EVIDENCE ON THE CARCINOGENICITY OF

N-NITROSOMETHYL-*n*-ALKYLAMINES

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Reproductive and Cancer Hazard Assessment Branch
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PREFACE

Proposition 65[1] requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. 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 OEHHA 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. In 2011 OEHHA brought N-nitrosomethyl-*n*-alkylamines (NMAs) to the CIC for prioritization and ranking for future listing consideration. OEHHA subsequently selected NMAs for consideration for listing by the CIC. Upon selection, the public was given the opportunity to submit information relevant to the assessment of the evidence on their carcinogenicity. No information was submitted.

OEHHA developed this document with information on the evidence on the carcinogenicity of NMAs to assist the CIC in its deliberations on whether or not NMAs as a group, or chemicals within the group, should be added to the Proposition 65 list as causing cancer. The original papers discussed in the document are also provided to the CIC as part of the hazard identification materials. In addition, comments on this hazard identification document received during the public comment period also form part of the hazard identification materials, and are provided to the CIC members prior to their formal deliberations.

On November 19, 2014, the CIC is scheduled to deliberate on the carcinogenicity of NMAs as a group, and individual chemicals within the group. The CIC may choose to list under Proposition 65 the group, or individual chemicals within the group that are not already on the list. Two NMAs, N-nitrosodimethylamine and N-nitrosomethylethylamine are already on the Proposition 65 list, so committee deliberations and listing decisions will not affect the status of these two chemicals. A transcript of the meeting will be available at www.oehha.ca.gov after the meeting.

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[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

This document summarizes the evidence of carcinogenicity for the chemical group "N-nitrosomethyl-*n*-alkylamines (NMAs)." Two NMAs, N-nitrosodimethylamine (NMA-C1) and N-nitrosomethylethylamine (NMA-C2), have been listed under Proposition 65 as causing cancer since October 1, 1987 and October 1, 1989, respectively. This document summarizes direct evidence of carcinogenicity for eleven other NMAs that are not on the Proposition 65 list.

NMAs are nitrosamines containing a second nitrogen to which a methyl and an alkyl group are attached. NMAs have been detected in personal care products, such as shampoos and conditioners, and household cleaning products, such as dishwashing liquids and surface cleaners. NMAs are not intentionally added to these products, but may form as a result of the reaction of nitrite with amine compounds.

The evidence for the carcinogenicity of NMAs as a group comes primarily from more than 90 experiments in animals with positive tumor findings. These studies were conducted in rats, hamsters, mice, and guinea pigs by various routes of exposure. Thirteen of the NMAs have been tested for carcinogenicity in animals, including the two NMAs currently listed under Proposition 65 (NMA-C1 and NMA-C2). Tumors were observed following treatment with each of the thirteen NMA compounds in at least one species. Many of the observed tumors are rare, including tumors of the nasal cavity, tongue, oropharynx, esophagus, forestomach, kidney, and bladder in rats; and tumors of the nasal cavity, lung, liver, and bladder in hamsters.

Positive findings from genotoxicity and DNA adduct studies indicate that NMAs are likely to operate through genotoxic mechanisms, and that metabolic activation by cytochrome P450 enzymes is required for activity. All twelve NMAs tested for the ability to alkylate DNA form DNA adducts in rats *in vivo*, all eleven NMAs tested for the ability to induce mutations in bacteria are mutagenic, and all three NMAs tested for the ability to induce mutations in mammalian cells *in vitro* are mutagenic. The most extensively tested NMA, NMA-C1, has been demonstrated to be mutagenic and clastogenic in multiple *in vitro* and *in vivo* assay systems.

NMAs are metabolized in a similar manner across chemicals and species, and common metabolites have been observed amongst all NMAs. Two of these common metabolites are listed under Proposition 65 as causing cancer, and are genotoxic (N-nitrososarcosine and formaldehyde). Three others are genotoxic and induce tumors in animals (*i.e.*, N-nitrosomethyl-3-carboxypropylamine, 4-hydroxy-nitrosomethyl-*n*-butylamine, and N-methyl-nitroso-2-oxopropylamine [MOP]). The NMAs and three structurally similar carcinogenic N-nitroso-dialkylamines listed under Proposition 65 share target tumor sites amongst species and chemicals, as well as positive findings of genotoxicity.

2. INTRODUCTION

This document summarizes the evidence of carcinogenicity for the chemical group "N-nitrosomethyl-*n*-alkylamines (NMAs)." Two NMAs, N-nitrosodimethylamine (NMA-C1) and N-nitrosomethylethylamine (NMA-C2), have been listed under Proposition 65 as causing cancer since October 1, 1987 and October 1, 1989, respectively. This document summarizes direct evidence of carcinogenicity for eleven other NMAs that are not on the Proposition 65 list. These other NMAs are:

- N-nitrosomethyl-*n*-propylamine (NMA-C3),
- N-nitrosomethyl-*n*-butylamine (NMA-C4),
- N-nitrosomethyl-*n*-pentylamine (NMA-C5),
- N-nitrosomethyl-*n*-hexylamine (NMA-C6),
- N-nitrosomethyl-*n*-heptylamine (NMA-C7),
- N-nitrosomethyl-*n*-octylamine (NMA-C8),
- N-nitrosomethyl-*n*-nonylamine (NMA-C9),
- N-nitrosomethyl-*n*-decylamine (NMA-C10),
- N-nitrosomethyl-*n*-undecylamine (NMA-C11),
- N-nitrosomethyl-*n*-dodecylamine (NMA-C12), and
- N-nitrosomethyl-*n*-tetradecylamine (NMA-C14).

For these specific NMAs, OEHHA found animal carcinogenicity studies in the literature. Before describing these studies and the results in Section 3, the identity, chemical and physical properties and occurrence and use of these compounds are briefly outlined.

2.1 Identity of N-nitrosomethyl-*n*-alkylamines (NMAs)

N-Nitrosomethyl-*n*-alkylamines (NMAs) are tertiary amines with two nitrogen atoms connected by a single bond, a nitroso group attached to the second nitrogen, and a methyl and an alkyl group attached to the second nitrogen. The identity and systematic name of the individual NMA is based on the actual alkyl chain attached to the second nitrogen (see Table 1).

The structures and some chemical and physical properties of these NMAs are presented in Table 1. These data indicate that with increasing chain length of the NMA alkyl group, water solubility and volatility decrease, and the boiling point increases.

Table 1. Identity and chemical characteristics of several N-nitrosomethyl-*n*-alkylamines (NMAs)

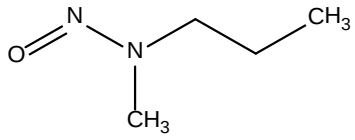
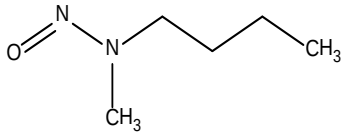
Alkyl Chain length (No. Car-bons)	NMA Chemical name & Synonyms	Molecular Formula & Structure	Molecular Weight [Da]	CAS Registry Number	Boiling Point (°C at 760 mmHg)	Vapor Pressure (mmHg at 25 °C)	Log P / Octanol-water co-efficient
C3	N-Nitrosomethyl-*n*-propylamine (NMA-C3) N-Methyl-N-nitroso-1-propanamine Methyl-n-propylnitrosamine Methylpropylnitrosamine Methylpropylnitrosoamine N-Methyl-N-propylnitrosamine N-Nitrosomethyl(N-propyl)amine N-Nitrosomethylpropylamine Nitrosomethylpropylamine	C4-H10-N2-O 	102	924-46-9	173.9	1.7	0.42
C4	N-Nitrosomethyl-*n*-butylamine (NMA-C4) N-Methyl-N-nitroso-1-butanamine Butylmethylnitrosamine MBNA, NMBA Methyl-butyl-nitrosamin Methyl-n-butylnitrosamine Methylbutylnitrosamine Methylbutylnitrosoamine N-Butyl-N-methylnitrosamine N-Methyl-N-nitrosobutylamine N-Nitroso-N-methyl-N-n-butylamine N-Nitrosomethyl-N-butylamine Nitrosomethyl-n-butylamine	C5-H12-N2-O 	116	7068-83-9	193.6	0.6	0.95

Table 1 (cont'd). Identity and chemical characteristics of NMAs

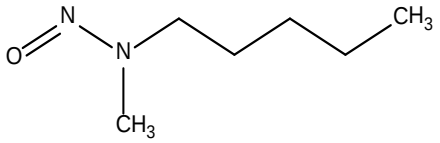
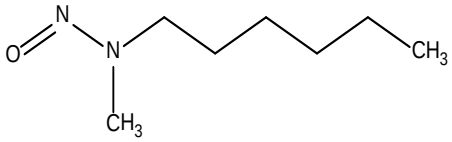
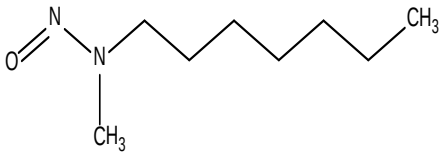
C5	N-Nitrosomethyl-*n*-pentylamine (NMA-C5) N-Methyl-N-nitroso-1-pentanamine Methyl-N-amylnitrosamine Methyl-N-pentylnitrosamine Methylamylnitrosamin Methylamylnitrosamine N-Amyl-N-methylnitrosamine N-Methyl-N-nitrosopentylamine N-Methyl-N-pentylnitrosamine N-Nitroso-N-methyl-n-amylamine Nitrosomethyl-n-amylamine Nitrosomethyl-n-pentylamine	C6-H14-N2-O 	130	13256-07-0	213.0	0.2	1.49
C6	N-Nitrosomethyl-*n*-hexylamine (NMA-C6) N-Methyl-N-nitroso-1-hexanamine N-Methyl-N-nitroso-hexylamine Nitroso-n-hexylmethylamine Nitrosomethyl-n-hexylamine	C7-H16-N2-O 	144	28538-70-7	232.0	0.092	2.05
C7	N-Nitrosomethyl-*n*-heptylamine (NMA-C7) N-Methyl-N-nitroso-1-heptanamine Heptylmethylnitrosamine Methylheptylnitrosamin Methylheptylnitrosamine N-Methyl-N-nitrosoheptylamine N-Nitroso-N-methylheptylamine N-Nitrosomethylheptylamine Nitrosomethyl-n-heptylamine Nitrosomethylheptylamin	C8-H18-N2-O 	158	16338-99-1	250.0	0.034	2.5

Table 1 (cont'd). Identity and chemical characteristics of NMAs

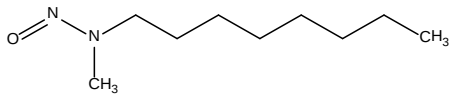
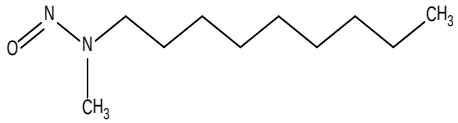
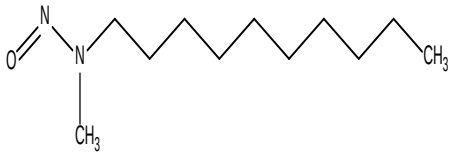
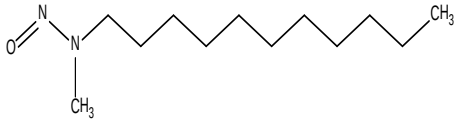
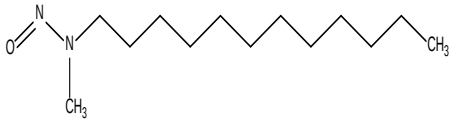
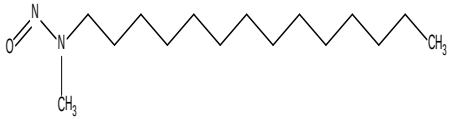
C8	N-Nitrosomethyl-*n*-octylamine (NMA-C8) N-Methyl-N-nitroso-1-octanamine N-Methyl-N-nitrosooctylamine Nitroso-N-methyl-n-octylamine Nitrosomethyl-n-octylamine	C9-H20-N2-O 	172	34423-54-6	268.5	0.013	3.07
C9	N-Nitrosomethyl-*n*-nonylamine (NMA-C9) N-Methyl-N-nitroso-1-nonanamine Nitroso-N-methyl-n-nonylamine Nitrosomethyl-n-nonylamine	C10-H22-N2-O 	186	75881-19-5	286.0	0.004	3.58
C10	N-Nitrosomethyl-*n*-decylamine (NMA-C10) N-Methyl-N-nitroso-1-decanamine N-Methyl-N-nitrosodecylamine Nitrosomethyl-n-decylamine	C11 H24-N2-O 	200	75881-22-0	303	0.002	4.09
C11	N-Nitrosomethyl-*n*-undecylamine (NMA-C11) N-Methyl-N-nitroso-1-undecanamine N-Methyl-N-nitrosoundecylamine Nitrosomethylundecylamine	C12-H26-N2-O 	214	68107-26-6	319.5	0	4.67

Table 1 (cont'd). Identity and chemical characteristics of NMAs

C12	N-Nitrosomethyl-*n*-dodecylamine (NMA-C12) N-Methyl-N-nitroso-1-dodecanamine Laurylamine N-methyl-N-nitroso- N-Dodecyl-N-methylnitrosamine N-Methyl-N-nitrosolaurylamine N-Nitroso-N-methyl-N-dodecylamin Nitrosomethyl-n-dodecylamine NMDA	C13-H28-N2-O 	228	55090-44-3	335.6	0	5.2
C14	N-Nitrosomethyl-*n*-tetradecylamine (NMA-C14) N-Methyl-N-nitroso-1-tetradecanamine N-Nitroso-N-methyl-n-tetradecylamine	C15-H32-N2-O 	256	75881-20-8	366.6	0	6.12

[a] Data at http://www.chemspider.com/

2.2 Occurrence and Use

No information on past or present commercial production of NMAs was identified, and no commercial uses of NMAs were identified. NMAs, including NMA -C12, -C14, -C16, and -C18 have been detected in personal care products, such as shampoos and conditioners, and household cleaning products, such as dishwashing liquids and surface cleaners (Morrison and Hecht, 1982; Hecht *et al.*, 1982, Kamp and Eisenbrand, 1991). NMAs are not intentionally added to these products, but may form as a result of the reaction of nitrite with amine compounds (Hecht *et al*., 1982; Morrison and Hecht, 1982; Kamp and Eisenbrand, 1991; Eisenbrand *et al.*, 1996). For example, NMAs can be derived from fatty amine oxide precursors, such as C12 or C14 dimethylamine oxides or corresponding tertiary or secondary amines (Hecht *et al.,* 1982; Ikeda and Migliorese, 1990). Fatty amine oxide precursors are used in consumer products as emulsifiers, detergents and thickening agents, and may be nitrosated by nitrite, the preservatives bronopol and bronidox, as well as environmental nitrogen oxides (Kamp and Eisenbrand, 1991).

Data published in 1982 (Morrison and Hecht, 1982; Hecht *et al*., 1982) indicate that four out of twelve liquid dishwashing detergents and one out of four household surface cleaners tested contained NMA-C12 and NMA-C14. Similarly, four out of six shampoos, one out of one hair conditioner, three out of four hair rinse products and two out of two hot oil treatments also contained NMA-C12 and NMA-C14. Levels of NMA-C12 in analyzed products ranged from 254 to 471 ppb in liquid dishwashing detergent, 16 to 203 ppb in shampoo, 11 to 83 ppb in hair rinse products, 604 to 873 in a hot oil treatment product, 112 ppb in a surface cleaner, and 11 ppb in a hair conditioner. Levels of NMA-C14 ranged from 67 to 108 ppb in liquid dishwashing detergent, 8 to 76 ppb in shampoo, 22 to 25 ppb in hair rinse products, 218 to 254 ppb in a hot oil treatment product, 46 ppb in a surface cleaner and 9 ppb in a hair conditioner.

Another study published in 1996 (Eisenbrand *et al*., 1996) analyzed 86 cosmetic products available on the German market and 45 products available on the U.S. and Japanese markets. Only one of the 86 German market products was contaminated with NMA-C12 (120 ppb), NMA-C14 (50 ppb), and NMA-C16 (20 ppb). Four out of 45 U.S. and Japanese market products analyzed contained levels of NMAs (combined) in the range of 150-250 ppb.

3. DATA ON CARCINOGENICITY

The parameters used in conducting literature searches on the carcinogenicity of NMAs are summarized in Appendix A.

3.1 Carcinogenicity Studies in Humans

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

3.2 Carcinogenicity Studies in Animals

The carcinogenicity of numerous individual NMAs not already listed under Proposition 65 has been studied in a variety of species, including rats, hamsters, mice, and guinea pigs. These studies are listed in Table 2. Study data from Lijinsky *et al.* (1981) using rats and Lijinsky and Kovatch (1988) using hamsters are presented in detail in Section 3.2.1. These two publications each contain carcinogenicity data for several NMAs, which enables comparisons between NMAs in the same set of studies. As indicated in Table 2, a substantial number of additional NMA carcinogenicity studies have also been reported. Findings from these other studies, along with information on key study design elements, are presented in tabular form in Section 3.2.2

Table 2. Overview of animal carcinogenicity studies of NMAs

Study No.	Species	Strain	Sex	Route	Reference
NMA-C3					
1	Rat	Fischer 344	F	drinking water	Lijinsky *et al.*, 1983a
2	Rat	Sprague Dawley	M	*s.c*. injection	Reznik *et al.*, 1975
3			F		
4	Hamster	Syrian golden	M	gavage	Lijinsky and Kovatch,1988
5			F		
6	Hamster	Syrian golden	M (F1)	in utero	Althoff and Grandjean, 1979
7			F (F1)		
8			F (pregnant)	*s.c*. injection	
9	Hamster	Syrian golden	M & F	*s.c*. injection	Pour *et al.*, 1974; 1975
11	Mouse	NMRI	F	*s.c.* injection	Dickhaus *et al.*, 1977
NMA-C4					
12	Rat	Fischer 344	M	drinking water	Lijinsky *et al.*, 1980; 1983b
13			F		Lijinsky *et al.*, 1980; 1983a
16	Rat	Fischer 344	M	drinking water	Koreeda *et al.*, 1999
17	Rat	Fischer 344	M	gavage	Lijinsky *et al.*, 1983b
18			F		
19	Rat	Fischer 344	F	gavage	Lijinsky *et al.*, 1991a
20				Intravesicular injection in bladder	
21	Hamster	Syrian golden	M	gavage	Lijinsky and Kovatch,1988
22			F		

Table 2 (continued). Overview of animal carcinogenicity studies of NMAs

Study No.	Species	Strain	Sex	Route	Reference
NMA-C5					
23	Rat	BD	Not Specified	drinking water	Druckery *et al.*, 1967
24	Rat	Wistar	M	drinking water	Matsufuji *et al.*, 1987
25	Rat	Wistar	M	drinking water	Sasajima *et al.*, 1982
26	Rat	Donyu	M (4- to 6-week old) 180-day study	drinking water	Iizuka *et al.*, 1980
27			M (4- to 6-week old) 322-day study		
28			M (8- to 9-week old)		
29			F (4- to 5-week old)		
30	Rat	Sprague Dawley	M	drinking water	Kuwayama and Eastwood, 1988
31	Rat	Sprague Dawley	M	drinking water	Kondoh *et al.*, 1990
32	Rat	MRC-Wistar	M & F (newborn)	*i.p*. injection	Mirvish *et al.*, 1996
33			M & F (3-day-old)		
34			M & F (6- to 8-week-old)		
35	Rat	MRC-Wistar	M	*i.p*. injection	Mirvish *et al.*, 1994a
36	Rat	MRC-Wistar	M (1 injection)	*i.p*. injection	Bulay and Mirvish, 1979
37			M (2 injections)		
38			M (6 injections)		
39			M (12 injections)		
40			F (1 injection)		
41			F (2 injections)		
42			F (6 injections)		
43			F (12 injections)		
44	Rat	MRC-Wistar	M	*i.p*. injection	Mirvish *et al.*, 1985a
45	Rat	Wistar	M	*i.p*. injection	Tanaka *et al.*, 1997
46	Rat	Fischer 344	M	*i.p*. injection	Yamaguchi *et al.*, 1989
47	Rat	Sprague Dawley	M	*i.p*. injection	Attwood *et al.*, 1992
48	Rat	Wistar	M & F	intramuscular	Luo *et al.*, 1987

Table 2 (continued). Overview of animal carcinogenicity studies of NMAs

Study No.	Species	Strain	Sex	Route	Reference
49	Rat	Wistar	M	*s.c*. injection	Seto *et al.*, 1991
50	Rat	BD	NS	*s.c*. injection	Druckery *et al.*, 1967
51	Hamster	Syrian golden	M	gavage	Lijinsky and Kovatch,1988
52			F		
53	Hamster	Syrian golden	M & F (newborn)	*i.p*. injection	Mirvish *et al.*, 1996
54			M & F (3-day-old)		
55			M & F (38-day-old)		
56			M & F (46- to 49-day-old)		
57	Mouse	101/N	M & F	drinking water	Kurooka *et al.*, 1998
58		STX/Le			
59		BXH-8			
60		C57BL/6J			
61	Mouse	Brca1 Δ11/Δ11 p53+/-	F	drinking water	Cao *et al.*, 2007
62		Brca1+/Δ11 p53+/-			
63	Mouse	C57BL/6 p53+/+ wild type 15-week study	M	drinking water	Shirai *et al.*, 2002a; 2002b
64		C57BL/6 p53+/- heterozygote 15-week study			
65		C57BL/6 p53-/- knockout 15-week study			
66		C57BL/6 p53+/+ wild type 25-week study			
67		C57BL/6 p53+/- heterozygote 25-week study			
68	Mouse	Swiss	M & F	*i.p.* injection	Mirvish *et al.*, 1996
NMA-C6					
69	Rat	Fischer 344	M	drinking water	Lijinsky *et al.*, 1983b
70				gavage	
70	Hamster	Syrian golden	M	gavage	Lijinsky and Kovatch,1988
72			F		

Table 2 (continued). Overview of animal carcinogenicity studies of NMAs

Study No.	Species	Strain	Sex	Route	Reference
NMA-C7					
73	Rat	Fischer 344	M	drinking water	Lijinsky *et al.*, 1983b
74				gavage	
75	Rat	BD	NS	*s.c*. injection	Druckery *et al.*, 1967
76	Hamster	Syrian golden	M	gavage	Lijinsky and Kovatch,1988
77			F		
78	Hamster	Syrian golden	M	gavage	Rehm and Lijinsky,1994
NMA-C8					
79	Rat	Fischer 344	M	gavage	Lijinsky *et al.*, 1981
80	Hamster	Syrian golden	M	gavage	Lijinsky and Kovatch,1988
81			F		
NMA-C9					
82	Rat	Fischer 344	M	gavage	Lijinsky *et al.*, 1981
NMA-C10					
83	Rat	Fischer 344	M	gavage	Lijinsky *et al.*, 1981
NMA-C11e					
84	Rat	Fischer 344	M	gavage	Lijinsky *et al.*, 1978
NMA-C12					
85	Rat	Fischer 344	M	gavage	Lijinsky *et al.*, 1981
86	Rat	Sprague Dawley	M	gavage	Lijinsky and Taylor, 1975
87			F		
88	Rat	Sprague Dawley	M	gavage	Lijinsky and Taylor, 1978
89			F		
90		Fischer 344	M		
91			F		
92	Rat	Fischer 344	F	gavage	Lijinsky *et al.*, 1983c
93	Hamster	Syrian golden	M	gavage	Althoff and Lijinsky, 1977
94			F		
95	Hamster	European Mhh:EPH	M	*s.c.* injection	Ketkar *et al.*, 1981
96			F		
97	Guinea Pig	Strain 2	M	gavage	Cardy and Lijinsky, 1980
NMA-C14					
98	Rat	Fischer 344	M	gavage	Lijinsky *et al.*, 1981

3.2.1 Oral Studies in Rats and Hamsters Comparing Several NMAs

Gavage studies of five NMAs in male Fischer F344 rats (Lijinsky et al., 1981)

NMA-C8, -C9, -C10, -C12, and -C14 were administered by gavage to groups of 20 male Fischer 344 rats twice a week (0.2 ml in corn oil) for 30 weeks at specific doses (Table 3). The untreated control group consisted of 20 animals. Animals were allowed to die naturally or were killed when moribund. In the treated groups, survival at 50 weeks was poor for animals treated with NMA-C8 and –C9, but all animals in other treatment groups survived to week 50 (Table 3). In the controls, 17 animals were alive at 80 weeks, and 12 animals were alive at 110 weeks. Eight survivors were killed at 126 weeks.

Table 3. Dose received and survival of male Fischer 344 rats administered NMAs by gavage twice/week for 30 weeks (Lijinsky *et al*., 1981)

Compound	Concentration (mg/ml)	Administered dose each occasion (mg)[1]	Total dose (mg)	Survival by week 40	Survival by week 50
NMA-C8	93	18.6	1120	5/20	0/20
NMA-C9	100	20	1200	11/20	1/20
NMA-C10	108	21.6	1300	20/20	20/20
NMA-C12	60	12	720	20/20	20/20
NMA-C14	140	28	1680	20/20	20/20
Untreated con-trols	---	---	---	20/20	20/20

[1] Calculated by OEHHA

Significant increases in tumors of various types were seen for each of the tested NMAs, as shown in Table 4. None of the control animals were observed with any of these tumor types. The only tumor seen in the control animals was interstitial cell lung tumors, which occurred in two control rats. No lung interstitial cell tumors were observed in any of the animals treated with NMAs.

