Proposition 65

Evidence on the Carcinogenicity of N-Nitrosohexamethyleneimine

August 2018

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

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

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PREFACE

Proposition 65[1] requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency maintains this list in its role as lead agency for implementing Proposition 65. The Carcinogen Identification Committee (CIC) advises and assists OEHHA in compiling the list of chemicals that cause cancer as required by Health and Safety Code section 25249.8. The Committee serves as the state's qualified experts for determining whether a chemical has been clearly shown to cause cancer.

In 2009, OEHHA brought N-nitrosohexamethyleneimine (NHEX) to the CIC for prioritization and ranking for future listing consideration. OEHHA subsequently selected NHEX 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 its carcinogenicity. No submissions were received.

On November 1, 2018, the CIC is scheduled to deliberate on the carcinogenicity of NHEX. OEHHA developed this document with information on the evidence of carcinogenicity of NHEX to assist the CIC in its deliberations. The original papers discussed in the document will also be provided to the CIC as part of the hazard identification materials. Comments on this hazard identification document received during the public comment period also form part of the hazard identification materials, and will be provided to the CIC members prior to their formal deliberations.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code 25249.5 *et seq*.)

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Acronyms and abbreviations

AhR	Aryl hydrocarbon receptor
AR	Androgen receptor
CA	Chromosomal aberrations
CCRIS	Chemical Carcinogenesis Research Information System
CHO	Chinese hamster ovary
C_{max}	Maximum serum concentration
CIC	Cancer Identification Committee
CO_2	Carbon dioxide
CTD	Comparative Toxicogenomic Database
DMBA	Dimethylbenz[*a*]anthracene
DMNM	2,6-Dimethylnitrosomorpholine
ECHA	European Chemicals Agency
EFSA	The European Food Safety Authority
ER	Estrogen receptor
F_0	Parent generation / exposed animals
F_1	First generation (offspring) of exposed animals
F_2	Second generation of exposed animals
F344	Fischer 344
GC	Gas chromatography
GD	Gestation day
HAWC	Health Assessment Workspace Collaborative
IARC	International Agency for Research on Cancer
iCSS	Interactive Chemical Safety for Sustainability
i.p. injection	Intraperitoneal injection
MN	Micronucleus
MS	Mass spectrometry
NHEX	N-Nitrosohexamethyleneimine
NHMI	Nitrosoheptamethyleneimine
NM	Nitrosomorpholine
NP	N-Nitrosopiperidine
NPYR	N-Nitrosopyrrolidine
NT	Not tested
NTP	National Toxicology Program
NZ strains	New Zealand strains
OECD	Organization for Economic Co-operation and Development
P65	Proposition 65
PB	Phenobarbital
PPAR	Peroxisome proliferator-activated receptors

PTP	Protein tyrosine phosphatase
PXR	Pregnane X receptor
QSAR	Quantitative structure activity relationship
S-9	Supernatant fraction from liver homogenate centrifuged at 9,000x g for 10 minutes
s.c. injection	Subcutaneous injection
S-D	Sprague-Dawley
SCE	Sister chromatid exchange
TLC	Thin-layer chromatography
UDS	Unscheduled DNA synthesis
µg	Microgram
µmol	Micromole

1. EXECUTIVE SUMMARY

N-Nitrosohexamethyleneimine (NHEX) is a heterocyclic nitrosamine. Nitrosamines are typically formed by the reaction between a secondary amine and a nitrosating agent or precursor to a nitrosating agent, such as nitrite. NHEX is not known to occur naturally. However, NHEX has previously been identified as a contaminant in at least some pharmaceutical preparations of the hypoglycemic agent marketed as Tolazamide. Additionally, NHEX may form in the acidic environment of the stomach following the interaction of ingested Tolazamide and nitrite from dietary sources. There is a paucity of information regarding current uses of NHEX. Historically, NHEX is known to have been used in at least one industrial chemical synthesis process. NHEX has also been manufactured by at least one producer in California for use as an explosive in ejector seats of military fighter jets.

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

Fifteen carcinogenicity studies of NHEX have been conducted in mice, seven in rats, and 11 in hamsters. These studies were conducted either by the oral or subcutaneous routes. Statistically significant increases in benign and/or malignant tumors, including many tumor types which are considered rare, have been observed at multiple sites in both sexes of multiple strains of mice and rats, and in the one hamster strain tested. NHEX treatment resulted in statistically significant increases in tumors of the oropharynx (including nasal cavity, tongue, and larynx; rare), esophagus (rare), lung, liver [hepatocellular adenoma or carcinoma; hemangioma or hemangiosarcoma (uncommon); cholangioma or cholangiocarcinoma (rare)], forestomach (rare), glandular stomach (rare), and reticuloendothelial lymphomas in mice; nasal cavity (rare), esophagus (rare), liver [hepatocellular adenoma or carcinoma (rare); hemangioma or hemangiosarcoma (rare)] in rats; and nasal cavity (rare), larynx (rare), trachea (rare) and lung (rare) in hamsters. In addition, marginally statistically significant increases ($p = 0.053$) in forestomach tumors were observed in hamsters, and increases in rare tongue, lung, forestomach and glandular stomach tumors were observed in rats.

NHEX, like many other nitrosamines, requires metabolic activation via cytochrome P450 enzymes in order to express genotoxic activity. Several reactive electrophilic intermediates and products of NHEX metabolism have been proposed, including a NHEX radical, a NHEX imminium ion, a carbonium ion metabolite, and nitrosonium ions (NO^+); other reactive or genotoxic NHEX metabolites have been detected in mammalian systems, including 1,6-hexanediol and β- and γ-hydroxy NHEX.

Evidence of NHEX genotoxicity comes from multiple *in vivo* and *in vitro* test systems. NHEX induces base-pair substitution mutations in *Salmonella typhimurium* and reverse mutations in *Escherichia coli* (primarily in the presence of metabolic activation) and X-linked recessive lethal mutations in *Drosophila melanogaster*. *In vitro*, NHEX induces 6-thioguanine and ouabain resistant mutations in primary rat hepatocyte-mediated assays in Chinese hamster V79 cells. NHEX covalently binds to RNA and DNA in rat liver following *in vivo* exposure, and the NHEX metabolite 1,6-hexanediol has been identified as one of the alkylating species. NHEX is postulated to form DNA and RNA adducts by O-alkylation of guanine, thymidine, and phosphate groups and by forming crosslinks within the RNA and DNA. Two other NHEX metabolites, β- and γ-hydroxy NHEX, induce base-pair substitution mutations in *Salmonella* in the presence of metabolic activation.

There are strong structure-activity similarities between NHEX and five comparison heterocyclic nitrosamines, 2,6-dimethylnitrosomorpholine (DMNM), nitrosoheptamethyleneimine (NHMI), nitrosomorpholine (NM), N-nitrosopiperidine (NP), and N-nitrosopyrrolidine (NPYR). All five comparison compounds are genotoxic and increase tumors in animal cancer bioassays. Nasal cavity, larynx and/or trachea and lung tumors are the common target tumor types observed between NHEX and five of the comparison nitrosamines. In addition, esophagus, forestomach, liver, tongue and glandular stomach tumors are observed between NHEX and some comparison nitrosamines. DMNM, NM, NP, and NPYR are each listed as Proposition 65 carcinogens. Additionally, several QSAR models predict that NHEX is both mutagenic and carcinogenic.

The mechanisms of carcinogenesis by which NHEX acts likely include the formation of electrophilic metabolites via cytochrome P450 activation and genotoxicity.

2. INTRODUCTION

2.1 Identity of N-Nitrosohexamethyleneimine

N-Nitrosohexamethyleneimine (NHEX), a heterocyclic nitrosamine, belongs to a class of N-nitroso compounds characterized by a nitroso group (-N=O) bonded to a nitrogen atom. The structure of N-nitrosohexamethyleneimine is given in Figure 1. Selected chemical and physical properties are given in Table 1.

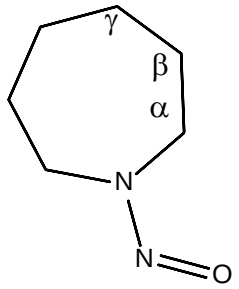


Figure 1. Structure of N-Nitrosohexamethyleneimine.
Carbon atoms are labeled (relative to the nitroso group) as α, β, and γ carbons.

Table 1. Chemical and physical properties of NHEX

Name	N-Nitrosohexamethyleneimine (NHEX)
IUPAC Systematic Name	1-nitrosoazepane
CAS Registry Number	932-83-2
Molecular Formula	$C_6H_{12}N_2O$
Molecular Weight	128.2 g/mol
Boiling Point	233°C[a]
Density	1.06 g/cm^3 at 25°C [a]
Selected Synonyms	1-nitrosoazepane, N-nitrosoazacycloheptane, N-nitrosohexahydroazepine, N-nitrosoperhydroazepine

[a] Sigma-Aldrich, 2014 (accessed May 29, 2018)

2.2 Synthesis, Occurrence, and Use of NHEX

Synthesis

Amines can undergo various reactions to form nitrosamines. Nitrosamines, including NHEX, are typically formed by the reaction between a secondary amine and a nitrosating agent or precursor to a nitrosating agent, such as nitrite (Brambilla and Martelli 2007; Mirvish 1975). NHEX can be synthesized by dissolving hexamethyleneimine in sulfuric acids, and then adding sodium nitrite (Goodall et al. 1968).

Occurrence

NHEX is not known to occur naturally. However, NHEX has previously been identified as a contaminant in at least some pharmaceutical preparations of the hypoglycemic agent 1-(azepan-1-yl)-3-(4-methylphenyl)sulfonylurea, marketed as Tolazamide (Severin 1987). Additionally, NHEX may form in the acidic environment of the stomach following the interaction of ingested Tolazamide, which is still approved for use as a prescription drug in the US, with nitrite from dietary sources (Eshraghi et al. 1990; Sakai et al. 1984; William Lijinsky, personal communication, 1996). NHEX may also form from Tolazamide via oxidation in the absence of a nitrating agent (Haseman et al. 1998).

Use

There is a paucity of information regarding current use of NHEX. Historically, NHEX is known to have been used by FMC Corporation in an in-plant synthetic process (Walker et al. 1976). The chemical has also been manufactured by at least one producer in California for use as an explosive in ejector seats of military fighter jets (Jon Rosenberg, personal communication, 1985). This California facility reported levels from a monitoring study in the 1-20 ppb range (Jon Rosenberg, personal communication, 1985).

3. DATA ON CARCINOGENICITY

3.1 Carcinogenicity Studies in Humans

No cancer epidemiological studies on the effects of human exposure to NHEX were identified in a recent systematic literature search conducted by OEHHA (Appendix A).

3.2 Carcinogenicity Studies in Animals

A review of the carcinogenicity studies of NHEX in experimental animals identified more than 30 studies conducted in three species (mouse, rat and hamster) under various study protocols. Of the fifteen studies conducted in eight different strains of mice, NHEX was administered via drinking water in ten, via gavage in three, and via subcutaneous (*s.c*.) injection in two. Of the seven studies conducted in four strains of rats, NHEX was administered via drinking water in six and *s.c*. injection in one. Of the 11 studies conducted in Syrian golden hamsters, NHEX was administered via transplacental exposure in four and via *s.c.* injection in seven. A number of these studies were limited by small numbers of animals per group, lack of concurrent controls, limited duration of exposure, and/or inadequate reporting.

This section is organized as follows:

- An overview of these studies is provided in Table 2, which lists each study by species, strain, sex, route of administration, and exposure duration.
- Table 3 presents a high-level summary of the tumor findings (*e.g.* statistically significant and/or rare tumors) in each of these studies.
- Studies conducted in mice are briefly discussed and summarized in the text. This is followed by Table 4, which presents information on several aspects of study design, dosing, tumor site, type and incidence, and other relevant issues, for each of the mouse studies.
- Studies conducted in rats are similarly discussed and summarized in the text, and information on study design, dosing, tumor site, type and incidence, and other relevant issues for these studies is presented in Table 5.
- Studies conducted in hamsters are similarly discussed and summarized in the text, and information on study design, dosing, tumor site, type and incidence, and other relevant issues for these studies is presented in Table 6.

Table 2. Overview of NHEX animal carcinogenicity studies

Study No.	Species	Strain	Sex (M, F)	Route of Administration	Exposure	Reference
1	Mouse	NZO/ BIGd	M	Drinking water	8 weeks	Goodall and Lijinsky 1984a & 1984b
2					32 weeks	
3			F		8 weeks	
4					32 weeks	
5		NZB/ BIGd	M		8 weeks	Goodall and Lijinsky 1984b
6			F			
7		NZC/ BIGd	M			
8			F			
9		NZY/ BIGd	M			
10			F			
11		BALB/c	F	Gavage	30 weeks	Strickland *et al*. 1988
12		CD-1				
13		SENCAR				
14		Swiss	M	*s.c.* injection	1 injection	Althoff *et al*. 1972
15			F			
16	Rat	MRC-Wistar	M	Drinking water	8 weeks	Goodall *et al*. 1968
17					Up to 60 weeks	
18			F		8 weeks	
19					Up to 60 week	
20		S-D	M		30 weeks	Lijinsky and Taylor 1979
21		Fischer 344	F		28 weeks	Lijinsky and Reuber 1981
22		BR46	M	*s.c.* injection	70 injections	Schmähl 1968
23	Hamster	Syrian golden	M	*s.c.* injection	1 injection	Althoff *et al*. 1972
24			F			
25			M		Multiple weekly injections until death	Althoff *et al*. 1973
26			F			
27			F (F_0*)		1 injection	Althoff *et al*. 1976
28					2-8 injections	
29			M & F offspring (F_1*)	Transplacental exposure	A single *s.c.* injection to pregnant dams	
30					2-8 *s.c.* injections to pregnant dams	
31			F (F_0*)	*s.c.* injection	1 injection	Althoff & Grandjean 1979
32			M offspring (F_1*)	Transplacental exposure	A single *s.c.* injection to pregnant dams	
33			F offspring (F_1*)			

*Two-generation carcinogenicity studies. F_0: pregnant dams. F_1: first generation.

Table 3. Observations of tumors in mice, rats and hamsters exposed to NHEX

Species / Target tumor site	Mice (M)		Rats (R)		Hamsters (H)			
							Two-generation study	
	Male	Female	Male	Female	Male	Female	F (F_0)[1]	M & F (F_1)[2]
Nasal cavity M (r), R (r), H (r)		X	X*[3]	X	X*	X*	X	
"Oropharynx" (Includes nasal cavity, tongue, and larynx) M (r), R (r)	X*	X*	X[4]	X[4]				
Esophagus M (r), R (r)	X*	X*	X*[3]	X*				
Larynx H (r)					X	X	X*	X*
Trachea H (r)					X*, t+	X*, t+	X*	X*
Lung R (r), H (r)	X*	X*	X		X*	X	X	X
Liver Hepatocellular adenoma/carcinoma R (r)	X*	X*	X*[3]	X*				
Liver Hemangioma/ hemangiosarcoma M (uncommon), R (r)	X*	X*	X*[3]	X*				
Liver Cholangioma / cholangiocarcinoma M (r)	X*	X*						
Forestomach M (r)[5], R (r)	X*	X*	X	X	X[6]	X[6]		
Glandular stomach M (r), R (r)	X*	X*		X				
Reticuloendothelial system	X*	X*						

"X" denotes observation of tumors, "*" statistically significant ($p < 0.05$) increase in tumor incidence by Fisher pairwise comparison, "t+" indicates significant positive results from exact trend test (performed by OEHHA); (r) = rare tumor

[1]F (F_0): Female pregnant hamsters (parent generation)

[2]M&F (F_1): Male, female, and combined male and female offspring hamsters (first generation)

[3]The studies did not include a specific concurrent control group; however, a continuous series of unexposed rats from the same animal colony was maintained in the same facility.

