male Syrian golden hamsters (gavage: 5 studies)
    - While not statistically significant, these types of tumors were also seen in one additional study in male Syrian golden hamsters (gavage).

- Adenocarcinomas
    - While not statistically significant, these types of tumors were seen in female Fischer rats (gavage).
- Combined benign and malignant tumors
    - Male Sprague Dawley rats (*s.c.* injection)
    - Female Sprague Dawley rats (*s.c.* injection)
    - Male Wistar rats (*i.p.* injection)
    - Male Syrian golden hamsters (gavage)
    - Female Syrian golden hamsters (gavage)
    - Syrian hamsters (*s.c.* injection)
    - Male European hamsters (gavage; *s.c*. injection)
    - Female European hamsters (gavage; *s.c*. injection)

Tongue tumors (rare in rats)

- Carcinomas
    - Female Fischer 344 rats (drinking water)
    - While not statistically significant, these types of tumors were also seen in additional studies in female Fischer 344 rats (drinking water: 2 studies).
- Combined papillomas and carcinomas
    - Female Fischer 344 rats (drinking water: 3 studies)

Esophageal tumors (rare in rats)

- Squamous cell papillomas
    - Male Sprague Dawley rats (drinking water)
    - Female Fischer rats (drinking water)
- Squamous cell and/or basal cell carcinomas
    - Male Fischer rats (gavage)
    - Female Fischer rats (drinking water: 3 studies; gavage)
- Combined squamous cell papillomas and carcinomas
    - Male Sprague Dawley rats (drinking water; *s.c*. injection)
    - Female Sprague Dawley rats (drinking water; *s.c*. injection)
    - Male Fischer rats (gavage)
    - Female Fischer rats (drinking water: 3 studies; gavage; intravesicular injection)

Forestomach tumors (rare in rats, uncommon in hamsters)

- Benign tumors
    - While not statistically significant, these types of tumors were seen in female Fischer rats (drinking water) and male Syrian golden hamsters (gavage).
- Squamous cell carcinomas
    - While not statistically significant, these types of tumors were seen in female Fischer rats (drinking water: 2 studies).
- Combined squamous cell papillomas and carcinomas
    - Male Syrian golden hamsters (gavage)
    - Female Syrian golden hamsters (gavage)
    - Syrian golden hamsters (*s.c.* injection)
    - While not statistically significant, these types of tumors were also seen in male Sprague Dawley rats (drinking water), female Fischer 344 rats (drinking water: 3 studies; gavage).

Glandular stomach tumors

- Papillomas
    - Rainbow trout (diet)

Liver tumors

- Vascular tumors
    - Angiosarcomas, hemangiosarcomas, or hemangioendotheliomas
        - Male Sprague Dawley rats (*s.c*. injection)
        - Female Sprague Dawley rats (*s.c*. injection)
        - Male Syrian golden hamsters (gavage: 4 studies)
        - Male European hamsters (gavage)
        - Female European hamsters (gavage)
        - Male strain 2 guinea pigs (gavage)
        - Male random-bred guinea pigs (gavage)
        - While not statistically significant, these types of tumors were also seen in one additional study in male Syrian golden hamsters (gavage).
- Cholangiocellular/biliary tumors
    - Cholangiomas
        - Male random-bred guinea pigs (gavage)
        - While not statistically significant, these types of tumors were also seen in male Syrian golden hamsters (gavage: 5 studies).
    - Combined cholangiomas and cholangiocarcinomas

        - Male Syrian golden hamsters (gavage)
        - Female Syrian golden hamsters (gavage)
- Hepatocellular tumors
    - Hepatocellular adenomas
        - Rainbow trout (diet)
- Combined hepatocellular and hepatocholangiocellular tumors
    - Combined hepatocellular carcinomas and hepatocholangiocellular carcinomas
        - Rainbow trout (diet)
    - Combined hepatocellular adenomas, carcinomas, and hepatocholangiocellular carcinomas
        - Rainbow trout (diet)

Gallbladder tumors (rare in hamsters)

- Benign tumors
    - Syrian golden hamsters (*s.c*. injection)
- Combined benign and malignant tumors
    - Male Syrian golden hamsters (gavage)
    - Female Syrian golden hamsters (gavage)

Pancreas tumors (rare/uncommon in hamsters)

- Adenomas or cystadenomas
    - Male Syrian golden hamsters (gavage: 4 studies)
    - Female Syrian golden hamsters (gavage)
    - While not statistically significant, these types of tumors were also seen in one additional study in male Syrian golden hamsters (gavage).
- Carcinomas
    - Male Syrian golden hamsters (gavage: 5 studies)
- Combined benign and malignant tumors
    - Male Syrian golden hamsters (gavage: 2 studies)
    - Female Syrian golden hamsters (gavage)
    - Syrian golden hamsters (*s.c.* injection)

Kidney tumors (rare in hamsters)

- Renal tubular adenomas

    - While not statistically significant, these types of tumors were seen in male Syrian golden hamsters (gavage) and Syrian golden hamsters (*s.c*. injection).
- Renal tubular carcinomas
    - Male Syrian golden hamsters (gavage: 3 studies)
    - While not statistically significant, these types of tumors were seen in one additional study in male Syrian golden hamsters (gavage).
- Renal pelvis/urinary bladder transitional cell carcinomas
    - While not statistically significant, these types of tumors were seen in male European hamsters (*s.c*. injection) and female European hamsters (*s.c*. injection).

