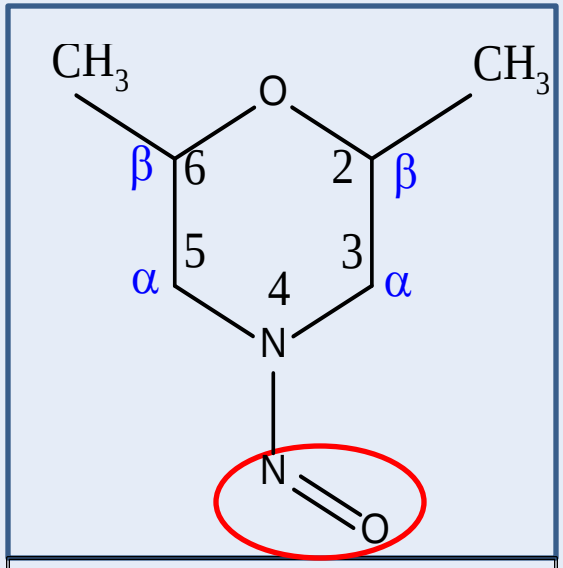
Evidence on the Carcinogenicity of 2,6-Dimethyl-N-Nitrosomorpholine (DMNM)

January 25, 2013

Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

2,6-Dimethyl-N-Nitrosomorpholine (DMNM)

- **Chemical properties:**
  - **Heterocyclic nitrosamine**
  - **Water solubility: 1.24 x 10^5 mg/L (@ 24°C)**
  - **Vapor pressure: 0.024 mmHg (@ 25°C)**
  - **Stereoisomer mixture of *cis*- & *trans*-DMNM**
- **Formed in industrial environments with amines and nitrosating agents (*e.g.*, fluid oil, rubber industry)**
- **Used as a model compound in carcinogenesis research (α- or β-deuterated DMNM)**



CAS NO: 1456-28-6

Carcinogenicity Studies

- Human: None
- Animal: Multiple positive bioassays in four species

Species	No. of Routes	No. of Strains	No. of Experiments
Rat	4	3	13
Hamster	2	2	13
Guinea Pig	1	2	2
Trout	1	1	1

Studies in Rats

- 3 strains: Sprague Dawley; Wistar; Fischer
- Studied in males and females
- 4 routes of exposure: drinking water; subcutaneous, intraperitoneal, and intravesicular bladder injections
- Generally small number of animals per dose group (N= 9-20); one study with 50 animals per group
- 4 studies with concurrent controls; some studies compared to continuous series of untreated animals maintained at investigator's facility

Tumor Findings in Rats- Overview

- Multiple sites by different routes
- Rare tumors:
  - Nasal cavity
  - Forestomach
  - Esophagus
  - Tongue
  - Lung
  - Trachea (benign only)
- Other tumors
  - Liver hemangiosarcomas

Tumor incidence in Sprague Dawley rats receiving weekly subcutaneous injections for life (Ovelar *et al*., 1981)

Tumor Site & Type		Sex	DMNM (mg/kg/week)				Trend test P value
			0	23.4	46.8	93.6	
Eso-phagus	Combined squamous cell papilloma & carcinoma	M	0/15	**14/14****	**14/14****	**9/14****	**0.004**
		F	0/15	**14/14****	**15/15****	**14/15****	**<0.001**
Lung	Combined adenoma & squamous cell carcinoma	M	0/15	**9/14****	**5/14***	**10/14****	**<0.001**
		F	0/15	**8/14****	**8/15****	**8/15****	**<0.001**
Liver	Hemangiosarcoma	M	0/15	0/14	**5/14***	**4/14***	**<0.01**
		F	0/15	0/14	2/15	**5/15***	**<0.01**

* p< 0.05; ** p<0.001 by pairwise comparison

Note: all treated animals had short survival compared to untreated rats (18 or 20 vs 43 or 44 weeks)

Tumor findings in female Fischer rats - drinking water (Lijinsky & Reuber, 1980 & 1982)

Tumor Site & Type		DMNM in drinking water (mg/L) [Total dose (mg)]		
		DMNM		Control [1]
		20 [60]	50 [110]	0 [0]
Nasal cavity	Carcinoma	**14/20***	0/20	0/20
	Combined papilloma & carcinoma	**15/20***	0/20	0/20
Tongue	Carcinoma	**8/20***	0/20	0/20
	Combined papilloma & carcinoma	**11/20***	0/20	0/20
Esophagus	Carcinoma	**16/20***	**12/20***	0/20
	Combined papilloma & carcinoma	**19/20***	**20/20***	0/20
Forestomach	Carcinoma	0/20	3/20	0/20
	Combined papilloma & carcinoma	3/20	3/20	0/20