[4]Squamous cell papillomas and carcinomas of the tongue (r) were reported.

[5] In mice squamous cell carcinomas of the forestomach are rare.

[6]The incidence of squamous cell papilloma of the forestomach, which is not considered rare in hamsters, was increased, but did not reach statistical significance.

3.2.1 Studies conducted in mice

NHEX was tested via the oral and *s.c.* injection routes of administration in 15 mouse studies. Administration was via drinking water in ten studies (4 strains; male and female; Goodall and Lijinsky 1984a, 1984b), via gavage in three studies (3 strains; female only; Strickland et al. 1988), and via *s.c.* injection in two studies (one strain; male and female; Althoff et al. 1972; no control incidences) (Table 4). No apparent sex differences in target tumor sites were observed in NHEX-treated mice.

Studies via drinking water

NHEX administered in drinking water for 8 weeks (8 studies in NZB/NZC/NZO/NZY strains) or 32 weeks (2 studies in NZO strain) induced treatment-related increases in tumors in multiple New Zealand inbred strains of both sexes, including rare "oropharynx"[2], esophagus, liver (cholangioma or cholangiocarcinoma), forestomach, and glandular stomach tumors. Treatment-related increases in tumors not considered rare were also observed in several of these mouse strains, including tumors of the lung, liver (hepatocellular carcinoma; hemangioma or hemangiosarcoma) and reticuloendothelial system (Goodall and Lijinsky 1984a and 1984b).

Rare squamous cell papillomas and carcinomas of the esophagus and forestomach[3] were statistically significantly increased in both sexes of four strains of mice. Increases in oropharynx tumors and hepatocellular carcinomas were statistically significant in almost all strains and sexes tested, with a few exceptions (*e.g.*, oropharynx tumors were not increased in NZY mice). Rare cholangiomas and cholangiocarcinomas of the liver bile duct were observed in NZO (male and female), NZC (male and female) and NZY (male) mice, and the increases were statistically significant in NZO (male and female), NZC (male), and NZY (male) mice. Differences in glandular stomach, lung, liver hemangiosarcoma, and hepatic bile duct tumor responses to NHEX were observed between the four New Zealand mouse strains. For example, increases in lung tumors were statistically significant in NZB (male and female) and NZC (male) mice, but not in the NZO strain, which has a high spontaneous incidence of lung tumors.

In two sets of studies in the NZO strain, two different dosing schemes were used, in which the same total target dose of NHEX (80 mg/mouse) was received via drinking water, but the doses were administered at different exposure rates (high intensity/short duration: 200 mg/L for 8 weeks vs. low intensity/long duration: 50 mg/L for 32 weeks). Under these two dosing schemes, statistically significant increases in squamous cell

[2] Includes tumors of the nasal cavity, tongue, and larynx
[3] Squamous cell carcinomas, but not papillomas of the forestomach, are rare.

papillomas and carcinomas of the oropharynx, esophagus, forestomach, and glandular stomach were observed in mice of both sexes. Comparing the two dosing schemes, higher tumor incidences were observed in the lung (female only), liver, and reticuloendothelial system in the high intensity/short duration exposure studies than in the low intensity/long duration studies.

Studies via gavage

NHEX administered by gavage twice per week for 30 weeks (total dose of 60 mg/mouse) to female BALB/c, CD-1, and SENCAR mice resulted in statistically significant increases in lung tumors (CD-1 and SENCAR), liver hepatocellular adenomas (BALB/c and CD-1), uncommon liver hemangiosarcomas (BALB/c and CD-1), and rare forestomach tumors (SENCAR) (Strickland et al. 1988). In all three strains, rare nasal cavity tumors (marginal significance with p=0.053 for BALB/c and SENCAR), rare esophageal tumors (marginal significance with p=0.053 for SENCAR), and rare liver cholangiomas (marginal significance with p=0.053 for BALB/c) were observed with NHEX treatment. Survival was poor in the treated groups in all three strains as compared to the control groups.

Studies via s.c. injection

A single *s.c.* injection of NHEX to male and female Swiss mice at doses of 4, 8, 16, 32, or 64 milligram per kilogram bodyweight (mg/kg-bw) did not result in statistically significant increases in tumors at any site (Althoff et al. 1972). Non-statistically significant increases in rare lung adenomas were observed in treated animals of both sexes; treatment had no effect on lung tumor latency. The doses used in these studies were low; the maximum dose administered in these studies was approximately 2 mg/mouse.

Table 4. Summary of study design, exposure and tumor incidences in mouse bioassays of NHEX

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0	200	
Goodall and Lijinsky (1984a & 1984b) Species: Mouse Strain: NZO/BIGd Sex: Male Age at start of exposure: 50 days old (7 week (wk) old) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 194 controls N = 20 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg; achieved dose: 52 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.	Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	0/194 (0%)	4/20*** (20%)	Survival: Survival in the treated group was better or similar to controls. Bodyweight (BW): No significant difference in bw between treated (35.7 g) and controls (34.5 g). Other tumor sites of interest: One hemangiosarcoma of the liver (uncommon); 1 transitional cell papilloma of the bladder (r); 1 salivary duct adenoma (r); 1 fibrosarcoma (uncommon); 1 adenocarcinoma of the kidney (uncommon). Other comments: Most of the moribund treated animals had obstructive and invasive squamous cell carcinoma of the upper alimentary tract.
		Esophagus (r)	Squamous cell papilloma and carcinoma	0/194 (0%)	7/20*** (35%)	
		Lung	Alveolar adenoma and carcinoma	53/194 (27%)	8/20 (40%)	
		Liver	Hepatocellular carcinoma	3/194 (2%)	10/20*** (50%)	
			Cholangioma (r) and cholangiocarcinoma (r)	0/194 (0%)	6/20*** (30%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/194 (0%)	14/20*** (70%)	
		Glandular stomach (r)	Mostly benign, adenomatous	0/194 (0%)	2/20** (10%)	
		Reticuloendothelium	Lymphoma	10/194 (5%)	8/20*** (40%)	

**p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[§] Includes nasal cavity, tongue, larynx
[$]Reported as "squamous stomach tumor" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0	50	
Goodall and Lijinsky (1984a) Species: Mouse Strain: NZO/BIGd Sex: Male Age at start of exposure: 50 days old (7 wk old) based on Goodall and Lijinsky (1984b) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 194 controls: N = 10 treated	Route: Drinking water Concentration: 0, 50 mg/L (target total dose: 80 mg; achieved dose: 52 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 32 wk.	Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	0/194 (0%)	4/10*** (40%)	Survival: Similar survival between treated and control groups until 1.5 years, after which higher mortality was observed in the treated group due to neoplasms. BW: Not reported Other tumor sites of interest: None Other comments: Most of the moribund treated animals had obstructive and invasive squamous cell carcinoma of the upper alimentary tract.
		Esophagus (r)	Squamous cell papilloma and carcinoma	0/194 (0%)	9/10*** (90%)	
		Lung	Alveolar adenoma and carcinoma	53/194 (27%)	5/10 (50%)	
		Liver	Hemangioma (uncommon)	0/194 (0%)	1/10* (10%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/194 (0%)	8/10*** (80%)	
		Glandular stomach (r)	Mostly benign, adenomatous	0/194 (0%)	2/10** (20%)	

*p<0.05; **p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[§] Includes nasal cavity, tongue, larynx
[$] Reported as "squamous stomach tumor" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0	200	
Goodall and Lijinsky (1984a & 1984b) Species: Mouse Strain: NZO/BIGd Sex: Female Age at start of exposure: 50 days old (7 wk old) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 113 controls N = 20 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg; achieved dose: 52 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.	Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	0/113 (0%)	3/20** (15%)	Survival: Survival in the treated group was similar up to 400 days; higher mortality was observed in the treated group at 600 days. BW: No significant difference in bw between treated (34.7 g) and controls (35.9 g). Other tumor sites of interest: One fibrosarcoma (uncommon). Other comments: Most of the moribund treated animals had obstructive and invasive squamous cell carcinoma of the upper alimentary tract.
		Esophagus (r)	Squamous cell papilloma and carcinoma	0/113 (0%)	12/20*** (60%)	
		Lung	Alveolar adenoma and carcinoma	39/113 (35%)	11/20 (55%)	
		Liver	Hepatocellular carcinoma	4/113 (4%)	8/20*** (40%)	
			Cholangioma (r)	0/113 (0%)	2/20* (10%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/113 (0%)	15/20*** (75%)	
		Glandular stomach (r)	Mostly benign, adenomatous	0/113 (0%)	3/20** (15%)	
		Reticuloendothelium	Lymphoma	11/113 (10%)	8/20** (40%)	

*p<0.05; **p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[§] Includes nasal cavity, tongue, larynx
[$] Reported as "squamous stomach tumor" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0	50	
Goodall and Lijinsky (1984a) Species: Mouse Strain: NZO/BIGd Sex: Female Age at start of exposure: 50 days old (7 wk old) based on Goodall and Lijinsky (1984b) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 113 controls N = 13 treated	Route: Drinking water Concentration: 0, 50 mg/L (target total dose: 80 mg; achieved dose: 52 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 32 wk.	Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	0/113 (0%)	2/13** (15%)	Survival: Similar survival between treated and control groups until 1.5 years, after which higher mortality was observed in the treated group due to neoplasms. BW: Not reported Other tumor sites of interest: None Other comments: Most of the moribund treated animals had obstructive and invasive squamous cell carcinoma of the upper alimentary tract.
		Esophagus (r)	Squamous cell papilloma and carcinoma	0/113 (0%)	10/13*** (77%)	
		Liver	Hepatocellular carcinoma	4/113 (4%)	4/13** (31%)	
			Cholangioma (r) and cholangiocarcinoma (r)	0/113 (0%)	2/13** (15%)	
		Forestomach[$](r)	Squamous cell papilloma and carcinoma	0/113 (0%)	12/13*** (92%)	
		Glandular stomach (r)	Mostly benign, adenomatous	0/113 (0%)	3/13*** (23%)	

**p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[§] Includes nasal cavity, tongue, larynx
[$] Reported as "squamous stomach" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
Goodall and Lijinsky (1984b) Species: Mouse Strain: NZB/BIGd Sex: Male Age at start of exposure: 50 days old (7 wk old) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 276 controls N = 19 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.			Concentration (mg/L) 0	 200	Survival: Survival in the treated group was similar to controls at 200 days; higher mortality was observed in the treated group at 400 days. BW: No significant difference in bw between treated (30.7 g) and controls (30 g). Other tumor sites of interest: None Other comments: Tumor incidences were estimated by OEHHA by the reported percent of animals with the specific tumor sites.
		Esophagus (r)	Squamous cell papilloma and carcinoma	0/276 (0%)	6/19*** (32%)	
		Lung	Alveolar adenoma and carcinoma	0/276 (0%)	2/19** (10%)	
		Liver	Hepatocellular carcinoma	0/276 (0%)	2/19** (10%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/276 (0%)	8/19*** (42%)	

**p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[$] Reported as "squamous stomach" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
Goodall and Lijinsky (1984b) Species: Mouse Strain: NZB/BIGd Sex: Female Age at start of exposure: 50 days old (7 wk old) Study duration Observed until death or killed when moribund. Control: Concurrent N = 259 controls N = 19 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.			Concentration (mg/L) 0	 200	Survival: Survival in the treated group was similar to controls at 200 days; higher mortality was observed in the treated group at 400 days. BW: No significant difference in bw between treated (25.8 g) and controls (25.4 g). Other tumor sites of interest: None Other comments: Tumor incidences were estimated by OEHHA by the reported percent of animals with the specific tumors.
		Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	0/259 (0%)	3/19*** (16%)	
		Esophagus (r)	Squamous cell papilloma and carcinoma	0/259 (0%)	6/19*** (31%)	
		Lung	Alveolar adenoma and carcinoma	5/259 (2%)	5/19*** (26%)	
		Liver	Hepatocellular carcinoma	0/259 (0%)	5/19*** (26%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/259 (0%)	7/19*** (37%)	
		Glandular stomach (r)	Mostly benign, adenomatous	0/259 (0%)	2/19** (10%)	
		Reticuloendothelium	Lymphoma	44/259 (17%)	10/19*** (53%)	

**p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[§] Includes nasal cavity, tongue, larynx
[$] Reported as "squamous stomach" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
Goodall and Lijinsky (1984b) Species: Mouse Strain: NZC/BIGd Sex: Male Age at start of exposure: 50 days old (7 wk old) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 163 controls N = 26 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.			Concentration (mg/L) 0	 200	Survival: Survival in the treated group was better or similar to controls up to 600 days. BW: No significant difference in bw between treated (28.3 g) and controls (29.9 g). Other tumor sites of interest: None. Other comments: Tumor incidences were estimated by OEHHA by the reported percent of animals with the specific tumors.
		Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	3/163 (2%)	5/26** (19%)	
		Esophagus (r)	Squamous cell papilloma and carcinoma	0/163 (0%)	21/26*** (81%)	
		Lung	Alveolar adenoma and carcinoma	15/163 (9%)	8/26** (31%)	
		Liver	Hepatocellular carcinoma	2/163 (1%)	7/26*** (27%)	
			Hemangiosarcoma (uncommon)	0/163 (0%)	2/26* (8%)	
			Cholangioma (r) and cholangiocarcinoma (r)	0/163 (0%)	6/26*** (23%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/163 (0%)	16/26*** (61%)	

*p<0.05; **p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[§] Includes nasal cavity, tongue, larynx
[$] Reported as "squamous stomach tumor" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0	200	
Goodall and Lijinsky (1984b) Species: Mouse Strain: NZC/BIGd Sex: Female Age at start of exposure: 50 days old (7 wk old) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 43 controls N = 23 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.	Oropharynx[§] (r)	Squamous cell papilloma and carcinoma and other tumors	1/43 (2%)	3/23 (13%)	Survival: Survival in the treated group was better or similar to controls up to 600 days. BW: No significant difference in bw between treated (25.7 g) and controls (24.3 g). Other tumor sites of interest: One glandular stomach tumor (r); 2 cholangioma (r) and cholangiocarcinoma (r); ovarian granulosa cell tumors [11/23 (48%) in treated vs 12/43 (28%) in controls]. Other comments: Tumor incidences were estimated by OEHHA by the reported percent of animals with the specific tumors.
		Esophagus (r)	Squamous cell papilloma and carcinoma	1/43 (2%)	11/23*** (47%)	
		Lung	Alveolar adenoma and carcinoma	6/43 (14%)	7/23 (30%)	
		Liver	Hepatocellular carcinoma	4/43 (9%)	9/23** (39%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/43 (0%)	14/23*** (61%)	