Table 4. Overview of rare or significantly increased tumor sites in gavage studies in male Fischer F344 rats (Lijinsky *et al.*, 1981)

Tumor site	Tumor type[1]	NMA-C8	NMA-C9	NMA-C10	NMA-C12	NMA-C14
Nasal cavity	Papilloma (r)	+	+			
	Carcinoma (r)	+*	+	+		
Trachea	Papilloma (r)	+*				
Lung	Adenoma					
	Adenocarcinoma (r)	+*	+*	+*	+	+
	Squamous cell carcinoma (r)		+			
Forestomach	Papilloma (r)			+	+	
	Carcinoma (r)			+	+*	
Liver	Hepatocellular carcinoma	+*	+*			
	Cholangiocarcinoma (r)		+*			
	Hemangiosarcoma (r)	+				
Pancreas	Islet cell carcinoma				+*	
Kidney	Carcinoma (r)					+
Bladder	Transitional cell carcinoma (r)	+*		+*	+*	+*

* Statistically significant ($p < 0.05$) increases of tumor incidence by Fisher pairwise comparison (conducted by OEHHA)
[1] (r) = rare

Tumors of the nasal cavity, lung, forestomach, liver and bladder were seen in treatment groups for at least two different NMAs. Nasal cavity papillomas and carcinomas, lung adenocarcinomas and squamous cell carcinomas, forestomach papillomas and carcinomas, and bladder transitional cell carcinomas are rare in male Fischer 344 (F344) rats (Frith *et al*., 1995, Frantz *et al.,* 1991; Schwartz *et al.*, 1994; Haseman *et al.*, 1998; Boorman *et al.*, 1990). Significant increases in tracheal papilloma were seen after treatment with NMA-C8, and in pancreatic islet cell carcinoma after treatment with NMA-C12. Tracheal papillomas are also rare in male F344 rats (Schwartz *et al.*, 1994; Frith *et al.*, 1995; Frantz *et al.*, 1991; Haseman *et al.*, 1998). Rare liver hemangiosarcomas and kidney carcinomas (Boorman *et al*., 1990) were seen with NMA-C8 treatment, and rare cholangiocarcinomas (Boorman *et al*., 1990) were seen following treatment with NMA-C9.

Incidences of the tumors seen in the Lijinsky *et al*. (1981) studies are presented separately below for each of the NMA treatment groups. The tumors that are rare are denoted by "r" in the tables.

NMA-C8 treatment resulted in the observation of tumors at multiple sites, including nasal cavity (papillomas and carcinomas), trachea (papillomas), lung (adenomas and adenocarcinomas), liver (hepatocellular carcinomas and hemangiosarcomas), and bladder (transitional cell carcinomas) (Table 5). There was a statistically significant increase by pairwise comparison with the control group of nasal cavity carcinomas, tracheal papillomas, lung adenocarcinomas, hepatocellular carcinomas, and urinary bladder transitional cell carcinomas.

Table 5. Tumor incidence in male Fischer 344 rats administered NMA-C8 by gavage twice/week for 30 weeks and observed for life (Lijinsky *et al*., 1981)

Tumor Site and Type		Control	Total dose: 1120 mg/animal[1]
Nasal Cavity	Papilloma (r)	0/20	3/20
	Carcinoma (r)	0/20	14/20***
Trachea	Papilloma (r)	0/20	5/20*
Lung	Adenoma	0/20	4/20
	Adenocarcinoma (r)	0/20[2]	8/20**
Liver	Hepatocellular carcinoma	0/20	13/20***
	Hemangiosarcoma (r)	0/20	4/20
Bladder	Transitional cell carcinoma (r)	0/20	14/20***

[1] Treated group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)
[2] Two lung interstitial cell carcinomas observed in controls
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C9 treatment resulted in the observation of tumors at multiple sites, including the nasal cavity, lung, and liver. Nasal tumors observed included papillomas and carcinomas; lung tumors included adenomas, adenocarcinomas, and squamous cell carcinomas; and liver tumors included hepatocellular carcinomas and cholangiocarcinomas (Table 6). Treated animals had statistically significant increases for lung adenocarcinomas, hepatocellular carcinomas, and liver cholangiocarcinomas.

Table 6. Tumor incidence in male Fischer 344 rats administered NMA-C9 by gavage twice/week for 30 weeks and observed for life (Lijinsky *et al*., 1981)

Tumor Site and Type		Control	Total dose: 1200 mg/animal[1]
Nasal Cavity	Papilloma (r)	0/20	4/20
	Carcinoma (r)	0/20	4/20
Lung	Adenoma	0/20	1/20
	Adenocarcinoma (r)	0/20[2]	9/20***
	Squamous cell Carcinoma (r)	0/20	2/20
Liver	Hepatocellular carcinoma	0/20	14/20***
	Cholangiocarcinoma (r)	0/20	6/20**

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)
[2] Two lung interstitial cell carcinomas observed in controls
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C10 treatment resulted in the observation of tumors at multiple sites, including the nasal cavity (carcinomas), lung (adenomas and adenocarcinomas), forestomach (papillomas and carcinomas), liver (hepatocellular carcinomas), and bladder (transitional cell carcinomas) (Table 7). Statistically significant increases were observed for adenocarcinomas of the lung and transitional cell carcinomas of the bladder. Additional tumors observed in treated animals but not presented in table 7 include two sebaceous gland carcinomas, one spleen hemangiosarcoma, one heart sarcoma, one carcinoma of the head, and one leiomyosarcoma of the ear. Both spleen hemangiosarcoma and sebaceous gland carcinoma are rare in male F344 rats (Haseman *et al*., 1998).

Table 7. Tumor incidence in male Fischer 344 rats administered NMA-C10 by gavage twice/week for 30 weeks and observed for life (Lijinsky *et al*., 1981)

Tumor Site and Type		Control	Total dose: 1300 mg/animal[1]
Nasal Cavity	Carcinoma (r)	0/20	1/20
Lung	Adenoma	0/20	3/20
	Adenocarcinoma (r)	0/20[2]	5/20*
Forestomach	Papilloma (r)	0/20	1/20
	Carcinoma (r)	0/20	1/20
Liver	Hepatocellular carcinoma	0/20	2/20
Bladder	Transitional cell carcinoma (r)	0/20	17/20***

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)
[2] Two lung interstitial cell carcinomas observed in controls
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

For NMA-C12, the primary target organ was the bladder, where transitional cell carcinomas were observed in all but one animal. Other tumor sites included the lung, forestomach, liver, and pancreas (Table 8). Statistically significant increases by pairwise comparison with controls were observed for forestomach carcinomas, pancreatic islet cell carcinomas, and bladder transitional cell carcinomas.

Table 8. Tumor incidence in male Fischer 344 rats administered NMA-C12 by gavage twice/week for 30 weeks and observed for life (Lijinsky *et al*., 1981)

Tumor Site and Type		Control	Total dose: 720 mg/animal[1]
Lung	Adenoma	0/20	1/20
	Adenocarcinoma (r)	0/20[2]	4/20
Forestomach	Papilloma (r)	0/20	2/20
	Carcinoma (r)	0/20	5/20*
Liver	Hepatocellular carcinoma	0/20	3/20
Pancreas	Islet cell carcinoma	3/20	10/20*
Bladder	Transitional cell carcinoma (r)	0/20	19/20***

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)
[2] Two lung interstitial cell carcinomas observed in controls
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

For NMA-C14, the primary target organ was also the bladder, where 100 percent (20/20) tumor incidence was observed. The tumors were described as transitional cell carcinomas and were invasive. Two adenomas and one adenocarcinoma of the lung and two kidney carcinomas were also observed in treated rats (Table 9).

Table 9. Tumor incidence in male Fischer 344 rats administered NMA-C14 by gavage twice/week for 30 weeks and observed for life (Lijinsky *et al.*, 1981)

Tumor Site and Type		Control	Total dose: 1680 mg/animal[1]
Lung	Adenoma	0/20	2/20
	Adenocarcinoma (r)	0/20[2]	1/20
Kidney	Carcinoma (r)	0/20	2/20
Bladder	Transitional cell carcinoma (r)	0/20	20/20***

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)
[2] Two lung interstitial cell carcinomas observed in controls
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

***Gavage studies of six NMAs in male and female Syrian golden hamsters* (Lijinsky and Kovatch, 1988)**

NMA-C3, -C4, -C5, -C6, -C7, and –C8 were administered in 0.2 ml of ethyl acetate:corn oil (1:2) by gavage once a week to groups of male and female Syrian golden hamsters. The dosing regimens for studies in males are presented in Table 10, and for studies in females in Table 11. Treatment group size was 12 animals/sex/group, including the vehicle controls, except for the study of NMA-C6 in females, where 20 animals were in the treatment group. Treatment duration varied for the different groups, as shown in Tables 10 and 11. Controls were given the vehicle for 75 weeks. Animals were allowed to live until natural death occurred or until moribund. Average survival times for treated animals are also presented in Table 10 (males) and Table 11 (females).

Table 10. Dose received and survival of male Syrian golden hamsters administered NMAs by gavage once/week for 23–50 weeks (Lijinsky and Kovatch, 1988)

Compound	Treatment duration (weeks)	Administered dose (mg/week)	Total dose (mmol [mg[1]])	Average survival (weeks)
NMA-C3	23-30	2.1	0.6 [61.2]	49
NMA-C4	23-30	2.5	0.65 [75.4]	49
	23-30	5.0	1.0 [116]	27
NMA-C5	23-30	5.6	1.1 [143]	34
NMA-C6	50	6.2	2.1 [302]	51
NMA-C7	40	6.8	2.1 [332]	52
NMA-C8	40	7.4	2.1 [361]	53
Vehicle control	75	0	0	100

[1] Converted by OEHHA (total mmol dose provided by authors)

Table 11. Dose received and survival of female Syrian golden hamsters administered NMAs by gavage once/week for 23–50 weeks (Lijinsky and Kovatch, 1988)

Compound	Treatment duration (weeks)	Administered dose (mg/week)	Total dose (mmol [mg[1]])	Average survival (weeks)
NMA-C3	23-30	2.1	0.6 [61.2]	44
NMA-C4	23-30	2.5	0.65 [75.4]	37
	23-30	5.0	1.0 [116]	25
NMA-C5	23-30	5.6	1.1 [143]	38
NMA-C6	50	6.2	2.1 [302]	57
NMA-C7	40	6.8	2.1 [332]	57
NMA-C8	40	7.4	2.1 [361]	54
Control	75	0	0	87

[1] Converted by OEHHA (total mmol dose provided by authors)

Significant increases in tumors of various types were seen for each of the tested NMAs in male and female hamsters, as shown in Table 12. No tumors were reported in the male or female vehicle controls, with the exception of one liver cholangioma in each. Liver cholangiomas were observed in several animals of each sex treated with NMAs.

Table 12. Overview of rare or significantly increased tumor sites in gavage studies in male and female Syrian golden hamsters (Lijinsky and Kovatch, 1988)

Tumor site	Tumor type[1]	NMA-C3	NMA-C4[2]	NMA-C5	NMA-C6	NMA-C7	NMA-C8
Nasal mucosa	Adenoma (r)	M[3]	M*,F*	F[4]	M	M*,F	M,F
	Carcinoma (r)	M*,F*	M*,F*	M,F*	M	M,F	
Lung	Alveolar/bronchial adenoma (r)	M	M,F*	M,F*	M*,F*	M*,F*	M*,F*
	Alveolar/bronchial carcinoma (r)		M		F*	M*,F*	M,F
	Squamous cell carcinoma (r)		M	M	M		
	Adenosquamous carcinoma (r)			F	F	M, F	
Forestomach	Squamous cell papilloma		M*,F*	F*	M*	F*	M*,F*
	Squamous cell carcinoma (r, M)		M*	M*, F*	M*,F*	M*	M*
Liver	Hepatocellular adenoma (r)	M*,F*	M*,F*	M, F	M	M, F	M*,F*
	Hepatocellular carcinoma (r)		M			M	M, F
	Hemangiosarcoma (r)	M*,F*	M*		M	M*	M
	Cholangioma				F*	M*,F*	
	Cholangiocarcinoma (r)			M		M	
Bladder	Transitional cell papilloma (r)				M,F		M
	Transitional cell carcinoma (r)				M,F		
	Hemangioma (r)						M
	Hemangiosarcoma (r)						M
	Undifferentiated sarcoma (r)						M, F

* Statistically significant ($p < 0.05$) increases of tumor incidence by Fisher pairwise comparison (conducted by OEHHA)
[1] (r) = rare
[2] Results for low and high dose are not distinguished here
[3] M = Male
[4] F= Female

Tumors of the nasal mucosa, lung, forestomach, liver and bladder were observed in treatment groups for at least two different NMAs. Nasal mucosa tumors, lung tumors, hepatocellular adenomas and carcinomas, liver cholangiocarcinomas, liver hemangiomas and hemangiosarcomas, and urinary bladder tumors are rare in Syrian golden hamsters (IARC, 1996; Pour *et al*., 1976a; 1976b). Forestomach squamous cell carcinomas are rare in male (but not female) Syrian golden hamsters (IARC, 1996).

Incidences of the tumors seen in the Lijinsky and Kovatch (1988) studies are presented separately below for each of the NMA treatment groups. The tumors which are rare are denoted by "r" in the tables.

NMA-C3 treatment of male hamsters resulted in the observation of tumors at multiple sites. Tumors observed included nasal mucosa (adenomas and carcinomas), lung (alveolar/bronchial adenomas), and liver (hepatocellular adenomas, hemangiosarcomas, one cholangioma) (Table 13). Statistically significant increases were observed in nasal mucosa carcinomas and liver hepatocellular adenomas and hemangiosarcomas compared to controls.

Table 13. Tumor incidence in male Syrian golden hamsters administered NMA-C3 by gavage once/week for 23 - 30 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor site and type		Vehicle Controls	2.1 mg/week Total dose: 0.6 mmol[1] (61.2 mg)
Nasal mucosa	Adenoma (r)	0/12	3/12
	Carcinoma (r)	0/12	10/12***
Lung	Alveolar/bronchial adenoma (r)	0/12	2/12
Liver	Hepatocellular adenoma (r)	0/12	6/12**
	Hemangiosarcoma (r)	0/12	10/12***

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

Tumor sites observed in the study of female hamsters exposed to NMA-C3 included the nasal mucosa (carcinomas) and the liver (hepatocellular adenomas, hemangiosarcomas) (Table 14). The incidences of each of these tumors were statistically significant by pairwise comparison with the controls.

Table 14. Tumor incidence in female Syrian golden hamsters administered NMA-C3 by gavage once/week for 23 - 30 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor site and type		Vehicle Controls	2.1 mg/week Total dose: 0.6 mmol[1] (61.2 mg)
Nasal mucosa	Carcinoma (r)	0/12	10/12***
Liver	Hepatocellular adenoma (r)	0/12	4/12*
	Hemangiosarcoma (r)	0/12	4/12*

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C4 was tested at two doses in male hamsters, and tumors were observed at multiple sites in the treated groups. Tumor sites observed included nasal mucosa (adenomas and carcinomas), lung (alveolar/bronchial adenomas and carcinomas, and one squamous cell carcinoma), forestomach (squamous cell papillomas and carcinomas) and liver (hepatocellular adenomas and carcinomas, cholangiomas, hemangiosarcomas, and one hemangioma) (Table 15). Statistically significant increases by pairwise comparisons with controls were observed for nasal mucosa adenomas (low-dose group) and carcinomas (both low-dose and high-dose groups), forestomach squamous cell papillomas (low-dose and high-dose groups) and carcinomas (high-dose group), hepatocellular adenomas (low-dose group), and liver hemangiosarcomas (both low- and high-dose groups). Statistically significant increases by trend were observed for nasal mucosa carcinomas, forestomach squamous cell papillomas and carcinomas, and liver hepatocellular adenomas and hemangiosarcomas.

Table 15. Tumor incidence in male Syrian golden hamsters administered NMA-C4 by gavage once/week for 23 - 30 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor site and type		Vehicle Controls[1]	2.5 mg/week Total dose: 0.65 mmol[2] (75.4 mg)	5.0 mg/week Total dose: 1.0 mmol[2] (116 mg)
Nasal mucosa	Adenoma (r)	0/12	4/12*	0/12
	Carcinoma (r)	0/12**	5/12*	6/12**
Lung	Alveolar/bronchial adenoma (r)	0/12	3/12	3/12
	Alveolar/bronchial carcinoma (r)	0/12	0/12	1/12
	Squamous cell carcinoma (r)	0/12	0/12	1/12
Forestomach	Squamous cell papilloma	0/12**	5/12*	5/12*
	Squamous cell carcinoma (r)	0/12*	2/12	4/12*
Liver	Hepatocellular adenoma (r)	0/12*	5/12*	3/12
	Hepatocellular carcinoma (r)	0/12	1/12	1/12
	Hemangioma (r)	0/12	0/12	1/12
	Hemangiosarcoma (r)	0/12*	5/12*	4/12*

[1] Control incidences with asterisks indicate significant results from exact trend test (performed by OEHHA)

[2] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C4 was tested at two doses in female hamsters, and tumors were observed in the nasal mucosa (carcinomas, adenomas, and one undifferentiated sarcoma), lung (alveolar/bronchial adenomas), forestomach (squamous cell papillomas and carcinomas), and liver (hepatocellular adenomas, one cholangioma, one hemangiosarcoma, and one hemangioma) (Table 16). Significant increases by pairwise comparisons with the control were observed for nasal carcinomas (low- and high-dose groups) and nasal adenomas (low-dose group), lung alveolar/bronchial adenomas (high-dose group), forestomach squamous cell papillomas (low-dose group), and hepatocellular adenomas (low-dose group). A statistically significant dose-response was noted for nasal mucosa carcinomas, lung alveolar/bronchial adenomas, and forestomach squamous cell papillomas and carcinomas.

Table 16. Tumor incidence in female Syrian golden hamsters administered NMA-C4 by gavage once/week for 23 - 30 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor site and type		Vehicle Controls[1]	2.5 mg/week Total dose: 0.65 mmol[2] (75.4 mg)	5.0 mg/week Total dose: 1.0 mmol[2] (116 mg)
Nasal mucosa	Adenoma (r)	0/12	4/12*	1/12
	Carcinoma (r)	0/12***	7/12**	9/12***
	Undifferentiated sarcoma	0/12	1/12	0/12
Lung	Alveolar/bronchial adenoma (r)	0/12*	2/12	4/12*
Forestomach	Squamous cell papilloma	0/12*	5/12*	3/12
	Squamous cell carcinoma	0/12*	0/12	3/12
Liver	Hepatocellular adenoma (r)	0/12	4/12*	1/12

[1] Control incidences with asterisks indicate significant results from exact trend test (performed by OEHHA)

[2] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C5 treatment of male hamsters resulted in the observation of tumors in the nasal mucosa (carcinomas), lung (alveolar/bronchial adenomas, squamous cell carcinomas), forestomach (squamous cell carcinomas and papillomas), and liver (hepatocellular adenomas, cholangiocarcinomas, one cholangioma, and two hemangiosarcomas) (Table 17). Tumor incidences for forestomach squamous cell carcinomas were statistically significantly increased by pairwise comparison with controls.

Table 17. Tumor incidence in male Syrian golden hamsters administered NMA-C5 by gavage once/week for 23 - 30 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor site and type		Vehicle Controls	5.6 mg/week Total dose: 1.1 mmol[1] (143 mg)
Nasal mucosa	Carcinoma (r)	0/12	3/12
Lung	Alveolar/bronchial adenoma (r)	0/12	3/12
	Squamous cell carcinoma (r)	0/12	1/12
Forestomach	Squamous cell papilloma	0/12	2/12
	Squamous cell carcinoma (r)	0/12	7/12**
Liver	Hepatocellular adenoma (r)	0/12	3/12
	Cholangiocarcinoma (r)	0/12	1/12

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

In the study of female hamsters exposed to NMA-C5, tumors were observed at multiple sites, including the nasal mucosa (adenoma and carcinoma), lung (alveolar/bronchial adenoma and carcinoma), forestomach (squamous cell papilloma and carcinoma), and liver (one hepatocellular adenoma, two cholangiomas, one hemangioma, and one hemangiosarcoma) (Table 18). Statistically significant incidence increases by pairwise comparison with controls were noted for nasal mucosa carcinomas, lung alveolar/bronchial adenomas, and forestomach squamous cell carcinomas and papillomas.

Table 18. Tumor incidence in female Syrian golden hamsters administered NMA-C5 by gavage once/week for 23 - 30 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor site and type		Vehicle Controls	5.6 mg/week Total dose: 1.1 mmol[1] (143 mg)
Nasal mucosa	Adenoma (r)	0/12	3/12
	Carcinoma (r)	0/12	8/12***
Lung	Alveolar/bronchial adenoma (r)	0/12	8/12***
	Adenosquamous carcinoma (r)	0/12	1/12
Forestomach	Squamous cell papilloma	0/12	6/12**
	Squamous cell carcinoma	0/12	6/12**
Liver	Hepatocellular adenoma (r)	0/12	1/12

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C6 treatment of male hamsters resulted in the observation of tumors at multiple sites, including nasal mucosa (carcinoma and adenoma), lung (alveolar/bronchial adenomas and squamous cell carcinoma), forestomach (squamous cell carcinomas and papillomas), liver (hepatocellular adenomas, cholanigoma, one hemangioma, and hemangiosarcomas), and urinary bladder (transitional cell carcinoma and papillomas) (Table 19). Tumor incidences were significantly increased by pairwise comparison with controls for alveolar/bronchial adenomas and forestomach squamous cell carcinomas and papillomas.

Table 19. Tumor incidence in male Syrian golden hamsters administered NMA-C6 by gavage once/week for 50 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor Site and Type		Vehicle Controls	6.2 mg/week Total dose: 2.1 mmol[1] (302 mg)
Nasal mucosa	Adenoma (r)	0/12	3/12
	Carcinoma (r)	0/12	2/12
Lung	Alveolar/bronchial adenoma (r)	0/12	5/12*
	Squamous cell carcinoma (r)	0/12	1/12
Forestomach	Squamous cell papilloma	0/12	4/12*
	Squamous cell carcinoma (r)	0/12	7/12**
Liver	Hepatocellular adenoma (r)	0/12	2/12
	Cholangioma	1/12	4/12
	Hemangioma (r)	0/12	1/12
	Hemangiosarcoma (r)	0/12	2/12
Urinary bladder	Transitional cell papilloma (r)	0/12	3/12
	Transitional cell carcinoma (r)	0/12	1/12

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

In the study of female hamsters exposed to NMA-C6, tumors were observed at multiple sites, including lung (alveolar/bronchial carcinomas and adenomas, and one adenosquamous carcinoma), forestomach (squamous cell carcinomas and papillomas), liver (cholangiomas), and urinary bladder (transitional cell carcinomas and papillomas) (see Table 20). Tumor incidences were significantly increased by pairwise comparison with controls for alveolar/bronchial adenomas and carcinomas, forestomach squamous cell carcinomas, as well as liver cholangiomas.

Table 20. Tumor incidence in female Syrian golden hamsters administered NMA-C6 by gavage once/week for 50 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor Site and Type		Vehicle Controls	6.2 mg/week Total dose: 2.1 mmol[1] (302 mg)
Lung	Alveolar/bronchial adenoma (r)	0/12	6/20*
	Alveolar/bronchial carcinoma (r)	0/12	9/20**
	Adenosquamous carcinoma (r)	0/12	1/20
Forestomach	Squamous cell papilloma	0/12	2/20
	Squamous cell carcinoma	0/12	18/20***
Liver	Cholangioma	1/12	12/20**
Urinary bladder	Transitional cell papilloma (r)	0/12	2/20
	Transitional cell carcinoma (r)	0/12	3/20

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C7 treatment of male hamsters resulted in observations of tumors at multiple sites, including nasal mucosa (adenomas and carcinomas), lung (alveolar/bronchial adenomas and carcinomas, and adenosquamous carcinomas), forestomach (squamous cell papillomas and carcinomas), and liver (hepatocellular adenomas and carcinomas, hemangiosarcomas, cholangiomas, and one cholangiocarcinoma) (Table 21). Tumor incidences were significantly increased by pairwise comparison with controls for nasal adenomas, alveolar/bronchial adenomas and carcinomas, forestomach squamous cell carcinomas, and liver cholangiomas and hemangiosarcomas.