* $P < 0.05$ [1] compared to colony kept at facility

Short survival compared to control (35 vs. 130 weeks)

Tumor incidence in Fischer 344 rats following gavage treatment with DMNM (20 mg/week) (Lijinsky *et al*., 1982b)

Tumor Site & Type		Male	Female
Esophagus	Squamous cell & basal cell carcinoma	6/20	10/20
	Combined benign & malignant tumor	16/20	20/20
Nasal cavity	Squamous cell carcinoma	3/20	7/20
Forestomach	Carcinoma	0/20	1/20
	Combined benign & malignant tumor	0/20	4/20
Lung	Adenocarcinoma	1/20	4/20

Note: Short Survival (< 40 wk)

Studies in Hamsters

- Two strains: Syrian golden and European
- Two routes: Gavage and subcutaneous injection
- Studied in males and females
- Small number of animals (N= 7 - 30); one experiment (Lijinsky, 1982b) did not have concurrent controls.
- Multiple tumor sites:
  - Rare tumors: *Nasal cavity*, *lung*, gallbladder, pancreas, kidney, vagina (benign), skin (benign)
  - Other tumors: Trachea, larynx, forestomach, liver, Harderian gland (benign)

Weekly gavage for life in female Syrian golden hamsters (Mohr *et al.*, 1977; Reznik *et al.*, 1978)

Tumor Site & Type (B: benign, M: malignant)	DMNM (mg/kg/week)					Trend test P value
	0	9.19	18.37	36.74	73.48	
Nasal cavity- anterior (B & M)	0/13	0/15	0/13	5/14*	1/12	0.066
Nasal cavity- posterior (B & M)	0/13	0/15	1/13	7/14**	4/12*	≤0.05
Trachea (B)	0/13	2/15	3/13	3/14	8/12***	≤0.001
Lung (B & M)	0/13	3/15	5/13*	5/14*	2/12	N.S.
Forestomach (B & M)	0/13	1/15	3/13*	5/14*	3/12	≤0.05
Liver (B & M)	0/13	0/15	1/13	3/14	6/12**	≤0.001
Gallbladder (B & M)	0/13	0/15	5/13*	0/14	4/12*	≤0.05
Pancreas (B & M)	0/13	3/15	7/13**	6/14**	6/12**	≤0.01

* p≤0.05, ** p≤0.01, *** p≤0.001 by pairwise comparison; N.S.: not significant

Weekly gavage for life in female European hamsters

(Althoff *et al.*, 1985)

Tumor Site and Type		DMNM (mg/kg/week)			Trend test P value
		0	37	112	
Nasal cavity	Benign & malignant	0/15	10/13***	10/15***	≤0.001
Trachea	Benign & malignant	0/15	2/13	5/15*	≤0.05
Lung	Benign & malignant	0/15	1/13	7/15**	≤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 by pairwise comparison; N.S.: not significant

Weekly *s.c.* injection for life in Syrian golden hamsters (Althoff *et al.*, 1978)

Tumor Site and Type (B: benign, M: malignant)	DMNM (mg/kg/week)				
	0	8	16	32	Trend test p value
Nasal cavity (B & M)	0/30	24/30***	25/30***	29/30***	≤0.001
Larynx (B & M)	0/30	4/30	6/30*	8/30**	≤0.01
Trachea (B)	0/30	9/30***	9/30***	12/30***	≤0.001
Lung (B & M)	0/30	7/30**	7/30**	12/30***	≤0.001
Forestomach (B & M)	1/30	11/30***	11/30***	9/30**	≤0.05
Pancreas (B & M)	0/30	2/30	5/30*	9/30***	≤0.001
Gallbladder (B)	0/30	3/30	2/30	5/30*	≤0.05
Liver, bile duct & gallbladder (B & M)	1/30	4/30	8/30*	7/30*	≤0.05
Kidney (B)	0/30	0/30	2/30	3/30	≤0.05
Harderian gland (B)	0/30	5/30*	5/30*	5/30*	0.065
Skin (B)	0/30	1/30	1/30	3/30	≤0.05
Vagina (B)	0/15	13/15***	12/15***	8/15***	≤0.01

* p≤0.05; ** p≤0.01; *** p≤0.001 by pairwise comparison

Studies in Guinea Pigs

- Two strains: Strain 2 and random-bred
- Gavage
- Studied in males
- Small number of animals (N =18 - 20) in two experiments with concurrent controls
- Tumor site: liver hemangiosarcoma and cholangioma.

