Evidence on the Carcinogenicity of N-Nitrosohexamethyleneimine

Carcinogen Identification Committee

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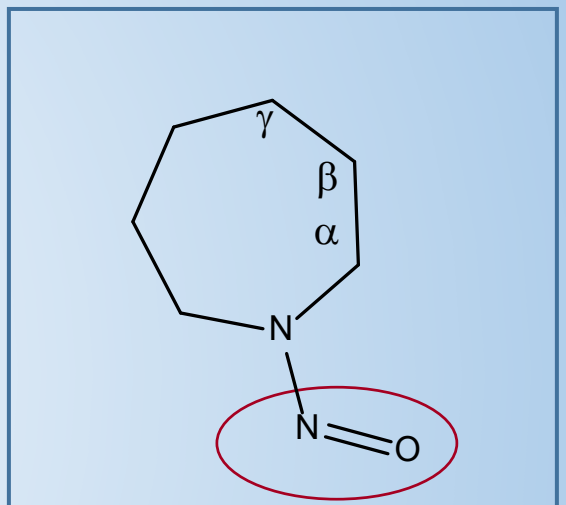
N-Nitrosohexymethyleneimine (NHEX)

- Heterocyclic nitrosamine formed by a secondary amine and a nitrosating agent
- Uses: industrial process or an explosive in ejector seats of military fighter jets
- Reviews by other entities
  - European Chemicals Agency (ECHA): 1B carcinogen



CAS NO: 932-83-2

$C_6H_{12}N_2O$

Carcinogenicity Studies

- Human: None
- Animal: 33 bioassays

Species	Routes of administration	Strains	Experiments
Mouse	2	8	15
Rat	2	4	7
Hamster	2	1	11

Overview of mouse bioassays (N = 15)

Strain	Route: No. of study (No. of strain)	Tumor findings (rare; *statistically significant)
NZO NZB NZC NZY BALB/c CD-1 SENCAR Swiss	▪ Drinking water: 10 studies (4 strains) ▪ Gavage: 3 studies (3 strains) ▪ Subcutaneous injection: 2 studies (1 strain)	nasal cavity (F) oropharynx (M*, F*) esophagus (M*, F*) lung (M*, F*) hepatocellular adenoma/carcinoma (M*, F*) liver hemangioma/hemangiosarcoma (M*, F*) liver cholangioma/cholangiocarcinoma (M*, F*) forestomach (M*, F*) glandular stomach (M*, F*) reticuloendothelial lymphoma (M*, F*)

M: male; F: female

Male NZO mice – Drinking water (Goodall & Lijinsky 1984a, 1984b)

Tumor site	Tumor type	Tumor incidence	
		Concentration (mg/L)	
		0	200
Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	0/194	4/20***
Esophagus (r)	Squamous cell papilloma and carcinoma	0/194	7/20***
Liver	Hepatocellular carcinoma	3/194	10/20***
	Cholangioma (r) and cholangiocarcinoma (r)	0/194	6/20***
Forestomach (r)	Squamous cell papilloma and carcinoma	0/194	14/20***
Glandular stomach (r)	Mostly benign, adenomatous	0/194	2/20**
Reticuloendothelium	Lymphoma	10/194	8/20***

[§] Includes nasal cavity, tongue, larynx

**p<0.01; ***p<0.001; r: rare tumor

Female SENCAR mice - gavage (Strickland *et al.*, 1988)

Tumor site	Tumor type	Tumor incidence	
		Total Dose (mg/mouse)	
		0	60
Nasal cavity (r)	Adenoma or mucosa-carcinoma	0/20	4/20#
Esophagus (r)	Squamous cell papilloma	0/20	4/20#
Lung	Adenoma	NR	15/20***
	Adenocarcinoma	NR	6/20*
	All	1/20	17/20***
Liver	Hepatocellular adenoma	NR	6/20
	Hepatocellular carcinoma	NR	3/20
	Hemangiosarcoma	NR	3/20
	Cholangioma (r)	NR	3/20
	All	3/20	12/20**
Forestomach	Squamous cell papilloma	NR	1/20
	Squamous cell carcinoma (r)	NR	9/20**
	All	1/20	10/20**

#p=0.053; *p<0.05; **p<0.01; ***p<0.001; NR: not reported; r: rare tumor

Overview of rat bioassays (N = 6)