Table 21. Tumor incidence in male Syrian golden hamsters administered NMA-C7 by gavage once/week for 40 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor Site and Type		Vehicle Controls	6.8 mg/week Total dose: 2.1 mmol[1] (332 mg)
Nasal mucosa	Adenoma (r)	0/12	5/12*
	Carcinoma (r)	0/12	3/12
Lung	Alveolar/bronchial adenoma (r)	0/12	9/12***
	Alveolar/bronchial carcinoma (r)	0/12	4/12*
	Adenosquamous carcinoma (r)	0/12	2/12
Forestomach	Squamous cell papilloma	0/12	2/12
	Squamous cell carcinoma (r)	0/12	6/12**
Liver	Hepatocellular adenoma (r)	0/12	1/12
	Hepatocellular carcinoma (r)	0/12	1/12
	Hemangiosarcoma (r)	0/12	5/12*
	Cholangioma	1/12	10/12***
	Cholangiocarcinoma (r)	0/12	1/12

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

Tumors were observed at multiple sites in female hamsters treated with NMA-C7, including nasal mucosa (adenomas and carcinomas), lung (alveolar/bronchial adenomas and carcinomas, adenosquamous carcinomas), forestomach (squamous cell papillomas), and liver (one hepatocellular adenoma, cholangiomas) (Table 22). Tumor incidences were significantly increased by pairwise comparison with controls for alveolar/bronchial adenomas and carcinomas, forestomach squamous cell papillomas, and liver cholangiomas.

Table 22. Tumor incidence in female Syrian golden hamsters administered NMA-C7 by gavage once/week for 40 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor Site and Type		Vehicle Controls	6.8 mg/kg/week Total dose: 2.1 mmol[1] (332 mg)
Nasal mucosa	Adenoma (r)	0/12	2/12
	Carcinoma (r)	0/12	3/12
Lung	Alveolar/bronchial adenoma (r)	0/12	8/12***
	Alveolar/bronchial carcinoma (r)	0/12	5/12*
	Adenosquamous carcinoma (r)	0/12	2/12
Forestomach	Squamous cell papilloma	0/12	7/12**
Liver	Hepatocellular adenoma (r)	0/12	1/12
	Cholangioma	1/12	9/12**

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

NMA-C8 treatment of male hamsters resulted in observations of tumors at multiple sites, including nasal mucosa (adenomas), lung (alveolar/bronchial adenomas and one carcinoma), forestomach (squamous cell papillomas and carcinomas), liver (hepatocellular adenomas and one carcinoma, cholangiomas, and hemangiosarcomas), and urinary bladder (transitional cell papillomas, hemangiomas, one hemangiosarcoma, and one undifferentiated sarcoma) (Table 23). Tumor incidences were significantly increased by pairwise comparison with controls for alveolar/bronchial adenomas, forestomach squamous cell papillomas and carcinomas, and hepatocellular adenomas.

Table 23. Tumor incidence in male Syrian golden hamsters administered NMA-C8 by gavage once/week for 40 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor Site and Type		Vehicle Controls	7.4 mg/week Total dose: 2.1 mmol[1] (361 mg)
Nasal mucosa	Adenoma (r)	0/12	2/12
Lung	Alveolar/bronchial adenoma (r)	0/12	6/12**
	Alveolar/bronchial carcinoma (r)	0/12	1/12
Forestomach	Squamous cell papilloma	0/12	5/12*
	Squamous cell carcinoma (r)	0/12	6/12**
Liver	Hepatocellular adenoma (r)	0/12	5/12*
	Hepatocellular carcinoma (r)	0/12	1/12
	Cholangioma	1/12	4/12
	Hemangiosarcoma (r)	0/12	3/12
Urinary bladder	Transitional cell papilloma (r)	0/12	2/12
	Hemangioma (r)	0/12	2/12
	Hemangiosarcoma (r)	0/12	1/12
	Undifferentiated sarcoma (r)	0/12	1/12

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

In the study of female hamsters exposed to NMA-C8, tumors were observed at multiple sites, including nasal mucosa (adenomas), lung (alveolar/bronchial adenomas and one carcinoma), forestomach (squamous cell papillomas), liver (hepatocellular adenomas and one carcinoma, one cholangioma, and one histiocytic sarcoma), and urinary bladder (undifferentiated sarcomas) (Table 24). Tumor incidences were significantly increased by pairwise comparison with controls for alveolar/bronchial adenomas, forestomach squamous cell papillomas, and liver adenomas.

Table 24. Tumor incidence in female Syrian golden hamsters administered NMA-C8 by gavage once/week for 40 weeks and observed for life (Lijinsky and Kovatch, 1988)

Tumor Site and Type		Vehicle Controls	7.4 mg/ week Total dose: 2.1 mmol[1] (361 mg)
Nasal mucosa	Adenoma (r)	0/12	3/12
Lung	Alveolar/bronchial adenoma (r)	0/12	7/12**
	Alveolar/bronchial carcinoma (r)	0/12	1/12
Forestomach	Squamous cell papilloma	0/12	6/12**
Liver	Hepatocellular adenoma (r)	0/12	8/12***
	Hepatocellular carcinoma (r)	0/12	1/12
	Histiocytic sarcoma	0/12	1/12
Urinary bladder	Undifferentiated sarcoma (r)	0/12	3/12

[1] Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (r) denotes a rare tumor. See text for details.

3.2.2 Additional Studies on NMAs

NMA-C3

The carcinogenicity of NMA-C3 was studied in rats, hamsters and mice. These studies are presented briefly below, with the exception of the two oral gavage studies conducted in male and female Syrian golden hamsters by Lijinsky and Kovatch (1988), discussed in Section 3.2.1 above.

Table 25. Summary of animal carcinogenicity studies of NMA-C3

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Fischer 344	Female	Drinking water	14 mg/liter in 20 ml water 5 days per week for 23 weeks; total dose: 0.3 mmol. Animals were observed until death. All animals died by 40 weeks and mean survival time was not reported. No concurrent controls	*Nasal cavity:* Carcinoma (r)	 N/A, 1/20	Lijinsky *et al*., 1983a[3]
				Tongue: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 2/20 N/A, 3/20	
				Epiglottis: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 2/20 N/A, 3/20	
				Oropharynx: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 5/20 N/A, 7/20	
				Esophagus: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 18/20 N/A, 19/20	
				Forestomach: Papilloma (r)	 N/A, 7/20	

[1] Rare tumor type denoted by (r); combined incidence of tumors denoted by "or" as in: "malignant or benign"

[2] Incidence listed first for control, then for treated groups in order of increasing dose; treatment group incidences with asterisks indicate significant results from Fisher pairwise comparison with controls; control incidences with asterisks indicate significant results from exact trend test (performed by OEHHA): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; N/A = not available

[3] While Lijinsky *et al*. (1983a) did not have concurrent controls, the tumors types tabulated here were not seen in a continuous series of untreated control animal groups from the same animal colony maintained at the same facility.

Table 25 (cont'd). NMA-C3 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/Tumor type[1]	Incidence[2]	Refer-ence
Rat, Sprague Dawley	Male	*Subcu-taneous (s.c.)*	All groups in 5 ml 0.9% NaCl once weekly for life. Animals killed when moribund Low dose: 5.3 mg/kg Total dose: 222 mg/kg. Average survival: 41.9 weeks. Mid-dose: 10.6 mg/kg Total dose: 373 mg/kg. Average survival: 35.2 weeks. High dose: 21.2 mg/kg Total dose: 568 mg/kg. Average survival: 26.8 weeks. Vehicle control: Average survival: 77.2 weeks.	***Nasal cavity/sinuses:*** *Maxilloturbinals, nasal turbinals, maxillary sinuses:* Squamous cell carcinoma (r) Squamous cell papilloma or carcinoma (r)	0/10, 0/10, 2/10, 1/9 1/10**, 4/10, 6/10*, 6/9*	Reznik *et al*., 1975
				Esophagus: Squamous cell carcinoma (r) Squamous cell papilloma or carcinoma (r)	0/10, 1/10, 2/10, 1/9 0/10***, 9/10***, 10/10***, 8/9***	
				Liver: Malignant hemangioendothelioma (r)	0/10***, 3/10, 9/10***, 6/9**	
	Female		All groups in 5 ml 0.9% NaCl once weekly for life. Animals killed when moribund Low dose: 5.3 mg/kg Total dose: 260 mg/kg. Average survival: 49.0 weeks. Mid-dose:10.6 mg/kg Total dose: 387 mg/kg. Average survival: 36.1 weeks. High dose: 21.2 mg/kg Total dose: 585 mg/kg. Average survival: 27.6 weeks. Vehicle control: Average survival: 86 weeks.	***Nasal cavity/sinuses:*** *Maxilloturbinals, nasal turbinals, maxillary sinuses:* Squamous cell carcinoma (r) Squamous cell papilloma or carcinoma (r)	0/10, 1/9, 0/8, 0/10 1/10, 6/9*, 5/8*, 3/10	
				Endoturbinals: Malignant tumor (r)	0/10, 1/9, 1/8, 0/10	
				Esophagus: Squamous cell carcinoma (r) Squamous cell papilloma or carcinoma (r)	0/10, 3/9, 0/8, 1/10 0/10***, 7/9***, 6/8**, 7/10**	
				Liver: Malignant hemangioendothelioma (r)	0/10**, 3/9, 6/8**, 5/10*	

Table 25 (cont'd). NMA-C3 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Hamster, Syrian golden	Pregnant females	*s.c.*	100 mg/kg in 0.9% NaCl (injection volume per animal was not reported) by single injection on GD 8, 10, 12, 14, or 15. Animals were observed for life. Average survival 58 weeks. Control: average survival 69 weeks.	*Nasal cavity:* Adenocarcinoma (r)	0/21, 2/19	Althoff & Grandjean, 1979[4]
				Trachea: Adenocarcinoma	0/21, 1/19	
				Lung: Bronchiogenic adenoma or adenocarcinoma (r)	1/21, 3/19	
	F_1 Male	*In utero*	F_1 animals from one-time maternal dosing (see above). Animals were observed for life with average survival of 68 weeks. Control: average survival 72 weeks.	*Nasal cavity:* Adenocarcinoma (r)	0/100, 2/88	
				Trachea: Adenocarcinoma	0/100, 1/88	
				Lung: Bronchiogenic adenoma or adenocarcinoma (r)	0/100, 3/88	
				Endocrine: Thyroid tumors	12/100, 21/88*	
	F_1 Female		F_1 animals from one-time maternal dosing (see above). Animals were observed for life with average survival of 60 weeks. Control: average survival 66 weeks.	*Digestive:* Combined	3/113, 14/89***	

[4] Althoff and Grandjean (1979) reported incidences as a percentage; OEHHA converted these to number of tumor bearing animals/total number of animals

Table 25 (cont'd). NMA-C3 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/Tumor type[1]	Incidence[2]	Refer-ence
Hamster, Syrian golden	Male and Female	*s.c.*	In olive oil (volume not reported) once weekly for life Low dose: 12.5 mg/kg Total dose: 425 mg/kg. Average survival: 34 weeks. Mid-dose: 25 mg/kg Total dose: 750 mg/kg. Average survival: 30 weeks. High dose: 50 mg/kg Total dose: 1200 mg/kg. Average survival: 24 weeks. Vehicle Control: Average survival: 49 weeks.	*Nasal cavity:* Carcinoma (r) Benign (epidermoid, muco-epidermoid and polypous) and carcinoma combined (r)	0/27***, 17/19***, 15/17***,18/19*** 0/27***, 18/19***, 15/17***, 18/19***	Pour *et al*.,1974; 1975
				Laryngo-tracheo-bronchial tract: Papillomas	0/27***, 15/19***, 14/17***,17/19***	
				Lung: Adenoma or carcinoma (r)	0/27, N/A, N/A, 10/19***	
				Liver: Hemangioendothelioma, cholangioma, or hepatocellular carcinoma (r)	0/27***, 1/19, 3/17, 17/19***	
Mouse, NMRI	Female	*s.c.*	In 0.9% NaCl (volume not reported) once weekly for life. Low dose: 3.9 mg/kg; Total dose: 187 mg/kg. Average survival 48±9 weeks (±SD). Mid-dose: 7.7 mg/kg Total dose: 331 mg/kg. Average survival: 43±9 weeks. High dose: 15.4 mg/kg Total dose: 508 mg/kg. Average survival 33±5 weeks. Vehicle control: average survival 78±14 weeks.	*Nasolacrimal duct:* Malignant Benign or malignant	0/14, 2/15, 0/13, 1/14 0/14*, 9/15***, 6/13**, 5/14*	Dick-haus *et al*., 1977
				Naso- and Maxilloturbinal: Malignant Benign or malignant	0/14*, 3/15, 2/13, 4/14* 0/14***, 8/15**, 10/13***, 9/14***	
				Endo- and ectoturbinal: Malignant Benign or malignant	0/14, 3/15, 3/13, 3/14 0/14*, 3/15, 3/13, 4/14*	
				Larynx-trachea-bronchial tract: Malignant Benign or malignant	0/14*, 2/15, 1/13, 4/14* 0/14***, 5/15*, 4/13*, 8/14***	
				Lung: Malignant Benign or malignant	3/14, 2/15, 2/13, 1/14 3/14, 15/15***, 11/13**, 5/14	
				Liver: Malignant Benign or malignant	0/14**, 0/15, 2/13, 5/14* 0/14***, 0/15, 2/13, 6/14**	

NMA-C4

The carcinogenicity of NMA-C4 was studied in rats and hamsters. These studies are presented briefly below, with the exception of the two oral gavage studies conducted in male and female Syrian golden hamsters by Lijinsky and Kovatch (1988), discussed in Section 3.2.1 above (see Table 15 and Table 16).

Table 26. Summary of animal carcinogenicity studies of NMA-C4

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Fischer 344	Male	Drinking water	Water consumption rate: 20 ml/day/rat. Low Dose: 6.25 mg/liter 5 days/week for 23 weeks; total dose: 0.12 mmol. All animals died by 45 weeks. High Dose: 16 mg/liter for 20 weeks; total dose: 0.28 mmol. All animals died by 35 weeks. No concurrent controls[3]	*Tongue:* Carcinoma (r) Papilloma or carcinoma (r)	 N/A, N/R, 3/20 N/A, 5/20, 8/20	Lijinsky *et al*., 1980; 1983b[3]
				Oropharynx: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, N/R, 2/20 N/A, 3/20, 3/20	
				Esophagus: Basal cell (BC) carcinoma (r) BC papilloma or carcinoma (r)	 N/A, N/R, 18/20 N/A, 20/20, 18/20	
				Forestomach: Carcinoma (r) Papilloma and carcinoma (r)	 N/A, N/R, 3/20 N/A, 5/20, 7/20	
	Female		Water consumption rate: 20 ml/day/rat. 16 mg/liter 5 days/week for 20 weeks; total dose: 0.3 mmol. All animals died by 40 weeks. No concurrent controls[3]	*Tongue:* Carcinoma (r)	 N/A, 2/20	Lijinsky *et al*., 1980; 1983a[3]
				Oropharynx: Carcinoma (r) Papilloma and carcinoma (r)	 N/A, 5/20 N/A, 8/20	
				Esophagus: Carcinoma (r) Papilloma or carcinoma(r)	 N/A, 13/20 N/A, 15/20	
				Forestomach: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 4/20 N/A, 7/20	

[1] Rare tumor type denoted by (r); combined incidence of tumors denoted by "or" as in: "papilloma or carcinoma"; [2] Incidence listed first for control, then for treated groups in order of increasing dose; treatment group incidences with asterisks indicate significant results from Fisher pairwise comparison with controls; control incidences with asterisks indicate significant results from exact trend test (performed by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; N/A: not available; N/R: not reported in the publication; [3] While Lijinsky *et al*. did not include concurrent controls, the tumor types tabulated here were not seen in a continuous series of untreated control animal groups from the same animal colony maintained at the same facility

Table 26 (cont'd). NMA-C4 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Fischer 344	Male	Drinking water	15 mg/liter in water (volume not reported) daily: for 17 weeks, then sacrificed; total dose: 0.4 mmol[4]. for 21 weeks, then sacrificed; total dose: 0.5 mmol[4]. No untreated controls.	*Esophagus:* Papilloma (r) Carcinoma (r)	 N/A, 27/27, 20/20 N/A, 24/27, 19/20	Koreeda *et al*., 1999
Rat, Fischer 344	Male	Gavage	In 0.2 ml corn oil twice per week for 20 weeks, 12 mg/ml; total dose 0.82 mmol. All animals died by 35 weeks. No concurrent controls[3].	*Nasal cavity:* Carcinoma (r)	N/A, 8/20	Lijinsky *et al*., 1983b[3]
				Tongue: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 1/20 N/A, 5/20	
				Oropharynx: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 1/20 N/A, 4/20	
				Esophagus: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 15/20 N/A, 16/20	
				Forestomach: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 3/20 N/A, 8/20	
	Female		In 0.2 ml corn oil twice per week for 20 weeks, 12 mg/ml; total dose 0.82 mmol. Animals were observed until death; all animals died by 30 weeks. No concurrent controls[3].	*Nasal cavity:* Carcinoma (r)	N/A, 3/20	
				Oropharynx: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 1/20 N/A, 2/20	
				Esophagus: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 7/20 N/A, 12/20	
				Forestomach: Carcinoma (r) Papilloma or carcinoma (r)	 N/A, 0/20 N/A, 2/20	

[3] While Lijinsky *et al*. did not include concurrent controls, the tumor types tabulated here were not seen in a continuous series of untreated control animal groups from the same animal colony maintained at the same facility

[4] Total NMA-C4 dose estimated by OEHHA assuming the daily water intake volume of 25 ml for male rats used for the Carcinogenic Potency Database (CPDB) (http://toxnet.nlm.nih.gov/cpdb/) (Gold *et al*., 1997)

Table 26 (cont'd). NMA-C4 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Fischer 344	Female	Gavage	In 0.2 ml 25% ethanol twice per week for 17 weeks: 3 mg; total dose 0.8 mmol. Animals were then observed until death. All treated animals died by 20 weeks. Vehicle control: mean survival 96 weeks.	*Esophagus:* Tumors (not otherwise specified) (r)	0/12, 7/12**	Lijinsky *et al*., 1991a[5]
		Intrave-sicular injection into bladder	In 0.2 ml 25% ethanol in deionized water twice per week for 17 weeks: 3 mg; total dose 0.9 mmol. Animals were observed until death. All treated animals died by 22 weeks. Vehicle control: mean survival 102 weeks.	*Esophagus:* Tumors (not otherwise specified) (r)	0/12, 12/12***	
				Liver: Tumors (not otherwise specified)	0/12, 3/12	

[5] Lijinsky *et al*. presented the incidence as a percentage; OEHHA converted these to number of tumor bearing animals/total number of animals

NMA-C5

The carcinogenicity of NMA-C5 was studied in rats, hamsters and mice. These studies are presented briefly below, with the exception of the two oral gavage studies conducted in male and female Syrian golden hamsters by Lijinsky and Kovatch (1988), discussed in Section 3.2.1 above (see Table 17 and Table 18).

Table 27. Summary of animal carcinogenicity studies of NMA-C5

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, BD	Not specified	Drinking water	Low dose: 1.5 mg/kg daily until death. High dose: 3 mg/kg daily until death. No controls. Study duration unknown.	*Esophagus*: Papilloma (r) Carcinoma (r)	 N/A, 3/12, N/A N/A, 12/15, 6/6	Druckery *et al*., 1967
Rat, Wistar	Male	Drinking water	0.003% in tap water daily Low dose: for 2 weeks; total dose: 22 mg. Mid-dose: for 4 weeks; total dose: 36.6 mg. High dose: for 12 weeks; total dose: 138.8 mg. All animals, including untreated controls were observed for 28 weeks.	*Esophagus:* Papilloma (r) Carcinoma (r)	 0/16***, 1/16, 10/16***, 15/16*** 0/16***, 0/16, 2/16, 15/16***	Matsufuji *et al*., 1987[3]
Rat, Wistar	Male	Drinking water	0.003% daily for 8 weeks and observed for up to week 25. Starting at week 1, three animals were killed per week and the tongue, pharynx, esophagus and stomach were examined. No controls.	*Esophagus:* Squamous cell papilloma (r) Squamous carcinoma (r)	 N/A, 47/75 N/A, 18/75	Sasajima *et al*., 1982

[1] Rare tumor type denoted by (r); combined incidence of tumors denoted by "or" as in: "papilloma or carcinoma"

[2] Incidence listed first for control, then for treated groups in order of increasing dose; treatment group incidences with asterisks indicate significant results from Fisher pairwise comparison with controls; control incidences with asterisks indicate significant results from exact trend test (performed by OEHHA): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; N/A: not available; N/R: not reported in the publication

[3] Matsufuji *et al*. (1987) presented the incidence as a percentage; OEHHA converted these to number of tumor bearing animals/total number of animals

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, Donryu	Male (4- to 6-week-old)	Drinking water	Dose 1 (study group 6): 0.003% for 14 days. Dose 2 (study group 7): 0.003% for 28 days. Dose 3 (study group 8): 0.003% for 56 days. Dose 4 (study group 9): 0.003% for 84 days. Five animals per dose group were killed at the end of dosing period, and remaining five animals per dose group were killed at 180 days. No controls.	*Esophagus:* Papilloma (r) Carcinoma (r)	N/A, 0/10, 1/8, 7/8, 5/10 N/A, 0/10, 0/8, 1/8, 5/10	Iizuka *et al*., 1980
			Dose 1 (study group 4): 0.0015% for 90 days; total dose: 48 mg. Dose 2 (study group 3): 0.003% for 60 days; total dose: 60 mg. Dose 3 (study group 1): 0.003% for 90 days; total dose: 97 mg. Surviving rats were killed at 322 days. No controls.	*Esophagus:* Papilloma (r) Carcinoma (r)	N/A, 3/14, 2/10, 21/49 N/A, 11/14, 8/10, 28/49	
	Male (8- to 9-week-old)		(Study group 2): 0.003% for 90 days; total dose: 124 mg. Surviving rats were killed at 322 days. No controls.	*Esophagus:* Papilloma (r) Carcinoma (r)	N/A, 0/12 N/A, 12/12	
	Female (4- to 5-week-old)		(Study group 5): 0.003% for 90 days; total dose: 72 mg. Surviving rats were killed at 322 days. No controls.	*Esophagus:* Papilloma (r) Carcinoma (r)	N/A, 5/11 N/A, 5/11	

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, Sprague Dawley	Male	Drinking water	***Experiment duration: 12 weeks*** 0.0015% for 12 weeks; animals were terminated at the end of dosing; total dose: 0.03 mmol[4]. Untreated controls received tap water for 12 weeks and were terminated at the same time as the treated group.	*Esophagus:* Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/16, 8/10*** 0/16, 1/10 0/16, 8/10***	Kuwayama & Eastwood, 1988
			Experiment duration: 16 weeks 0.0015% for 12 weeks; animals were terminated at 4 weeks after dosing; total dose: 0.03 mmol[4]. Untreated controls received tap water for 16 weeks and were terminated at the same time as the treated group.	*Esophagus:* Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/16, 8/10*** 0/16, 4/10* 0/16, 8/10***	
			Experiment duration: 20 weeks 0.0015% for 12 weeks; animals were terminated at 8 weeks after dosing; total dose: 0.03 mmol[4]. Untreated controls received tap water for 20 weeks and were terminated at the same time as the treated group.	*Esophagus:* Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/16, 9/10*** 0/16, 1/10 0/16, 9/10***	
Rat, Sprague Dawley	Male	Drinking water	0.003% for 10 weeks in groups of castrated and intact animals; total dose: 0.045 mmol[4]. Animals were killed at 20 weeks. No controls for either treatment group.	*Esophagus:* Papilloma or carcinoma (r)	N/A, 6/6 (intact) N/A, 3/6 (castrated)	Kondoh *et al*., 1990[5]

[4] Total NMA-C5 dose estimated by OEHHA assuming the daily water intake volume of 25 ml for male rats used for the Carcinogenic Potency Database (CPDB) (http://toxnet.nlm.nih.gov/cpdb/) (Gold *et al*., 1997)

[5] Reported as a meeting abstract

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, MRC-Wistar	Male and female (newborn)	Intraper-itoneal *(i.p.)*	6 mg/kg in 0.9% NaCl (injection volume was not reported) by single injection. Mean survival 82±13 weeks. Control: mean survival 72±11 weeks.	*Forestomach*: Papilloma (r)	0/25, 2/20	Mirvish *et al*., 1996
	Male and female (3-day-old)		12.5 mg/kg in 0.9% NaCl (injection volume was not reported) by single injection. Mean survival was 74±14 weeks. Control: mean survival 72±11 weeks.	*Esophagus:* Papilloma (r) Carcinoma (r)	1/25, 1/21 0/25, 1/21	
				Forestomach: Papilloma (r) Carcinoma (r)	0/25, 1/21 0/25, 1/21	
	Male and female (6- to 8-week-old)		50-70 mg/kg in 0.9% NaCl (injection volume was not reported) by single injection[6]. Mean survival was 63±27 weeks. Control: mean survival 72±11 weeks.	*Esophagus:* Papilloma (r) Carcinoma (r)	1/25, 2/19 0/25, 14/19***	
Rat, MRC-Wistar	Male	*i.p.*	25 mg/kg in 5 ml water per rat once weekly for 3 weeks; total dose: 75 mg/kg. Animals were observed until death. Mean survival for animals exhibiting esophagus tumors was 51-60 weeks, for nasal cavity or tongue tumors was 50-70 weeks, and for thyroid tumors was 54-59 weeks. Control: mean survival 45 weeks.	*Nasal cavity:* Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/9, 9/39 0/9, 15/39* 0/9, 20/39**	Mirvish *et al*., 1994a
				Tongue: Papilloma (r)	0/9, 3/39	
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/9, 27/39*** 0/9, 7/39 0/9, 28/39***	
				Thyroid: Adenoma	3/9, 17/39	