Weekly gavage in male strain 2 guinea pigs
(Cardy and Lijinsky, 1980)

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

* $p \leq 0.01$; ** $p \leq 0.001$ by pairwise comparison
[1] Four animals died on the 12th week and were not necropsied.
[2] One tumor was considered by the authors to be an osteosarcoma.

Weekly gavage for 23 weeks in male random-bred guinea pigs (Rao *et al.*, 1980)

Liver Tumor Type	Control	DMNM (mg/kg/week)	
		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 by pairwise comparison

Alternative model for environmental carcinogenesis research Rainbow Trout (*Oncorhynchus mykiss*)

- Sensitivity to many classes of carcinogens
- Well described tumor pathology
- Responsiveness to tumor promoters and inhibitors

Tumor incidence in rainbow trout administered DMNM in the diet (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	Malignant	0/66	**9/64****	0/113	**66/113****
	Combined benign & malignant	0/66	**11/64****	0/113	**78/113****
Glandular stomach	Benign	0/66	0/64	0/113	**53/113****
Swimbladder	Benign	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] Not reported. * p=0.06; ** p<0.001 by pairwise comparison

Non-mammalian Genotoxicity

- **Positive**
  - **Multiple *Salmonella typhimurium* reverse mutation assays**
    - **Base-pair substitution: TA 100, TA 1530, and TA 1535**
    - **Frameshift: TA 98**
  - **X-linked recessive lethal mutation in *Drosophila melanogaster***