**p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
§ Includes nasal cavity, tongue, larynx
[$] Reported as "squamous stomach" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0	200	
Goodall and Lijinsky (1984b) Species: Mouse Strain: NZY/BIGd Sex: Male Age at start of exposure: 50 days old (7 wk old) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 167 controls N = 15 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.	Esophagus (r)	Squamous cell papilloma and carcinoma	0/167 (0%)	8/15*** (53%)	Survival: Survival in treated group was similar or better up to 400 days; higher mortality was observed in the treated group at 600 days. BW: No significant difference in bw between treated (31.5 g) and controls (31.2 g). Other tumor sites of interest: One oropharynx tumor (r). Other comments: Tumor incidences were estimated by OEHHA by the reported percent of animals with the specific tumors.
		Lung	Alveolar adenoma and carcinoma	27/167 (16%)	4/15 (27%)	
		Liver	Hepatocellular carcinoma	3/167 (2%)	8/15*** (53%)	
			Hemangiosarcoma (uncommon)	0/167 (0%)	2/15** (13%)	
			Cholangioma (r) and cholangiocarcinoma (r)	0/167 (0%)	2/15** (13%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/167 (0%)	10/15*** (67%)	

**p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[$] Reported as "squamous stomach" by authors.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0	200	
Goodall and Lijinsky (1984b) Species: Mouse Strain: NZY/BIGd Sex: Female Age at start of exposure: 50 days old (7 wk old) Study duration: Observed until death or killed when moribund. Control: Concurrent N = 123 controls N = 16 treated	Route: Drinking water Concentration: 0, 200 mg/L (target total dose: 80 mg) Frequency and duration: 10 mL/mouse/day, 5 day/wk for 8 wk.	Esophagus (r)	Squamous cell papilloma and carcinoma	0/123 (0%)	9/16*** (56%)	Survival: Survival in the treated group was similar or better up to 400 days; higher mortality was observed in the treated group at 600 days. BW: No significant difference in bw between treated (27.7 g) and controls (27.9 g). Other tumor sites of interest: One glandular stomach tumor ("mostly benign, adenomatous") (r), 1 fibrosarcoma (uncommon) Other comments: Tumor incidences were estimated by OEHHA by the reported percent of animals with the specific tumors.
		Lung	Alveolar adenoma and carcinoma	19/123 (15%)	5/16 (31%)	
		Forestomach[$] (r)	Squamous cell papilloma and carcinoma	0/123 (0%)	9/16*** (56%)	

**p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor.
[$] Reported as "squamous stomach" by authors.

Table 4. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
Strickland et al. (1988) Species: Mouse Strain: BALB/c Sex: Female Age at start of exposure: 12 wk old Study duration: Observed until death or killed when moribund or at 92 wk Control: Concurrent N = 20/group	Route: Gavage Dose: 0, 2 mg/mouse/wk (total dose: 60 mg/mouse) Frequency and duration: NHEX dissolved in corn oil, twice a wk for 30 wk. Control received vehicle only.			Total Dose (mg/mouse)		Survival: Survival was poor in the treated group (median survival: 37 vs. 92 wk for control).
				0	60	
		Nasal cavity (r)	Adenoma or mucosa-carcinoma	0/19 (0%)	4/19[#] (21%)	
		Esophagus (r)	Squamous cell papilloma	0/19 (0%)	1/19 (5%)	
		Lung	Adenoma	NR	5/19 (26%)	
			Adenocarcinoma	NR	2/19 (11%)	
			All	4/19 (21%)	6/19 (32%)	
		Liver	Hepatocellular adenoma	0/19 (0%)	5/19* (26%)	
			Hepatocellular carcinoma	0/19 (0%)	3/19 (16%)	
			Hemangiosarcoma	0/19 (0%)	6/19** (32%)	
			Cholangioma (r)	0/19 (0%)	4/19[#] (21%)	
			All	0/19 (0%)	14/19*** (74%)	
		Forestomach (r)	Squamous cell papilloma	NR	1/19 (5%)	
			All	2/19 (21%)	1/19 (5%)	

*p<0.05; **p<0.01; ***p<0.001; pairwise comparison with control by Fisher exact test (Performed by OEHHA. In cases where control incidence of tumor subtypes was not reported, OEHHA used the control incidence for "all" tumors at that site to perform the pairwise comparison.); NR: not reported; r: rare tumor.
[#]p=0.053, marginally significant

Table 4. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Total Dose (mg/mouse)		
				0	60	
Strickland et al. (1988) Species: Mouse Strain: CD-1 Sex: Female Age at start of exposure: 12 wk old Study duration: Observed until death or killed when moribund or at 92 wk Control: Concurrent N = 20/group	Route: Gavage Dose: 0, 2 mg/mouse/wk (total dose: 60 mg/mouse) Frequency and duration: NHEX in corn oil, twice a wk for 30 wk. Control received vehicle only.	Nasal cavity (r)	Adenoma or mucosa-carcinoma	0/20 (0%)	1/20 (5%)	Survival: Survival was poor in the treated group (median survival 49 vs. approximately 70 wk for control). Other comments: 3 liver hemangiosarcomas and one forestomach squamous cell carcinoma metastasized.
		Esophagus (r)	Squamous cell papilloma	0/20 (0%)	2/20 (10%)	
		Lung	Adenoma	NR	9/20* (45%)	
			Adenocarcinoma	NR	0/20 (0%)	
			All	2/20 (10%)	9/20* (45%)	
		Liver	Hepatocellular adenoma	NR	8/20* (40%)	
			Hepatocellular carcinoma	NR	0/20 (0%)	
			Hemangiosarcoma	NR	12/20** (60%)	
			Cholangioma (r)	NR	3/20 (15%)	
			All	2/20 (10%)	17/20*** (85%)	
		Forestomach (r)	Squamous cell papilloma	NR	2/20 (10%)	
			Squamous cell carcinoma	NR	3/20 (15%)	
			All	1/20 (5%)	5/20 (25%)	

*p<0.05; **p<0.01; ***p<0.001, pairwise comparison with control by Fisher exact test (Performed by OEHHA. In cases where control incidence of tumor subtypes was not reported, OEHHA used the control incidence for "all" tumors at that site to perform the pairwise comparison.); NR: not reported; r: rare tumor.

Table 4. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Total Dose (mg/mouse)		
				0	60	
Strickland et al. (1988) Species: Mouse Strain: SENCAR Sex: Female Age at start of exposure: 12 wk old Study duration: Observed until death or killed when moribund or at 92 wk Control: Concurrent N = 20/group	Route: Gavage Dose: 0, 2 mg/mouse/wk (total dose: 60 mg/mouse) Frequency and duration: NHEX in corn oil, twice a wk for 30 wk. Control received vehicle only.	Nasal cavity (r)	Adenoma or mucosa-carcinoma	0/20 (0%)	4/20# (20%)	Survival: Survival was poor in the treated group (median survival: 61 vs. approximately 81 wk for control).
		Esophagus (r)	Squamous cell papilloma	0/20 (0%)	4/20# (20%)	
		Lung	Adenoma	NR	15/20*** (75%)	
			Adenocarcinoma	NR	6/20* (30%)	
			All	1/20 (5%)	17/20*** (85%)	
		Liver	Hepatocellular adenoma	NR	6/20 (30%)	
			Hepatocellular carcinoma	NR	3/20 (15%)	
			Hemangiosarcoma	NR	3/20 (15%)	
			Cholangioma (r)	NR	3/20 (15%)	
			All	3/20 (15%)	12/20** (60%)	
		Forestomach (r)	Squamous cell papilloma	NR	1/20 (5%)	
			Squamous cell carcinoma	NR	9/20** (45%)	
			All	1/20 (5%)	10/20** (50%)	

*p<0.05; **p<0.01; ***p<0.001; pairwise comparison with control by Fisher exact test (Performed by OEHHA. In cases where control incidence of tumor subtypes was not reported, OEHHA used the control incidence for "all" tumors at that site to perform the pairwise comparison.); NR: not reported; r: rare tumor.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Althoff et al. (1972) Species: Mouse Strain: Swiss Sex: Male Age at start of exposure: 8 wk old Study duration: Observed until death Control: Concurrent N = 20/group	Route: Subcutaneous (*s.c.*) injection Dose: 0, 4, 8, 16, 32, 64 mg/kg-bw Frequency and duration: Single dose of NHEX dissolved in 0.9% NaCl solution. Control received vehicle only.				Survival: No significant difference between the control and treated groups (average survival: 69 wk). Tumor findings: A non-significant increase in lung adenoma was observed in treated animals. Lung adenomas were found in 10% of the controls (males and females combined) and in the treated males the lung tumor incidence averaged 31%. Treatment did not decrease lung tumor latency. Other comments: The authors did not report tumor incidence data for mice.

Table 4. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Althoff et al. (1972) Species: Mouse Strain: Swiss Sex: Female Age at start of exposure: 8 wk old Study duration: Observed until death Control: Concurrent N = 20/group	Route: *s.c.* injection Dose: 0, 4, 8, 16, 32, 64 mg/kg-bw Frequency and duration: Single dose of NHEX dissolved in 0.9% NaCl solution. Control received vehicle only.				Survival: No significant difference between the control and treated groups (average survival: 75 wk). Tumor findings: A non-significant increase in lung adenoma was observed in treated animals. Lung adenomas were found in 10% of the controls (males and females combined) and in the treated females the lung tumor incidence averaged 20%. Treatment did not decrease lung tumor latency. The lymphoma incidence in control mice was 23% and in treated females it was 33%. Other comments: The authors did not report tumor incidence data for mice.

3.2.2 Studies conducted in rats

In rats, two routes of administration were studied: six drinking water studies (3 strains; male and/or female; Goodall et al. 1968; Lijinsky and Taylor 1979; Lijinsky and Reuber 1981) and one *s.c.* injection study (male only; Schmähl 1968) (Table 5). Oral administration is more potent than *s.c.* injection for the carcinogenicity of NHEX in rats (Schmähl 1968). No apparent sex differences in target tumor sites were observed in NHEX-treated rats.

Studies via drinking water

In the studies of male and female MRC-Wistar rats (Goodall et al. 1968), NHEX was administered in drinking water via two different dosing schemes (high intensity/short duration: 200 mg/L for 8 weeks and low intensity/long duration: 50 mg/L for life). These studies did not include control groups; however, elevated incidences of several rare tumors in this strain of rats were observed in treated animals. Specifically, in the high intensity/short duration studies, the combined incidence of rare hepatocellular carcinomas and endothelial sarcomas (hemangiosarcomas) was 100% in males and 73% in females. In the low intensity/long duration studies, the incidence of rare squamous cell papillomas and carcinomas of the tongue was 53% in males and 20% in females and the incidence of rare squamous cell papillomas and carcinomas of the esophagus was 87% in males and 73% in females (Goodall et al. 1968).

In male S-D rats administered NHEX at a concentration of 110 mg/L in drinking water five days per week for 30 weeks, statistically significant increases in rare nasal turbinate adenocarcinomas, rare esophageal papillomas, rare hepatocellular carcinomas and rare liver sarcomas (mostly hemangiosarcomas) were observed (Lijinsky and Taylor 1979). Additionally, increases in several rare tumors that did not reach statistical significance were observed in this study, *e.g.*, a tongue papilloma, two esophageal carcinomas, a forestomach papilloma, and a lung alveolar cell adenoma (Lijinsky and Taylor 1979; Lijinsky and Taylor 1976). Three of these same rare tumor types, namely esophageal carcinomas, hepatocellular carcinomas, and liver hemangiosarcomas, were also statistically significantly increased in female Fischer 344 rats administered similar doses of NHEX (112 mg/L in drinking water five days per week for 28 weeks) (Lijinsky and Reuber 1981).

Studies via s.c. injection

Reporting of the *s.c.* injection study by Schmähl (1968) was limited, and there is no mention of a control group. Three rare liver hemangioendotheliomas

(hemangiosarcomas) and one rare liver carcinoma were observed in 62 male BR46 rats that received weekly injections of NHEX at a dose of 25 mg/kg-bw per week for 70 weeks. The mean time to tumor occurrence was 16 months.

Table 5. Summary of study design, exposure and tumor incidences in rat bioassays of NHEX

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Goodall et al. (1968) Species: Rat Strain: MRC-Wistar Sex: Male Age at start of exposure: 8-9 wk old Study duration: Observed until death (up to 68 wk). Control: No control N = 15 treated	Route: Drinking water Concentration: 200 mg/L (equivalent to 4 mg/day; "high" dose group) (total dose: 160 mg) Frequency and duration: 20 mL/mouse/day, 5 day/wk for a total of 8 wk. After 6 wk of treatment, dosing was stopped for one month followed by two additional wk of treatment.			Dose 4 mg/day	Survival: Two animals died between wk 30-40. Five animals survived to wk 60 and all had died by wk 68. BW: Animals lost weight after 6 wk. Other tumor sites of interest: One squamous cell carcinoma of the tongue (r) Other comments: First appearance of liver tumor at 36 wk
		Nasal cavity (r)	Squamous cell carcinoma, undifferentiated carcinoma and neuroepithelial carcinoma	2/15 (13%)	
		Esophagus (r)	Squamous cell papilloma and carcinoma	4/15 (27%)	
		Liver (r)	Hepatocellular carcinoma and endothelial sarcoma (hemangiosarcoma)	15/15 (100%)	

r: rare tumor

Table 5. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Goodall et al. (1968) Species: Rat Strain: MRC-Wistar Sex: Male Age at start of exposure: 8-9 wk old Study duration: Observed until death (up to 60 wk). Control: No control N = 15 treated	Route: Drinking water Concentration: 50 mg/L (equivalent to 1 mg/day; "low" dose group) (total dose: 150-300 mg) Frequency and duration: 20 mL/mouse/day, 5 day/wk for lifetime			Dose 1 mg/day	Survival: One animal died between wk 30-40. 11 animals survived to wk 50 and all had died by wk 60. BW: No treatment-related loss of bw reported. Other tumor sites of interest: One nasal cavity tumor (61 wk, r); 1 carcinoma of the pancreas. Other comments: First appearance of liver tumor at 48 wk
		Tongue (r)	Squamous cell papilloma and carcinoma	8/15 (53%)	
		Esophagus (r)	Squamous cell papilloma and carcinoma	13/15 (87%)	
		Liver (r)	Hepatocellular carcinoma and endothelial sarcoma (hemangiosarcoma)	2/15 (13%)	
		Forestomach (r)	Squamous cell carcinoma	2/15 (13%)	

r: rare tumor

Table 5. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Goodall et al. (1968) Species: Rat Strain: MRC-Wistar Sex: Female Age at start of exposure: 8-9 weeks old Study duration: Observed until death (up to 68 wk) Control: No control N = 15 treated	Route: Drinking water Concentration: 200 mg/L (equivalent to 4 mg/day; "high" dose group) (total dose: 160 mg) Frequency and duration: 20 mL/mouse/day, 5 day/wk for a total of 8 wk. After 6 wk of treatment, dosing was stopped for one month followed by two additional wk of treatment.			Dose 4 mg/d	Survival: Two animals died at 6 wk, one at 13 wk, four animals survived to 60 wk, and all had died by 68 wk. BW: Animals lost weight after 6 wk. Other tumor sites of interest: One carcinoma of the glandular stomach (r). Other comments: First appearance of liver tumor at wk 16.
		Nasal cavity (r)	Squamous cell carcinoma, undifferentiated carcinoma or neuroepithelial carcinoma	2/15 (13%)	
		Esophagus (r)	Squamous cell papilloma and carcinoma	2/15 (13%)	
		Liver (r)	Hepatocellular carcinoma and endothelial sarcoma (hemangiosarcoma)	11/15 (73%)	