Harderian gland tumors

- Adenomas
    - Syrian hamster (*s.c.* injection)

Vaginal tumors (rare in hamsters)

- Papillomas
    - Female Syrian golden hamsters (*s.c.* injection)

Swimbladder tumors

- Papillomas
    - Rainbow trout (diet)

Increased incidences of rare skin tumors in hamsters (combined trichloepitheliomas and squamous cell papillomas) were also observed, that while not statistically significant, are likely biologically significant (Syrian golden hamsters: *s.c*. injection).

Evidence of DMNM genotoxicity comes from multiple test systems:
In vitro:

- DMNM induced base-pair substitution mutations in *Salmonella typhimurium*, primarily in the presence of metabolic activation.
- DMNM induced UDS in cultured primary rat hepatocytes and in isolated hamster main pancreatic ducts.
- DMNM binds to hamster pancreas DNA, RNA, and protein.

In vivo:

- DMNM induced X-linked recessive lethal mutations in *Drosophila.*

- DMNM induced DNA single strand breaks in hamster pancreas (acinar cells).
- DMNM binds to hamster and rat liver DNA, RNA, and protein, and forms N-7 methylguanine in hamster and rat liver, and O^6-methylguanine in hamster liver.

Metabolites of DMNM and structurally similar heterocyclic nitrosamines present concern regarding the carcinogenicity of DMNM:

- DMNM is structurally similar to the carcinogenic and genotoxic heterocyclic nitrosamines NM and NP.
    - NM and NP are IARC Group 2B carcinogens and are on the Proposition 65 list as causing cancer.
    - Like DMNM, NM and NP induce mutations in *Salmonella typhimurim,* mutations in mammalian cells *in vitro*, and UDS in hepatocytes *in vitro*.
    - Like DMNM, NM forms DNA adducts *in vivo*.
- The DMNM metabolites HPOP, BHP, and BOP are genotoxic.
    - All are mutagenic in *Salmonella typhimurim* and Chinese hamster V79 lung cells.
    - HPOP induces DNA single strand breaks.
    - All form DNA adducts *in vivo* in rat and hamster liver.
- The DMNM metabolites HPOP, BHP, and BOP each induce tumors, including rare tumors, in rodents at several of the same sites as does DMNM.
    - Like DMNM, BHP and BOP have been shown to induce tumors at multiple sites in multiple species.
    - HPOP induces tumors at multiple sites in the rat, the only species tested.
- The DMNM metabolites HPOP, BHP, and BOP and the structurally similar carcinogens NM and NP share species-specific target tumor sites with DMNM:
    - In the rat
        - DMNM, HPOP, BHP, BOP, NM, and NP induce liver and nasal cavity tumors.
        - DMNM, HPOP, BHP, BOP induce lung tumors.
        - DMNM, HPOP, NM, and NP induce esophagus tumors.
    - In the hamster
        - DMNM, BHP, BOP, NM, and NP induce liver tumors.
        - DMNM, BHP, BOP, and NP induce lung tumors.
        - DMNM, BHP, NM, and NP induce nasal cavity tumors.
        - DMNM, NP, BHP induce larynx tumors.
        - DMNM, BHP, and NM induce trachea tumors.
        - DMNM, BHP, and BOP induce pancreas tumors.

        - DMNM and NP induce forestomach tumors.
    - In the guinea pig
        - DMNM and BOP induce liver tumors.
    - In trout
        - DMNM and NM induce liver tumors.

6.2 Conclusions

Evidence for carcinogenicity of DMNM comes primarily from more than 20 animal cancer bioassays with positive tumor findings. These studies were conducted in rats, hamsters, guinea pigs, and trout by various routes of exposure. Moreover, many rare tumor types were induced by DMNM, including nasal cavity and lung tumors in rats and hamsters; tracheal, tongue, esophageal, and forestomach tumors in rats, and gallbladder, pancreas, kidney, vaginal, and skin tumors in hamsters.

Positive findings in *in vitro* and *in vivo* genotoxicity test systems indicate that DMNM is likely to operate through a genotoxic mechanism, and that metabolic activation through α- or β-hydroxylation is required for activity. DMNM induces mutations in *Salmonella* and *Drosophila*, UDS *in vitro* in rat liver and hamster pancreas cells, DNA single strand breaks *in vivo* in rat and hamster pancreas, and binds to DNA in *in vitro* and *in vivo* in the hamster and the rat, forming N-7 methylguanine and O^6-methylguanine adducts.

The DMNM metabolites HPOP, BHP, and BOP are genotoxic and each induces tumors at multiple sites in animal studies. HPOP, BHP, and BOP and NM and NP, two genotoxic heterocyclic nitrosamine carcinogens that are structurally similar to DMNM each share many species-specific target tumor sites with DMNM.

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