Strain	Route: (No. of strain)	Tumor findings (rare; *statistically significant)
MRC-Wistar (M, F) Sprague-Dawley (M) F344 (F)	Drinking water: 3 strains	nasal cavity (M*, F) tongue (F) esophagus (M*, F*) hepatocellular adenoma/carcinoma (M*, F*) liver hemangioma/hemangiosarcomas (M*, F*)

M: male; F: female

Note: One study included concurrent controls; 1 study used colony controls; 4 studies without controls but showed high incidences of rare tumors (*e.g.*, rare liver tumors in treated M (15/15) & F (11/15) in Goodall *et al*., 1968)

Male Sprague Dawley rats - drinking water (Lijinsky and Taylor, 1979)

Tumor site	Tumor type	Tumor incidence	
		Concentration (mg/L)	
		0#	110
Nasal turbinate (r)	Adenocarcinoma	0/26	7/15***
Esophagus (r)	Papilloma	0/26	9/15***
	Carcinoma	0/26	2/15
Liver (r)	Hepatocellular carcinoma	0/26	3/15*
	Sarcoma (mostly hemangiosarcoma)	0/26	5/15**

#: Colony controls;
*p<0.05; **p<0.01; ***p<0.001; r: rare tumor

Female F344 rats - drinking water (Lijinsky & Reuber, 1981)

Tumor site	Tumor type	Tumor incidence	
		Concentration (mg/L)	
		0	112
Esophagus (r)	Papilloma	0/20	4/20
	Carcinoma	0/20	14/20***
	Combined	0/20	18/20***
Liver (r)	Hepatocellular carcinoma	1/20	[a]6/20*
	Hemangiosarcoma	0/20	[a]13/20***

*p<0.05; ***p<0.001; r: rare tumor

a Three rats have both hepatocellular carcinomas and hemangiosarcomas

Overview of hamster bioassays (N = 11)

Strain	Route: (No. of study)	Tumor findings (rare; *statistically significant)	
Syrian golden	*s.c.* injection: 7 studies	nasal cavity (M*, F*) larynx (M, F) trachea (M*, F*) lung (M*)	
	Transplacental exposure: 4 studies	Single injection (10 mg/kg)	No treatment-related tumors
		Multiple-injection (20-80 mg/kg)	larynx (M/F combined*) trachea (M/F combined*)

M: male; F: female

Male Syrian hamsters - *s.c.* (Althoff *et al.*, 1973)

Tumor site	Tumor type	Tumor incidence					
		Dose (mg/kg-bw)					
		0	4	8	16	32	64
Nasal cavity (r)	Primarily adenocarcinoma	0/20	0/19	1/19	4/19*	10/15***	0/20
Larynx (r)	Papillomas, other	0/20	0/19	2/19	2/19	2/15	0/20
Trachea (r)	Papillary tumors	0/20^{t++}	4/19*	12/19***	13/19***	14/15***	10/20***
Lung-bronchi (r)	Papillary tumors, adenocarcinoma, other carcinomas	0/20	2/19	4/19*	1/19	0/15	0/20
Forestomach	Squamous cell papilloma	1/20	2/19	3/19	1/19	2/15	0/20

*p<0.05; ***p<0.001

t++: Trend test p<0.01; r: rare tumor

Pregnant female Syrian hamsters - *s.c.* (Althoff *et al.*, 1976)