r: rare tumor

Table 5. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Goodall et al. (1968) Species: Rat Strain: MRC-Wistar Sex: Female Age at start of exposure: 8-9 wk old Study duration: Observed until death (up to 60 wk) Control: No control N = 15 treated	Route: Drinking water Concentration: 50 mg/L (equivalent to 1 mg/day; "low" dose group) (total dose: 150-300 mg) Frequency and duration: 20 mL/mouse/day, 5 day/wk for lifetime			Dose 1 mg/day	Survival: One animal died between wk 30-40. Six animals survived to wk 50 and all had died by wk 60. BW: No treatment-related loss of bw reported. Other tumor sites of interest: None Other comments: First appearance of liver tumor at 41 wk.
		Tongue (r)	Squamous cell papilloma and carcinoma	3/15 (20%)	
		Esophagus (r)	Squamous cell papilloma and carcinoma	11/15 (73%)	
		Liver (r)	Hepatocellular carcinoma and endothelial sarcoma (hemangiosarcoma)	7/15 (47%)	

r: rare tumor

Table 5. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
				Concentration (mg/L)		
				0[a]	110	
Lijinsky & Taylor (1979) Species: Rat Strain: Sprague-Dawley (S-D) Sex: Male Age at start of exposure: 8-9 wk old Study duration: Observed until death or killed when moribund. Control: No concurrent control. Authors cited Lijinsky and Taylor (1976) for spontaneous tumor incidence from a continuous series of unexposed male rats from the same animal colony maintained in the same facility. N = 15 treated	Route: Drinking water Concentration: 110 mg/L (total dose: 330 mg). Frequency and duration: 20 mL/day, five days per wk for 30 wk	Nasal turbinate (r)	Adenocarcinoma	0/26 (0%)	7/15*** (47%)	Survival: Survival was poor in the treated group. Four animals were alive at wk 30, all died before wk 40. Other tumor sites of interest: One forestomach papilloma (r), 1 tongue papilloma (r) and 1 pharynx papilloma, 1 lung alveolar cell adenoma (r).
		Esophagus (r)	Papilloma	0/26 (0%)	9/15*** (60%)	
			Carcinoma	0/26 (0%)	2/15 (13%)	
		Liver (r)	Hepatocellular carcinoma	0/26 (0%)	3/15* (20%)	
			Sarcoma (mostly hemangiosarcoma)	0/26 (0%)	5/15** (33%)	

[a] The study did not include a concurrent control group. Authors referred to the spontaneous tumor incidence from a continuous series of unexposed male rats from the same animal colony maintained in the same facility (Lijinsky and Taylor 1976). The average lifespan of the unexposed rats was about 144 weeks. Pairwise comparison with reported colony control incidence by Fisher exact test (performed by OEHHA); *p<0.05; **p<0.01; ***p<0.001; r: rare tumor

Table 5. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
Lijinsky & Reuber (1981) Species: Rat Strain: Fischer 344 Sex: Female Age at start of exposure: 7-8 wk old Study duration: Observed until death or killed when moribund or at 132 wk Control: Concurrent N = 20/group	Route: Drinking water Concentration: 0, 112 mg/L (total dose: 314 mg). Frequency and duration: 20 mL/day, five days per wk for 28 wk			Concentration (mg/L)		Survival: Survival was poor in the treated group. Two females were alive at 30 wk, the last animal died at 37 wk. All control animals survived until 60 weeks; surviving controls were killed at 132 weeks. Other tumor sites of interest: One forestomach papilloma (r).
				0	112	
		Esophagus (r)	Papilloma	0/20 (0%)	4/20 (20%)	
			Carcinoma	0/20 (0%)	14/20*** (70%)	
			Combined	0/20 (0%)	18/20*** (90%)	
		Liver (r)	Hepatocellular carcinoma	1/20 (5%)	[a]6/20* (30%)	
			Hemangio-sarcoma	0/20 (0%)	[a]13/20*** (65%)	

*p<0.05; ***p<0.001; pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor

[a]3 animals have both tumor types

Table 5. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Schmähl (1968) Species: Rat Strain: BR46 Sex: Male Age at start of exposure: 3 months old Study duration: Not reported, assumed to be until death or killed when moribund. Control: No control reported N = 62 treated	Route: *s.c.* injection Dose: 25 mg/kg-bw (total dose 1750 mg/kg-bw) Frequency and duration: NHEX dissolved in oil, weekly *s.c.* injections for 70 wk				Survival: Not reported Tumor findings: Six rats had tumors. Three had liver hemangioma (r); a fourth had a hepatocellular carcinoma (r), a fifth had a retothel sarcoma of the pancreas, and the sixth had a squamous cell carcinoma of the paranasal sinus (r) Other comments: 1) Abstract published in German. 2) No reported controls. 3) The mean time to tumor occurrence was 16 months.

3.2.3 Studies conducted in hamsters

NHEX was administered to Syrian golden hamsters in seven studies by *s.c.* injection (Althoff et al. 1972; Althoff et al. 1973; Althoff et al. 1976; Althoff and Grandjean 1979) (Table 6). In an additional four studies, male and female F_1 offspring were exposed to NHEX via transplacental exposure as a result of *s.c.* injection of the pregnant dams between gestation days (GD) 8 to 15 (Althoff et al. 1976; Althoff and Grandjean 1979) (Table 6).

Studies via s.c. injection

A single *s.c.* injection (Althoff et al. 1972) or multiple *s.c.* injections (Althoff et al. 1973) of relatively low doses of NHEX (4 - 64 mg/kg-bw; less than 1 mg/hamster) were administered to male and female hamsters. Doses used in these hamster studies were much lower than those administered in the rat and mouse studies discussed above. Rare tracheal tumors were statistically significantly increased in males in the lowest dose group and in females in the highest dose group in the single injection studies (Althoff et al. 1972). In animals given multiple doses of *s.c.* injections, stronger tumor responses were seen, including statistically significant increases in rare tumors of the nasal cavity and trachea in both male and female hamsters. In addition, rare lung tumors were statistically significantly increased in males receiving multiple injections. In females receiving multiple injections, one rare lung tumor was observed in the highest dose group (Althoff et al. 1973).

Two-generation studies via s.c. injection

In two sets of hamster two-generation carcinogenicity studies designed to investigate susceptibility of the prenatal life stage to NHEX, either a single low “non-carcinogenic” dose (Althoff et al. 1976; Althoff and Grandjean 1979) or multiple doses were administered to pregnant dams (Althoff et al. 1976). As predicted, no treatment-related tumors were observed in the dams that received a single low dose of NHEX in either set of studies. Althoff *et al.* (1976) observed three rare benign tumors in the offspring exposed transplacentally to the single low dose of NHEX (two papillary polyps of the larynx and one papillary polyp of the trachea among 186 male and female offspring combined), while no tumors were reported in the 93 male or 93 female offspring transplacentally exposed to a single low dose of NHEX in the studies of Althoff and Grandjean (1979). In the multiple injection studies of Althoff *et al.* (1976) in which pregnant females received up to a total dose of 80 mg/kg-bw (~1 mg/dam), statistically significant increases in rare benign tumors (papillary polyps) of the larynx and trachea, two rare nasal cavity adenocarcinomas, and one rare papillary tumor of the lungs were

observed in the dams. The tumor response in the offspring (males and females combined) exposed transplacentally to multiple doses of NHEX was similar to that of the dams: statistically significant increases in rare benign tumors (papillary polyps) of the larynx and trachea, and one rare papillary tumor of the lungs.

Table 6. Summary of study design, exposure and tumor incidences in hamster bioassays of NHEX

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)						Comments
				Dose (mg/kg-bw)						
				0	4	8	16	32	64	
Althoff et al. (1972) Species: Hamster Strain: Syrian golden Sex: Male Age at start of exposure: 8 wk old Study duration: Observed until death Control: Concurrent N = 20/group	Route: *s.c.* injection Dose: 0, 4, 8, 16, 32, 64 mg/kg-bw Frequency and duration: Single dose of NHEX dissolved in 0.9% NaCl solution. Control received vehicle only.	Trachea (r)	Papillary polyps	0/17 (0%)	5/20* (25%)	2/17 (12%)	2/20 (10%)	3/19 (16%)	4/20 (20%)	Survival: No significant difference between the control and treated groups (average survival: 67 wk).

*p<0.05; pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor

Table 6. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)						Comments
				Dose (mg/kg-bw)						
				0	4	8	16	32	64	
Althoff et al. (1972) Species: Hamster Strain: Syrian golden Sex: Female Age at start of exposure: 8 wk old Study duration: Observed until death Control: Concurrent N = 20/group	Route: *s.c.* injection Dose: 0, 4, 8, 16, 32, 64 mg/kg-bw Frequency and duration: Single dose of NHEX dissolved in 0.9% NaCl solution. Control received vehicle only.	Trachea (r)	Papillary polyps	0/15 [t+++] (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	3/13 (23%)	5/19* (26%)	Survival: No significant difference between the control and treated groups (average survival: 50 wk).

*p<0.05; pairwise comparison with control by Fisher exact test (performed by OEHHA); [t]+++: Trend test p< 0.001; r: rare tumor

Table 6. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)						Comments
				Dose (mg/kg-bw)						
				0	4	8	16	32	64	
Althoff et al. (1973) Species: Hamster Strain: Syrian golden Sex: Male Age at start of exposure: 8 wk old Study duration: Observed until death or killed when moribund or dyspneic. Control: Concurrent N = 20/group	Route: *s.c.* injection Dose: 0, 4, 8, 16, 32, 64 mg/kg-bw Frequency and duration: NHEX dissolved in 0.9% NaCl solution, injected once a wk for life. Control received vehicle only.	Nasal cavity (r)	Primarily adenocarcinoma	0/20 (0%)	0/19 (0%)	1/19 (5%)	4/19* (21%)	10/15*** (67%)	0/20 (0%)	Survival: Dose-dependent decrease in survival observed in the 3 highest dose groups, compared to control. Other tumor sites of interest: Malignant schwannoma (r): none in controls, 1 each in the 4 mg/kg-bw and 8 mg/kg-bw groups. Lacrimal gland adenoma: none in controls, 1 in 8 mg/kg-bw, 1 in 16 mg/kg-bw, and 2 in 32 mg/kg-bw groups.
		Larynx (r)	Neoplasm[1]	0/20 (0%)	0/19 (0%)	2/19 (11%)	2/19 (11%)	2/15 (13%)	0/20 (0%)	
		Trachea (r)	Papillary tumors	0/20[t++] (0%)	4/19* (21%)	12/19*** (63%)	13/19*** (68%)	14/15*** (93%)	10/20*** (50%)	
		Lung (r) bronchi	Neoplasm[2]	0/20 (0%)	2/19 (11%)	4/19* (21%)	1/19 (5%)	0/15 (0%)	0/20 (0%)	
		Forestomach	Squamous cell papilloma	1/20 (5%)	2/19 (11%)	3/19 (16%)	1/19 (5%)	2/15 (13%)	0/20 (0%)	

[1] Not clearly specified, included squamous cell papillomas
[2] Not clearly specified, included papillary tumors, adenocarcinomas, and other carcinomas
*p<0.05; ***p<0.001; pairwise comparison with control by Fisher exact test (performed by OEHHA)
[t]++: Trend test p< 0.01; r: rare tumor

Table 6. Continued

Reference/study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)						Comments
				Dose (mg/kg-bw)						
				0	4	8	16	32	64	
Althoff et al. (1973) Species: Hamster Strain: Syrian golden Sex: Female Age at start of exposure: 8 wk old Study duration: Observed until death or killed when moribund or dyspneic Control: Concurrent N=20/group	Route: *s.c.* injection Dose: 0, 4, 8, 16, 32, 64 mg/kg-bw Frequency and duration: NHEX dissolved in 0.9% NaCl solution, injected once a wk for life. Control received vehicle only.	Nasal cavity (r)	Primarily adenocarcinoma	0/20 (0%)	0/20 (0%)	0/20 (0%)	2/19 (11%)	4/16* (25%)	0/20 (0%)	Survival: Dose-dependent decrease in survival observed in the 3 highest dose groups, compared to control. Other tumor sites of interest: Malignant schwannoma (r): none in controls, 1 each in 4 mg/kg-bw and 16 mg/kg-bw groups.
		Larynx (r)	Neoplasm[1]	0/20 (0%)	1/20 (5%)	0/20 (0%)	1/19 (5%)	0/16 (0%)	0/20 (0%)	
		Trachea (r)	Papillary tumors	0/20[t+] (0%)	7/20** (35%)	6/20* (30%)	10/19*** (53%)	16/16*** (100%)	6/20* (30%)	
		Lung (r) bronchi	Neoplasm[2]	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/19 (0%)	0/16 (0%)	1/20 (5%)	
		Forestomach	Squamous cell papilloma	0/20 (0%)	2/20 (10%)	1/20 (5%)	0/19 (0%)	1/16 (6%)	0/20 (0%)	

[1] Not clearly specified, included squamous cell papillomas
[2] Not clearly specified, included papillary tumors, adenocarcinomas, and other carcinomas
*p<0.05; **p<0.01; ***p<0.001; pairwise comparison with control by Fisher exact test (performed by OEHHA)
[t]+: Trend test p< 0.05; r: rare tumor

Table 6. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)			Comments
				Total Dose (mg/kg-bw)			
				0	10 (Single injection)	20-80 (Multiple injections)	
Althoff et al. (1976) Species: Hamster Strain: Syrian Sex: Pregnant females Age at start of exposure: 12 wk old (after mating); either a single or multiple *s.c.* injections between gestation day (GD) 8-15 Study duration: Observed until death. Control: Concurrent N = 20 controls N = 40 single dose group N = 35 multiple dose group	Route: *s.c.* injection Dose: 0, 10 mg/kg-bw per injection Frequency and duration: 1) Single dose of 10 mg/kg-bw between GD 8-15. 2) Multiple doses injected between GD 8-15 (2-8 doses per dam; total dose: 20-80 mg/kg-bw).	Nasal cavity (r)	Respiratory epithelium adenocarcinoma	0/20 (0%)	0/40 (0%)	2/35 (6%)	Survival: No significant differences; controls: average 57 wk (range: 14-91 wk), single injection: average 63 wk (range: 8-90 wk), multiple injections: average 64 wk (range: 4-88 wk). Other comments: Two *s.c.* studies, a single injection and a multiple injection study, are reported here. The single injection study employed a low "non-carcinogenic" dose, to investigate susceptibility of the prenatal life stage.
		Larynx (r)	Papillary polyps	0/20 (0%)	0/40 (0%)	7/35* (20%)	
		Trachea (r)	Papillary polyps	0/20 (0%)	0/40 (0%)	10/35** (29%)	
		Lung (r) bronchi	Papillary polyps	0/20 (0%)	0/40 (0%)	1/35 (3%)	