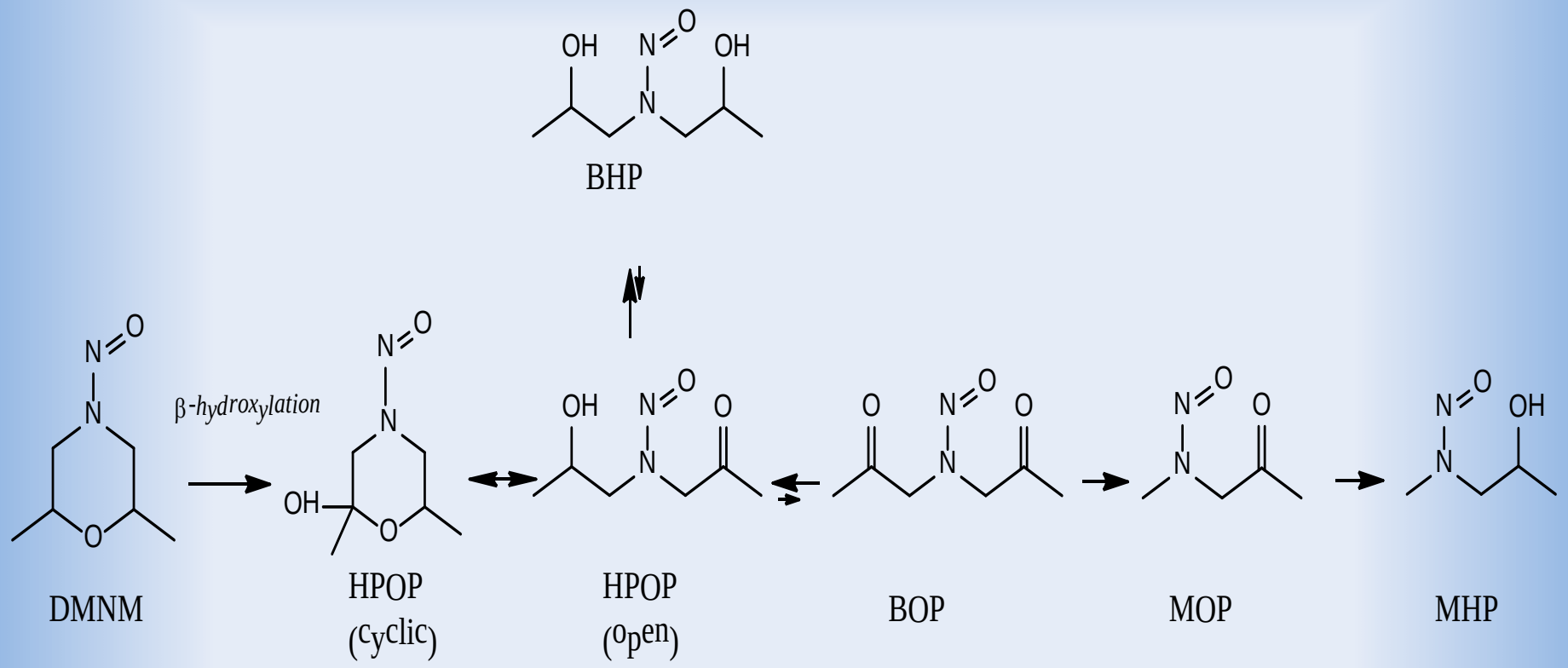
Mammalian Genotoxicity

- Positive- *in vitro*
  - Unscheduled DNA synthesis in rat hepatocyte primary culture and hamster isolated main pancreatic ducts
  - DNA, RNA and protein adduct formation in hamster pancreas
- Positive- *in vivo*
  - Single strand DNA breaks in hamster (but not rat) pancreatic acinar cells
  - DNA, RNA and protein adduct formation in hamster and rat liver

Pharmacokinetics and Metabolism

- Rapidly absorbed and distributed in several *in vivo* studies by gavage (rat and hamster; no significant species difference)
- Metabolism studied in *in vitro* and *in vivo* settings (rats, hamster, guinea pig, and rabbit)
  - Multiple metabolites
  - Multiple pathways (α- & β-hydroxylation) and enzyme systems (cytochrome P450s; *e.g.*, CYP2B4 and CYP2E1)
- Small amount (<2%) of DMNM excreted in the urine or feces after 24-hr of gavage in rat, hamster and guinea pig

DMNM Metabolic Pathways



α-hydroxylation

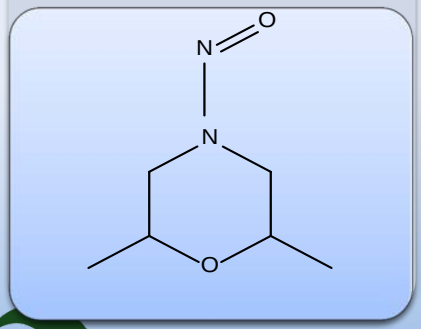
Nitrosamide
(reactive)

DMNM:	2,6-Dimethyl-N-nitrosomorpholine
HPOP:	N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine
BHP:	N-nitroso-bis(2-hydroxypropyl)amine
BOP:	N-nitroso-bis(2-oxopropyl)amine
MOP:	N-nitroso-methyl(2-oxopropyl)amine
MHP:	N-nitroso-methyl(2-hydroxypropyl)amine

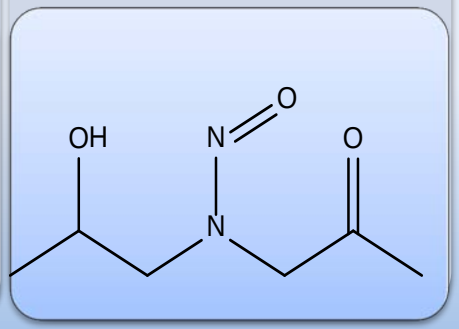
DMNM Metabolites- Common Tumor Sites

Chemical	Liver			Nasal cavity		Lung			Pancreas	
	R	M	H	R	H	R	M	H	R	H
DMNM	+	NT*	+	+	+	+	NT	+	-	+
HPOP	+	NT	NT	+	NT	+	NT	NT	-	NT
BHP	+	+	+	+	+	+	+	+	-	+
BOP	+	+	+	+	-	+	+	+	-	+

