

# **Evidence on the Carcinogenicity of N-Nitrosomethyl-*n*-Alkylamines (NMAs)**

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Reproductive and Cancer Hazard Assessment Branch**

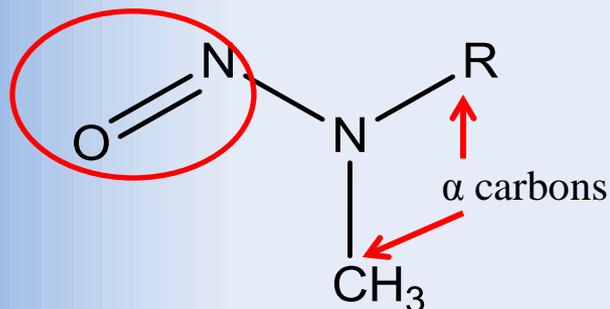


# Overview

- Chemical identity, use and occurrence
- Carcinogenicity evidence
  - Carcinogenicity studies in animals
  - Other relevant data
- Possible mechanisms of action
- Reviews by other agencies
- Summary



# N-Nitrosomethyl-*n*-Alkylamines (NMAs)



R= alkyl group  $(\text{CH}_2)_{(n-1)}\text{CH}_3$

## Chemical Structure

- NMAs contain a *nitroso* group and a second nitrogen to which a methyl and a linear alkyl group are attached.

## Occurrence & Uses

- NMAs have been detected in personal care products and household cleaning products.
- NMAs are not intentionally added to these products but can be formed by the reaction of nitrite with amine compounds.



# NMAs Studied in Animals

- N-Nitroso-dimethylamine (NMA-C1) Listed on Proposition 65
- N-Nitrosomethyl-ethylamine (NMA-C2) Listed on Proposition 65
- 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)
- N-Nitrosomethyl-*n*-tetradecylamine (NMA-C14)



# NMAs: Carcinogenicity Evidence

- No human epidemiology studies identified
- Carcinogenicity studies in animals
  - More than 90 bioassays
- Other relevant data
  - Genotoxicity
  - Pharmacokinetics and metabolism
  - Structure activity comparisons



# Animal Carcinogenicity Studies

## NMAs C3 - C14

Species	No. of strains	No. of routes	No. of studies
Rat	5	7	58
Hamster	2	4	26
Mouse	8	3	13
Guinea Pig	1	1	1



# Animal Studies - Overview

- Various dose levels, exposure and study durations
- Small group sizes ( $n \leq 20$ )
- Less than lifetime exposure and study durations
- Some studies lack concurrent controls
- Similar tumor findings reported in different laboratories
- Treatment-related tumors observed at multiple sites across species, strain, sex, or age at exposure



# Tumor Findings - Overview

- **Multiple tumor sites**
  - Rats, hamsters, mice
- **Major tumor sites:**
  - Nasal cavity
  - Tongue
  - Larynx, trachea, bronchial tract (L-T-B)
  - Lung
  - Esophagus
  - Forestomach
  - Liver
  - Bladder



# Target Tumor Sites in Rats

NMA-	Nasal cavity	Tongue	Lung	Esophagus	Forestomach	Liver	Bladder
C3	M, F	F		M, F	F	M, F	
C4	M, F	M, F		M, F	M, F		
C5	M, F	M		M, F	MF <sup>1</sup> , M		
C6	M	M	M	M	M	M	
C7	M	M	M	M		M	
C8	M		M			M	M
C9	M		M			M	
C10	M		M		M		M
C11			M	M	M	M	
C12			M, F	F	M		M, F
C14			M				M

<sup>1</sup> MF- tumor incidences were reported in males and females combined



# Target Tumor Sites in Hamsters

NMA-	Nasal cavity	L-T-B <sup>1</sup>	Lung	Esophagus	Forestomach	Liver	Bladder
C3	M, F	M, F	M, F			M, F	
C4	M, F		M, F		M, F	M, F	
C5	M, F		M, F	MF <sup>2</sup>	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

<sup>1</sup> Tumors of larynx, trachea, bronchial tract

<sup>2</sup> MF- tumor incidences were reported in males and females combined



# Target Tumor Sites in Mice

NMA-	Nasal cavity	Tongue	L-T-B <sup>1</sup>	Lung	Eso-phagus	Fore-stomach	Liver
C3	F		F	F			F
C5		M		MF <sup>2</sup>	M, F	F <sup>3</sup>	

<sup>1</sup> Tumors of larynx, trachea, bronchial tract

<sup>2</sup> MF- tumor incidences were reported as males and females combined.