*p<0.05; **p<0.01; pairwise comparison with control by Fisher exact test (performed by OEHHA); r: rare tumor

Table 6. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)			Comments
				Total Dose (mg/kg-bw)			
				0 mg/kg	10 (Single injection)	20-80 (Multiple injections)	
Althoff et al. (1976) Species: Hamster Strain: Syrian Sex: Male and female offspring from pregnant hamsters given either a single or multiple *s.c.* injections Age at start of exposure: *in utero* exposure on one or multiple days between GD 8-15 Study duration: Life-long, observed until death. Control: Concurrent N = 213 controls N = 186 single dose group N = 168 multiple dose group	Route: Transplacental exposure via *s.c.* injection to dams Dose: 0, 10 mg/kg-bw per injection Frequency and duration: Pups exposed via transplacental exposure. 1) Single *s.c.* injection between GD 8-15. 2) Multiple *s.c.* injections between GD 8-15 (2-8 doses per dam; total dose: 20-80 mg/kg-bw).	Larynx (r)	Papillary polyps	0/213 (0%)	2/186 (1%)	12/168*** (7%)	Survival: No significant differences; controls: average 59 wk (range: 6-121 wk), treated groups (single and multiple dose studies): average 62 wk (range: 6-129 wk). Other comments: Two *s.c.* studies, a single injection and a multiple injection study, are reported here. The single injection study employed a low "non-carcinogenic" dose, to investigate susceptibility of the prenatal life stage.
		Trachea (r)	Papillary polyps	0/213 (0%)	1/186 (1%)	13/168*** (8%)	
		Lung (r) bronchi	Papillary polyps	0/213 (0%)	0/186 (0%)	1/168 (1%)	

***p<0.001; pairwise comparison with control by Fisher exact test (performed by OEHHA) r: rare tumor

Table 6. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Althoff and Grandjean (1979) Species: Hamster Strain: Syrian golden Sex: Pregnant female Age at start of exposure: Sexually mature dams (after mating); single *s.c.* injection on GD 8, 10, 12, or 14 (in some instances GD 15) Study duration: Observed until death or killed when moribund Control: Concurrent N = 21 controls N = 20 treated	Route: *s.c.* injection Dose: 0, 10 mg/kg-bw Frequency and duration: Single dose of NHEX, dissolved in 0.9% NaCl solution, on GD 8, 10, 12, or 14 (in some instances GD 15).				Survival: No significant differences; controls: average 69 wk, treated: average 68 wk. Tumor findings: None Other comments: "Non-carcinogenic" single low dose selected by authors to investigate susceptibility of prenatal life stage.

Table 6. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Althoff and Grandjean (1979) Species: Hamster Strain: Syrian golden Sex: Male offspring from pregnant hamsters given a single *s.c.* injection Age at start of exposure: *in utero* exposure to a single dose on GD 8, 10, 12, or 14 (in some instances GD 15) Study duration: Life-long, observed until death Control: Concurrent N = 100 controls N = 93 treated	Route: Transplacental exposure via *s.c.* injection to dams. Dose: 0, 10 mg/kg-bw Frequency and duration: Pups exposed via transplacental exposure of a single *s.c.* injection at GD 8, 10, 12, or 14 (in some instances GD 15).				Survival: No significant differences; controls: average 72 wk, treated: average 70 wk. Tumor findings: None Other comments: "Non-carcinogenic" single low dose selected by authors to investigate susceptibility of prenatal life stage.

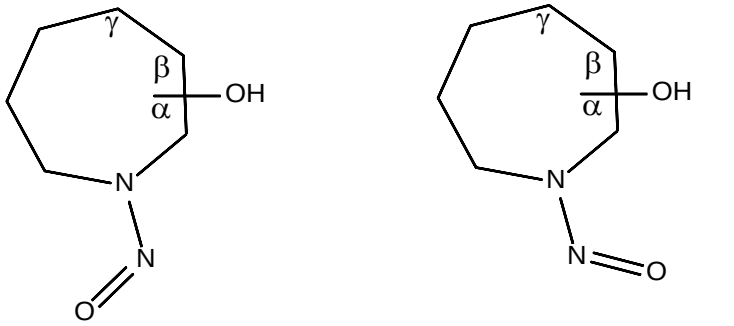
Table 6. Continued

Reference/ study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Althoff and Grandjean (1979) Species: Hamster Strain: Syrian golden Sex: Female offspring from pregnant hamsters given a single *s.c.* injection Age at start of exposure: *in utero* exposed a single dose in GD 8, 10, 12, or 14 (in some instances, GD 15) Study duration: Life-long, observed until death. Control: Concurrent control N = 113 controls N = 93 treated	Route: Transplacental exposure via *s.c.* injection to dams. Dose: 0, 10 mg/kg-bw Frequency and duration: Pups were exposed via transplacental exposure of a single *s.c.* injection at GD 8, 10, 12, or 14 (in some instances, GD 15).				Survival: No significant differences; controls: average 66 wk, treated: average 66 wk. Tumor findings: None Other comments: "Non-carcinogenic" single low dose selected by authors to investigate susceptibility of prenatal life stage.

3.3 Other Relevant Data

3.3.1 Pharmacokinetics and metabolism

The pharmacokinetics and metabolism of NHEX have been studied in animals *in vivo* and *in vitro.* No human data have been identified. Briefly, *in vivo* studies have been conducted in rats, mice, and hamsters, and *in vitro* metabolism studies have been conducted with rat and hamster liver and lung subcellular fractions. Orally administered NHEX is readily absorbed and rapidly distributed throughout the body. NHEX can be metabolized by hydroxylation of any of the carbon atoms (α, β, or γ to the nitroso group) to form either an E (*trans*) or Z (*cis*) conformer (see Figure 2 below). β- and γ-hydroxylated NHEX are stable and account for one-third of NHEX metabolites. α-Hydroxylated NHEX is a reactive intermediate and accounts for the majority of hydroxylated NHEX metabolites. In addition to α-, β-, and γ-hydroxylation of ring carbons, other metabolic pathways have been proposed that also involve production of reactive metabolites of NHEX. In animals, NHEX is completely metabolized and is excreted in the urine and as expired CO_2.



NHEX Z conformer NHEX E conformer

Figure 2. E (*trans*) and Z (*cis*) conformers of hydroxylated NHEX. Carbon atoms are labeled (relative to the nitroso group) as α, β, and γ carbons.

3.3.1.1 Absorption and Distribution

NHEX is quickly absorbed following administration by oral gavage in rats and subcutaneous injection in hamsters and mice (Althoff et al. 1977; Grandjean 1976; Abdurakhmanov 1983). Following a single oral gavage dose of ^{14}C-NHEX in rats, expired $^{14}CO_2$ was detected within 4 hours (Grandjean 1976). Following subcutaneous injection to pregnant Syrian hamsters, NHEX was observed in both maternal tissues, including maternal blood, placenta, and amniotic fluid, and in fetal tissues after 15 minutes (Althoff et al. 1977). Following subcutaneous injection of ^{3}H-NHEX to mice,

radioactivity was detected in all tissues examined (liver, kidneys, spleen, brain, gut epithelium, muscle, and plasma) within 30 minutes (Abdurakhmanov 1983).

NHEX and its metabolites are distributed throughout the body in rats and mice (Grandjean 1976; Abdurakhmanov 1983). In rats, ^{14}C-NHEX and its metabolites were distributed in all tissues examined (liver, lung, kidney, spleen, and blood) following a single oral dose. The majority of NHEX is distributed to the liver in rats. Peak radioactivity detection in the liver occurred at approximately 24 hours and slowly decreased afterwards. Radioactivity was still detectable in the liver, lung, and kidneys after 10 days (Grandjean 1976). In mice, the majority of NHEX and its metabolites were distributed to the liver and gut epithelium, followed by the kidneys, spleen, brain, and muscles after subcutaneous injection (Abdurakhmanov 1983).

Pregnant Syrian hamsters given a subcutaneous injection of 391 µmoles (or 50 mg/kg bw) NHEX on gestation day 14 exhibited measurable amounts of unchanged NHEX in maternal blood, placenta, amniotic fluid, and the fetus as early as 15 minutes following injection (Althoff et al. 1977). NHEX reached a maximum concentration in maternal blood 30 minutes after subcutaneous injection and after one hour in the placenta, amniotic fluid, and fetus. After two hours, only traces of unchanged NHEX were observed in most samples. Maximal levels in the amniotic fluid were more than twice the maximal levels found in the placenta and fetus. Thirty to 60 minutes post-injection, approximately one-sixth of the amount measured in maternal blood was measured in the fetus.

3.3.1.2 Metabolism

A number of NHEX metabolites have been identified in studies conducted in rats and mice that analyzed urine or liver samples, and in studies conducted *in vitro* with rat or hamster liver or lung microsomes with or without cytosol (Tables 7 and 8). Metabolites identified in rats and mice *in vivo* include ϵ-caprolactam, ϵ-aminocaproic acid (ϵ-aminocaproate), ϵ-aminocaprohydroxamic acid, adipic acid, β-oxidized derivatives (β-alcohol and β-ketone), γ-oxidized derivatives (γ-alcohol), 1,6-hexanediol, ω-oxycaproic acid, α-hydroxylated NHEX, and carbon dioxide (CO_2) (see Table 7) (Grandjean 1976; Ross and Mirvish 1977; Singer and MacIntosh 1984; Abdurakhmanov 1983). Additional metabolites that have been identified *in vitro* include β-hydroxylated NHEX (E, Z conformers), γ-hydroxylated NHEX (E, Z conformers), 6-hydroxyhexanal, and ϵ-hydroxycaproate (ϵ-hydroxycaproic acid) (see Table 8) (Hecker and Saavedra 1980; Hecker and McClusky 1982; Hecker et al. 1983; Hecker et al. 1984; Farrelly and Hecker 1984). Other metabolites of NHEX have been observed in rats *in vivo* and *in vitro*, but their structures have not been determined (Snyder et al. 1977; Hecker and

McClusky 1982). Several additional chemicals are proposed to be chemical intermediates of NHEX metabolism, including hexamethyleneimine, hexamethyleneimeine, diazohydroxide, 6-aminohexanal, a nitrosonium ion (NO^+), a carbonium ion, a NHEX imminium ion and a NHEX radical (see Figures 3 and 4; Grandjean 1976; Hecker et al. 1984).

NHEX is metabolized by cytochrome P450 enzymes, forming heme-iron-bound activated oxygen. It has been proposed that the NHEX-P450 complex, which contains the activated oxygen, eventually results in the formation of a NHEX radical or other activated species, such as a NHEX imminium ion, and formation of NO+ as a result of denitrosation (Hecker et al. 1984; see Figure 3). Hydroxylation of NHEX may occur at the α, β, or γ carbons. ^{14}C-NHEX-labeling studies have shown that CO_2 is produced from hydroxylated α, β, and γ carbons of NHEX, and exhaled via the lungs (Figure 3).

While hydroxylation of NHEX occurs at either the α, β, or γ carbons, the primary pathway of NHEX metabolism is through α-hydroxylation. After α-hydroxylation, the ring structure is cleaved between the α-carbon and the ring nitrogen to form diazohydroxide (Figure 4). Diazohydroxide can be further metabolized via a proposed unstable intermediate carbonium ion to form 6-hydroxyhexanal. After a reduction reaction, 6-hydroxyhexanal is converted to 1,6-hexanediol. Further metabolism of 1,6-hexanediol has been shown to yield ϵ-hydroxycaproate (ϵ-hydroxycaproic acid).

Additional pathways by which α-hydroxy NHEX is metabolized to adipic acid, ϵ-caprolactam, ϵ-aminocaproate, and ω-oxycaproic acid are shown in Figure 4. This figure also shows an additional postulated pathway for the formation of ω-oxycaproic acid from the proposed NHEX metabolite hexamethyleneimeine.

Table 7. Summary of NHEX metabolites detected *in vivo*

Sex/ species/ strain	Dose	Biological sample (analytical methods)	Metabolites	Reference
Male MRC-Wistar rats	12 mg/kg-bw (10-21 μCi) [2-^{14}C] NHEX/rat	Urine (GC, TLC, MS)	ϵ-Aminocaprohydroxamic acid, ϵ-Aminocaproic acid, ϵ-Caprolactam, CO_2	Grandjean (1976)
Male Fischer 344 rats	12.6 mg of [2-^{14}C] NHEX/rat	Urine (GC, GC/thermal energy analysis, GC/MS)	Adipic acid, β-Oxidized derivatives (β-alcohol and β-ketone), γ-Oxidized derivatives (γ-alcohol)	Singer and MacIntosh (1984)
Male Wistar rats	60 mg/kg-bw (500 μCi) [2-^{14}C] NHEX or 60 mg/kg bw (2.8 mCi) [^{3}H] NHEX/ rat	Liver (cation exchange, anion exchange, TLC and GC)	1,6-Hexanediol (bound to rat liver nucleic acid)	Ross and Mirvish (1977)
Female Sprague-Dawley rats	2 or 130 mg [α-^{14}C] NHEX/rat	Urine and excreted CO_2 (methods not specified)	CO_2	Snyder *et al.* (1977)
Mice, unspecified strain	25 mg/kg-bw (10 μCi/mg) [^{3}H] NHEX/mouse	Urine from non-tumor-bearing mice (methods not specified)	ε-Aminocaproic acid, ω-Oxycaproic acid	Abdurakhmanov (1983)
		Urine from tumor-bearing mice (methods not specified)	α-Hydroxylated NHEX, 1,6-Hexanediol	

GC: gas chromatography; TLC: thin-layer chromatography; MS: mass spectrometry

Table 8. Summary of NHEX metabolites detected *in vitro*

Test system	Compound	Metabolites detected	Reference
Male Fischer 344 rat liver microsomes +/- post-microsomal supernatant	[α-^{14}C] NHEX	β-Hydroxylated NHEX (E and Z conformers), γ-Hydroxylated NHEX (E and Z conformers)	Hecker and Saavedra (1980)
Male Fischer 344 rat liver and lung microsomes +/- cytosol	[α-^{14}C] NHEX	β-Hydroxylated NHEX (E and Z conformers), γ-Hydroxylated NHEX (E and Z conformers), ε-Hydroxycaproate, ε-Aminocaproic acid, 6-Hydroxyhexanal, 1,6-Hexanediol	Hecker and McClusky (1982)
Rat & hamster liver (uninduced or induced with PB or Aroclor 1254) S8 or S9 fraction and cytosol	[α-^{14}C] NHEX	β-Hydroxylated NHEX (E and Z conformers), γ-Hydroxylated NHEX, ε-Hydroxycaproate, ε-Aminocaproate, 6-Hydroxyhexanal, 1,6-Hexanediol	Hecker *et al*. (1983)
Male Fischer 344 rat liver induced with PB	[β-^{14}C] NHEX	β-Hydroxylated NHEX, γ-Hydroxylated NHEX, ε-Hydroxycaproate, ε-Aminocaproate	Hecker *et al*. (1984)
Male Fischer 344 and Sprague-Dawley rat liver induced with Aroclor 1254 or PB	[α-^{14}C] NHEX	β-Hydroxylated NHEX (E and Z conformers), γ-Hydroxylated NHEX (E and Z conformers)	Farrelly and Hecker (1984)