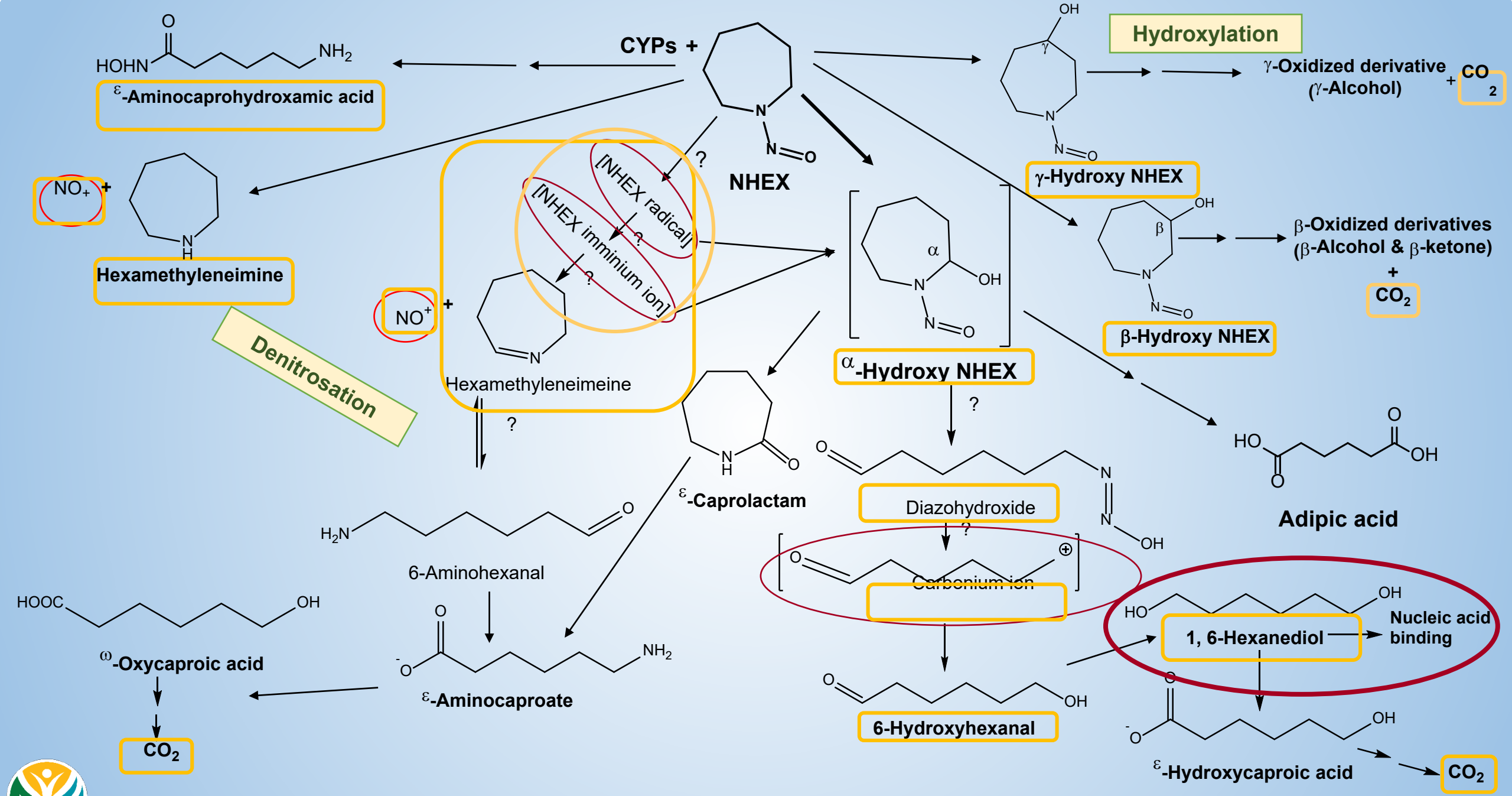
Tumor site	Tumor type	Tumor incidence		
		Total Dose (mg/kg-bw)		
		0	10 (Single injection)	20 - 80 (Multiple injections)
Nasal cavity (r)	Respiratory epithelium adenocarcinoma	0/20	0/40	2/35
Larynx (r)	Papillary polyps	0/20	0/40	7/35*
Trachea (r)	Papillary polyps	0/20	0/40	10/35**

*p<0.05; **p<0.01; r: rare tumor

Pharmacokinetics and metabolism

- Rapidly absorbed, distributed, and completely metabolized
- Excreted in the urine and as expired CO_2
- Can be metabolized by CYPs to form a number of metabolites:
  - 18 identified metabolites, *e.g.* α-, β-, γ- hydroxylated NHEX, 1,6-hexanediol, hexamethyleneimine
  - 7 proposed metabolites, *e.g.* diazohydroxide, 6-aminohexanal
  - Some unknown metabolites

Overview of NHEX Metabolism


CYPs +
NHEX
Hydroxylation
γ-Oxidized derivative (γ-Alcohol)
+ CO2
γ-Hydroxy NHEX
β-Oxidized derivatives (β-Alcohol & β-ketone)
+
CO2
β-Hydroxy NHEX
α-Hydroxy NHEX
ε-Aminocaprohydroxamic acid
NO+ +
Hexamethyleneimine
Denitrosation
NO+ +
[NHEX radical]
[NHEX imminium ion]
Hexamethyleneimeine
ε-Caprolactam
Diazohydroxide
Carbenium ion
Adipic acid
6-Aminohexanal
ω-Oxycaproic acid
CO2
ε-Aminocaproate
6-Hydroxyhexanal
1, 6-Hexanediol
Nucleic acid binding
ε-Hydroxycaproic acid
CO2