<sup>3</sup> Esophagus and forestomach tumors were combined



# Rare Tumors

- *Rats*: nasal cavity, tongue, oropharynx, lung, esophagus, forestomach, liver cholangiocarcinoma, kidney, and bladder
- *Hamsters*: nasal cavity, lung, forestomach, liver (hepatocellular, cholangiocellular, hemangioma and hemangiosarcoma), and bladder
- *Mice*: nasal cavity, tongue, esophagus, and forestomach



# Statistically Significant or Rare Tumor Sites

	Nasal cavity			Tongue		Oro-pharynx	Lung			Esophagus			Fore-stomach			Liver			Kidney		Bladder		
	R	H	M	R	M	R	R	H	M	R	H (i)	M	R	H	M	R	H	M	R	M	R	H	
C1	X*	X					X		X*							X*	X*	X*	X*	X*			
C2	X	X*					X									X*	X*						
C3	X*	X*	X*	X		X		X*	X*	X*			X			X*	X*	X*					
C4	X	X*	NT	X	NT	X		X*	NT	X*		NT	X	X*	NT		X*	NT			NT		
C5	X*	X*		X*	X			X*	X*	X*	X	X*	X	X*	X		X						
C6	X	X	NT	X	NT		X1	X*	NT	X1		NT	X	X*	NT	X1	X*	NT			NT	X	
C7	X	X*	NT	X	NT		X1	X*	NT	X1		NT		X*	NT	X1	X*	NT			NT		
C8	X*	X	NT		NT		X*	X*	NT			NT		X*	NT	X*	X*	NT			NT	X*	X
C9	X	NT	NT		NT		X*	NT	NT		NT	NT		NT	NT	X*	NT	NT			NT	NT	
C10	X	NT	NT		NT		X*	NT	NT		NT	NT	X	NT	NT		NT	NT			NT	X*	NT
C11		NT	NT		NT		X	NT	NT	X	NT	NT	X	NT	NT	X	NT	NT			NT	NT	
C12		X	NT		NT		X*	X*	NT	X		NT	X*		NT			NT			NT	X*	X*
C14		NT	NT		NT		X	NT	NT		NT	NT		NT	NT		NT	NT	X	NT	X*	NT	



Rare	i Infrequent	X Tumor observed	X* p < 0.05	X1 No concurrent control, but tumor incidence ≥ 90%	NT Not tested
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# Genotoxicity

- Mutagenicity assays *in vitro*
  - All NMAs tested are mutagenic in bacterial assays (NMA-C1 to -C4; -C6 to -C12)
  - All NMAs tested are mutagenic in mammalian cells (NMA-C1 to -C3)
- DNA adduct studies in rats *in vivo*
  - All NMAs tested form DNA adducts (NMA-C1 to -C12)



# Pharmacokinetics & Metabolism

- *In vivo* ADME studies in rats
- *In vitro* studies with tissue preparations or microsomes from humans, rats, mice, hamsters, or guinea pigs



# Pharmacokinetics & Metabolism

- NMAs are rapidly absorbed, distributed, metabolized and eliminated within 24 hours, following oral exposure or *ip* injection in rats
- NMAs are absorbed to a limited extent *in vitro* using human skin (0.75% of applied dose after 48 h)
- Cytochrome P450 enzymes are required for activation
  - $\alpha$ -oxidation; diazonium ions
  - other oxidation products; nitrite
- Similar metabolism across species
- Common metabolites formed across NMAs
  - carcinogenic and mutagenic metabolites



# Carcinogenic and Genotoxic Metabolites

- Formaldehyde (Proposition 65 carcinogen)
- N-Nitrososarcosine (Proposition 65 carcinogen)
- 4-Hydroxy-nitrosomethyl-*n*-butylamine
- N-Nitrosomethyl-3-carboxypropylamine
- N-Methyl-nitroso-2-oxopropylamine (MOP)