PB: phenobarbital

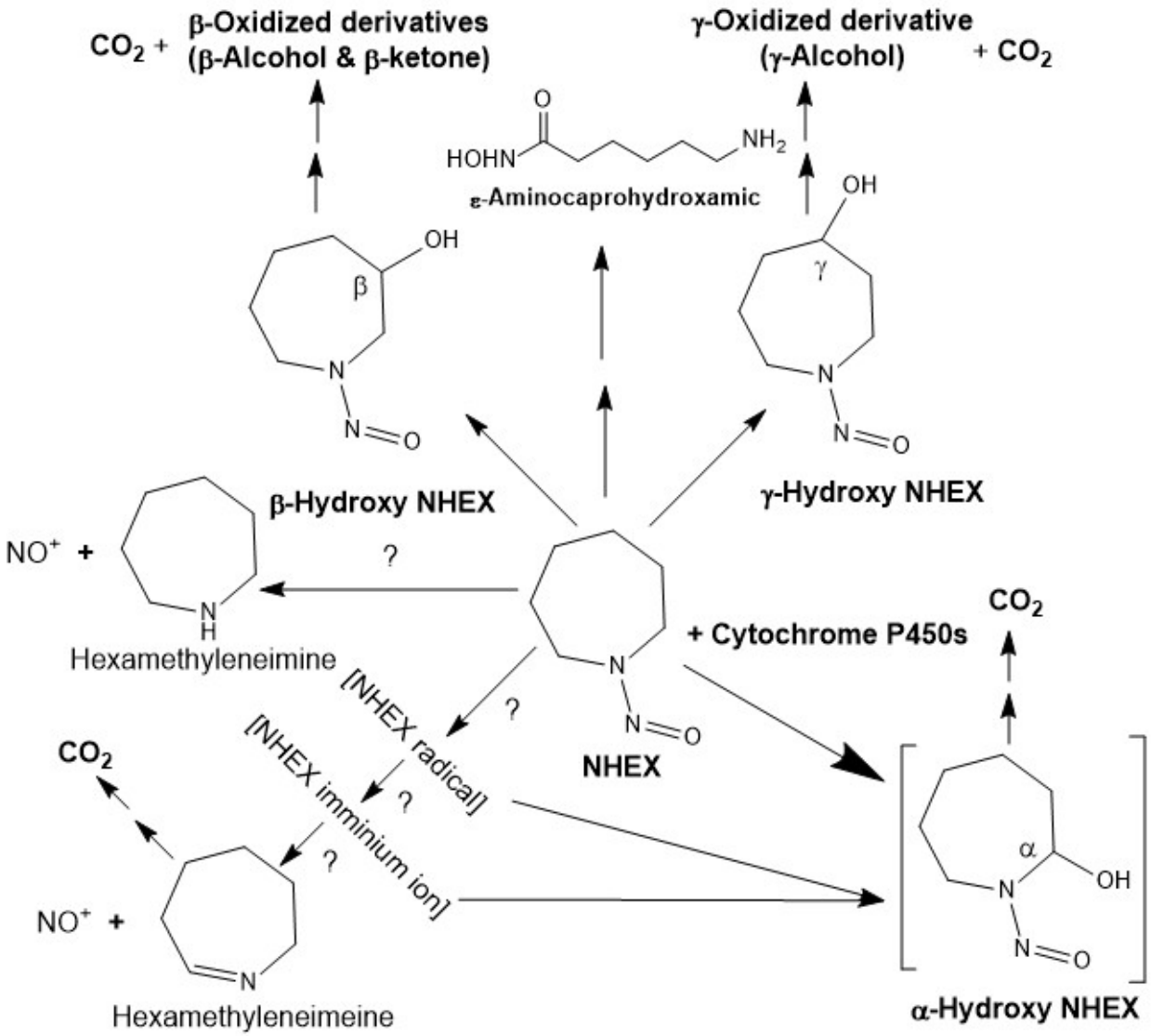


Figure 3. Overview of NHEX metabolism

Chemical names in bold indicate detection in mammalian systems. Reactive intermediates are in brackets. Question marks indicate proposed reactions/pathways. Modified from Hecker *et al*. (1984), Grandjean (1976), and Singer and MacIntosh (1984).

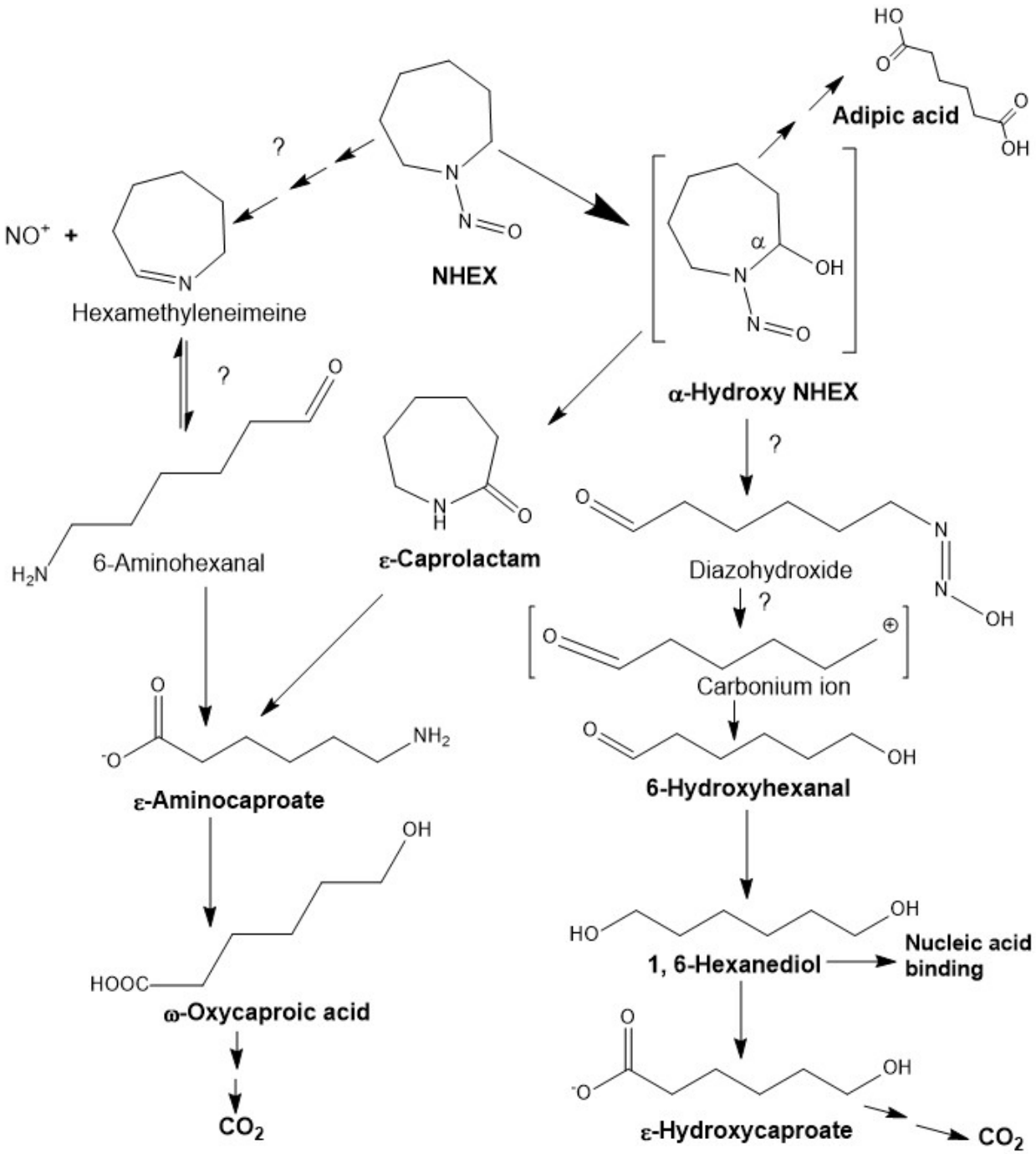


Figure 4. Proposed metabolic pathways for α-hydroxy NHEX and hexamethyleneimeine

Chemical names in bold indicate detection in mammalian systems. Reactive intermediates are in brackets. Question marks indicate proposed reactions/pathways. Modified from Hecker *et al*. (1984), Grandjean (1976), Abdurakhmanov (1983), and Singer and MacIntosh (1984).

3.3.1.3 Excretion

In rats, excretion of NHEX and its metabolites is rapid and proceeds primarily via the urine and exhalation as CO_2 from the lungs (Grandjean 1976; Singer and MacIntosh 1984; Snyder et al. 1977).

The urinary excretion of NHEX has been studied in rats administered NHEX via gavage in two studies. In one study, 36.7% of the administered dose (approximately 2 to 4 mg/rat) of radioactivity was estimated to be excreted in the urine in the first 24 hours (Grandjean 1976). In another study, 31% of the administered ^{14}C-NHEX (12.6 mg/rat) was excreted in the urine in the first 24 hours, and an additional 18% within 48 hours (Singer and MacIntosh 1984).

Exhalation as CO_2 has been observed in two studies in rats administered NHEX at doses of 2 to 4 mg/rat via gavage. In one study, expired $^{14}CO_2$ was detected at 4 hours and increased linearly for about 24 hours, at which point it leveled off. The amount of expired CO_2 accumulated for 24 hours was 21.6% of the administered dose. After 24 hours, an additional 18.1% of the administered dose was expired as $^{14}CO_2$ (Grandjean 1976). In another study, 43% of the administered NHEX was converted to CO_2 after 24 hours (Snyder et al. 1977).

3.3.2 Genotoxicity

Studies evaluating the genotoxic potential of NHEX consist of mutagenicity assays in *Salmonella typhimurium (S. typhimurium)*, *Escherichia coli (E. coli)*, mammalian cells *in vitro*, and *Drosophila melanogaster (D. melanogaster)*, and DNA damage and DNA and RNA binding studies in rats *in vivo*. Five metabolites of NHEX have also been tested in *S. typhimurium* and a variety of other genotoxicity assays. The findings from these studies are presented below.

3.3.2.1 Mutagenicity studies of NHEX in bacteria

As shown in Table 9, the mutagenicity of NHEX was tested in reverse mutation assays in *S. typhimurium* strain TA1535, which detects base-pair substitutions, in multiple studies, and in *E. coli* WU 3610 in one study. NHEX induced mutations in *S. typhimurium* TA1535 in the presence of metabolic activation (*e.g.*, addition of S-9) Farrelly and Hecker 1984; Hecker et al. 1983; Rao et al. 1979; Zeiger and Sheldon 1978). Mutagenic activity was observed with liver S-9 preparations from mice, rats, and hamsters. The studies of Hecker et al. (1983) reported greater mutagenic activity of NHEX in the presence of Aroclor 1254- or phenobarbital (PB)-induced hamster liver S-9 compared to uninduced hamster liver S-9 and in the presence of PB-induced rat liver S-9 compared to uninduced rat liver S-9. The authors found there was a strong correlation between the amount of α-hydroxylation products formed with the various S-9 preparations and the mutagenicity activity observed. Weak mutagenic activity of NHEX in *S. typhimurium* in the absence of S-9 was reported only by Farrelly and Hecker (1984).

NHEX tested positive in the *E. coli* WU 3610 reverse mutation assay following incubation with PB-induced rat liver S-9 (Elespuru and Lijinsky 1976).

Table 9. Mutagenicity studies of NHEX in bacteria

Test system	Strain	Concentration tested	Results		Activation system	References
			- S-9	+ S-9		
***S. typhimurium* reverse mutation assay**	TA1535 suspension	0.01-1 μmoles/mL	-	+	Mouse and rat liver S-9	Zeiger and Sheldon 1978
	TA1535 plate incorp-oration	0.01-1 μmoles/plate	-	+	Mouse and rat liver S-9	
	TA1535	10-200 μg/plate	-	+	Rat liver S-9: PB-induced	Rao *et al.* 1979
	TA1535 plate test	100-1000 μg/plate	NT	+	Rat and hamster liver S-9: uninduced; PB-induced; and Aroclor 1254-induced	Hecker *et al.* 1983
	Strain not specified	2.5-1000 μg/plate	(+)	+	Rat liver S-9: Aroclor 1254-induced	Farrelly and Hecker 1984
***E. coli* reverse mutation assay**	WU 3610 (*tyr⁻, leu⁻)*	16.7 mM	-	+	Rat liver S-9: PB-induced	Elespuru and Lijinsky 1976

NT: not tested; +: positive result; −: negative result; (+): weakly or marginally positive; S-9: supernatant fraction from liver homogenate centrifuged at 9,000 g for 10 minutes

3.3.2.2 Genotoxicity studies of NHEX in mammalian cells in vitro

NHEX was mutagenic to Chinese hamster lung fibroblast V79 cells co-cultured with primary Fischer rat hepatocytes in 6-thioguanine and ouabain resistance mutation assays (Table 10) (Jones et al. 1981).

Table 10. Genotoxicity studies of NHEX in mammalian cells *in vitro*

Endpoint	Test system	Results	Reference
6-Thioguanine resistance mutation	Primary rat hepatocyte-mediated mutagenesis assay in Chinese hamster V79 cells	+	Jones *et al*. 1981
Ouabain resistance mutation	Primary rat hepatocyte-mediated mutagenesis assay in Chinese hamster V79 cells	+	Jones *et al*. 1981

+: positive result

3.3.2.3 Genotoxicity studies of NHEX in vivo

As shown in Table 11, the available *in vivo* genotoxicity studies of NHEX consist of a mutagenicity study in *D. melanogaster*, a DNA damage study in rats, and three RNA and DNA binding studies in rats.

NHEX tested positive in the *D. melanogaster* X-linked recessive-lethal mutation assay conducted by Nix et al. (1980).

Swenberg (1981) reported results of alkaline elution assays conducted on liver, lung, kidney, and duodenum tissue from rats administered NHEX either orally or by intraperitoneal (*i.p.*) injection at doses of 25-250 mg/kg-bw for three weeks. The alkaline elution assay measures DNA damage by detecting single strand breaks and alkali-labile sites. In these studies, NHEX did not induce DNA damage in any of the tissues examined.

NHEX has been shown in multiple studies to bind covalently to RNA and DNA in the liver of rats administered a single oral dose of either [^{3}H]NHEX or [2-^{14}C]NHEX (Lijinsky and Ross 1969; Ross and Mirvish 1977; Ross and Lawson 1982). Lijinsky and Ross (1969) reported binding of [^{3}H]NHEX to rat liver RNA and DNA; however, they did not detect alkylation of either guanine (*i.e.*, 7-methylguanine) or adenine. Ross and Mirvish (1977) reported covalent binding of [^{3}H]NHEX and [2-^{14}C]NHEX to rat liver RNA and DNA. The study also used cation exchange chromatography to identify 5 radiolabeled peaks in rat liver RNA following acid hydrolysis. A similar profile was observed in rat liver DNA. The authors identified one of the peaks, present in both RNA and DNA, as 1,6-hexanediol or its benzoate derivative. Equivalent amounts of covalently bound 1,6-hexanediol were detected in the RNA with [^{3}H]NHEX and [2-^{14}C]NHEX, and the authors proposed that 1,6-hexanediol was binding to an oxygen atom, such as O^{6}-guanine, O^{4}-thymidine (in DNA), or the tertiary phosphate hydroxyl (to form phosphotriesters). In the study by Ross and Lawson (1982), [^{3}H]NHEX was shown to bind to rat liver DNA and RNA, and the radioactivity remained bound following depurination of the DNA and RNA. The authors suggested that in addition to interacting with oxygen atoms on guanine, thymidine, and phosphate subunits, NHEX may form crosslinks within the RNA or DNA.