Genotoxicity Studies of NHEX

Bacteria			
Endpoint	**Strain**	**Results**	
		- S-9	**+ S-9**
***S. typhimurium* reverse mutation**	TA1535	**-**	**+**
	not specified	**(+)**	**+**
***E. coli* reverse mutation**	WU 3610 (*tyr-, leu-*)	**-**	**+**

In Vitro		
Endpoint	**Target**	**Results**
6-Thioguanine or ouabain resistance mutation	Chinese hamster V79 cells	**+**

In Vivo		
***D. melanogaster* X-linked recessive-lethal mutation**	F_2 generation	**+**
RNA binding in rats	liver	**+**
DNA binding in rats		**+**
DNA damage in rats	liver, lung, kidney, duodenum	**-**

+ : positive

- : negative

(+) : weakly positive

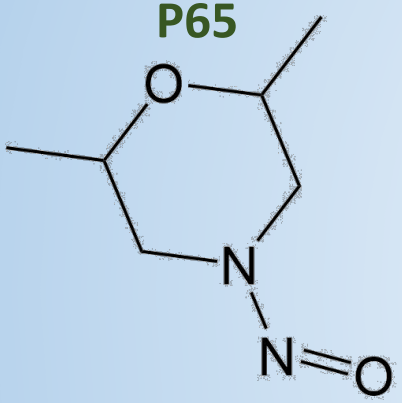
Genotoxicity Studies of NHEX Metabolites

- β-Hydroxy NHEX and γ-hydroxy NHEX induced base-pair substitution mutations in *S. typhimurium*.
- 1,6-Hexanediol covalently bound to liver DNA/RNA in rats *in vivo*.
- ϵ-Caprolactam: mixed, primarily negative in a wide range of assays
- Adipic acid: negative in mutagenicity assays

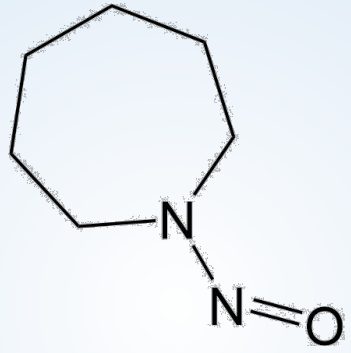
Structure Activity Considerations

2,6-Dimethylnitroso-morpholine (DMNM)

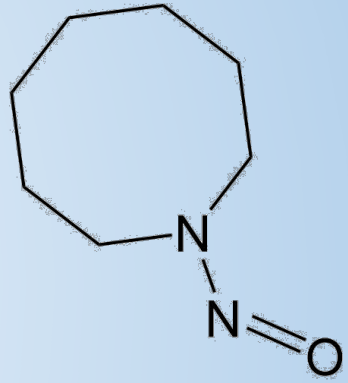
P65



NHEX

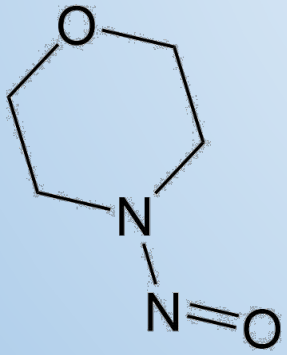


N-Nitrosohepta-methyleneimine (NHMI)



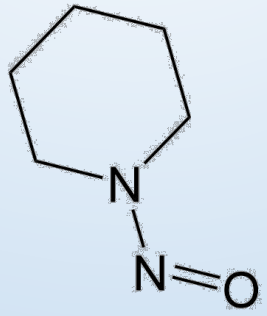
Nitrosomorpholine (NM)

P65, IARC, NTP



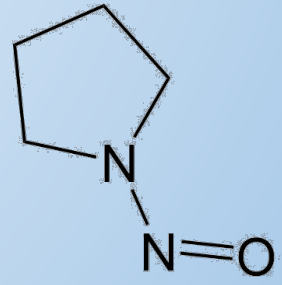
N-Nitrosopiperidine (NP)

P65, IARC, NTP



N-Nitrosopyrrolidine (NPYR)