[6] Mirvish *et al*. (1996) injected doses of NM-C5 to 42- to 50-day-old MRC-Wistar rats (10 males and 9 females) as follows: 50 mg/kg of to 7 females; 60 mg/kg to 2 females; and 70 mg/kg to 10 males

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, MRC-Wistar	Male	*i.p.*	*Animals were ip injected in 10 ml water/kg bodyweight, and killed when moribund. Controls received 12 weekly water injections (10 ml/kg-bw), had a mean survival of 84 wks.* ***Single Injection:*** Low dose: 50 mg/kg . Mean survival: 75 wks. High dose: 85 mg/kg . Mean survival: 43 wks	*Nasal cavity:* Papilloma (r) Carcinoma (r)	 0/20, 1/17, 0/4 0/20, 2/17, 0/4	Bulay & Mirvish, 1979
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	 0/20, 1/17, 1/4 0/20**, 1/17, 2/4* 0/20**, 2/17, 2/4*	
			Two injections: Treated group: 50 mg/kg once per week for 2 weeks; Total dose: 100 mg/kg. Mean survival was 67 weeks.	*Nasal cavity:* Papilloma (r) Carcinoma (r)	 0/20, 1/18 0/20, 2/18	
				Esophagus: Papilloma (r) Papilloma or carcinoma (r)	 0/20, 3/18 0/20, 3/18	
			Six injections: Treated group: 25 mg/kg once per week total dose: 150 mg/kg. Mean survival was 42 weeks.	*Nasal cavity:* Papilloma (r) Carcinoma (r)	 0/20, 5/20* 0/20, 3/20	
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	 0/20, 20/20*** 0/20, 11/20*** 0/20, 20/20***	
			Twelve injections: Low dose: 12.5 mg/kg once per week total dose: 150 mg/kg. Mean survival was 28 weeks. High dose: 25 mg/kg once per week total dose: 300 mg/kg. Mean survival was 25 weeks.	*Nasal cavity:* Papilloma (r) Carcinoma (r)	 0/20*, 4/20, 5/20* 0/20, 3/20, 2/20	
				Trachea: Papilloma (r) Carcinoma (r)	 0/20**, 1/20, 5/20* 0/20, 2/20, 1/20	
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	 0/20***, 20/20***, 17/20*** 0/20***, 13 /20***, 13/20*** 0/20***, 20/20***, 18/20***	

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, MRC-Wistar	Female	*i.p.*	*Animals were ip injected in 10 ml water/kg bodyweight, and killed when moribund. Controls received 12 weekly water injections (10 ml/kg-bw), had a mean survival of 98 wks.* ***Single injection:*** Low dose: 50 mg/kg, Mean survival: 77 wks. High dose: 85 mg/kg , Mean survival 32 wks.	*Nasal cavity:* Carcinomas (r)	0/22, 2/18, 0/3	Bulay & Mirvish, 1979
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/22***, 1/18, 3/3*** 0/22***, 2/18, 3/3*** 0/22***, 3/18, 3/3***
			Two injections: 50 mg/kg in 10 ml water per kg bw once per week for 2 weeks; total dose: 100 mg/kg. Mean survival was 68 weeks.	*Nasal cavity:* Carcinomas (r)	0/22, 3/20
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/22, 2/20 0/22, 1/20 0/22, 2/20
			Six injections: 25 mg/kg once per week total dose: 150 mg/kg. Mean survival was 50 weeks.	*Nasal cavity:* Papilloma (r) Carcinoma (r)	0/22, 5/20* 0/22, 5/20*
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/22, 19/20*** 0/22, 8/20** 0/22, 19/20***
			Twelve injections: Low dose: 12.5 mg/kg once per week total dose: 150 mg/kg. Mean survival was 31 weeks. High dose: 25 mg/kg bw once per week total dose: 300 mg/kg. Mean survival was 30 weeks.	*Nasal cavity:* Papilloma (r) Carcinoma (r)	0/22, 7/20**, 1/19 0/22, 2/20, 3/19
				Trachea: Papilloma (r) Carcinoma (r)	0/22*, 0/20, 3/19 0/22, 1/20, 1/19
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	0/22***, 19/20***, 19/19*** 0/22***, 13/20***, 11/19*** 0/22***, 19/20***, 19/19***

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, MRC-Wistar	Male	*i.p.*	25 mg/kg in 5 ml water per kg bw once per week for 3 weeks; total dose 75 mg/kg. Untreated controls. 5 rats were killed 28-30 weeks after the start of treatment, 12 rats were killed after 52 weeks, some died at intermediate times, and the remaining animals were killed at 70 weeks.	*Nasal cavity:* Papilloma (r) Carcinoma (r)	 0/30, 6/58 0/30, 12/58**	Mirvish *et al*., 1985a[7]
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	 0/30, 40/58*** 0/30, 14/58** 0/30, 45/58***	
				Forestomach: Papilloma or carcinoma (r)	 0/30, 2/58	
Rat, Wistar	Male	*i.p.*	12.5 mg/kg in dimethyl sulfoxide (12.5 mg/kg bw/injection) weekly for 12 weeks; total dose: 150 mg/kg. Untreated controls. Study terminated at 20 weeks.	*Tongue:* Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	 0/8, 4/12 0/8, 2/12 0/8, 6/12*	Tanaka *et al*., 1997[7]
				Esophagus: Papilloma (r) Carcinoma (r) Papilloma or carcinoma (r)	 0/8, 12/12*** 0/8, 9/12** 0/8, 12/12***	
Rat, Fischer 344	Male	*i.p.*	25 mg/kg once per week for 3 weeks, injection volume and solvent were not reported; total dose: 75 mg/kg. All surviving animals were sacrificed at 52 weeks. Untreated controls.	*Nasal cavity:* Papilloma (r)	 N/R[8], 3/11	Yamaguchi *et al*., 1989
				Tongue: Papilloma (r)	 N/R[8], 1/11	
				Esophagus: Papilloma (r)	 N/R[8], 2/11	

[7] Mirvish *et al*. (1985a) and Tanaka *et al*. (1997) presented the incidences as a percentage; OEHHA converted these to number of tumor bearing animals/total number of animals

[8] N/R = not reported; the authors did not report on the presence or absence of any tumors in the control group, noting only that the incidence of forestomach hyperplasia in untreated controls was 0%

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, Sprague Dawley	Male	*i.p.*	25 mg/kg in saline (injection volume: 0.5 to 1 ml) once per week for 3 weeks; total dose: 75 mg/kg. Animals were killed at 30 weeks. Untreated controls.	*Esophagus:* Benign diffuse papillomastosis (r) Squamous carcinoma (r)	0/16, 2/10 0/16, 3/10*	Attwood *et al*., 1992
Rat, Wistar	Male and female	*intra-muscu-lar*	5 mg/kg in rape seed oil weekly for 29 weeks, injection volume not available; total dose: 145 mg/kg. Vehicle controls. All animals were sacrificed at 86 weeks.	*Esophagus:* Papilloma and carcinoma combined (r)	0/34, 17/36***	Luo *et al*., 1987
				Forestomach: Papilloma and carcinoma combined (r)	0/34, 3/36	
Rat, Wistar	Male	*Subcu-taneous (s.c.)*	12.5 mg/kg in water (injection volume was not available) weekly for 8 weeks; total dose: 100 mg/kg. Animals were observed for 6 weeks. No controls.	*Esophagus:* Papilloma (r)	N/A, 11/18	Seto *et al*., 1991
Rat, BD	Not specified	*s.c.*	10 or 20 mg/kg once weekly until death. Solvent and injection volume not reported; study duration unknown. No controls.	*Esophagus:* Papilloma (r) Carcinoma (r)	N/A, 3/20 N/A, 17/20	Druckery *et al*., 1967[9]

[9] 20 rats were treated with either 10 or 20 mg/kg once per week. No treatment duration was reported; authors report combined results of both treatment groups (Druckery *et al.*, 1967)

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Hamster, Syrian golden	Male and female (Newborn)	*i.p.*	3 mg/kg (in 5 mg/ml 0.9% NaCl) by single injection. Animals were maintained until death. Mean survival was 57±19 weeks. Control: mean survival 57±15 weeks.	*Esophagus:* Papilloma (infrequent)	0/26, 1/28	Mirvish *et al*., 1996
				Forestomach: Papilloma	0/26, 2/28	
	Male and female (3-day-old)		6 mg/kg (in 5 mg/ml 0.9% NaCl) by single injection. Animals were maintained until death. Mean survival was 75±15 weeks. Control: mean survival 57±15 weeks.	*Esophagus:* Papilloma (infrequent) Carcinoma (infrequent)	0/26, 3/26 0/26, 2/26	
				Forestomach: Papilloma	0/26, 2/26	
	Male and female (38-day-old)		75 mg/kg (in 5 mg/ml 0.9% NaCl) once per week for 6 weeks; total dose: 450 mg/kg. Animals were maintained until death. Mean survival was 33±11 weeks. Control: mean survival 43±8 weeks.	*Nasal cavity:* Benign or malignant (r)	0/20, 10/23***	
				Lung: Adenoma (r)	0/20, 15/23***	
				Esophagus: Papilloma (infrequent) Carcinoma (infrequent)	2/20, 5/23 1/20, 0/23	
				Forestomach: Papilloma	3/20, 19/23***	
				Liver: Benign or malignant (r)	0/20, 4/23	
	Male and female (46- to 49-day-old)		70 mg/kg to 3 males and 100 mg/kg to 11 females (in 5 mg/ml 0.9% NaCl) by single injection. Animals were maintained until death. Mean survival was 43±15 weeks. Control: mean survival 57±15 weeks.	*Esophagus:* Papilloma (infrequent)	0/26, 2/14	
				Forestomach: Papilloma	2/26, 3/14	

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Mouse, 101/N	Male and Female	Drinking Water	0.0015% daily for 8 weeks; total dose: 0.003 mmol[4]. Animals were observed for 16 weeks after dosing. Untreated controls.	*Esophagus:* Benign or malignant (r)	0/146, 135/135***	Kurooka *et al*., 1998
Mouse, STX/Le					0/67, 75/76***	
Mouse, BXH-8					0/99, 101/101***	
Mouse, C57BL/6J					0/103, 64/64***	
Mouse, *Brca1* $^{\Delta 11/\Delta 11}$ *p53* $^{+/-}$	Female	Drinking Water	10 ppm daily for 8 weeks; total dose: 0.002 mmol[4]. Animals were kept for 7 weeks after dosing and killed between weeks 15 and 25. No controls.	*Esophagus and forestomach:* Hyperplasia Carcinoma (r)	N/A, 7/7 N/A, 4/7	Cao *et al*., 2007
Mouse, *Brca1* $^{+/\Delta 11}$ *p53* $^{+/-}$				*Esophagus and forestomach:* Hyperplasia Carcinoma (r)	N/A, 5/7 N/A, 0/7	
Mouse, C57BL/6 p53 $^{+/+}$ wild type	Male	Drinking Water	***Experiment termination: 15 weeks*** Low dose: 5 ppm daily for 8 weeks Total dose: 0.001 mmol[4]. High dose: 15 ppm daily for 8 weeks Total dose: 0.003 mmol[4]. Untreated controls (p53 $^{+/+}$ or p53 $^{+/-}$ as appropriate).	*Esophagus:* Papilloma (r) Squamous cell carcinoma (r)	0/5***, 7/13, 10/11** 0/5, 0/13, 1/11	Shirai *et al*., 2002a; 2002b
Mouse, C57BL/6 p53 $^{+/-}$ heterozygote				*Esophagus:* Papilloma (r) Squamous cell carcinoma (r)	0/5**, 12/15**,13/14*** 0/5, 1/15, 2/14	
Mouse, C57BL/6 p53 $^{-/-}$ knockout			5 ppm daily for 8 weeks Total dose: 0.001 mmol[4]. Untreated p53 $^{-/-}$ controls	*Lingual:* Papilloma (r) Squamous cell carcinoma (r)	0/4, 2/12 0/4, 5/12	
				Esophagus: Papilloma (r) Squamous cell carcinoma (r)	0/4, 10/12** 0/4, 10/12**	

[4] Total NMA-C5 dose estimated by OEHHA assuming the daily water intake volume of 25 ml for male rats used for the Carcinogenic Potency Database (CPDB) (http://toxnet.nlm.nih.gov/cpdb/) (Gold *et al*., 1997)

Table 27 (cont'd). NMA-C5 animal carcinogenicity studies

Species, Strain	Sex	Route	Dose and Duration	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Mouse, C57BL/6 $p53^{+/+}$ wild type	Male	Drinking water	***Experiment termination: 25 weeks*** Low dose: 5 ppm daily for 8 weeks; total dose: 0.001 mmol[4], terminated at 25 weeks. High dose: 15 ppm daily for 8 weeks; total dose: 0.003 mmol[4], terminated at 25 weeks. Untreated controls ($p53^{+/+}$ or $p53^{+/-}$, as appropriate).	*Lingual:* Carcinoma *in situ*	0/5, 1/13, N/R[10]	Shirai *et al*., 2002a; 2002b
				Esophagus: Papilloma (r) Squamous cell carcinoma (r)	0/5***, 13/13***, 10/10*** 0/5, 1/13, 2/10	
Mouse, C57BL/6 $p53^{+/-}$ heterozygote				*Lingual:* Papilloma (r) Carcinoma *in situ*	0/6, 1/16, N/R[10] 0/6, 1/16, N/R[10]	
				Esophagus: Papilloma (r) Squamous cell carcinoma (r)	0/6**, 15/16***, 13/14*** 0/6*, 7/16, 8/14*	
Mouse, Swiss	Male and female	*i.p.*	15 mg/kg (in 5 mg/ml 0.9% NaCl) once per week for 3 weeks; total dose: 45 mg/kg. Animals were maintained until death. Mean survival was 57±12 weeks. Control: mean survival 79±17 weeks.	*Lung:* Adenoma (r)	3/20, 27/28***	Mirvish *et al*., 1996
				Esophagus: Papilloma (r)	0/20, 9/28**	

[4] Total NMA-C5 dose estimated by OEHHA assuming the daily water intake volume of 25 ml for male rats used for the Carcinogenic Potency Database (CPDB) (http://toxnet.nlm.nih.gov/cpdb/) (Gold *et al*., 1997)

[10] N/R = not reported

NMA-C6

The carcinogenicity of NMA-C6 was studied in rats and hamsters. Two studies in rats are presented briefly below. Two oral gavage studies conducted in male and female Syrian golden hamsters were discussed in Section 3.2.1 above.

Table 28. Summary of animal carcinogenicity studies of NMA-C6

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/ Tumor type[1]	Incidence[2]	Reference
Rat, Fischer 344	Male	Drinking water	1 mg in 20 ml drinking water/rat/day, 5 days/week for 21 weeks; total dose: 105 mg; 0 animals alive at 40 weeks. Animals allowed to die naturally unless moribund. No concurrent controls.	*Tongue*: Papilloma (r) Carcinoma (r)	 N/A, 1/20 N/A, 8/20	Lijinsky *et al.*, 1983b
				Esophagus: Papilloma (r) Carcinoma (r)	 N/A, 1/20 N/A, 19/20	
				Forestomach: Papilloma (r) Carcinoma (r)	 N/A, 2/20 N/A, 2/20	
		Gavage	In 0.2 ml corn oil daily: Low dose: 13 mg/kg for 32 weeks; total dose: 170 mg; 6 animals alive at 70 weeks. Mid-dose: 31 mg/kgfor 28 weeks; total dose: 360 mg; 0 animals alive at 35 weeks. High dose: 78 mg/kg for 16 weeks; total dose: 500 mg; 0 animals alive at 30 weeks. Animals allowed to die naturally unless moribund. No concurrent controls.	*Nasal cavity*: Carcinoma (r)	 N/A, 0/20, 1/20, 1/20	
				Tongue: Papilloma (r) Carcinoma (r)	 N/A, 0/20, 2/20, 0/20 N/A, 0/20, 1/20, 0/20	
				Lung (r)*:* Adenoma Carcinoma (r)[3]	 N/A, 2/20, 2/20, 10/20 N/A, 16/20, 7/20, 3/20	
				Esophagus: Papilloma (r) Carcinoma (r)	 N/A, 5/20, 5/20, 12/20 N/A, 5/20, 14/20, 7/20	
				Forestomach: Papilloma (r) Carcinoma (r)	 N/A, 0/20, 1/20, 2/20 N/A, 6/20, 1/20, 0/20	
				Liver: Sarcoma Carcinoma	 N/A, 8/20, 18/20, 1/20 N/A, 12/20, 6/20, 10/20	

[1] Rare tumor type denoted by (r)
[2] Control incidence listed first, subsequent incidences correspond to treated groups in order of increasing dose; N/A = not available
[3] Carcinomas, including adenocarcinoma

NMA-C7

The carcinogenicity of NMA-C7 was studied in rats and hamsters. Three studies in rats and one in hamsters are presented in the table below (Table 29). Two oral gavage studies conducted in male and female Syrian golden hamsters by Lijinsky and Kovatch (1988) are discussed in Section 3.2.1 above (see Table 21 and Table 22).

Table 29. Summary of animal carcinogenicity studies of NMA-C7

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Fischer 344	Male	Drinking water	2.8 mg in 20 ml drinking water/rat/day, 5 days/week for 34 weeks; total dose: 490 mg; 0 animals alive at 55 weeks. Animals allowed to die naturally unless moribund. No concurrent controls.	*Nasal cavity:* Carcinoma (r)	N/A, 2/20	Lijinsky *et al.*, 1983b
				Tongue: Papilloma (r)	N/A, 2/20	
				Lung: Adenoma Carcinoma (r)[3]	N/A, 2/20 N/A, 11/20	
				Esophagus: Adenoma (r) Carcinoma (r)	N/A, 4/20 N/A, 15/20	
				Liver: Sarcoma Carcinoma	N/A, 0/20 N/A, 6/20	
		Gavage	In 0.2 ml corn oil daily: Low dose: 35 mg/kg for 30 weeks; total dose: 420 mg; 0 animals alive at 65 weeks. High dose: 86 mg/kg for 25 weeks; total dose: 860 mg; 0 animals alive at 40 weeks. Animals allowed to die naturally unless moribund. No concurrent controls.	*Nasal cavity*: Carcinoma (r)	N/A, 1/20, 2/20	
				Lung: Adenoma Carcinoma (r)[3]	N/A, 20/20, 7/20 N/A, 2/20, 2/20	
				Liver: Sarcoma Carcinoma	N/A, 11/20, 12/20 N/A, 20/20, 10/20	

[1] Rare tumor type denoted by (r)
[2] Control incidence listed first, subsequent incidences correspond to treated groups in order of increasing dose; N/A = not available; N/R = not reported
[3] Carcinomas, including adenocarcinoma

Table 29 (cont'd). NMA-C7 animal carcinogenicity studies

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, BD	Not specified	Subcu-taneous	40 or 80 mg/kg once weekly until death. Results reported for animals receiving either treatment. Solvent and injection volume not reported; study duration unknown. No controls.	*Lung:* Squamous cell carcinoma (r)	N/A, 1/20	Druckery *et al*., 1967
Hamster, Syrian golden	Male	Gavage	6.8 mg/animal in 0.2 ml ethyl acetate:corn oil (1:2) once weekly for 35 weeks; total dose: 236 mg. Necropsies performed on animals euthanized at various time intervals and on those found moribund or dead. Vehicle controls.	*Lung:* Adenosquamous carcinoma (r) Squamous cell carcinoma (r)	N/R, 20/36 N/R, 2/36	Rehm & Lijinsky,1994

NMA-C11

The carcinogenicity of NMA-C11 was studied in male rats by gavage. The study is presented briefly below.

Table 30. Summary of animal carcinogenicity studies of NMA-C11

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Fischer 344	Male	Gavage	23 mg/animal 0.2 ml olive oil twice per week for 30 weeks; total dose: 1380 mg. 1 treated animal alive at 60 weeks. Animals were allowed to die naturally.	*Lung*: Alveolar cell adenoma Alveolar cell carcinoma Squamous cell carcinoma (r)	 3%, 6/19 (32%) 2%, 10/19 (53%) <1%, 10/19 (53%)	Lijinsky *et al*., 1978
				Esophagus: Squamous cell carcinoma (r)	 0%, 2/19 (11%)	
				Forestomach: Papilloma (r)	 0%, 2/19 (11%)	
				Liver: Hepatocellular carcinoma Cholangiocarcinoma (r)	 1%[3], 10/19 (53%) 0%, 8/19 (42%)	

[1] Rare tumor type denoted by (r)
[2] Control incidence listed first, subsequent incidences correspond to treated groups in order of increasing dose; incidence numbers converted to percentage by OEHHA
[3] Authors reported tumor incidence of controls as percentage; treated animals were compared to colony of male control rats kept at the study facilty during the same time period.

NMA-C12

The carcinogenicity of NMA-C12 was studied in rats, hamsters, and guinea pigs. These studies are described in the table below (Table 31), with the exception of the oral gavage study conducted in male Fischer 344 rats by Lijinsky *et al.* (1981), discussed in Section 3.2.1 above (see Table 8).