# Proposed Routes of NMA-C4 Metabolism in Rats

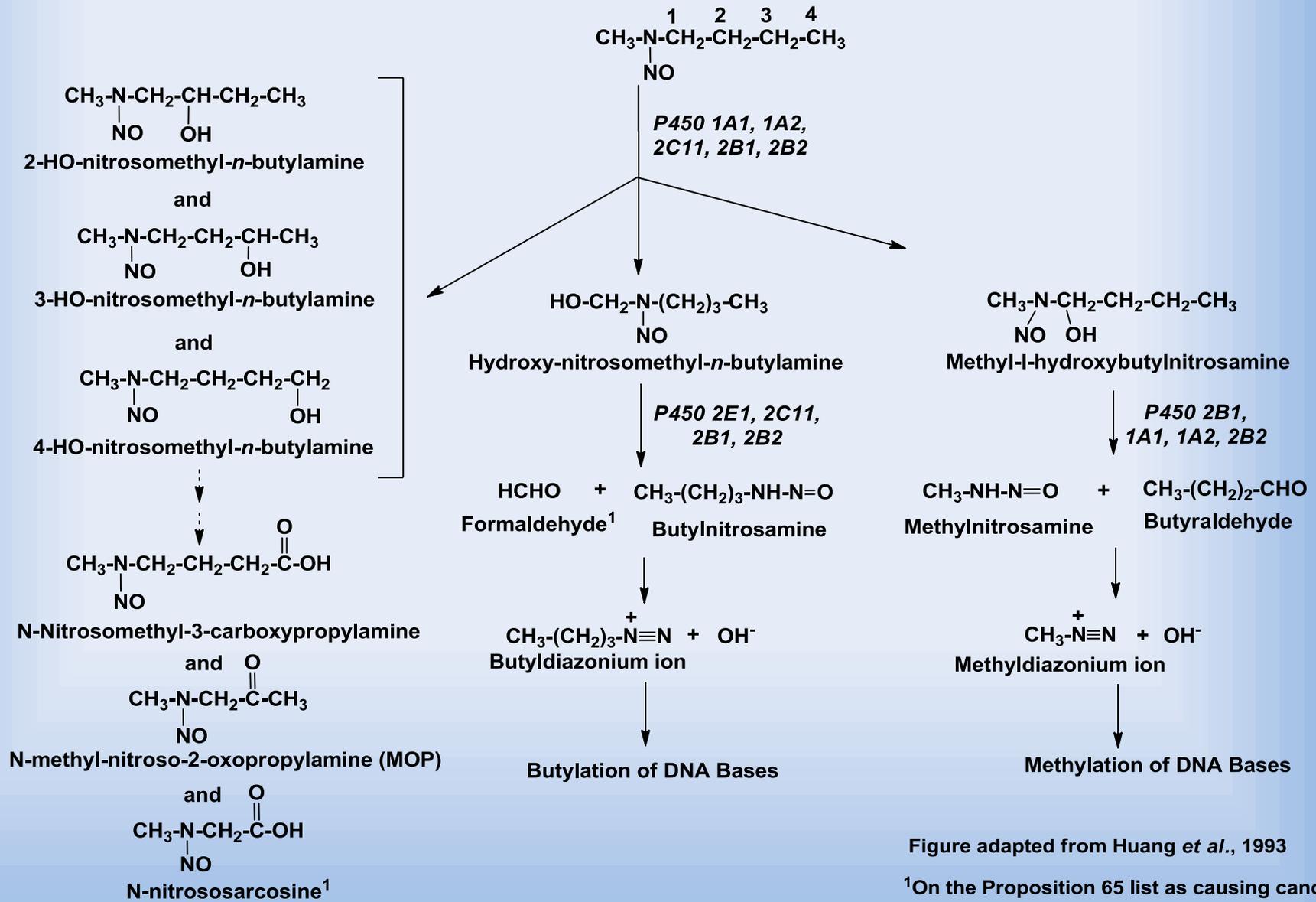
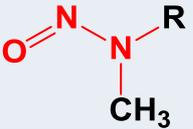
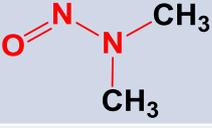
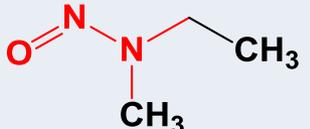
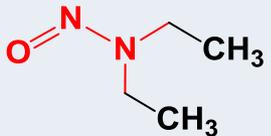
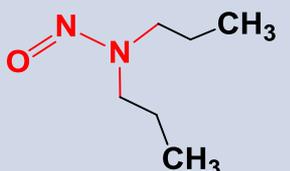
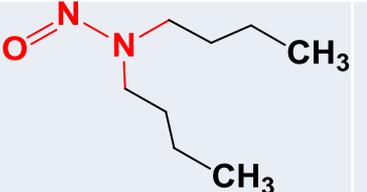