P65, IARC, NTP



Structure Activity Considerations: Tumor Sites in Animals

Chemical	Nasal cavity	Larynx and/or trachea	Esophagus	Lung	Liver	Fore-stomach
NHEX	R M H	H	R M	R M H	R M	R M H
DMNM	R H	R H	R H	R H	R H	R H
NHMI	R H	R H	R H	R H		H
NM	R H	R H	R	M	R M	H
NP	R H	H	R H	M H	R M H	M H
NPYR	H	H		M	R	

R: rat M: mouse H: hamster

Structure Activity Considerations: Genotoxicity

Chemical	Mutagenicity in bacteria		*In vitro* genotoxicity (mammalian cells)		*In vivo* genotoxicity	
	S. typhimurium	*E. coli*	Mutation	DNA/chromosomal damage/binding	X-linked recessive-lethal mutation assay in *D. melanogaster*	DNA/RNA binding
NHEX	+	+	+	NT	+	+
DMNM	+	NT	NT	+	+	+
NHMI	+	+	NT	+	NT	NT
NM	+	+	+	+	+	+
NP	+	+	+	+	+	NT
NPYR	+	+	+	+	+	+

NT: Not tested

QSAR Predictions for NHEX (ECHA, 2018)

Predicted carcinogen

- QSAR Toolbox
- VEGA QSAR platform
  - CAESAR model
  - ISS model

Predicted mutagen

- QSAR Toolbox
- VEGA QSAR platform
  - CAESAR model
  - ISS model
  - SarPy model
  - KNN model

IARC's Key Characteristics of Carcinogens[1]

Key Characteristic	Relevant evidence for NHEX
1. Is electrophilic or can be metabolically activated	Forms electrophilic metabolites • 1,6-hexanediol • NHEX radical • NHEX imminium ion • Carbonium ion metabolite • NO^+
2. Is genotoxic	Mutagenicity in bacteria, mammalian cells *in vitro*, and *Drosophila*; DNA and RNA binding *in vivo*
3. Alters DNA repair or causes genomic instability	
4. Induces epigenetic alterations	
5. Induces oxidative stress	
6. Induces chronic inflammation	
7. Is immunosuppressive	
8. Modulates receptor-mediated effects	
9. Causes immortalization	
10. Alters cell proliferation, cell death, or nutrient supply	

[1]Smith et al. (2016) *Environ Health Perspect*. 2016 Jun; 124(6): 713–721.

Summary: Animal Studies

Tumors observed in multiple species, strains and often both sexes.

Tumor site	Mice	Rats	Hamsters
Nasal cavity	F	M*, F	M*, F*
Lung	M*, F*	M	M*, F
Forestomach	M*, F*	M, F	
Esophagus	M*, F*	M*, F*	
Glandular stomach	M*, F*	F	
Liver hepatocellular adenoma/carcinoma	M*, F*	M*, F*	
Liver hemangioma/hemangiosarcoma	M*, F*	M*, F*	
Liver cholangioma/cholangiocarcinoma	M*, F*		
Oropharynx	M*, F*		
Reticuloendothelium system	M*, F*		
Tongue		M, F	
Larynx			M, F
Trachea			M*, F*

: Rare tumor sites
*: Sign't increase by pairwise comparison
M: Male
F: Female

Summary: Other relevant data

- NHEX is bioactivated by CYPs to form several electrophilic and/or genotoxic metabolites:
  - β- and γ-Hydroxy NHEX induce mutations in *Salmonella*
  - 1,6-Hexanediol alkylates liver RNA and DNA in rats
  - Several proposed electrophilic metabolites, *e.g.* NO^+, carbonium ion metabolite, NHEX radical, NHEX iminium ion
- Genotoxicity evidence for NHEX:
  - Mutagenic in *Salmonella* and *E. coli*, in Chinese hamster V79 cells *in vitro* and in *Drosophila in vivo*
  - Covalent binding to RNA and DNA in liver of NHEX-treated rats *in vivo*

Summary: Other relevant data (cont'd)

- Strong structure-activity similarities between NHEX and five comparison nitrosamines (four are Proposition 65 carcinogens).
  - Several QSAR models predict that NHEX is both mutagenic and carcinogenic.
- Mechanistic findings for NHEX are associated with two key characteristics of carcinogens:
  - Is electrophile or can form electrophilic metabolites
  - Is genotoxic