Table 31. Summary of animal carcinogenicity studies of NMA-C12

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Sprague Dawley	Male	Gavage	0.2 ml of 60 mg/ml twice per week for 50 weeks; vehicle: olive oil; total dose: 1200 mg[3]. Male: 0 survivors at 80 weeks. Female: last survivor killed at 96 weeks. All animals allowed to die naturally or killed when moribund. No controls.	*Lung:* Adenoma	N/A, 1/15	Lijinsky & Taylor, 1975
				Bladder: Transitional cell carcinoma (r)	N/A, 15/15	
	Female			*Lung:* Adenoma	N/A, 6/15	
				Esophagus: Papilloma (r)	N/A, 1/15	
				Bladder: Transitional cell carcinoma (r)	N/A, 15/15	

[1] Rare tumor type denoted by (r)
[2] Control incidence listed first, subsequent incidences correspond to treated groups in order of increasing dose; treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (performed by OEHHA); control incidences with asterisks indicate significant results from exact trend test (performed by OEHHA): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; N/A = not available
[3] Chemical preparation contained about 10% of impurity (aliphatic N-oxide)

Table 31 (cont'd). NMA-C12 animal carcinogenicity studies

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Rat, Sprague Dawley	Male	Gavage	0.2 ml of 125 mg/ml twice per week for 30 weeks; vehicle: olive oil; total dose: 1500 mg. 0 animals alive at 70 weeks post-treatment. No controls.	*Bladder:* Transitional cell carcinoma (r)	N/A, 13/13	Lijinsky & Taylor, 1978
	Female			*Bladder:* Transitional cell carcinoma (r)	N/A, 11/11	
Rat, Fischer 344	Male	Gavage	0.2 ml of 125 mg/ml twice per week for 30 weeks; vehicle: olive oil; total dose: 1500 mg. 0 animals alive at 60 weeks post-treatment. No controls.	*Bladder:* Transitional cell carcinoma (r)	N/A, 4/6	
	Female			*Bladder:* Transitional cell carcinoma (r)	N/A, 6/6	
Rat, Fischer 344	Female	Gavage	0.2 ml of 60 mg/ml twice per week for 30 weeks; vehicle: corn oil; total dose: 720 mg. 0 animals alive at 70 weeks. Untreated controls.	*Lung:* Tumors (not otherwise specified)	0/20, 12/20***	Lijinsky *et al.*, 1983c
				Bladder (r)*:* Tumors (not otherwise specified)	1/20, 17/20***	

Table 31 (cont'd). NMA-C12 animal carcinogenicity studies

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Hamster, Syrian golden	Male	Gavage	Low dose: 15 mg/kg/week for life; total average dose: 75 mg/animal/year; vehicle: olive oil; male average survival 67 weeks; female average survival 49 weeks. Mid-dose: 30 mg/kg/week; total average dose: 150 mg/animal/year; male average survival 65 weeks; female average survival 59 weeks. High dose: 60 mg/kg/week; total average dose 300 mg/animal/year; male average survival 58 weeks; female average survival 54 weeks. Two treatments missed in all dose groups. Vehicle controls: male average survival was 72 weeks; female average survivial was 56 weeks.	*Respiratory tract:* Tumors (not otherwise specified)	0/15, 1/15, 1/15, 0/15	Althoff & Lijinsky, 1977
				Digestive tract: Tumors (not otherwise specified)	5/15, 8/15, 3/15, 6/15	
				Urinary tract: Tumors (not otherwise specified)	0/15***, 3/15, 5/15*, 13/15***	
	Female			*Respiratory tract:* Tumors (not otherwise specified)	0/15***, 0/15, 0/15, 6/15**	
				Digestive tract: Tumors (not otherwise specified)	3/15, 1/15, 3/15, 2/15	
				Urinary tract: Tumors (not otherwise specified)	0/15***, 0/15, 4/15*, 13/15***	

Table 31 (cont'd). NMA-C12 animal carcinogenicity studies

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Hamster, European (Strain Mhh:EPH)	Male	*s.c.*	Animals injected once per week for life; initial dose: 360 mg/kg bw for 4 weeks, followed by 90 mg/kg bw for an average of 25 weeks; vehicle: olive oil; average total dose: 132 mg[4]. Average survival time: 29 weeks. Vehicle controls.	*Lung:* Adenoma Mucoepidermoid carcinoma Squamous cell carcinoma (r)	 0/10, 4/10* 0/10, 5/10* 0/10, 6/10**	Ketkar *et al.*, 1981
				Bladder: Transitional cell carcinoma (r) Papilloma (r)	 0/10, 4/10* 0/10, 2/10	
				Site of injection: Anaplastic sarcoma Squamous cell carcinoma (r) Fibrosarcoma Osteosarcoma (r) Liposarcoma (r)	 0/10, 3/10 0/10, 1/10 0/10, 1/10 0/10, 1/10 0/10, 1/10	
	Female		Animals injected once per week for life; initial dose: 360 mg/kg bw for 4 weeks, followed by 90 mg/kg bw for an average of 25 weeks; vehicle: olive oil; average total dose: 141 mg[5]. Average survival time: 24 weeks. Vehicle controls.	*Nasal cavity:* Papilloma (r)	 0/10, 1/10	
				Salivary gland: Haemangoendothelial sarcoma	 0/10, 1/10	
				Lung: Adenoma Mucoepidermoid carcinoma Sqamous cell carcinoma (r)	 0/10, 2/10 0/10, 6/10** 0/10, 4/10*	
				Bladder: Transitional cell carcinoma (r) Papilloma (r)	 0/10, 3/10 0/10, 2/10	
				Uterus Leiomyosarcoma	 0/10, 1/10	
				Site of injection: Anaplastic sarcoma Squamous cell carcinoma Fibrosarcoma	 0/10, 3/10 0/10, 3/10 0/10, 1/10	

[4] First tumor appeared at week 5 after initial treatment
[5] First tumor appeared at week 6 after initial treatment

Table 31 (cont'd). NMA-C12 animal carcinogenicity studies

Species/ Strain	Sex	Route	Exposure duration and total dose	Tumor Site/Tumor type[1]	Incidence[2]	Reference
Guinea Pig, Strain 2	Male	Gavage	1.0 ml/kg of 100 mg/ml, 2 treatments/week for 40 weeks; total dose: 8000 mg; olive oil vehicle; 0 animals alive at 80 weeks. Vehicle control; 0 controls alive at 120 weeks	*Liver:* Hemangiosarcoma Bile duct carcinoma (Cholangiocarcinoma) Hepatocellular carcinoma	0/20, 12/20*** 0/20, 3/20 0/20, 1/20	Cardy & Lijinsky, 1980
				Pancreas: Hemangiosarcoma	0/20, 1/20	

3.3 Other Relevant Data

3.3.1 Genotoxicity

Studies evaluating the genotoxic potential of the NMAs consist primarily of mutagenicity assays in bacteria and DNA adduct studies in rats. Specifically, eleven NMAs have been tested in bacterial mutagenicity assays (NMA-C1 – C4, NMA-C6 – C12), and *in vivo* DNA adduct formation has been studied in rats with twelve NMAs (NMA-C1 – C12) The majority of NMAs have not been studied in other gentoxicity assay systems. The exceptions are NMA-C3, which has been tested for mutagenicity in Chinese hamster V79 cells, and NMA-C1, which has been tested in a number of different genotoxicity assays.

Since NMA-C1 and –C2 are already listed under Proposition 65 as causing cancer, the genotoxicity findings for these two chemicals are only briefly summarized here. Specifically, NMA-C1 and NMA-C2 both induce mutations in *Salmonella typhimurium* and Chinese hamster V79 cells (CCRIS, 2014; US EPA, 2003a), and form DNA adducts in rats *in vivo* (Von Hofe *et al*., 1987; Kleihues *et al*., 1987). NMA-C1 has tested positive for genotoxicity in numerous additional *in vitro* and *in vivo* assays assessing a number of different endpoints, including reverse mutations in *Escherichia coli*, forward mutations in mammalian assays *in vitro* and *in vivo*, unscheduled DNA synthesis (UDS) in human fibroblasts and animal hepatocytes *in vitro*, and DNA strand breaks, sister chromatid exchange, chromosomal aberrations, and micronuclei formation in rodents *in vitro* and *in vivo* (CCRIS, 2014; OEHHA, 2006). Thus the most well-studied NMA, NMA-C1, has been shown to induce mutations, to be clastogenic, and to form DNA adducts *in vitro* and *in vivo*. The less studied NMA-C2 has also been shown to induce mutations in bacteria and mammalian cells *in vitro*, and forms DNA adducts *in vivo*. Findings from the gentoxicity studies of the other NMAs are presented in some detail below.

Bacterial Mutagenicity

The genotoxicity of several individual NMAs (NMA-C3 – C4, NMA-C6 – C12 compounds) was tested in bacterial mutation assays with various *S. typhimurium* and *E. coli* strains (see Table 32). *S. typhimurium* strains TA100, TA1530 and TA1535, and *E. coli* strain WP2 uvrA are sensitive to base-pair substitutions (Camus *et al*., 1976; Gatehouse, 2012; Eastmond *et al*., 2009). *S. typhimurium* strain TA98 is sensitive to frameshift substitutions (Mortelmans and Zeiger, 2000).

In general, NMAs tested positive for mutagenicity in test strains TA100, TA1530 and TA1535, primarily when tested in the presence of rat liver S9 induced with phenobarbital (PB), Aroclor 1254, or polychlorinated biphenyl (PCB), or in the presence of a modified Fenton's reagent (H_2O_2, Fe^{2+} and Cu^{2+}, a reactive oxygen generating system) (Inami *et al*., 2010; Andrews and Lijinsky, 1980; Camus *et al.*, 1976; Tsutsumi *et al.*, 2010; Suzuki *et al.*, 1985; Yahagi *et al.*, 1977). No mutagenicity was observed with test strain TA98 in the absence of S9, but some mutagenicity was observed in the presence of induced S9 (Sugimura *et al*., 1976; Inoue *et al*., 1980). Specific findings from the bacterial mutagenicity studies presented in Table 32 are described in more detail below.

NMA-C3 was mutagenic in bacterial mutagenicity assays using test strain TA1530 with phenobarbital (PB)-induced hamster liver S9 but not with PB-induced rat liver S9 (Camus *et al.,* 1976). It was also positive in test strain TA1535 with Aroclor 1254-induced rat liver S9 (Andrews and Lijinsky, 1980), and in test strains TA 1535 and *E. coli* WP2 uvrA in the presence of a modified Fenton's reagent (Inami *et al*., 2010).

NMA-C4 induced mutagenicity (primarily with PB- or PCB-induced rat liver S9 extract) in test strains TA100 and TA1535, as well as *E. coli* WP2 hcr$^-$ (Tsutsumi *et al.*, 2010; Suzuki *et al.*, 1985; Yahagi *et al.*, 1977; Sugimura *et al*., 1976). Andrews and Lijinsky (1980) found that NMA-C4 was mutagenic to test strain TA1535 in both the presence and absence of Aroclor 1254-induced rat liver S9. It was also mutagenic to test strains TA 1535 and *E. coli* WP2 uvrA in the presence of a modified Fenton's reagent (Inami *et al*., 2010; Tsutsumi *et al*., 2010). NMA-C4 was not mutagenic in the presence or absence of rat liver S9 in test strain TA98 (Sugimura *et al*., 1976; Yahagi *et al*., 1977).

Several NMAs (NMA-C6 – C11) induced mutations in test strain TA1535 in the presence but not the absence of Aroclor 1254-induced rat liver S9 (Andrews and Lijinsky, 1980).

NMA-C12 induced mutations in test strain TA100 in the presence of PB or PCB-induced rat liver S9 but not in test strain TA98 in the presence or absence of metabolic activation (Inoue *et al.*, 1980; Yahagi *et al.*, 1977; Sugimura *et al*., 1976).

No bacterial mutagenicity assays were identified for NMA-C5 or NMA-C14.

Table 32. Bacterial gene mutation assays

NMA	Strain[1]	Concentration tested	Results[2]		Activation system	Reference
			- S9	+ S9		
C3	TA1530	0 - 5 µmol/plate	NT	+	PB[3] induced hamster liver S9	Camus *et al*., 1976
			NT	-	PB induced rat liver S9	
	TA1535		+	NT	Modified Fenton's reagent[4]	Inami *et al*., 2010
	E. coli WP2 uvrA		+	NT		
C4	TA98	Not stated	-	-	PB or PCB[5] induced rat liver S9	Sugimura *et al*., 1976
	TA100	Not stated	-	+		
	TA98	Not stated	-	-	PB or PCB induced rat liver S9	Yahagi *et al.*, 1977
	TA100	0 - 40 µmol/plate	-	+		
	TA1535	0 - 15 µmol/plate	(+)[6]	+	PB or PCB induced rat liver S9	Suzuki *et al.*, 1985
			(+)[6]	+	PB or PCB induced rat liver S9 with or without DMSO	
	E. coli WP2 hcr$^-$	0 - 15 µmol/plate	(+)[6]	+	PB or PCB induced rat liver S9	
			(+)[6]	+	PB or PCB induced rat liver S9 rat with or without DMSO	
	TA1535	0 - 5 µmol/plate	+	NT	Modified Fenton's reagent plus nitric oxide	Tsutsumi *et al.,* 2010
			-	NT	Modified Fenton's reagent plus DMPO[7]	
			-	NT	Modified Fenton's reagent plus CarboxyPTIO[8]	
	TA1535	0 - 5 µmol/plate	+	NT	Modified Fenton's reagent	Inami *et al*., 2010
			+	NT	H_2O_2 plus Cu^{2+}	
	E. coli WP2 uvrA	0 - 5 µmol/plate	+	NT	Modified Fenton's reagent	

Table 32 (cont'd). Bacterial gene mutation assays

NMA	Strain[1]	Concentration tested	Results[2]		Activation system	Reference
			- S9	+ S9		
C3	TA1535	0 - 1000 µg/plate	-	+	Aroclor 1254 induced rat liver S9	Andrews and Lijinsky, 1980
C4			+	+		
C6			-	+		
C7			-	+		
C8			-	+		
C9			-	+		
C10			-	+		
C11			-	+		
C12	TA98	Not stated	-	-	PB or PCB induced rat liver S9	Sugimura *et al*., 1976
	TA100	Not stated	-	+		
	TA98	Not stated	-	-	PB or PCB induced rat liver S9	Yahagi *et al.*, 1977
	TA100	0 - 1 µmol/plate	-	+		
	TA98	50 - 500 µg/plate	-	-	PCB induced rat liver S9	Inoue *et al*., 1980
	TA100	50 - 500 µg/plate	-	+		

[1] Test strains used are of the genus *Salmonella typhimurium* unless otherwise noted in table
[2] Not tested (NT)
[3] Phenobarbital (PB)
[4] Modified Fenton's reagent (H_2O_2, Fe^{2+} and Cu^{2+}, a reactive oxygen generating system)
[5] Polychlorinated biphenyl
[6] "weakly positive" as stated by author
[7] 5,5-Dimethyl-1-pyrroline N-oxide (DMPO)
[8] 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3 oxide (CarboxyPTIO)

In vitro mammalian mutagenicity

NMA-C3 was mutagenic in V79 Chinese hamster lung cells in the presence of a rat liver S15 fraction (a post-mitochondrial fraction from male BD VI rat livers centrifuged at 15000 x g for 20 minutes) (Kuroki *et al*., 1977). NMA-C3 was also mutagenic in V79 Chinese hamster lung cells in the presence, but not absence, of hamster hepatocytes (Langenbach, 1986) (Table 33).

Table 33. *In vitro* mammalian genotoxicity tests

Chemical	Endpoint	Test system	Result	Reference
C3	Mutagenicity; Azaguanine resistance	V79 Chinese hamster cells	+ with S15[1] from PB treated rats	Kuroki *et al*., 1977
	Mutagenicity; Ouabain resistance	V79 Chinese hamster cells	+ in presence of hamster hepatocytes	Langenbach, 1986

[1] A post-mitochondrial fraction from male rat BD VI livers centrifuged at 15000 x g for 20 minutes

Nucleic acid alkylation adducts

NMAs have been shown to form DNA and RNA adducts through alkylation in target tissues *in vivo* and *in vitro*.

Nucleic acid alkylation in vivo – NMA-C1 – C12
Twelve NMAs, from NMA-C1 through NMA-C12, have been shown to form DNA adducts as a result of alkylation of the DNA of target tissues (lung, esophagus, liver, kidney) in male Fischer rats *in vivo* (Von Hofe *et al.*, 1987; Kleihues *et al.*, 1987) (see Table 34). In these studies rats were treated with a single oral dose of 0.1 mmol/kg of the respective NMA and sacrificed 6 hours later. DNA was extracted from frozen tissues and the amounts of 7-methylguanine and O^6-methylguanine were determined using high performance liquid chromatography. All NMAs tested formed DNA adducts in rat liver (see Table 34). Additional sites of 7-methylguanine and O^6-methylguanine formation for the individual NMA compounds were as follows: NMA-C1 and NMA-C2 formed DNA adducts in the lung and kidney; NMA-C3 and NMA-C4 formed DNA adducts in the esophagus, lung, and kidney; NMA-C5 formed DNA adducts in the esophagus and the lung; and NMA-C10, -C11, and -C12 formed DNA adducts in the lung. The extent of formation of methylated guanine adducts was greatest in liver tissue following administration of short chain alkyl nitrosamines; levels of methylated guanine adducts in the liver decreased with increasing alkyl chain length. The tissue with the second largest amount of methylated guanine adducts was the esophagus, with the highest levels seen with NMA-C4 and NMA-C5, followed by NMA-C3. Both kidney and lung tissues had similar levels of methylated guanine adducts; in kidney tissues NMA-C1 – C4 formed methylated guanine adducts, with levels decreased with increasing alkyl chain length. While only 7-methylguanine and O^6-methylguanine were measured in this set of

studies, NMAs also form other types of DNA adducts (Von Hofe *et al.*, 1987; Kleihues *et al.*, 1987). For example, Kleihues *et al*. (1987) reported on a separate study of rats exposed to NMA-C2, where several ethyl and hydroxyethyl DNA adducts were identified.

Nucleic acid and protein alkylation in vivo – NMA-C4
Studies by Magee and Lee (1964) demonstrated that the methyl group of NMA-C4 binds to protein and RNA of rat liver and kidney tissues to a greater extent than its butyl group. ^{14}C-labeled NMA-C4 (either N-butyl-[^{14}C]methylnitrosamine or N[^{14}C]butylmethylnitrosamine) was injected intraperitoneally into Wistar rats, and rats were sacrificed 6 or 7 hours post-injection. RNA and protein from the liver, kidneys, pancreas, and spleen were extracted and examined for radioactivity. Liver RNA had the highest radioactivity (measured as radioactive 7-methylguanine), followed by kidneys, spleen, and pancreas (Table 34). Nucleic acids and protein were labeled to a greater extent when N-butyl-[^{14}C]methylnitrosamine rather than N-[^{14}C]butylmethylnitrosamine was administered.

Nucleic acid alkylation in vitro - NMA-C5
Alkylation of liver and esophageal rat DNA was observed after incubating tissue slices of rat esophagus and liver with ^{3}H-labeled NMA-C5 (Mirvish *et al*., 1987) (Table 34). DNA was isolated and analyzed for apurinic acid, N7-methylguanine, and O^6-methylguanine. Esophageal DNA contained 19 times more O^6-methylguanine than liver DNA. This is consistent with the bioassay findings for NMA-C5, which significantly increased the incidence of esophageal, nasal, forestomach, and tracheal tumors but not liver tumors in rats (as presented in Section 3.2).

Nucleic acid alkylation in vivo – NMA-C5
Adult male F344 rats were injected with a single dose of N-nitroso[methyl^{14}C]-pentanamine (NMA-C5) (0.1mmol/kg), and DNA was extracted from individual organs six hours later (Koenigsmann *et al*., 1988). The concentration of methylated purines (guanine and adenine) was measured. Levels of alkylated DNA (measured as methylated purines) were highest in esophageal tissue, followed by nasal epithelium and liver, with lower levels in trachea, lung, forestomach, and kidney (Table 34).

Table 34. Nucleic acid and protein adducts formed by NMAs

NMA-	*In vivo/ in vitro*	Type of Adduct	Animal Test Species	Result	Reference
C4	*In vivo*	RNA and protein	Wistar rat	+ liver, kidney, pancreas, spleen	Magee and Lee, 1964
C5	*In vitro*	DNA	MRC-Wistar rat	+ esophagus, liver	Mirvish *et al*., 1987
	In vivo	DNA	Fischer rat	+ esophagus, nose,liver, trachea, lung, forestomach, kidney	Koenigsmann *et al*., 1988
C1	*In vivo*	DNA	Fischer rat	+ liver, lung, kidney	Von Hofe *et al.,* 1987; Kleihues *et al.*, 1987
C2				+ liver, lung, kidney	
C3				+ esophagus, liver, lung, kidney	
C4				+ esophagus, liver, lung, kidney	
C5				+ lung, esophagus, liver	
C6				+ liver	
C7				+ liver	
C8				+ liver	
C9				+ liver	
C10				+ lung, liver	
C11				+ lung, liver	
C12				+ lung, liver	

In summary, all twelve NMAs tested form DNA adducts in rats *in vivo*, all eleven NMAs tested are mutagenic in bacteria, all three NMAs tested are mutagenic in Chinese hamster V79 cells, and NMA-C1, the most extensively tested compound in the group, has been shown to be mutagenic and clastogenic in multiple *in vitro* and *in vivo* test systems.

3.3.2 In Vitro Cell Transformation Assays

NMA-12 was tested in an *in vitro* cell transformation assay using Syrian golden hamster embryo cells (Inoue *et al*., 1980). Cells were observed for randomly oriented three-dimensional growth with extensive crossing-over of the cells at the periphery of the colony. NMA-C12 was negative in this test system at media concentrations of 1, 10 and 100 µg/ml. However, the response to the concurrent positive control 3-methylcholanthrene (3-MC) was low (0.2% transformation in 1 µg/ml treatment group) compared to the response to the same 3-MC concentration during cell line characterization (>1.5% transformation).

3.3.3 Pharmacokinetics and Metabolism

The pharmacokinetics and metabolism of NMAs have been studied in several animal species and in humans. *In vivo* metabolism studies have been conducted in rats (Blattmann *et al.*, 1974; Mirvish *et al.*, 1985b; 1987; 1989; Suzuki *et al.,* 1981; Okada *et al.*, 1976a; Singer *et al.*, 1981, 1982). *In vitro* studies have been conducted with tissue preparations from liver, esophagus, trachea, lung, nose, and kidney of rats, mice, hamsters, and guinea pigs, (Lee *et al.*, 1989; Kawanishi *et al.*, 1983; Farrelly and Stewart, 1982; Farrelly *et al.*, 1982; Huang *et al.*, 1992, 1993; Lorr *et al.*, 1982; Mirvish *et al.*, 1985b, 1987, 1988, 1989, 1991a, 1991b, 1994b; Ji *et al*., 1989) as well as with human liver and esophageal microsomes (Huang *et al*., 1992; Mirvish *et al*., 1994b).

NMAs are metabolized across species in a similar manner, and common metabolites are formed *in vivo* and *in vitro*. Metabolites identified include hydroxy-, oxo- and carboxy- intermediates as well as the Proposition 65 listed carcinogens formaldehyde and N-nitrososarcosine, and the animal tumorigens N-nitrosomethyl-3-carboxypropylamine, 4-hydroxy-nitrosomethyl-*n*-butylamine, and N-methyl-nitroso-2-oxopropylamine (MOP) (Bellec *et al*., 1996; Koenigsmann *et al*., 1988; Farrelly and Stewart, 1982; Mirvish *et al*., 1985b, 1989, 1991a, 1991b, 1994b; Huang *et al*., 1993; Lai and Arcos, 1980; Lorr *et al*., 1982).

3.3.3.1 Absorption and Distribution

Dermal absorption studies using an *in vitro* human skin model system were performed with NMA-C12. In these studies NMA-C12 was absorbed to a limited extent by human skin when applied either in isopropyl myristate, an oil-in-water emulsion, or a shampoo vehicle (Walters *et al*., 1997). Different doses of NMA-C12 and different study protocols were used with each vehicle, thus comparison of results across vehicles is not straightforward. Application of the chemical to the skin in a shampoo formulation for 10 minutes, followed by a rinse, resulted in absorption of 0.75% of the applied dose after 48 hours. Application of the chemical to the skin in the oil-in-water emulsion (with no rinsing) resulted in absorption of 4.66% after 48 hours. Application of the chemical to the skin in isopropyl myristate resulted in absorption of 0.098% after 48 hours.

In rats, NMAs were absorbed rapidly and metabolites were detected in blood and urine (Blattmann *et al*., 1974; Mirvish *et al*., 1985b; 1987; 1989; Suzuki *et al*., 1981; Okada *et al*., 1976a; Singer *et al*., 1981; 1982). Levels of NMA-C5 in the blood were determined in studies by Mirvish and colleagues. NMA-C5 was rapidly absorbed and distributed following a single *i.p*. injection in male MRC Wistar rats of 25 mg/ml water/kg, with NMA-C5 levels of 11.2 µg/ml (86 nmol/ml) measured in the blood after 15 minutes (Mirvish *et al.*, 1987). In a subsequent experiment, male MRC Wistar rats were exposed to 25 mg NMA-C5/10 ml water/kg by *i.p.* injection (192 µmol NMA-C5/kg bodyweight) (Mirvish *et al*., 1989). Blood levels of NMA-C5 reached 86 nmol/ml blood 15 minutes post-injection, and then declined to 40 nmol/ml blood at 1 hour post-injection, 10 nmol/ml blood at 2 hours post-injection, and to undetectable levels at 5 hours post-injection.

3.3.3.2 Metabolism

Initial steps in bioactivation and metabolism of NMAs involve hydroxylation of the alkyl chain by cytochrome P450 dependent enzyme systems, oxidative dealkylation, and reductive denitrosation. Hydroxylation at the α carbon is thought to form an unstable intermediate, leading to the formation of a diazonium ion, which in turn can react with DNA and lead to DNA alkylation (Magee, 1980; Lorr *et al*., 1982; Kawanishi *et al*., 1992; Bellec *et al*., 1996). Hydroxylation at non-α carbons also occurs, ultimately resulting in formation of DNA reactive products. Dinitrosation of NMAs occurs via the combined actions of cytochrome P450 and NADPH-dependent P450 reductase, but can also occur via hydroxyl radicals (Appel *et al*., 1986).