Table 11. Genotoxicity studies of NHEX *in vivo*

Endpoint	Study design	Target	Results[1]	Reference
X-linked recessive-lethal mutation	5-30 mM NHEX to F_0 male *Drosophila melanogaster* for 48 hours; F_1 females from treated F_0 were mated with untreated males; assessed X-linked lethals in F_2 generation	F_2 generation	+	Nix *et al.* 1980
DNA damage[2]	25-250 mg/kg-bw NHEX in Sprague-Dawley rats via *i.p*. or oral routes for three weeks	liver lung kidney duodenum	-	Swenberg 1981
RNA binding	550 μCi (41 mg per rat) [^{3}H]NHEX in male Wistar rats by gavage and killed 12-16 hours later	liver	+[3]	Lijinsky and Ross 1969
DNA binding			+[3]	
RNA binding	500 μCi (60 mg /kg-bw) [2-^{14}C]NHEX and 2.8 mCi (60 mg /kg bw) [^{3}H]NHEX in male Wistar rats by gavage and killed 16 hours later	liver	+[4]	Ross and Mirvish 1977
DNA binding			+[4]	
RNA binding	13 mCi/mmol (60 mg/kg-bw) [^{3}H]NHEX in male MRC-Wistar rats by gavage and killed 17 hours later	liver	+[5]	Ross and Lawson 1982
DNA binding			+[5]	

[1] +: positive result; −: negative result
[2] Alkaline elution assay.
[3] Binding observed; however, alkylated bases, assessed as alkylated guanine (7-methylguanine) and adenine, were not detected.
[4] Covalent binding observed as 5 radioactive peaks for RNA and 4 peaks for DNA; 1 peak in both RNA and DNA identified as 1,6-hexanediol. Postulated O-alkylation targets: O^6 of guanine, O^4 of thymidine (DNA only), or the tertiary phosphate hydroxyl (forming phosphotriesters).
[5] Covalent binding observed in apurinic RNA and DNA. Postulated alkylation of phosphates or pyrimidines, or formation of cross-links.

In summary, NHEX was mutagenic in *S. typhimurium* and *E. coli* in the presence of metabolic activation, and in a primary rat hepatocyte-mediated mutagenesis assay in Chinese hamster V79 cells *in vitro*. NHEX was also mutagenic in *D. melanogaster*, inducing X-linked recessive-lethal mutations. NHEX did not induce DNA damage as assessed in the alkaline elution assay in multiple tissues of rats following *in vivo* exposure. NHEX binds to RNA and DNA in rat liver following *in vivo* exposure, and the NHEX metabolite 1,6-hexanediol has been identified as one of the alkylating species. NHEX is postulated to interact with oxygen atoms present in guanine, thymidine, and phosphate subunits and to form crosslinks within the RNA and DNA.

3.3.2.4 Genotoxicity studies of NHEX metabolites

Table 12 presents the findings from genotoxicity studies of five NHEX metabolites, namely β-hydroxy NHEX, γ-hydroxy NHEX, 1,6-hexanediol, adipic acid, and ϵ-caprolactam. No genotoxicity studies were available for other NHEX metabolites or intermediates, including ϵ-aminocaprohydroxamic acid, hexamethyleneimine, the carbonium ion metabolite, 6-aminohexanal, ϵ-aminocaproate, ϵ-hydroxycaproate, 6-hydroxyhexanal, β-oxidized derivatives (β-alcohol and β-ketone), γ-oxidized derivative (γ-alcohol), ω-oxycaproic acid, and α-hydroxylated NHEX.

All five metabolites with genotoxicity data have been tested in *S. typhimurium* reverse mutation assays; β-hydroxy NHEX and γ-hydroxy NHEX were positive when tested in the presence of metabolic activation (Hecker et al. 1983; Farrelly and Hecker 1984). The other three metabolites tested negative in *S. typhimurium*.

β-Hydroxy NHEX and γ-hydroxy NHEX have not been tested in other genotoxicity assay systems besides the *S. typhimurium* reverse mutation assay.

1,6-Hexanediol has been tested in one additional assay system (a mutation assay in *E. coli*) in which it was negative (Table 12). In addition, as discussed earlier, 1,6-hexanediol has been identified in studies of rats administered NHEX as covalently bound to liver RNA and DNA (Ross and Mirvish 1977).

Adipic acid has been tested in two additional assay systems (mutation assays in *E. coli* and mouse lymphoma cells); it was negative in each (Table 12).

ϵ-Caprolactam, which is classified by the International Agency for Research on Cancer (IARC) as a Group 4 chemical, i.e., "*probably not carcinogenic to humans"*, has been tested extensively in numerous *in vitro* and *in vivo* genotoxicity assays (IARC 1999a). As shown in Table 12, ϵ-caprolactam has tested negative for a wide range of genotoxicity endpoints in the majority of assays conducted in human and other mammalian systems and tested positive in a handful of studies (IARC 1999a). Specifically, ϵ-caprolactam was weakly mutagenic in a somatic mutation assay and in the X-linked recessive mutation assays in *D. melanogaster.* It induced micronuclei in newt larvae, gene mutations or conversions in yeast, and chromosomal aberrations (CA) and aneuploidy in human lymphocytes *in vitro*.

Table 12. Genotoxicity studies of NHEX metabolites

Chemicals	Assay system	Results[1]	References
β-Hydroxy NHEX	*S. typhimurium* TA 1535 mutagenicity assay (hamster liver S-9; uninduced, PB-induced or Aroclor 1254-induced)	+ (+ S-9)	Hecker *et al*. 1983
	S. typhimurium TA 1535 mutagenicity assay (rat liver S-9; uninduced, PB-induced or Aroclor 1254-induced)	+ (+ S-9)	
γ-Hydroxy NHEX	*S. typhimurium* TA 1535 mutagenicity assay (hamster liver S-9; uninduced, PB-induced or Aroclor 1254-induced)	+ (+ S-9)	
	S. typhimurium TA 1535 mutagenicity assay (rat liver S-9; uninduced, PB-induced or Aroclor 1254-induced)	- (+ S-9)	
	S. typhimurium (strain not specified) mutagenicity assay (rat liver S-9; Aroclor 1254-induced)	(+) (+ S-9)	Farrelly and Hecker 1984
		- (- S-9)	
1,6-Hexanediol	*S. typhimurium* mutagenicity assay	-	CCRIS 2004
	E. coli WP2UVRA reverse gene mutation assay	-	
	[Identified as covalently bound to rat liver RNA and DNA in rats exposed to NHEX]	+	Ross and Mirvish 1977
Adipic acid	*S. typhimurium* mutagenicity assay	-	Prival *et al*. 1991; CCRIS 2009
	E. coli WP2UVRA reverse gene mutation assay	-	Brusick *et al.* 1980; CCRIS 2009
	Mouse lymphoma L5178Y(TK^+/TK^-) forward mutation assay	-	CCRIS 2009
ϵ-Caprolactam	Newt larvae MN test	+	Fernandez *et al*. 1989
	D. melanogaster somatic genotoxicity assay	(+)	Henderson and Grigliatti 1992; IARC 1999a[2]
	D. melanogaster X-linked recessive mutation assay	(+)/-	IARC 1999a[2]
	S. typhimurium mutagenicity assay	-	
	Yeast gene conversion or mutation	+/-	
	CA in human lymphocytes *in vitro*	+	
	Aneuploidy in human lymphocytes *in vitro*	+	
	UDS and DNA strand breaks in rats *in vivo*	-	
	SCE, MN and CA in mice *in vivo*	-	
	SCE in Chinese hamster cells and human lymphocytes *in vitro*	-	
	Mouse lymphoma L5178Y(TK^+/TK^-) forward mutation assay	-	
	Chinese hamster lung V79/ or ovary cell 6-thioguanine resistance mutation assay	-	
	UDS in rat hepatocytes	-	
	CA, MN and DNA strand breaks in CHO cells	-	
	Gene mutation in human lymphocytes *in vitro*	-	

[1] +: positive result; −: negative result; (+): weakly or marginally positive; +/-: positive and negative results
[2] This is a partial list; there are at least 29 additional negative genotoxicity findings for ϵ-caprolactam. Refer to Table 1 in IARC (1999a) for a more complete listing of ϵ-caprolactam genotoxicity studies.
MN: micronucleus; CA: chromosomal aberrations; UDS: unscheduled DNA synthesis;
SCE: sister chromatid exchange; CHO: Chinese hamster ovary

3.3.3 Animal Tumor Pathology

This section describes the relevant pathology details for the tumor types observed in the animal cancer bioassays of NHEX, by rodent species tested.

3.3.3.1 Mouse

In mice treated with NHEX via drinking water, gavage, or *s.c.* injection, increases in tumors of the nasal cavity, “oropharynx” (including nasal cavity, tongue, and larynx per Goodall and Lijinsky, 1984a and 1984b), esophagus, lung, liver, forestomach, glandular stomach, and reticuloendothelial system were observed (Goodall and Lijinsky 1984a and 1984b; Strickland et al. 1988).

Nasal cavity

The nasal tumors observed in Strickland et al. (1988) were described as adenomas and mucosa-carcinomas (one metastasized). Nasal cavity tumors are rare in untreated mice (Leininger and Jokinen 1994).

“Oropharynx” (including nasal cavity, tongue, and larynx)

Oropharynx tumors were reported in Goodall and Lijinsky (1984a and 1984b). These tumors were identified by examining the pharynx, which was opened to see tumors of the tongue, and by decalcifying the skull and facial bones and then examining serial coronal sections of the orofacial region to detect nasal cavity tumors. The authors reported that most of the tumors occurring in the “oropharynx” were squamous cell papillomas and carcinomas, and that some were invasive.

Esophagus

Esophageal tumors observed in Goodall and Lijinsky (1984a and 1984b) were squamous cell papillomas and carcinomas, and at least some of these tumors were invasive. Tumors of the esophagus grew unexpectedly large (often > 10 mm in diameter) before causing partial obstruction or weight loss or death in some animals. All esophageal tumors observed in Strickland et al. (1988) were squamous cell papillomas. Esophageal squamous cell papillomas are considered to have the potential to progress to carcinomas (McConnell et al. 1986). Esophagus tumors are rare in untreated mice (Leininger and Jokinen 1994).

Lung

Lung tumors observed in Goodall and Lijinsky (1984a and 1984b) were alveolar adenomas, and a few carcinomas or invasive adenocarcinomas. Strickland et al. (1988) reported mostly lung adenomas and adenocarcinomas. Alveolar/bronchiolar adenomas in mice are considered to have the potential to progress to carcinomas, and are aggregated when evaluating study results (McConnell et al. 1986).

Liver

Liver tumors observed in NHEX-treated mice include hepatocellular adenomas and carcinomas, hemangiomas, hemangiosarcomas (or "hemangioendothelial sarcomas"), cholangiomas (or "adenomas of the bile duct"), and cholangiocarcinomas (or "carcinomas of the bile duct") (Goodall and Lijinsky, 1984a and 1984b; Strickland et al., 1988). Among these various types of liver tumors, hemangiomas and hemangiosarcomas are considered uncommon in NZO (Goodall et al. 1973) and BALB/c mice (MTB 2018), and cholangiomas and cholangiocarcinomas are considered rare in mice (Thoolen et al. 2010).

Hepatocellular carcinoma was observed in four New Zealand strains of mice (Goodall and Lijinsky, 1984a and 1984b). Hepatocellular adenoma or carcinoma was observed in three additional strains of mice (Strickland et al. 1988). Hemangioendothelioma (angiosarcoma) or hemangioma of the liver was observed in five strains of mice (Goodall and Lijinsky 1984a and 1984b; Strickland et al. 1988). Rare cholangiomas and cholangiocarcinomas were observed in six strains of mice (Goodall and Lijinsky 1984b; Strickland et al. 1988).

Hepatocellular adenomas are considered to have the potential to progress to hepatocellular carcinomas (McConnell et al. 1986). Hemangiomas and hemangiosarcomas are of the same cell type and can be grouped together for cancer evaluation (McConnell et al. 1986). Likewise, cholangiomas and cholangiocarcinomas can be grouped together for cancer evaluation (Thoolen et al. 2010).

Forestomach

Forestomach tumors (reported as "squamous stomach tumors") in Goodall and Lijinsky (1984a and 1984b) were squamous cell papillomas and carcinomas, and some of them were invasive. Forestomach tumors observed in Strickland et al. (1988) were primarily squamous cell carcinomas, with some papillomas. The forestomach epithelium is composed of keratinized stratified squamous cells. Tumors of the forestomach could grow large, cause partial obstruction, or cause weight loss or death. Forestomach

squamous cell papillomas are considered to have the potential to progress to carcinomas (McConnell et al. 1986). Forestomach carcinomas are rare in mice (Leininger and Jokinen 1994).

Glandular stomach

Tumors of the glandular stomach were mostly of benign adenomatous appearance (Goodall and Lijinsky, 1984a and 1984b). Glandular adenomas of the stomach are considered to have the potential to progress to adenocarcinomas (McConnell et al. 1986). Glandular tumors of the stomach are rare in untreated mice (Leininger and Jokinen 1994).

Reticuloendothelial system

Statistically significant increases in malignant lymphomas were observed in two strains of New Zealand mice, in the studies of Goodall and Lijinsky (1984a; 1984b).

3.3.3.2 Rat

In rats treated with NHEX via drinking water, increases in tumors of the nasal cavity, tongue, esophagus, lung, liver, forestomach, and glandular stomach were observed (Goodall et al. 1968; Lijinsky and Taylor 1979; Lijinsky and Reuber 1981).

Nasal cavity

NHEX induced nasal cavity tumors (Goodall et al. 1968; Lijinsky and Taylor 1979), including adenocarcinomas, squamous cell carcinomas, undifferentiated carcinomas with occasional tubular arrangements of the cells, and neuroepithelial carcinomas. These tumors originated from the posterior and upper nasal cavity and were highly invasive, progressing to the eye socket or the brain. Adenocarcinomas may arise from respiratory epithelium of the anterior naso- or maxillary turbinates, the septal glands, Bowman's glands, or Steno's gland (Schwartz et al. 1994). Squamous cell carcinomas can arise from the squamous epithelium of the anterior nose or from metaplastic squamous epithelium in the respiratory or olfactory portions of the nasal cavity. All types of nasal cavity tumors are rare in rats (Schwartz et al. 1994).

Tongue

NHEX induced squamous cell papillomas and carcinomas of the tongue, which were reported as "benign and malignant epitheliomas of the tongue" in Goodall et al. (1968);

a single papilloma was reported in Lijinsky and Taylor (1979). Tumors of the tongue arise principally from the stratified squamous epithelium, but can also arise from the connective tissue. Squamous cell papilloma and carcinoma are considered to have the potential to progress from benign to malignant phenotypes (Whiteley et al. 1996; McConnell et al. 1986). Neoplasms of the tongue are rare in untreated rats (Whiteley et al. 1996).