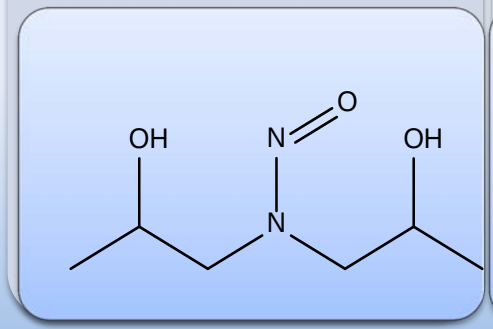
DMNM



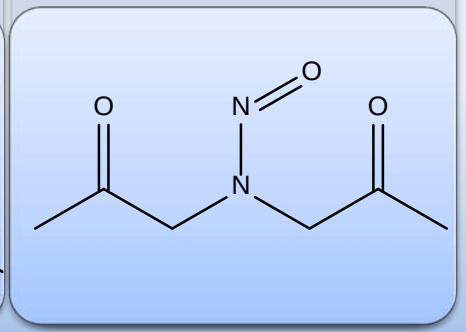
HPOP



BHP



BOP



* Not tested

Genotoxicity of DMNM Metabolites

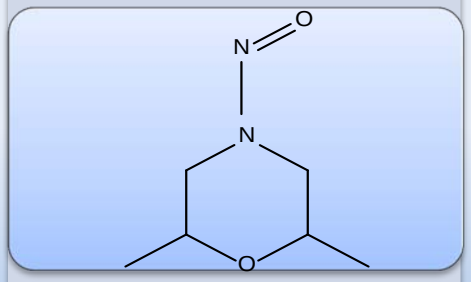
Chemical	Mutagenicity in *Salmonella typhimurium*	Mutagenicity in mammalian cells	UDS (*in vitro*)	DNA adduct formation
DMNM	+	NT*	+	+ (*in vivo*)
HPOP	+	+	NT	+ (*in vitro* & *in vivo*)
BHP	+	+	NT	+ (*in vitro* & *in vivo*)
BOP	+	+	NT	+ (*in vitro* & *in vivo*)

* NT Not tested

Structurally Related Chemicals: Common Tumor Sites

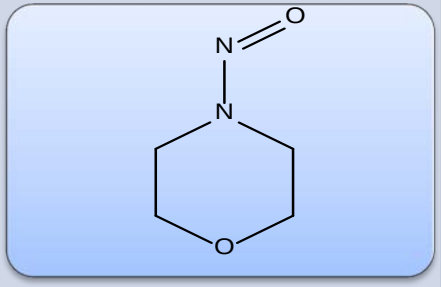
Chemical	Liver			Nasal cavity		Lung			Eso-phagus		Forestomach		
	R	M	H	R	H	R	M	H	R	H	R	M	H
DMNM	+	NT*	+	+	+	+	NT	+	+	-	+	NT	+
NM	+	+	+	+	+	-	+	-	+	-	-	-	+
NP	+	+	+	+	+	-	+	+	+	+	-	+	+

DMNM



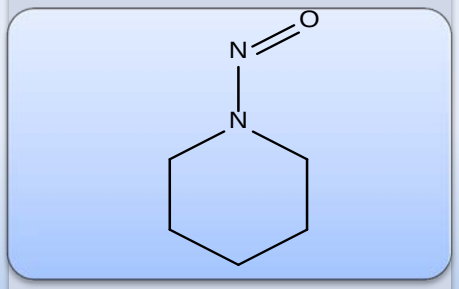
Under consideration

Nitrosomorpholine (NM)



Prop 65 listed

Nitrosopiperidine (NP)



Prop 65 listed

* Not Tested

Genotoxicity of Structurally Related Chemicals

Chemical	Mutagenicity in *Salmonella typhimurium*	Mutagenicity in mammalian cells	UDS (*in vitro*)	DNA adduct formation
DMNM	**+**	**NT***	**+**	**+ (*in vivo*)**
NM	**+**	**+**	**+**	**+ (*in vivo*)**
NP	**+**	**+**	**+**	**NT**

*** NT Not tested**

Possible Mechanisms of Action

- DMNM is likely to induce tumors through genotoxic mechanisms.
- Metabolic activation is required for genotoxic and carcinogenic activity.

Summary of Evidence

- DMNM induces tumors in animal bioassays
  - at multiple sites
    - More than 10 rare tumor sites/types
  - in multiple species & strains
  - in males and females
  - by multiple routes of exposure

Shared Tumor Sites among Species

Tumor site	Rat	Hamster	Guinea Pig	Trout
Nasal cavity	√	√		
Trachea	√	√		
Lung	√	√		
Forestomach	√	√		
Liver	√	√	√	√

√: Rare tumor

Additional Tumor Sites

- Rat: tongue, esophagus
- Hamster: gallbladder, pancreas, kidney, vagina, skin, Harderian gland, larynx
- Trout: swimbladder, glandular stomach

Other Relevant Data

- DMNM tested positive in
  - *In vitro* mutagenicity in *Salmonella* and *in vivo* mutagenicity in *Drosophila*
  - *in vitro* UDS in rat and hamster
  - DNA single strand breaks *in vivo* in hamster
  - DNA adducts *in vitro* and *in vivo* in rat and hamster
- DMNM metabolites are genotoxic.
- DMNM metabolites and structural analogues share many tumor sites with DMNM.