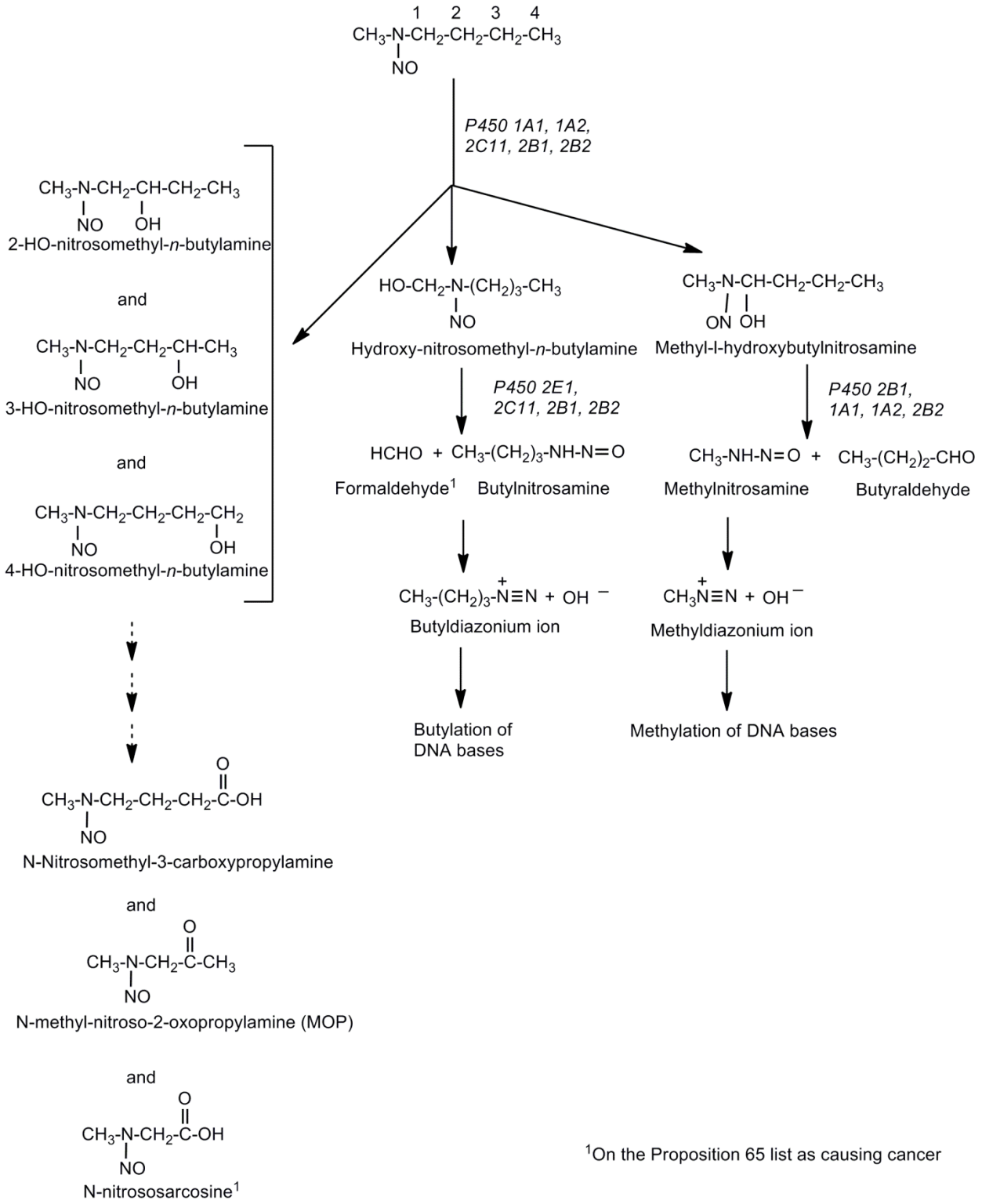
Different cytochrome P450 enzyme systems (CYP) are involved in the initial metabolic reactions, with CYP 1A shown to catalyze the α-hydroxylation of the alkyl chain and CYP 2E1 shown to catalyze the oxidation of the methyl group (Bellec *et al*., 1996). Further hydroxylation of NMAs, thought to occur mainly via ω- and/or β-oxidation, results in the generation of 2-, 3-, 4- and/or 5-hydroxy-NMAs (Blattmann *et al*., 1974; Mirvish *et al*., 1991a, 1991b, 1994b; Huang *et al.*, 1993). CYP 2C11 and 2C12 catalyze the 4-hydroxylation of NMA-C5, while CYP 1A1, 1A2, 2B1, 2B2 and 2E1 catalyze hydroxylation at different positions of the alkyl chain (Mirvish *et al*., 1991a; 1991b). CYP 2E1 has also been shown to form formaldehyde through α-oxidation of the methyl groups of NMA-C3 and NMA-C4 (Bellec *et al*., 1996).

The main metabolites formed include various aldehydes, carboxylated- and hydroxylated-NMAs, and nitrite, as well as several metabolites that are genotoxic and are classified as carcinogens (formaldehyde, N-nitrososarcosine), or that have been shown to induce tumors in animals (N-nitrosomethyl-3-carboxypropylamine, 4-hydroxy-nitrosomethyl-*n*-butylamine, MOP) (Lijinsky *et al*., 1983a; Hasegawa *et al*., 1998; Okada *et al*., 1976b; Druckery *et al*., 1967; Thomas *et al*., 1988, Sugimura *et al*., 1976; Bellec *et al*., 1996; Koenigsmann *et al*., 1988; Farrelly and Stewart, 1982; Mirvish *et al*., 1985b, 1989, 1991a, 1991b, 1994b; Ji *et al*., 1989; Huang *et al*., 1993; Lai and Arcos, 1980; Lorr *et al*., 1982).

The *in vitro* studies indicate that the metabolism of NMAs may be qualitatively similar across several animal species (rats, mice, hamsters, and guinea pigs) and humans.

Possible routes of NMA metabolism with NMA-C4 as an example are presented in Figure 1 on the next page.

Figure 1. Proposed routes of NMA-C4 metabolism in rats (adapted from Huang *et al*., 1993)



Results from metabolism studies with individual NMAs are summarized below.

NMA-C4

NMA-C4 was metabolized by human liver microsomes, and identified metabolites include formaldehyde, butyraldehyde and nitrite (Yoo *et al*., 1988). In rats, four metabolites were identified in urine at 48 hours following a single dose of orally administered NMA-C4 (130 mg/kg), namely 4-hydroxy-nitrosomethyl-*n*-butylamine, N-nitrosomethyl-3-carboxypropylamine, methyl-(3-carboxy-2-hydroxypropyl)-nitrosamine, and N-nitrososarcosine (Blattmann *et al.*, 1974). *In vitro*, formaldehyde, butyraldehyde and nitrite are formed with microsomal enzyme systems (Farrelly *et al*., 1982; Lee *et al*., 1989; Lorr *et al.*, 1982).

Demethylation of NMA-C4 to formaldehyde occurred primarily via the acetone inducible CYP 2E1 whereas debutylation occurred via a phenobarbital (PB) inducible CYP 2B1 enzyme, indicating alkyl group selectivity by the P450 enzymes (Lee *et al*., 1989). Induction of rat microsomes with isopropanol, PB or 3-methylcholanthrene (MC) increased dealkylation as measured by increased formaldehyde and butyraldehyde formation compared to uninduced controls (Kawanishi *et al*., 1983; Lorr *et al*., 1982). Use of specific P450 antibodies indicated that rat CYP 2C11 mainly catalyzes demethylation and ω-1 hydroxylation, CYP 1A1 or 1A2 catalyze 3-hydroxylation and debutylation, CYP 2E1 catalyzes demethylation, and CYP 2B1 or 2B2 mainly perform ω-1 hydroxylation, demethylation, and debutylation reactions (Huang *et al*., 1993). Other metabolites of NMA-C4 include 2-, 3-, and 4-hydroxy-nitrosomethyl-*n*-butylamine (Mirvish *et al.*, 1994b; Huang *et al*., 1993).

Nitrite generation from NMA-C4 was also observed in an artificial hydroxy radical generating system (Appel *et al*., 1986). This mechanism is likely to involve the generation of hydroxyl radicals via the formation of superoxide anion by cytochrome P450 or NADPH cytochrome P450 reductase in the presence of iron.

NMA-C5

The metabolism of NMA-C5 was studied using segments of human esophagi, as well as human liver and esophageal microsomes (Huang *et al*., 1992; Mirvish *et al*., 1994b). Similar to metabolism in animals, NMA-C5 metabolism involves hydroxylation and dealkylation reactions. Segments of human esophagi (harvested within 6 hours of death and subsequently incubated with NMA-C5 for 6 hours) yielded several hydroxylated metabolites. The main hydroxylated metabolite was 5-hydroxy-N-nitrosomethyl-*n*-pentylamine, and trace amounts of 2-, 3-, and 4-hydroxy-N-nitrosomethyl-*n*-pentylamine were observed (Huang *et al.,* 1992). Microsomes harvested from human esophagi and liver tissues also yielded 2-, 3-, 4-, and 5-hydroxy-N-nitrosomethyl-*n*-pentylamine, with the 2-hydroxy-N-nitrosomethyl-*n*-pentylamine being predominant (Huang *et al*., 1992; Mirvish *et al*., 1994b). Other metabolites identified were formaldehyde and pentaldehyde. The demethylation reaction was inhibited in the presence of carbon monoxide, indicating the involvement of cytochrome P450 enzymes. Human liver microsomes

were more active than esophageal microsomes with regard to depentylation of NMA-C5.

In rats, metabolites were identified in 24- or 48-hour urine collected from animals which had received a single *i.p*. injection of 25 mg or 30 mg NMA-C5 (Mirvish *et al*., 1985b, 1989). Urine contained parent compound and the metabolites 2-, 3-, and 4-hydroxy-N-nitrosomethylpentanamine and 4-oxo-methyl-N-pentan-nitrosamine. The principal blood metabolites were 2- and 4-hydroxy- and 4-oxo-nitrosomethyl-*n*-pentylamine (Mirvish *et al*., 1987; 1989). Blood levels of 4-hydroxy- and 4-oxo-nitrosomethyl-*n*-pentylamine, peaked after an *i.p*. injection of 25 mg/10 ml water /kg at 13 nmol/ml at 30 minutes and 21-22 nmol/ml at 60 to 120 minutes, respectively (Mirvish *et al*., 1989).

In vitro, NMA-C5 was dealkylized to formaldehyde and pentaldehyde by rat esophageal and liver microsomes (Mirvish *et al*., 1994b; Huang *et al.*, 1992). The demethylation reactions were inhibited by carbon monoxide (CO), indicating that P450 enzymes are involved. In addition to pentaldehyde and formaldehyde, 2-, 3-, 4-, and 5-hydroxy-N-nitrosomethylpentanamine were formed, with 5-hydroxy-N-nitrosomethylpentanamine being produced predominantly (Huang *et al*., 1992). The two keto-nitrosamines, 3-, and 4-oxo-N-nitrosomethylpentanamine, which are formed *in vivo*, were also identified (Ji *et al*., 1989; Mirvish *et al*., 1987, 1988, 1989).

NMA-C5 is metabolized in a similar manner across species, including human tissues, and many of the same metabolites are formed both *in vivo* and *in vitro*. The 2-, 3-, 4-, and 5-hydroxy-N-nitrosomethylpentanamine and one keto-nitrosamine were identified in various tissues of mouse, hamster, and guinea pig, as well as in human tissues (Mirvish *et al*., 1985, 1994b). The positional hydroxylation reaction, as well as the formation of formaldehyde (demethylation) and pentaldehyde (depentylation) are catalyzed by P450 isozymes (Mirvish *et al*., 1991a, 1991b). Inhibition studies with monoclonal P450 antibodies indicated CYP 2C11, 2EI and 2B1 as major P450s in uninduced microsomes from male rats, and CYP 2C12 from female rats. Enzymes CYP 2C11 and CYP 2C12 were deemed to catalyze most of the 4-hydroxylation reactions (Mirvish *et al*., 1991a, 1991b).

NMA-C12

Male Wistar rats were exposed to NMA-C12 by gavage (average dose 143 mg/rat), and urine was collected from the animals over a 48-hour period. Four metabolites were detected in the pooled rat urine, with the primary metabolite being N-nitrososarcosine (14.5%), followed by N-nitrosomethyl-n-(2-hydroxy-3-carboxy)propylamine (3.5%), N-nitrosomethyl-3-carboxypropylamine (2.5%), and MOP (0.7%) (Okada *et al*., 1976a; Suzuki *et al*., 1981).

C4-C14 NMAs

Metabolites of a series of NMAs, ranging from NMA-C4 to NMA-C14, were extracted from rat urine 24 hours after gavage with 0.52 mM of each individual NMA (Singer *et al*., 1981, 1982). The principle metabolites of NMAs with even-numbered carbons were different compared to NMAs with odd-numbered carbons. Compounds with even-numbered carbons were metabolized to approximately equal amounts of N-nitrososarcosine and N-nitrosomethyl-3-carboxypropylamine. Compounds with odd-numbered carbons were metabolized to nitroso-methyl-2-carboxyethyl-amine and small amounts of N-nitrosomethyl-3-carboxypropylamine. MOP was formed from all NMAs studied, but levels were higher with NMAs with even-numbered carbons compared to NMAs with odd-numbered carbons. The proportion of MOP increased with the length of the even-numbered alkyl chains, ranging from 0.01% for NMA-C4 to 0.53% for NMA-C14. Very little MOP along with nitroso-3-methylamino-2-hydroxypropane was formed with odd-numbered alkyl chains, with levels ranging from 0.02% to 0.04% and from 0.01% to 0.03%, respectively.

An overview of the metabolism studies is presented in Table 35 below.

Table 35. Metabolism studies of NMAs

NMA-	*In vitro/ in vivo*	Species	Test system	Reaction products/Metabolites observed	Reference
C4	*In vitro*	Human	Liver microsomes	Formaldehyde, butyraldehyde, nitrite	Yoo *et al.*, 1988
		Fischer rats	Liver microsomes	Oxidative de-alkylation; formaldehyde, butyraldehyde	Farrelly *et al.*, 1982
		Sprague Dawley rats	Liver microsomes; induced by isopropanol, pyrazole, PB, or MC	Nitrite, formaldehyde	Lorr *et al*., 1982
		Wistar rats	Liver microsomes; induced by PB or MC	Oxidative de-alkylation; metabolites: formaldehyde, butyraldehyde	Kawanishi *et al.*, 1983
		Sprague Dawley rats	Liver microsomes; P450 enzymes; induced by PB, safrole, or MC	Oxidative de-alkylation; substrate specificity and alkyl group specificity of P450 enzymes; formaldehyde, butyraldehyde (reported as demethylation and debutylation reactions)	Lee *et al.*, 1989
		NMRI mice; Artificial system (XOD/hypoxanthine)	Liver microsomes; induced with PB	Formaldehyde and Nitrite formation	Appel *et al.*, 1986
		Sprague Dawley rats	Liver microsomes; Induced with PB and b-Naphthoflavon	Demethylation and debutylation	Kawanishi *et al*., 1992
		MRC-Wistar rats; Sprague Dawley rats	MRC W rats- lung, liver, trachea, and esophagus slices; SD rats- microsomes (induced by PB, MC, or isoniazid)	Dealkylation and hydroxylation; metabolite yield; substrate specificity of P 450; 2-, 3-and 4- hydroxy- nitrosomethyl-*n*-butylamine; formaldehyde, butyraldehyde	Huang *et al.*, 1993; Mirvish *et al.*, 1994b
	In vivo	Rats (unknown)	Oral route; single dose 130mg/kg; 48 h urine analysis	Metabolites: 4-hydroxy- nitrosomethyl-*n*-butylamine; N-nitrosomethyl-3-carboxypropylamine; Methyl-(carboxymethyl)-nitrosamine (N-Nitrososarcosine)	Blattmann *et al.*, 1974

Table 35 (cont'd). Metabolism studies of NMAs

NMA-	*In vitro/ in vivo*	Species	Test system	Reaction products/Metabolites observed	Reference
C5	*In vitro*	Sprague Dawley rats	Liver microsomes; induced by MC, PB, or Aroclor 1254; isoniazid	Metabolites: 2-, 3-, 4-, and 5-hydroxy-N-nitrosomethylpentylamine; 2-, 3-, 4-, and 5-hydroxymethyl-pentan-nitrosamine, Formaldehyde, Pentaldehyde; P 450 specificity	Mirvish *et al.*, 1991a
		MRC-Wistar rats; Sprague Dawley rats; Swiss mice; Syrian hamsters; guinea pigs	Tissue preparations (esophagus,- tracheal, liver, lung, kidney, and nasal tissue)	Metabolites: 2,-3-, 4- hydroxylmethyl-pentan-nitrosamine and oxo- N-methyl-pentan-nitrosamine found in all species, most tissues; 5- hydroxy-methyl-pentan-nitrosamine only with Wistar-rat tissues	Mirvish *et al.*, 1985b
		MRC-Wistar rats; Syrian hamsters	Tissues ranging from fetuses two days before term to tissues from young adults; esophagus, forestomach, whole stomach, liver lung, skin, kidney	Rat: 2-, 3-, and 4- hydroxy-methyl-pentan-nitrosamine; 3-, and 4-oxo- N-methyl -pentan nitrosamine. Maximum metabolism of esophagus at 9 d of age; forestomach at 3 days; none in adults. Hamster: newborn esophagus active; 3 d old hamster forestomach active; none in adults	Mirvish *et al.,* 1987
		MRC-Wistar rats; Syrian hamsters	Tissue slices (esophagus, stomach, intestine, trachea, and liver); neonatal to adult	Metabolites: 2-, 3-, 4-, and 5- hydroxy-nitrosomethyl-*n*-pentylamine; 4-oxo-nitrosomethyl-*n*-pentylamine	Mirvish *et al.*, 1988
		Sprague Dawley rats	Liver preparations; Induced by PB, MC, clofibrate, or isoniazid	Metabolites:2-, 3-, 4-, 5- hydroxylmethyl-pentan-nitrosamine;	Mirvish *et al.*, 1989
		Sprague Dawley rats	Liver slices and microsomes; Induced by PB, MC, Aroclor 1254 clofibrate, or isoniazid	Metabolites: Nitrite, formaldehyde, pentaldehyde, 2-, 3-, 4-, and 5- hydroxyl- methyl-pentan-nitrosamine, 4-oxo- methyl-pentan-nitrosamine	Ji *et al*., 1989

Table 35 (cont'd). Metabolism studies of NMAs

NMA-	*In vitro/ in vivo*	Species	Test system	Reaction products/Metabolites observed	Reference
C5		Sprague Dawley rats	Induced and uninduced liver microsomes; Monoclonal AB to P450 enzymes to identify P450s involved	Metabolites in uninduced microsomes:4- hydroxy-methyl-pentan nitrosamine, formaldehyde, pentaldehyde, nitrite formation; Induced microsomes: 2-, 3-4-, and 5-hydroxy-methyl-pentan-nitrosamine, pentaldehyde, formaldehyde	Mirvish *et al*., 1991b
		Human; Sprague Dawley rats	Microsomes from esophagus and liver	Metabolites (human): formaldehyde; pentaldehyde; 5-HO-methyl- pentan-nitrosamine. Very low levels of 2,-3-, 4- HO-methyl- pentan-nitrosamine. Metabolites (rat): formaldehyde, pentaldehyde; Methyl-hydroxy-pentan-nitrosamine (HO positions not specified)	Huang *et al.*, 1992; Mirvish *et al*., 1994b
	In vivo	MRC-Wistar rats	Blood & urine analysis; *i.p*. injection of 25 mg C5/10ml water/kg; 5 min – 5 h blood samples	Metabolites: 2-,3-, and 4- hydroxy-methyl-pentan-nitrosamine and 4-oxo-N-methyl-pentan nitrosamine in urine as β glucuronides; low levels 2 HO-, 3-HO- and higher levels of 4-HO- and 4-oxo N-methyl-pentan-nitrosamine in blood. Rapid elimination within 2 h, undetectable at 5 h.	Mirvish *et al.*, 1989
		MRC-Wistar rats	Blood & urine analysis; *i.p*. injection (25 mg/ml water/kg)	Blood: rapid absorption and distribution of C5 (11.2 µg/ml blood at 15 minutes; one-compartment first order kinetics); half-life 21 minutes; main metabolites were 4-HO- and 4-oxo N-methyl –pentan-nitrosamine; 80% elimination in urine within 6 h.	Mirvish *et al*., 1987
		MRC-Wistar rats	Urine analysis (48 h); *i.p.* injection (30 mg/ml water/kg)	Metabolites: 2-, 3-, 4-, and 5- methyl-hydroxy-pentyl-nitrosamine; Oxo- N-methyl-pentan nitrosamine	Mirvish *et al.*, 1985b
C12	*In vivo*	Wistar rats	Urine analysis(48 h); gastric intubation (125-159 mg/rat)	Metabolites: N-nitrosomethyl-3-carboxypropylamine, N-methyl-N-(2-hydroxy-3-carboxypropyl)nitrosamine, N-methyl-N-(carboxymethyl)nitrosamine (N-Nitrososarcosine), MOP.	Suzuki *et al.*,1981
		Wistar rats	Urine analysis 48 h; gavage; 143 mg/rat	Metabolites- see above	Okada *et al.*,1976a

Table 35 (cont'd). Metabolism studies of NMAs

NMA-	*In vitro/ in vivo*	Species	Test system	Reaction products/Metabolites observed	Reference
C3-C6	*In vitro*	Sprague Dawley rats	Microsomes; induced with acetone; pyrazole, PB, pyridine, 3-MC, β-naphthoflavone, indole-3-carbinol	Formaldehyde; propionaldehyde; butyraldehyde; pentaldehyde	Bellec *et al*., 1996
C3, C5-C7	*In vitro*	Fischer rats	Microsomes	Formaldehyde	Farrelly & Stewart, 1982
C4-C14	*In vivo*	Fischer rats	Gavage (12 mg; 2/week for 30 weeks) Urine analysis; 24 h	*Odd*-numbered nitroso - alkylamine metabolites: nitroso-methyl-carboxy-ethylamine; nitroso-methyl-2-oxopropylamine (small amount only). Even-numbered nitroso-alkylamine metabolites: N-nitrososarcosine, nitroso-methyl-carboxypropylamine; All: nitroso-methyl-2-oxopropylamine (increasing amounts with increasing even C number)	Singer *et al.*, 1982
		Fischer rats	Gavage, (0.2 ml of 0.52 mM 2/week) Urine analysis; 24 h	Main metabolites from *odd* numbered compounds: Nitroso-methyl-2-carboxy-ethylamine. Nitroso-3-methylaminopropionic acid; Small amounts of nitrosomethyl-aminoalkyl-carboxylic acids; Main metabolites from *even* numbered compounds: N-nitrososarcosine, N-nitrosomethyl-3-carboxypropylamine (NMCP); nitrosomethyl-aminoalkyl-carboxylic acids; Other metabolites: MOP; methyl 3-(nitrosomethylamino)-propionic acid; methyl 4-(nitrosomethyl-amino)butyric acid; 5-(nitrosomethylamino) pentanoic acid; 6-(nitroso-methylamino)hexanoid acid; MOP	Singer *et al.*, 1981

3.3.3.3 Excretion

Based on the clearance of NMA-C5 from the blood of rats following a single *i.p*. injection, Mirvish *et al.* (1987) concluded that the elimination of NMA-C5 follows a one-compartment first order kinetics model, with a half-life of 21 minutes, an absorption rate constant of 5.25/hour, and an elimination rate constant of 2.0/hours.

NMAs are excreted into urine, primarily as metabolites, regardless of the route of administration (*i.e*., gavage or *i.p* injection). NMA-C12 was extensively metabolized and excreted into urine at about 20% of the dose as nitroso metabolites within 48 hours following a single gavage exposure. No parent compound was detected in urine, and no additional nitroso metabolites were detected in urine beyond 48 hours (Suzuki *et al*., 1981).

Male Wistar rats received *i.p.* injections of NMA-C5 (Mirvish *et al*., 1985b) or were induced with MC and then 48 hours later injected with NMA-C5 (Mirvish *et al*., 1989). About 0.08% of the administered dose of NMA-C5 was excreted unchanged in urine and total nitroso metabolite excretion was 1.1%. The urinary nitroso metabolites occurred as their β-glucuronides.

3.3.3.4 Summary

NMAs are rapidly absorbed regardless of the route of administration, distributed via the blood, rapidly metabolized, and eliminated via urine. Metabolism occurs primarily via cytochrome P450 enzymes, resulting in various oxidation products (alcohols, ketones, carboxylic acids) and nitrite. *In vitro* experiments with human and animal tissues have identified the common metabolites formaldehyde, nitrite, and butyr- or pentaldehyde. *In vivo*, common metabolites include the Proposition 65 listed carcinogen N-nitrososarcosine, and the animal tumorigens 4-hydroxy-nitrosomethyl-*n*-butylamine, N-nitrosomethyl-3-carboxypropylamine and MOP.

3.3.4 Animal Tumor Pathology

Rat

Table 36 lists tumor types which are rare or significantly increased in incidence or both resulting from exposure of rats to NMAs with alkyl chain lengths ranging from three to fourteen carbons. Tumors of the nasal cavity, tongue, lung, esophagus, forestomach, liver, bladder and several other organs were observed in rats.

Table 36. Tumor sites in rats treated with NMAs of various alkyl chain lengths

NMA-	Nasal cavity	Tongue	Lung	Esophagus	Forestomach	Liver	Bladder	Others
C3	**M, F**	**F**		**M, F**	**F**	**M, F**		**F[1,2]**
C4	**M, F**	**M, F**		**M, F**	**M, F**			**M[1], F[1]**
C5	**M, F**	**M**		**M, F**	**MF[3], M**			**M[4], F[4]**
C6	**M**	**M**	**M**	**M**	**M**	**M**		
C7	**M**	**M**	**M**	**M**		**M**		
C8	**M**		**M**			**M**	**M**	**M[4]**
C9	**M**		**M**			**M**		
C10	**M**		**M**		**M**		**M**	
C11			**M**	**M**	**M**	**M**		
C12			**M, F**	**F**	**M**		**M, F**	**M[5]**
C14			**M**				**M**	**M[6]**

[1] Oropharyngeal tumors (rare)
[2] Epiglottis tumors (rare)
[3] MF- tumor incidences were reported in males and females combined
[4] Trachea tumors (papilloma, carcinoma; rare)
[5] Pancreatic islet cell carcinoma
[6] Kidney carcinoma (rare)

Nasal cavity tumors were observed in male rats treated with NMA-C3 (Reznik *et al*., 1975), NMA-C4 (Lijinsky *et al*., 1983b), NMA-C5 (Mirvish *et al*., 1985a, 1994a; Bulay and Mirvish, 1979; Yamaguchi *et al*., 1989), NMA-C6 (Lijinsky *et al*., 1983b), NMA-C7 (Lijinsky *et al*., 1983b), NMA-C8 (Lijinsky *et al*., 1981), NMA-C9 (Lijinsky *et al.*, 1981) and NMA-C10 (Lijinsky *et al*., 1981), and in female rats treated with NMA-C3 (Lijinsky *et al*., 1983a; Reznik *et al*., 1975), NMA-C4 (Lijinsky *et al*., 1980, 1983a, 1983b), and NMA-C5 (Bulay and Mirvish, 1979). All types of nasal tumors are rare in rats (Boorman *et al*., 1990; Schwartz *et al*., 1994) in one or more of the following strains: Sprague Dawley (SD), F344, and MRC-Wistar.