Figure adapted from Huang *et al.*, 1993

<sup>1</sup>On the Proposition 65 list as causing cancer



# Structure-Activity Comparisons

NMA Structures		Cancer Classification	
		Proposition 65	Other
NMA general structure		<b>Currently under evaluation</b>	Not evaluated
NMA-C1		<b>Listed</b>	IARC 2A; NTP RA EPA B2
NMA-C2		<b>Listed</b>	IARC 2B EPA B2
<b>Related Structures</b>			
N-nitrosodiethylamine		<b>Listed</b>	IARC 2A; NTP RA EPA B2
N-nitrosodipropylamine		<b>Listed</b>	IARC 2A; NTP RA EPA B2
N-nitrosodibutylamine		<b>Listed</b>	IARC 2A; NTP RA EPA B2



# SAR - Target Tumor Sites - Rat

Chemical	Nasal cavity (r)	Tongue (r)	Oro- Pharynx (r)	Lung (r)	Eso- phagus (r)	Fore- stomach (r)	Liver	Kidney (r)	Bladder (r)
<b>NMAs</b>									
C1	X*			X			X*	X*	
C2	X			X			X		
C3	X*	X	X		X*	X	X*		
C4	X	X	X		X*	X			
C5	X*	X*			X*	X			
C6	X	X		X	X	X	X		
C7	X	X		X	X		X		
C8	X*			X*			X*		X*
C9	X			X*			X*		
C10	X			X*		X			X*
C11				X	X	X	X		
C12				X*		X*	X		X*
C14				X				X	X*
<b>Related compounds</b>									
NDEA	X				X*		X*	X	
NDPA	X*	X		X	X		X*		
NDBA		X		X	X*		X*		X*

\* = p<0.05



# SAR - Target Tumor Sites - Hamster

Chemical	Nasal cavity (r)	Lung (r)	Esophagus (infrequent)	Forestomach (r)	Liver (r)	Bladder (r)
<b>NMAs</b>						
C1	X				X*	
C2	X*				X*	
C3	X*	X*			X*	
C4	X*	X*		X*	X*	
C5	X*	X*	X	X*	X	
C6	X	X*		X*	X*	X
C7	X*	X*		X*	X*	
C8	X	X*		X*	X*	X
C9				NT		
C10				NT		
C11				NT		
C12	X	X*				X*
C14				NT		
<b>Related compounds</b>						
NDEA	X*	X*	X*	X*	X*	
NDPA	X*	X*				
NDBA		X*		X*		X*

\* = p<0.05



# Possible Mechanisms of Action

## NMAs act via genotoxic mechanisms

- Mutagenic in bacterial systems
- Mutagenic in mammalian cells
- CYP450 activation
  - Electrophilic intermediates (*i.e.*, an alkyl-diazonium ion)
  - DNA alkylation
  - Formation of carcinogenic and genotoxic metabolites



# Reviews by Other Agencies

- NMA-C1 and NMA-C2
  - U.S. EPA (both as Group B2: probable human carcinogen)
  - NTP (both as “reasonably anticipated to be a human carcinogen”)
  - IARC (NMA-C1 as Group 2A: probably carcinogenic to humans; NMA-C2 as Group 2B: possibly carcinogenic to humans)
- All other NMAs have not been classified



# Summary of Evidence

- Positive evidence from over 90 animal studies
  - Most studies have small group sizes ( $n \leq 20$ ) with a range of dose levels, exposure and study durations
  - Several studies lack concurrent controls; but NMAs have been tested in different laboratories, with similar tumor findings reported across studies
  - Tumors were observed with all NMAs tested
  - Positive tumor findings with multiple exposure routes
  - Significant increases in tumors were observed in multiple species, strains and at multiple sites
  - Many rare tumor sites and tumor types
  - Common tumor sites across species & NMAs



# Summary of Evidence, continued

- Positive genotoxicity studies
- Formation of DNA adducts *in vivo*
- Similar metabolism across chemicals and species
  - Formation of carcinogenic and genotoxic metabolites
- NMAs share common tumor sites with structurally similar carcinogens