The nasal cavity tumors observed with the NMAs include squamous cell papillomas and carcinomas, olfactory adenocarcinomas and basal cell carcinomas that originate from the respiratory epithelium of the nasoturbinals and maxilloturbinals or from the maxillary sinuses and the olfactory epithelium (Lijinsky *et al*., 1983a; Reznik *et al*., 1975; Bulay and Mirvish, 1979; Yamaguchi *et al*., 1989; Lijinsky *et al*., 1981).

The rat nasal cavities are composed of two compartments that are divided by a septum. Each has three turbinates (synonymous with turbinals) projecting into the lumen: nasoturbinates (most apical and superior), maxilloturbinates (inferior to the nasoturbinates), and ethmoid turbinates (most caudal). The olfactory organ is located posteriorly, near the ethmoid turbinals. There is one pair of maxillary sinuses in the lateral wall of the nasal cavity, which are lined by ciliated columnar epithelium with few goblet cells. Half the surface area of the nasal passage is lined with *olfactory* epithelium, half by *respiratory* and transitional epithelium, and 4% by *squamous* epithelium (Boorman *et al*., 1990).

The *olfactory* epithelium covers a portion of the nasal septum, most of the dorsal meatus, parts of the nasal wall, and the ethmoid turbinates. Olfactory epithelium is composed of pseudostratified columnar cells of three types: sustentacular, sensory, and basal cells. Olfactory adenocarcinomas were observed in the nasal cavity (Lijinsky *et al*., 1983a). *Respiratory* epithelium extends from the nasal vestibule to the nasopharynx, covering the lateral wall, maxilloturbinate, the majority of the nasoturbinate, and part of the ethmoid turbinate. Six different cell types make up the respiratory epithelium: ciliated and non-ciliated columnar cells, goblet cells, brush cells, cuboidal cells, and basal cells. Tumors were observed in the apical nasal cavities that originated from the respiratory epithelium of the nasoturbinates and maxilloturbinates and maxillary sinuses (Reznik *et al.*, 1975; Lijinsky *et al*., 1981). The nares and nasal vestibule are lined by lightly keratinized stratified *squamous* epithelium. Squamous cell papillomas and carcinomas were observed in the nasal cavity (Lijinsky *et al.*, 1983a; 1981). Squamous cell papilloma may progress to overt squamous cell carcinoma, which is marked by invasive rather than expansile growth (Boorman *et al*., 1990). Observed carcinomas were either basal cell or a mixture of basal and squamous cell with keratin (Lijinsky *et al*., 1981).

Tongue tumors were observed in male rats treated with NMA-C4 (Lijinsky *et al*., 1980; 1983b), NMA-C5 (Mirvish *et al*., 1994a; Tanaka *et al*., 1997; Yamaguchi *et al*., 1989), NMA-C6 (Lijinsky *et al*., 1983b) and NMA-C7 (Lijinsky *et al*., 1983b) in one or more of the following strains: F344, MRC-Wistar, ad Wistar, and in female F344 rats exposed to NMA-C3 (Lijinsky *et al*., 1983a) and NMA-C4 (Lijinsky *et al*., 1980; 1983a).

The tongue tumors were squamous cell papillomas and carcinomas, which arise principally from the stratified squamous epithelium but can also arise from the connective tissue. Neoplasms of the tongue are rare in SD rats and F344 rats (Baldrick, 2005; Haseman *et al*., 1998; Whiteley *et al*., 1996). Tongue squamous cell papillomas are considered to have the potential to progress to carcinomas (Whiteley *et al*., 1996; McConnell *et al*., 1986).

Tumors of the trachea were observed in male and female MRC-Wistar rats following NMA-C5 exposure (Bulay and Mirvish, 1979) and in male F344 rats following NMA-C8 (Lijinsky *et al.*, 1981) exposure. The tumors observed in rats treated with NMA-C5 were papillomas and carcinomas, while the tumors observed after treatment with NMA-C8 were papillomas. Trachea tumors are rare in rats (Boorman *et al*., 1990).

Lung tumors were observed in male rats treated with several NMAs: NMA-C6 (Lijinsky *et al*., 1983b), NMA-C7 (Lijinsky *et al*., 1983b), NMA-C8 (Lijinsky *et al*., 1981), NMA-C9 (Lijinsky *et al*., 1981), NMA-C10 (Lijinsky *et al*., 1981), NMA-C11 (Lijinsky *et al.*, 1978), NMA-C12 (Lijinsky *et al*., 1981;), and NMA-C14 (Lijinsky *et al*., 1981) and in females treated with NMA-C12 (Lijinsky and Taylor, 1975; Lijinsky *et al*., 1983c) in one or more of the following strains: F344 and SD. The lung tumors observed include alveolar/bronchial adenomas, adenocarcinomas, 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). Adenocarcinomas and squamous cell carcinomas of the lung are rare in rats (Boorman *et al.*, 1990; Schwartz *et al*., 1994). The origin of squamous cell carcinomas is uncertain; they may arise from bronchi, bronchioles, or alveoli, but may also arise from keratinizing epidermal cysts originating in the alveoli (Boorman *et al*., 1990).

Esophageal tumors were observed in both sexes of rats treated with NMAs: NMA-C3 (Lijinsky *et al*., 1983a; Reznik *et al*., 1975), NMA-C4 (Lijinsky *et al*., 1980, 1983a, 1983b, 1991a; Koreeda *et al.*, 1999), NMA-C5 (Sasajima *et al*., 1982; Druckery *et al*., 1967; Mirvish *et al*., 1985a, 1996, 1994a; Bulay and Mirvish, 1979; Luo *et al*., 1987; Tanaka *et al*., 1997; Yamaguchi *et al*., 1989; Attwood *et al*., 1992; Seto *et al*., 1991; Matsufuji *et al*., 1987; Iizuka *et al*., 1980; Kuwayama and Eastwood, 1988; Kondoh *et al*., 1990), NMA-C6 (Lijinsky *et al.,* 1983b [males only]), NMA-C7 (Lijinsky *et al.,* 1983b [males only]), NMA-C11 (Lijinsky *et al*., 1978), and NMA-C12 exposure (Lijinsky and Taylor, 1975 [females only]). Tumors were observed in >20 studies in rats of F344, SD, Wistar and Donryu strains and in young Wistar rats following NMA-C5 exposure.

The esophageal tumors were squamous cell papillomas and carcinomas, and basal cell papillomas and carcinomas. 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). Esophageal tumors are rare in rats (Whiteley *et al*., 1996; Brown and Hardisty, 1990; Haseman *et al*., 1998). Esophageal squamous cell papillomas are considered to have the potential to progress to carcinomas (McConnell *et al*., 1986).

Forestomach tumors were observed in female rats treated with NMAs: NMA-C3 (Lijinsky *et al*., 1983a) and NMA-C4 (Lijinsky *et al*., 1980, 1983a, 1983b) and in male rats treated with NMA-C4 (Lijinsky *et al*., 1980, 1983b), NMA-C5 (Mirvish *et al*., 1996, 1985a; Luo *et al*., 1987), NMA-C6 (Lijinsky *et al*., 1983b), NMA-C8 (Lijinsky *et al.*, 1981), NMA-C10 (Lijinsky *et al*., 1981), NMA-C11 (Lijinsky *et al.*, 1978), and NMA-C12 (Lijinsky *et al.*, 1981).

The forestomach tumors were squamous cell papillomas and carcinomas in F344, MRC-Wistar, and Wistar rats. Forestomach tumors are rare in rats (Frantz *et al*., 1991; Haseman *et al*., 1998). Squamous cell carcinomas arise through proliferation of the non-glandular epithelium, and are locally invasive. Forestomach squamous cell papillomas are considered to have the potential to progress to carcinomas (McConnell *et al*., 1986; Frantz *et al*., 1991).

Liver tumors were observed in female SD rats treated with NMA-C3 (Reznik *et al*., 1975) and in male rats treated with NMA-C3 (Reznik *et al*., 1975), NMA-C6 (Lijinsky *et al*., 1983b), NMA-C7 (Lijinsky *et al*., 1983b), NMA-C8 (Lijinsky *et al.*, 1981), NMA-C9 (Lijinsky *et al.*, 1981) and NMA-C11 (Lijinsky *et al*., 1978) in one or more of the following strains: F344 and SD.

The liver tumors were hepatocellular adenomas and carcinomas, hemangioendotheliomas (hemangiomas), hemangiosarcomas, and cholangiocarcinomas. Hepatocellular adenomas are considered to have the potential to progress to carcinomas (McConnell *et al*., 1986). Hemangiomas (synonyms: benign hemangioendothelioma; benign hemangioma) are benign tumors of the endothelial cells lining blood vessels that are well-demarcated and do not exhibit local invasion (Mitsumori, 1990). They can progress to malignant hemangiosarcomas (synonymous with malignant hemangioendotheliomas; angiosarcomas), which are a proliferation of atypical endothelial cells consisting of irregular, poorly formed vascular channels (IARC, 1996; Mitsumori, 1990). They tend to metastasize to other organs, especially the lungs (Bannasch and Zerban, 1990). Hemangiosarcomas are rare in Fischer rats (Boorman *et al.*, 1990). Cholangiocarcinomas appear as firm nodules frequently distributed in the liver in a multinodular fashion and they arise from cholangiofibromas (Boorman and Everitt, 2006). Cholangiocarcinomas are rare in Fischer rats (Boorman *et al*., 1990).

Significant increases in pancreatic islet cell carcinomas were observed in male F344 rats treated with NMA-C12 (Lijinsky *et al*., 1981). The pancreas is comprised of the exocrine and endocrine pancreas. The endocrine pancreas is made up of the islets of Langerhans (*i.e.,* the islets), which are collections of cells distributed throughout the pancreas and comprise about 1-2% of the total pancreatic tissue. Islet cell carcinomas display marked cellular anaplasia and pleomorphism, with occasional alpha and delta cells scattered throughout the neoplastic mass. Carcinomas also display varied growth patterns and may invade the fibrous capsule, lymphatics and blood vessels. Occasionally they metastasize, usually to the liver or lung. These tumors are considered uncommon but not rare in F344 rats (Boorman *et al*., 1990).

Kidney tumors were observed in male F344 rats treated with NMA-C14 (Lijinsky *et al*., 1981). Lijinsky *et al.* (1981) did not specify the carcinoma cell type. Kidney carcinomas may arise from tubule cells, transitional cells, or squamous cells. Carcinomas of all three cell types are rare in male F344 rats (Boorman *et al*., 1990). Tubular cell carcinomas arise from the tubules of the nephron, most frequently the proximal tubule. They are often locally invasive, but rarely metastasize. Transitional cell carcinomas arise from the renal pelvis, which is part of the collecting duct system. They may be difficult to distinguish from tubular cell carcinomas and can only be identified by the location of the tumor. Squamous cell carcinomas may also arise from the renal pelvis, and although uncertain, may have arisen from transitional epithelium that has undergone squamous metaplasia (Boorman *et al*., 1990).

Bladder tumors were observed in male rats treated with NMA-C8 (Lijinsky *et al.*, 1981), NMA-C10 (Lijinsky *et al*., 1981), NMA-C12 (Lijinsky *et al*., 1981; Lijinsky and Taylor,

1975, 1978) and NMA-C14 exposure (Lijinsky *et al*., 1981) in one or more of the following strains: F344 and SD, and in female F344 rats treated with NMA-C12 (Lijinsky and Taylor, 1975, 1978; Lijinsky *et al*., 1983c).

The bladder tumors were transitional cell papillomas and carcinomas, and undifferentiated sarcomas. Bladder tumors are rare in male rats (Goodman *et al*., 1979; Boorman and Everitt, 2006). In rats, most urinary bladder transitional cell carcinomas arise from papillomas and can be exophytic, endophytic, or both (Boorman *et al*., 1990). The endophytic carcinomas can invade the bladder wall and are generally more malignant. The differentiation of transitional cell carcinoma from papilloma is based primarily on invasion, and then to a lesser extent on growth patterns and cell atypia (Boorman *et al*., 1990). Transitional cell papillomas may progress to malignant carcinomas (McConnell *et al*., 1986).

Hamster

Table 37 lists tumor types which are rare, infrequent or significantly increased in incidence or both resulting from exposure of hamsters to NMAs with various chain lengths. Tumors of the nasal cavity, laryngeal-tracheal-bronchial tract, lung, esophagus, forestomach, liver, bladder, thyroid and digestive system were observed in hamsters.

Table 37. Tumor sites in hamsters treated with NMAs of various alkyl chain lengths

NMA-	Nasal cavity	L-T-B[1]	Lung	Esophagus	Forestomach	Liver	Bladder	Others
C3	**M, F**	**M, F**	**M, F**			**M, F**		**M[2], F[3]**
C4	**M, F**		**M, F**		**M, F**	**M, F**		
C5	**M, F**		**M, F**	**MF[4]**	**M, F**	**M, F**		
C6	**M**		**M, F**		**M, F**	**M, F**	**M, F**	
C7	**M, F**		**M, F**		**M, F**	**M, F**		
C8	**M, F**		**M, F**		**M, F**	**M, F**	**M, F**	
C12	**F**		**M, F**				**M, F**	

[1] Tumors of larynx, trachea, bronchial tract
[2] Thyroid tumors
[3] Described only as digestive system tumors
[4] MF- tumor incidences were reported in males and females combined

Nasal cavity tumors were observed in male and female hamsters treated with NMA-C3 (Pour *et al*., 1974; Lijinsky and Kovatch, 1988; Althoff and Grandjean, 1979), NMA-C4 (Lijinsky and Kovatch, 1988), NMA-C5 (Mirvish *et al*., 1996; Lijinsky and Kovatch,

1988), NMA-C6 (Lijinsky and Kovatch, 1988), NMA-C7 (Lijinsky and Kovatch, 1988), NMA-C8 (Lijinsky and Kovatch, 1988) and NMA-C12 exposure (Ketkar *et al.*, 1981).

Nasal cavity tumors were seen both in the anterior and posterior regions, including nasal cell papillomas, adenomas, adenocarcinomas and carcinomas. Adenocarcinomas may originate either from the lining epithelium or submucosal glands (IARC, 1996). All types of nasal 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." Hamster nasal cavities are composed of four turbinates: atrioturbinates, nasal turbinates, maxillary turbinates, and ethmoturbinates. Atrioturbinates are the most apical part of the nasal cavity and are lined by non-keratinizing squamous epithelium. The nasoturbinates and maxilloturbinates form the intermediate region and are lined by pseudo-stratified respiratory epithelium, containing ciliated, basal, mucous, and neuroendocrine cells. The ethmoturbinates are the most caudal of the nasal cavities, and are made up of four ectoturbinates and three endoturbinates. They are lined by olfactory epithelium, containing basal, olfactory sensory, sustentacular, and neuroendocrine cells. The hamster has a maxillary sinus which extends into the nasal lumen and is coated with respiratory epithelium (IARC, 1996).

Laryngeal, tracheal and bronchial tract (L-T-B) tumor incidences were increased significantly after treatment with NMA-C3 (Pour *et al*., 1974; Althoff and Grandjean, 1979 [malignant tracheal tumors]). These structures are sometimes grouped together because the larynx, trachea and extrapulmonary bronchi are lined by the same types of cells. This pseudo-stratified or one-layered epithelium is made up of ciliated cells and various types of non-ciliated secretory goblet cells. Tumors arising from these areas showed papillary growth lined by cuboidal mucus-secreting or squamous cells, or both. Tumors of the L-T-B tract have been reported occasionally in control hamsters (IARC, 1996).

Lung tumors were observed in hamsters after treatment with the following NMAs: NMA-C3 (Pour *et al*., 1974; Althoff and Grandjean, 1979; Lijinsky and Kovatch, 1988), NMA-C4 (Lijinsky and Kovatch, 1988), NMA-C5 (Mirvish *et al*.,1996; Lijinsky and Kovatch, 1988), NMA-C6 (Lijinsky and Kovatch, 1988), NMA-C7 (Lijinsky and Kovatch, 1988; Rehm and Lijinsky, 1994), NMA-C8 (Lijinsky and Kovatch, 1988) and NMA-C12 (Ketkar *et al*., 1981; Althoff and Lijinsky, 1977).

The histology of the bronchiolo-alveolar region of the lower respiratory tract in hamsters is strikingly different from that of the larynx, trachea and extrapulmonary bronchi; it is a mosaic of attenuated lining cells and cuboidal cells (IARC, 1996). Lung tumors included alveolar/bronchial adenomas and carcinomas, squamous cell carcinomas, adenosquamous carcinomas and mucoepidermoid carcinomas. Squamous cell papillary tumors rarely invade adjacent tissues but may progress to carcinomas (IARC, 1996). Adenosquamous carcinomas originate from squamous differentiation of neoplastic Clara cells or undifferentiated neoplastic cells in bronchioloalveolar carcinomas, particularly those with an acinar adenocarcinomatous pattern (Rehm and Lijinsky, 1994). Lung

tumors are rare in untreated Syrian golden hamsters, with a spontaneous incidence of no more than 0.1-0.5% (IARC, 1996).

Statistically non-significant increases in esophageal squamous cell papillomas and carcinomas were observed in hamsters by i.p. injection to NMA-C5, as newborns, 3-day olds and 38-day olds (Mirvish *et al*., 1996). The spontaneous occurrences of esophageal squamous cell papillomas or carcinomas are infrequent in hamsters (IARC, 1996).

Forestomach tumors were observed in male and female hamsters exposed to NMA-C4 (Lijinsky and Kovatch, 1988), NMA-C5 (Mirvish *et al*., 1996; Lijinsky and Kovatch, 1988), NMA-C6, -C7 and -C8 (Lijinsky and Kovatch, 1988). 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 papillomas of the forestomach listed by IARC were 4.1% in female hamsters and 6.1% in males, and the incidence of squamous cell carcinoma in males was 0.5%. Squamous cell carcinomas may either develop from benign papillomas or evolve directly from atypical epithelium (IARC, 1996).

Liver tumors were observed in both male and female hamsters exposed to NMA-C3 (Pour *et al*., 1974; Lijinsky and Kovatch, 1988), NMA-C4 (Lijinsky and Kovatch, 1988), NMA-C5 (Mirvish *et al*., 1996; Lijinsky and Kovatch, 1988), NMA-C6 (Lijinsky and Kovatch, 1988), NMA-C7 (Lijinsky and Kovatch, 1988), and NMA-C8 (Lijinsky and Kovatch, 1988) and in females exposed to NMA-C6 (Lijinsky and Kovatch, 1988). Liver tumors included vascular tumors (hemangioendotheliomas [hemangiomas] and hemangiosarcomas), biliary/cholangiocellular tumors (cholangiomas and cholangiocarcinomas), hepatocellular adenomas and carcinomas, and histiocytic sarcomas. Similar to rats, hemangiosarcomas commonly metastasize in hamsters, especially to the lungs. Progression from cholangioma to cholangiocarcinoma is likely (IARC, 1996). The incidences of spontaneous epithelial (combined hepatocellular and cholangiocellular) carcinomas in untreated Syrian hamsters were less than 1%, as were the incidences of spontaneous hemangiomas and hemangioendotheliomas (IARC, 1996).

Bladder tumors were observed in male and female hamsters exposed to NMA-C6 (Lijinsky and Kovatch, 1988) and NMA-C12 (Ketkar *et al.*, 1981; Althoff and Lijinsky, 1977 [identified as urinary tract tumors]). Urinary bladder tumors were transitional cell papillomas and carcinomas, and hemangioendotheliomas or hemangiomas. No spontaneously occurring epithelial neoplasms of the hamster urinary bladder have been reported except for two cases of malignant lymphoma (IARC, 1996). Therefore, the occurrences of the above tumor types are considered to be rare in hamsters.

Mouse

Table 38 lists tumor types which are rare or significantly increased in incidence or both resulting from exposure of mice to NMAs with various chain lengths. Tumors of the nasal cavity, tongue, larynx-trachea-bronchial tract, lung, esophagus, forestomach, and liver were observed in mice.

Table 38. Tumor sites in mice treated with NMAs of various alkyl chain lengths

NMA-	Nasal cavity	Tongue	L-T-B[1]	Lung	Esophagus	Forestomach	Liver
C3	F		F	F			F
C5		M		MF[2]	M, F	F[3]	

[1] Tumors of larynx, trachea, bronchial tract
[2] MF- tumor incidences were reported as males and females combined.
[3] Esophagus and forestomach were combined

Nasal cavity tumors were observed in female NMRI mice treated with NMA-C3 (Dickhaus *et al*., 1977), and were mainly squamous cell papillomas and carcinomas. Squamous cell papillomas of the nasal cavity have the potential to progress to malignant carcinomas (McConnell *et al*., 1986; Maronpot, 1999). The nasal anatomic structure and histology of the mouse is similar to that of the rat (Maronpot, 1999). Nasal cavity tumors are considered rare in mice (Haseman *et al*., 1998).

Tumors of the tongue were observed in male C57BL/6 mice exposed to NMA-C5 (Shirai *et al*., 2002a, 2002b), and were mainly papillomas and squamous cell carcinomas. Tongue tumors are considered rare in mice (Haseman *et al*., 1998).

L-T-B tumors were observed in female NMRI mice exposed to NMA-C3 (Dickhaus *et al.*, 1977). Squamous cell carcinomas plus a carcinoma *in situ* and a papilloma were observed in the larynx. Squamous cell carcinomas and papillomas were observed in the trachea. Squamous cell papillomas also occurred in the intrapulmonary large bronchi.

Lung tumors were observed in female NMRI mice exposed to NMA-C3 (Dickhaus *et al.*, 1977) and in male and female Swiss mice exposed to NMA-C5 (Mirvish *et al*., 1996). Lung tumors were mostly adenomas with some incidence of malignant adenocarcinomas and one incidence of squamous carcinoma. Mouse lung adenomas are considered to have the potential of progressing to malignant carcinomas (McConnell *et al*., 1986).

Esophageal and forestomach tumors were observed in male and female Swiss, 101/N, STX/Le, BXH-8, C57BL/6, and Brca mice after treatment with NMA-C5 (Mirvish *et al*., 1996; Kurooka *et al*., 1998; Cao *et al*., 2007; Shirai *et al*., 2002a, 2002b). Esophageal and forestomach tumors are considered rare in mice (Haseman *et al*., 1998). The tumor types in those organs were squamous cell papillomas and carcinomas. Squamous cell papillomas are considered to have the potential to progress to malignant carcinomas (McConnell *et al*., 1986).

Liver tumors were observed in female NMRI mice exposed to NMA-C3 (Dickhaus *et al*., 1977). Most neoplasms were hemangioendotheliosarcomas (hemangiosarcomas).

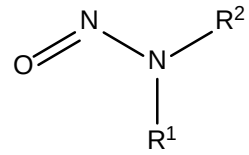
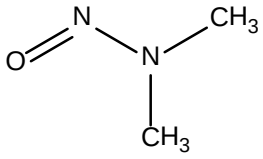
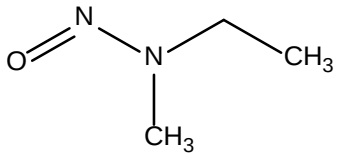
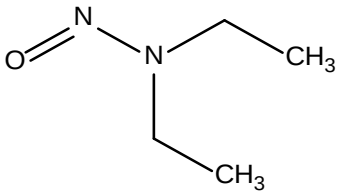
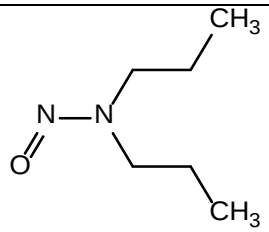
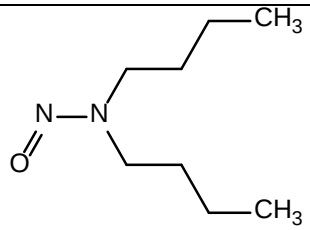
Guinea Pig
Liver tumors were observed in male guinea pigs exposed to NMA-C12. The incidence of hemangioendothelial sarcomas (hemangiosarcomas) was significantly increased (Cardy and Lijinsky, 1980). Hemangiosarcoma is a malignant tumor of blood vessel cells and often metastasizes to other organs such as lungs and spleen. In addition, three cholangiocarcinomas or bile duct carcinomas, one hepatocellular carcinoma, and one pancreatic hemangiosarcoma were also observed (Cardy and Lijinsky, 1980).

3.3.5 Structure – Activity Comparisons

NMAs are tertiary amines that share structural similarities with other carcinogenic alkylated nitrosamines. Three structurally similar N-nitroso-dialkylamine compounds-- N-nitrosodiethylamine (NDEA), N-nitrosodi-*n*-propylamine (NDPA), and N-nitrosodi-*n*-butylamine (NDBA), are shown in Table 39, along with the general structure for NMAs, and NMA-C1 and NMA-C2.

NMAs share close structural similarities with N-nitroso-dialkylamines. All compounds within each group contain a nitroso group, a second N atom, and two alkyl groups. The difference between the dialkylamines and NMAs is that the two alkyl groups in the dialkylamine compounds are identical, making the molecule symmetrical, while for the NMAs one of the alkyl groups is a methyl group, and the length of the second alkyl group may range from one to fourteen (or more) carbons.

Table 39. NMAs and related structures

Chemical (CAS number)	Structure	Cancer Classification	
		Proposition 65	Other
NMAs			
General structure R1= (CH3); R2= (CH2)n		Currently under evaluation	Not Evaluated
NMA-C1 (62-75-9)		Listed	IARC 2A[1] NTP RA[2] EPA B2[3]
NMA- C2 (10595-95-6)		Listed	IARC 2B[1] EPA B2[4]
Related structures			
N-Nitrosodiethylamine (55-18-5) (NDEA)		Listed	IARC 2A[1] NTP RA[2] EPA B2[5]
N-Nitrosodi-*n*-propylamine (621-64-7) (NDPA)		Listed	IARC 2B[1] NTP RA[2] EPA B2[6]
N-Nitrosodi-*n*-butylamine (924-16-3) (NDBA)		Listed	IARC 2B[1] NTP RA[2] EPA B2[7]

[1] IARC, 1987
[2] National Toxicology Program (NTP): reasonably anticipated (RA) to be a human carcinogen (RA) (NTP, 1981)
[3] US EPA, 2002b
[4] US EPA, 2003a; [5]US EPA, 2003c; [6]US EPA, 2002a; [7]US EPA, 2003b

As indicated in Table 39, the two smallest NMAs (NMA-C1 and NMA-C2) and each of the three N-nitroso-dialkylamines (NDEA, NDPA, and NDBA) are classified as carcinogens by IARC, the National Toxicology Program (NTP), and US EPA, and each is on the Proposition 65 list as causing cancer.

Tables 40 and 41 present the target tissue sites that have been observed in rats (Table 40) and hamsters (Table 41) for thirteen NMA compounds (C1 – C12, C14) and the three N-nitroso-dialkylamines (NDEA, NDPA, and NDBA), allowing comparisons across chemicals. As shown in Tables 40 and 41, tumors were observed at multiple sites in rats and hamsters following treatment with each of these chemicals. Moreover, several of the tumor sites were common to many of the NMAs, and many of these sites are also common target tumor sites of NDEA, NDPA and NDBA.

In addition to sharing similar findings of carcinogenic activity in animals, the NMAs and the three N-nitroso-dialkylamines also share positive findings of genotoxic activity. As summarized in Section 3.3.1, all twelve NMAs tested for the ability to alkylate DNA form DNA adducts in rats *in vivo*, all eleven NMAs tested for the ability to induce mutations in bacteria are mutagenic, and all three NMAs tested for the ability to induce mutations in mammalian cells *in vitro* are mutagenic. The most extensively tested NMA, NMA-C1, has been demonstrated to be mutagenic and clastogenic in multiple *in vitro* and *in vivo* assay systems. The evidence for the NDEA, NDPA, and NDBA includes the following:

- NDEA is mutagenic in *Salmonella* and *E. coli*, as well as in mammalian assays *in vitro*. It induces UDS in rat hepatocytes and induces micronuclei formation in human cell lines (CCRIS, 2014).
- NDPA is mutagenic in *Salmonella* and mammalian assays *in vitro* (CCRIS, 2014).
- NDBA is mutagenic in *Salmonella* and *E. coli*, and mammalian assays *in vitro* (CCRIS, 2014).

In summary, tumors were observed in multiple species and at multiple sites following treatment with NMAs and three close structural analogues, and many tumor sites are shared amongst species and chemicals. All tested compounds are mutagenic in *Salmonella* and several are positive in mammalian assay systems for genotoxicity.

Table 40. Structure Activity Comparisons: Target tumor sites in rats

Chemical	Nasal cavity (r)	Tongue (r)	Oro-Pharynx (r)	Lung (r)[1]	Esoph-agus (r)	Fore-stomach (r)	Liver	Kidney (r)	Bladder (r)	Others
NMAs										
C1[2]	x[3]			x			x[3]	x[3]		Bile duct
C2[4]	x			x			x			
C3	x[3]	x	x		x[3]	x	x[3]			Epiglottis (r)
C4	x	x	x		x[3]	x				
C5	x[3]	x[3]			x[3]	x				Trachea (r)[3]
C6	x	x		x[5]	x[5]	x	x[5]			
C7	x	x		x[5]	x[5]		x[5]			
C8	x[3]			x[3]			x[3,6]		x[3]	Trachea (r)[3]
C9	x			x[3]			x[3,7]			
C10	x			x[3]		x			x[3]	
C11				x	x	x	x[7]			
C12				x[3]		x[3]	x		x[3]	Pancreas[3]
C14				x				x	x[3]	
Related compounds										
NDEA[2]	x				x[3]		x[3]	x		Leukemia
NDPA[2]	x[3]	x		x	x		x[3]			
NDBA[8]		x		x	x[3]		x[3]		x[3]	

"x" denotes observation of tumors, (r) = rare tumors
[1] Adenocarcinoma and squamous cell carcinoma of the lung are rare in rats.
[2] IARC (1978)
[3] Statistically significant ($p<0.05$) increases of tumor incidence by Fisher pairwise comparison (conducted by OEHHA)
[4] Lijinsky *et al*. (1991b); Murai *et al*. (1991)
[5] No concurrent control, but tumor incidence ≥90%
[6] Hemangiosarcoma of the liver observed in males, this tumor type is rare in male rats
[7] Cholangiocarcinoma of the liver observed in males, this tumor type is rare in male rats
[8] IARC (1974)

Table 41. Structure Activity Comparisons: Target tumor sites in hamsters

Chemical	Nasal cavity (r)	Lung (r)	Esophagus (infrequent)	Forestomach (r[1])	Liver (r)	Bladder (r)	Others
MNAs							
C1[2]	x				x[4]		
C2[3]	x[4]				x[4]		
C3	x[4]	x[4]			x[4]		Digestive system[4] and thyroid[4] with *in utero* exposure, larynx-trachea-bronchi[4]
C4	x[4]	x[4]		x[4]	x[4]		
C5	x[4]	x[4]	x	x[4]	x		
C6	x	x[4]		x[4]	x[4]	x	
C7	x[4]	x[4]		x[4]	x[4]		
C8	x	x[4]		x[4]	x[4]	x	
C9	No studies identified						
C10	No studies identified						
C11	No studies identified						
C12	x	x[4]				x[4]	
C14	No studies identified						
Related compounds							
NDEA[2]	x[4]	x[4]	x[4]	x[4]	x[4]		
NDPA[2]	x[4]	x[4]					
NDBA[5]		x[4]		x[4]		x[4]	

"x" denotes observation of tumors, (r) = rare tumors
[1] Forestomach squamous cell carcinomas are rare in males
[2] IARC (1978)
[3] Lijinsky and Kovatch (1988)
[4] Statistically significant ($p<0.05$) increases of tumor incidence by Fisher pairwise comparison (conducted by OEHHA)
[5] IARC (1974)

4. MECHANISMS

Tumors have been seen in multiple species and strains at multiple sites after treatment with NMAs (Tables 5-9, 13-31). A body of evidence suggests that NMAs act via a genotoxic mechanism or mechanisms. As discussed in Section 3.3.1 (Genotoxicity), all twelve NMAs tested form DNA adducts in rats *in vivo* (Table 34), eleven NMAs have been tested and shown to induce mutations in bacteria (Table 32, CCRIS, 2014), three have been tested and shown to induce mutations in mammalian cells *in vitro* (Table 33, CCRIS, 2014; US EPA, 2003a) and the most extensively tested NMA, NMA-C1, has been demonstrated to be mutagenic and clastogenic in multiple *in vitro* and *in vivo* assay systems (CCRIS, 2014).

NMAs, like many other nitrosamines, require metabolic activation for genotoxic and carcinogenic activity. Activation of nitrosamines is thought to occur primarily via mixed function oxidase-catalyzed hydroxylation. Hydroxylation of NMAs at the carbon alpha to the nitroso group results in formation of carbonyl compounds, such as the carcinogen formaldehyde, and an electrophilic intermediate (*i.e*., an alkyl-diazonium ion). The alkyl-diazonium ion has been proposed to react with DNA or other nucleophilic molecules giving rise to alkylation products and one molecule of N_2 (Magee, 1980; Bellec *et al*., 1996; Kawanishi *et al*., 1992; Farrelly and Stewart, 1982; Lorr *et al*., 1982; Lee *et al*., 1989). Hydroxylation of NMAs at a non-alpha carbon ultimately yields a series of carboxylated and hydroxylated products, including the genotoxic carcinogen N-nitrososarcosine (for NMA-C2 and NMAs with longer alkyl chains) and three other genotoxic compounds that are tumorigenic in animals: MOP (for NMA–C3 and NMAs with longer alkyl chains), 4-hydroxy-nitrosomethyl-*n*-butylamine and N-nitrosomethyl-3-carboxypropylamine (for NMA-C4 and NMAs with longer alkyl chains).

In summary, there is strong evidence that NMAs act through one or more genotoxic mechanisms, as a result of metabolic activation via cytochrome P450 enzymes to form mutagenic and carcinogenic metabolites.

5. REVIEWS BY OTHER AGENCIES

NMAs as a chemical group have not been classified as to their potential carcinogenicity by the U.S. EPA, the U.S. Food and Drug Administration, NTP, the National Institute for Occupational Safety and Health, or IARC. The two smallest NMAs (NMA-C1 and NMA-C2), which have been listed under Proposition 65 as causing cancer since October 1, 1987 and October 1, 1989, respectively, have been classified by the U.S. EPA (both as Group B2: probable human carcinogen), NTP (both as “reasonably anticipated to be a human carcinogen”), and IARC (NMA-C1 as Group 2A: probably carcinogenic to humans; NMA-C2 as Group 2B: possibly carcinogenic to humans).

6. SUMMARY AND CONCLUSION

6.1 Summary of Evidence

No epidemiology studies were identified that investigated the risk of cancer associated with exposure to NMAs.

Evidence for carcinogenicity of NMAs comes from multiple animal studies in which significant increases in malignant and combined malignant and benign tumors, including rare tumors, have been observed. Tumors were observed at multiple sites, in multiple species and strains, by various routes of exposure, and tumor sites were shared amongst species and across NMAs. Specifically, NMAs have been tested in rats, hamsters, mice and guinea pigs. Rats were exposed via drinking water, gavage, *s.c.* injection, *i.p.* injection, intravesicular injection into the bladder, and intramuscular injection. Hamsters were exposed via drinking water, gavage, *s.c.* injection, *i.p.* injection and *in utero* exposure. Mice were exposed via *s.c.* injection and *i.p.* injection. Guinea pigs were exposed by gavage. The main results of the bioassays for NMA-C3 through NMA-C14 are summarized below[3] and a summary of the target tumor sites for NMA-C1 through NMA-C14 by species is presented in Table 42.

- NMA-C3: Statistically significant increases in the incidence of nasal cavity, esophageal and liver tumors, and increases in the incidence of rare tumors in the tongue, epiglottis, oropharynx and forestomach in rats. Statistically significant increases in the incidence of nasal cavity, larynx-trachea-bronchial tract, lung, thyroid, liver, and digestive system tumors in hamsters. Statistically significant increases in the incidence of nasal cavity, larynx-trachea-bronchial tract, lung and liver tumors in mice.
- NMA-C4: Statistically significant increases in the incidence of esophageal tumors, and increases in the incidence of rare tumors in the nasal cavity, tongue, oropharynx and forestomach in rats. Statistically significant increases in the incidence of nasal cavity, lung, forestomach and liver tumors in hamsters.
- NMA-C5: Statistically significant increases in the incidence of nasal cavity, tongue, and esophageal tumors, and increases in the incidence of rare tumors in the trachea and forestomach in rats. Statistically significant increases in the incidence of nasal cavity, lung, and forestomach tumors, and increases in the incidence of rare tumors in the liver and infrequent tumors in the esophagus in hamsters. Statistically significant increases in the incidence of lung, esophageal and combined esophageal and forestomach tumors, and increases in the incidence of rare tongue tumors in mice.

[3]NMA-C1 and NMA-C2 induce tumors in animals, are genotoxic, and are listed under Proposition 65 as causing cancer.

- NMA-C6: Greater than 90% incidence of liver tumors, and increases in the incidence of rare tumors in the nasal cavity, tongue, lung, esophagus, and forestomach in rats. Statistically significant increases in the incidence of lung, forestomach and liver tumors, and increases in the incidence of rare tumors in the nasal cavity and bladder in hamsters.
- NMA-C7: Greater than 90% incidence of liver tumors, and increases in the incidence of rare tumors in the nasal cavity, tongue, lung, and esophagus in rats. Statistically significant increases in the incidence of nasal cavity, lung, forestomach and liver tumors in hamsters.
- NMA-C8: Statistically significant increases in the incidence of nasal cavity, trachea, lung, liver and bladder tumors in rats. Statistically significant increases in the incidence of lung, forestomach and liver tumors, and increases in the incidence of rare tumors in the nasal cavity and bladder in hamsters.
- NMA-C9: Statistically significant increases in the incidence of lung and liver tumors, and increases in the incidence of rare tumors in the nasal cavity in rats.
- NMA-C10: Statistically significant increases in the incidence of lung and bladder tumors, and increases in the incidence of rare tumors in the nasal cavity and forestomach in rats.
- NMA-C11: Increases in the incidence of rare tumors in the lung, esophagus, forestomach and liver in rats.
- NMA-C12: Statistically significant increases in the incidence of lung, forestomach, pancreatic and bladder tumors, and increases in the incidence of rare tumors of the esophagus in rats. Statistically significant increases in the incidence of lung and bladder tumors, and increases in the incidence of rare tumors in the nasal cavity in hamsters. Statistically significant increases in the incidence of hemangiosarcomas in guinea pigs.
- NMA-C14: Statistically significant increases in the incidence of bladder tumors, and increases in the incidence of rare tumors in the lung and kidney in rats.

Table 42. Observations of statistically significant tumor sites and increased rare tumor sites in rats, hamsters, mice and guinea pigs[1] exposed to NMAs

	Target tumor site	Nasal cavity			Tongue			Oro-Pharynx			Lung			Esophagus			Fore-stomach			Liver			Kidney			Bladder		
	Species[2]	R (r)	H (r)	M (r)	R (r)	H	M (r)	R (r)	H	M	R (r)[3]	H (r)	M	R (r)	H (i)	M (r)	R (r)	H (r)[4]	M (r)	R (r)[5]	H (r)	M	R (r)	H	M	R (r)	H (r)	M
NMA-	C1[6,7]	x*	x								x		x							x*	x*	x	x*		x			
	C2[6]	x	x*								x									x	x*							
	C3[8]	x*	x*	x*	x			x				x*	x*	x*			x			x*	x*	x*						
	C4	x	x*	NT	x		NT	x		NT		x*	NT	x*		NT	x	x*	NT		x*	NT			NT			NT
	C5[9]	x*	x*		x*		x					x*	x*	x*	x	x*	x	x*	x[10]		x							
	C6	x	x	NT	x		NT			NT	x[11]	x*	NT	x[11]		NT	x	x*	NT	x[11]	x*	NT			NT		x	NT
	C7	x	x*	NT	x		NT			NT	x[11]	x*	NT	x[11]		NT		x*	NT	x[11]	x*	NT			NT			NT
	C8[12]	x*	x	NT			NT			NT	x*	x*	NT			NT		x*	NT	x*	x*	NT			NT	x*	x	NT
	C9	x	NT	NT		NT	NT		NT	NT	x*	NT	NT		NT	NT		NT	NT	x*	NT	NT		NT	NT		NT	NT
	C10	x	NT	NT		NT	NT		NT	NT	x*	NT	NT		NT	NT	x	NT	NT		NT	NT		NT	NT	x*	NT	NT
	C11		NT	NT		NT	NT		NT	NT	x	NT	NT	x	NT	NT	x	NT	NT	x[13]	NT	NT		NT	NT		NT	NT
	C12[14]		x	NT			NT			NT	x*	x*	NT	x		NT	x*		NT			NT			NT	x*	x*	NT
	C14		NT	NT		NT	NT		NT	NT	x	NT	NT		NT	NT		NT	NT		NT	NT	x	NT	NT	x*	NT	NT

"x" denotes observation of tumors, * statistically significant ($p < 0.05$) increases of tumor incidence by Fisher pairwise comparison (conducted by OEHHA); (r) = rare tumor; (i) = infrequent tumor; NT = not tested

[1] Hemangiosarcomas* of the liver were observed in male guinea pigs exposed to NMA-C12

[2] R (rat); H (hamster); M (mouse)

[3] Adenocarcinoma and squamous cell carcinoma of the lung are rare in rats.

[4] Squamous cell carcinoma of the forestomach are rare in male hamsters

[5] Cholangiocarcinoma and hemangiosarcoma of the liver are rare in rats.

[6] IARC (1978)

[7] Bile duct [rat]

[8] Epiglottis (r) [rat]; digestive system* and thyroid* with *in utero* exposure, larynx-trachea-bronchial tract * [hamster]; larynx-trachea-bronchial tract* [mouse]

[9] Trachea* (r) [rat]

[10] Esophagus and forestomach combined (Cao *et al*., 2007)

[11] No concurrent control, but tumor incidence ≥ 90%

[12] Trachea* (r), liver hemangiosarcomas (r) [rat]

[13] Cholangiocarcinoma of the liver observed in males, this tumor type is rare in male rats, [14] Pancreas* [rat],

Additional evidence for the carcinogenicity of NMAs stems from genotoxicity studies. All NMAs tested (NMA-C1 – C4, NMA-C6 – C12) have demonstrated positive mutagenicity in at least one *Salmonella* test strain, generally in the presence of exogenous metabolic activation. NMA-C1, -C3 and -C4 were tested and found to be mutagenic in *E. coli*. In mammalian systems NMA-C1 – C3 have demonstrated mutagenicity *in vitro* in V79 Chinese hamster lung cells. The most well-studied NMA, NMA-C1, has tested positive for a number of additional genotoxicity endpoints, including induction of mutations in rodents *in vivo*, UDS in human and rodent cells *in vitro*, and DNA strand breaks, sister chromatid exchange, chromosomal aberrations, and micronuclei formation in rodents *in vitro* and *in vivo*.

NMAs have also been shown to form DNA, RNA, and protein adducts in target tissues *in vivo* and *in vitro*. Specifically, NMA-C4 has been shown to form methyl adducts with RNA and protein *in vivo* in rat liver, kidney, pancreas and spleen. NMA-C1 – C12 have all been shown to form DNA adducts in rat liver *in vivo*. DNA methyl adducts have been measured in several additional target tissues in rats exposed to NMAs, including lung (NMA-C1 – C5, NMA-C10 – C12), esophagus (NMA-C3 – C5), kidney (NMA-C1 – C5), and nasal cavity, trachea and forestomach (NMA-C5).

NMAs require metabolic activation for genotoxic and carcinogenic activity. Metabolism studies indicate that NMAs are metabolized in a similar manner (across chemicals), forming various carboxylated and hydroxylated products, and share several common metabolites, including some that are carcinogenic and genotoxic. The carcinogenic metabolites identified are the Proposition 65 listed genotoxic carcinogens N-nitrososarcosine and formaldehyde, as well as the genotoxic animal tumorigens N-nitrosomethyl-3-carboxypropylamine, 4-hydroxy-nitrosomethylbutylamine, and MOP. *In vitro* metabolism studies with human and rodent tissue preparations reveal common metabolites and similar activation mechanisms across NMAs and across species.

NMAs share structural similarities with other alkylated nitrosamines, including the N-nitroso-dialkylamines NDEA, NDPA, and NDBA. These three N-nitroso-dialkylamines are listed under Proposition 65 and are classified as carcinogens by IARC, NTP, and US EPA, as are NMA-C1 and NMA-C2[4]. The three N-nitroso-dialkylamines each induce tumors, including rare tumors, at multiple sites, in several species, and share common target sites amongst each other and with the NMAs. In addition to sharing similar findings of carcinogenic activity in animals, the NMAs and the three N-nitroso-dialkylamines also share positive findings of genotoxic activity. Specifically, NDEA is mutagenic in *Salmonella* and *E. coli*, as well as in mammalian assays *in vitro* and induces UDS in rat hepatocytes and micronuclei formation in human cell lines. NDPA is

[4] NMA-C2 has not been classified by NTP.

mutagenic in *Salmonella* and mammalian assays *in vitro*. NDBA is mutagenic in *Salmonella* and *E. coli*, and in mammalian assays *in vitro*.

A body of evidence suggests that NMAs act via a genotoxic mechanism or mechanisms.

6.2 Conclusion

The evidence for the carcinogenicity of NMAs as a group comes primarily from more than 90 experiments in animals with positive tumor findings. These studies were conducted in rats, hamsters, mice, and guinea pigs by various routes of exposure. Tumors were observed following treatment with all thirteen of the NMAs that have been tested for carcinogenicity in animals, including the two NMAs currently listed under Proposition 65 (NMA-C1 and NMA-C2). Tumors were observed in multiple species or strains for many of the NMAs. Several of the observed tumors are rare, including tumors of the nasal cavity, tongue, oropharynx, esophagus, forestomach, kidney, and bladder in rats; and tumors of the nasal cavity, lung, liver, and bladder in hamsters.

Positive findings from genotoxicity and DNA adduct studies indicate that NMAs are likely to operate through genotoxic mechanisms, and that metabolic activation by cytochrome P450 enzymes is required for activity. All twelve NMAs tested for the ability to alkylate DNA form DNA adducts in rats *in vivo*, all eleven NMAs tested for the ability to induce mutations in bacteria are mutagenic, and all three NMAs tested for the ability to induce mutations in mammalian cells *in vitro* are mutagenic. The most extensively tested NMA, NMA-C1, has been demonstrated to be mutagenic and clastogenic in multiple *in vitro* and *in vivo* assay systems.

NMAs are metabolized in a similar manner across chemicals and species, and common metabolites have been observed amongst all NMAs. Two of these common metabolites are listed under Proposition 65 as causing cancer, and are genotoxic (N-nitrososarcosine and formaldehyde). Three others are genotoxic and induce tumors in animals (*i.e.*, N-nitrosomethyl-3-carboxypropylamine, 4-hydroxy-nitrosomethyl-*n*-butylamine, and MOP). The NMAs and three structurally similar carcinogenic N-nitroso-dialkylamines listed under Proposition 65 share target tumor sites amongst species and chemicals, as well as positive findings of genotoxicity.

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Appendix A: Parameters for Literature Searches on the Carcinogenicity of "N-Nitrosomethyl-*n*-alkylamines"

Searches of the literature on the carcinogenicity of "N-Nitrosomethyl-*n*-alkylamines" were conducted under contract by the University of California at Berkeley (Charleen Kubota, M.L.I.S.). The goal was to identify peer-reviewed open source and proprietary journal articles, print and digital books, reports and gray literature that potentially reported relevant toxicological and epidemiological information on the carcinogenicity of these chemicals. The search sought to specifically identify all literature relevant to the assessment of evidence on cancer.

Databases

The literature search utilized the following search platforms/database vendors:

- PubMed (National Library of Medicine)
- EMIC (National Library of Medicine)
- SciFinder®: CAS (Chemical Abstracts Service)
- TOXNET (National Library of Medicine): Toxicology Literature Online (TOXLINE), Genetic Toxicology Data Bank (GENE-TOX)
- Web of Knowledge: BIOSIS Previews®, Web of Science® (Thomson-Reuters, Inc.)

Search Process

Relevant subject terms were entered into the PubMed Search Builder to execute a search.

The following is a typical chemical search strategy used to search PubMed:
("*chemical name*" [MeSh] OR CAS *registry number*[RN]) AND ("bioassay"[MeSh] OR "carcinogenicity"[MeSh] OR "cancer"[MeSh] OR "tumor"[MeSH]) OR "neoplasm"[MeSH]) OR "genotoxicity"[MeSH]) OR "mutagenicity"[MeSH]) OR "metabolism"[MeSH]) OR "absorbtion"[MeSH]) OR "pharmacokinetics"[MeSH]) OR "structure activity relationship"[MeSH])

In PubMed, MeSH (Medical Subject Headings) terms at the top of hierarchical lists of subject headings are automatically "exploded" in a search to retrieve citations with more specific MeSH terms. For example, the heading "carcinogenicity" includes broad conditions that are related to cancer induction in animals and humans.

Additional databases listed above were then searched. The search strategies were tailored according to the search features unique to each database. Web of Science, for example, was searched by entering chemical terms and refining the search by applying Web of Science categories Toxicology and/or Public, Environmental and Occupational Health. The search term used includes either the CAS registry number or the chemical name and its available synonyms. Sometimes other databases not listed here were searched as needed.

Additional focused searches were performed by OEHHA as needed. Relevant literature was also identified from citations in individual articles.