Esophagus

The esophageal tumors induced by NHEX include squamous cell papillomas and carcinomas ("benign and malignant epitheliomas of the esophagus"). These tumors often occurred in multiples, were distributed along the entire length of the esophagus, and were invasive (Goodall et al. 1968; Lijinsky and Taylor 1979; Lijinsky and Reuber 1981). Proliferative lesions of the esophagus typically arise from the stratified squamous epithelium, and carcinomas may arise from epithelium or within papillomas (Whiteley et al. 1996). Squamous cell papillomas and carcinomas are considered to have the potential to progress from benign to malignant phenotypes (McConnell et al. 1986). Esophageal tumors are rare in untreated rats (Brown and Hardisty 1990; Whiteley et al. 1996; Haseman et al. 1998).

Lung

One alveolar cell adenoma was observed in 15 male S-D rats following exposure to NHEX via drinking water (Lijinsky and Taylor 1979). Bronchioalveolar adenomas are considered to have the potential to progress to carcinomas (McConnell et al. 1986). Bronchioalveolar adenomas and carcinomas of the lung are considered rare in male S-D rats (Chandra et al. 1992; McMartin et al. 1992).

Liver

Significant increases in hepatocellular carcinomas and "endothelial sarcomas" (hemangioendotheliosarcoma) were observed in female Fischer 344, male S-D, and male and female MRC-Wistar rats treated with NHEX in drinking water (Goodall et al. 1968; Lijinsky and Taylor 1979; Lijinsky and Reuber 1981). Hemangioendotheliosarcoma refers to the predominant growth of endothelial cells in some areas that replaces large parenchymatous areas and shows sarcomatous patterns (Goodall et al. 1968). Hepatocellular carcinomas and hemangiosarcomas often occurred simultaneously, with one or the other dominating in some treated animals. In the *s.c.* injection study by Schmähl (1968), three liver hemangiomas and one hepatocellular carcinoma were observed in 46 male BR46 rats. Spontaneous

tumors of the liver are relatively rare in rats (Bannasch and Zerban 1990), although spontaneous incidence varies by rat strain and by liver tumor type.

Forestomach

One squamous cell papilloma of the forestomach was observed in a male S-D rat (Lijinsky and Taylor 1979). Two squamous cell carcinomas of the forestomach (reported as "squamous stomach carcinomas" by Goodall et al. 1968) were observed in male Wistar rats, and one forestomach papilloma was observed in a female Fischer 344 rat (Lijinsky and Reuber 1981) following exposure to NHEX via drinking water. Forestomach squamous cell papillomas are considered to have the potential to progress to carcinomas (McConnell et al. 1986). Spontaneous tumors of the forestomach in untreated rats are very rare (Takahashi and Hasegawa 1990).

Glandular stomach

One carcinoma of the glandular stomach (reported as "carcinoma of the stomach" by Goodall et al. 1968) was observed in a female MRC-Wistar rat following exposure to NHEX via drinking water. Spontaneous tumors of the glandular stomach in untreated rats are very rare (Takahashi and Hasegawa 1990).

3.3.3.3 Hamster

In hamsters treated with NHEX by *s.c.* injection, increases in tumors of the nasal cavity, larynx, trachea, lung, and forestomach were observed (Althoff et al. 1972, 1973 & 1976; Althoff and Grandjean 1979).

Nasal cavity

Nasal cavity tumors observed in NHEX-treated hamsters or their offspring exposed *in utero* were mainly adenocarcinomas (Althoff et al. 1973 and 1976). Adenocarcinomas originated from the respiratory epithelium and sometimes showed invasive growth into the orbit, maxilla, and ethmoid bones, and through the cribriform plate into the brain (frontal and parietal lobes). Nasal cavity tumors are rare in untreated Syrian golden hamsters (Schuller 1996). Schuller (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…. The vast majority of experimentally induced nasal cavity tumors in hamsters has been caused by nitrosamines."

Larynx

Larynx tumors observed in NHEX-treated hamsters or their offspring exposed *in utero* (Althoff et al. 1972, 1976) were characterized as squamous cell papillomas or papillary polyps. Two of these tumors were diagnosed as squamous cell papillomas without keratinization (Althoff et al. 1973). Squamous cell papillomas have the potential to progress to carcinomas (Mohr et al. 1996). Larynx tumors are considered rare, as the laboratory historical incidence of laryngotracheal tract tumors was about 1% in Syrian golden hamsters (Pour et al. 1976).

Trachea

Trachea tumors reported in NHEX-treated hamsters or their offspring exposed *in utero* (Althoff et al. 1972, 1973, 1976, 1979) were characterized as benign papillary polyps or papillary tumors. The tracheal epithelium of most NHEX-treated hamsters had undergone focal or diffuse hyperplasia and dysplasia. The neoplasms of the trachea were multiple and more frequent in the laryngeal part than in the middle part or at the bifurcation (Althoff et al. 1973). Tumors at the distal part of the laryngeal fold or papillary tumors in the proximal (extrapulmonary) part of the stem bronchi were considered tracheal tumors (Althoff et al. 1973). Benign papillomas may progress to carcinomas (Mohr et al. 1976). Trachea tumors are considered rare, as the laboratory historical incidence of laryngotracheal tract tumors was about 1% in untreated Syrian golden hamsters (Pour et al. 1976).

Lung

Lung tumors reported in NHEX-treated hamsters or their offspring exposed *in utero* (Althoff et al. 1973 & 1976) included papillary polyps, adenomas, adenocarcinomas, hemangioendotheliomas, squamous cell carcinomas, and anaplastic carcinomas. Squamous cell carcinomas may arise from benign papillomas (Mohr et al. 1976). Lung tumors are rare in untreated Syrian golden hamsters, with a spontaneous incidence of no more than 0.1-0.5% (Mohr et al. 1976). Laboratory historical incidence of lung tumors was 0.6-0.7% in Syrian golden hamsters (Pour et al. 1976).

Forestomach

Modest increases in squamous cell papillomas were observed in male and female hamsters treated with NHEX (Althoff et al. 1973). Squamous cell papillomas have the potential to progress to carcinomas (Takahashi and Okamiya 1996). Spontaneous neoplasms of the forestomach are infrequent in the hamster; the incidences of

spontaneous squamous cell papilloma of the forestomach were 4.1% in female hamsters and 6.1% in males (IARC 1999b).

3.3.4 Structure Activity Considerations

NHEX is a heterocyclic nitrosamine that is characterized by a nitroso group bonded to a nitrogen atom. NHEX shares structural similarities with other carcinogenic cyclic nitrosamines. The structures of five structurally similar compounds and information on the carcinogenicity findings from animal studies of these chemicals are briefly described below, and summarized in Table 13. Information on genotoxicity findings for these five comparison chemicals is also described below, and summarized in Table 14.

2,6-Dimethylnitrosomorpholine (DMNM) is on the Proposition 65 list of chemicals known to cause cancer. DMNM induces tumors at multiple sites in multiple species, and shares several common species-specific target tumor sites with NHEX, including the nasal cavity (rat, hamster), tongue (rat), larynx/trachea (hamster), esophagus (rat), forestomach (rat, hamster), lung (rat, hamster), and liver (rat [hemangioma/ hemangiosarcoma]) (OEHHA 2012). DMNM is mutagenic in *S. typhimurium*, and induces UDS in rat hepatocytes *in vitro*, X-linked recessive mutations in *Drosophila melanogaster*, and DNA single-strand breaks in pancreatic acinar cells in hamsters *in vivo*. DMNM also forms DNA adducts in rats and hamsters *in vivo* (OEHHA 2012).

N-Nitrosoheptamethyleneimine (NHMI) induced tumors at multiple sites in hamsters (Lijinsky et al. 1970; Reznik et al. 1978) and rats (Garcia and Lijinsky 1972; Lijinsky and Ross 1969; Schreiber et al. 1972; Taylor and Lijinsky 1985; Terzaghi et al. 1981; Yarita and Nettesheim 1979; Yoshida et al. 1992), and shares several common species-specific target tumor sites with NHEX, including the nasal cavity (rat, hamster), larynx/trachea (hamster), esophagus (rat), lung (rat, hamster), and forestomach (hamster). NHMI was mutagenic in *S. typhimurium* (Dahl et al. 1990) and *E. coli* (Elespuru and Lijinsky 1976), and increased DNA damage and UDS in Clara cells from rabbit lung (Becher et al. 1993; Dahl et al. 1990).

Nitrosomorpholine (NM) is on the Proposition 65 list of chemicals known to cause cancer. It is an IARC Group 2B carcinogen (IARC 1987), and is listed as “reasonably anticipated to be a human carcinogen” in the NTP Report on Carcinogens (NTP 2016). NM induces tumors at multiple sites in multiple species, and shares several common species-specific target tumor sites with NHEX, including the nasal cavity (rat, hamster), trachea/larynx (hamster), esophagus (rat), lung (mouse), liver (rat [hepatocellular adenoma/carcinoma, hemangioma/hemangiosarcoma, and cholangioma/ cholangiocarcinoma], mouse [hepatocellular adenoma/carcinoma and hemangioma/ hemangiosarcoma]), and forestomach (hamster) (IARC 1978; Klein et al. 1990; Lijinsky and Reuber 1982). NM is mutagenic in *Salmonella* and *E. coli*, and induces

8-azaguanine resistant mutations in Chinese hamster V79 lung and BHK-21 cells, DNA damage in CHO cells (IARC 1978; Robichova et al. 2004; Wagner et al. 2012), CAs in human HepG2 hepatoma cells and Chinese hamster V79 lung cells, and UDS in rat hepatocytes (Mitchell et al. 1983). It induces X-linked recessive lethal mutations and translocations in *Drosophila melanogaster* (IARC 1978; Knasmuller et al. 1990), and forms DNA adducts *in vivo* in rats (Loeppky and Goelzer 2002).

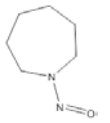
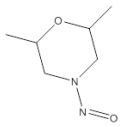
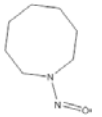
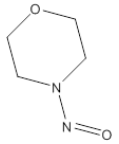
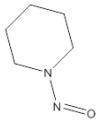
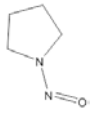
N-Nitrosopiperidine (NP) is on the Proposition 65 list of chemicals known to cause cancer. It is an IARC Group 2B carcinogen (IARC 1987), and is listed as "reasonably anticipated to be a human carcinogen" in the NTP Report on Carcinogens (NTP 2016). NP induces tumors at multiple sites in multiple species, and shares several common species-specific target tumor sites with NHEX, including nasal cavity (rat, hamster), larynx (hamster), esophagus (rat), lung (mouse, hamster), liver (rat [hepatocellular adenoma/carcinoma], mouse [hepatocellular adenoma/carcinoma and hemangioma/hemangiosarcoma]), and forestomach (mouse, hamster) (IARC 1978; NTP 2016). NP is mutagenic in *S. typhimurium, E. coli,* and *S. cerevisiae,* induced mutations in Chinese hamster V79 lung cells and mouse lymphoma L5178Y TK cells, induced UDS in Syrian hamster embryo cells *in vitro* (Tsutsui et al. 1984), forms DNA adducts in human Caco-2 cells (Hebels et al. 2011; IARC 1978; Larimer et al. 1978; Seifried et al. 2006; Wangenheim and Bolcsfoldi 1988), and binds to calf thymus DNA (Wang et al. 1995). NP also induced X-linked recessive-lethal mutations in *Drosophila melanogaster* (Nix et al. 1979; Yoon et al. 1985; Zimmering 1982).

N-Nitrosopyrrolidine (NPYR) is on the Proposition 65 list of chemicals known to cause cancer. It is an IARC Group 2B carcinogen (IARC 1987), and is listed as "reasonably anticipated to be a human carcinogen" in the NTP Report on Carcinogens (NTP 2016). NPYR induces tumors at multiple sites in multiples species. It shares several common species-specific target tumor sites with NHEX, including nasal cavity (hamster), larynx/trachea (hamster), lung (mouse), and liver (rat [hepatocellular adenoma/ carcinoma]) (IARC 1978; McCoy et al. 1994). NPYR is mutagenic in *S. typhimurium* and *E. coli,* induced mutations in Chinese hamster V79 lung and CHO cell assays, induced UDS in rat hepatocytes *in vitro* (Mitchell et al. 1983), forms DNA adducts in human Caco-2 cells (Hebels et al. 2011; IARC 1978; Oberly et al. 1990), and binds to calf thymus DNA (Wang et al. 1992; Wang et al. 1995). NPYR also induced X-linked recessive-lethal mutations in *Drosophila melanogaster* (Knasmuller et al. 1990) and DNA adducts in rats *in vivo* (Chung et al. 1989a; Chung et al. 1989b; Diaz Gomez et al. 1986).

As shown in Table 13, many of the tumor types that are increased in animal studies of NHEX are also increased in animal studies of the comparison chemicals. Specifically,

increases in nasal cavity tumors and larynx and/or trachea tumors were seen with all five chemicals: esophagus tumors were seen with DMNM, NHMI, NM, and NP; lung tumors were seen with all five chemicals; hepatocellular adenomas and/or carcinomas were seen with NM, NP, and NPYR; liver hemangiomas and/or hemangiosarcomas were seen with DMNM, NM, and NP; liver cholangiomas and/or cholangiocarcinomas were seen with DMNM, NM, and NPYR; and forestomach tumors were seen with DMNM, NHMI, NM, and NP. Additionally, tongue tumors were seen with DMNM and NHMI, pharynx tumors were seen with NHMI and NP, and glandular stomach tumors were seen with DMNM.

Table 13. Comparison of target tumor sites across species[1] for NHEX and structurally related cyclic nitrosamines

Chemical (Cancer classification[2])	Structure	Nasal cavity			Larynx and/or trachea			Esophagus			Lung			Liver									Fore-stomach			Other
														HC[3] adenoma/ carcinoma			Hemangioma/ sarcoma			Cholangioma/ carcinoma						
		R	M	H	R	M	H	R	M	H	R	M	H	R	M	H	R	M	H	R	M	H	R	M	H	
N-Nitrosohexa-methyleneimine **(NHEX)**		+	+	+			+	+	+		+	+	+	+	+		+	+			+		+	+	+	Tongue (R) "Oropharynx" (M) Glandular stomach (M, R) Lymphoma (M)
2,6-Dimethyl-nitrosomorpholine **(DMNM)** (P65)		+		+	+		+	+		+	+		+				+		+			+	+		+	Tongue (R, H) , Glandular stomach (T), Harderian gland (H), Liver (G), Pancreas (H), Kidney (H), Gall bladder (H) Renal Pelvis/urinary bladder (H) Swim bladder (T), Vagina (H), Skin (H)
N-Nitrosohepta-methyleneimine **(NHMI)**		+		+	+		+	+		+	+		+												+	Tongue (H) Pharynx (H)
Nitrosomorpholine **(NM)** (P65, IARC, NTP)		+		+	+		+	+				+		+	+		+	+		+					+	Thyroid (R) Kidney (R)
N-Nitrosopiperidine **(NP)** (P65, IARC, NTP)		+		+			+	+		+		+	+	+	+	+		+							+	+	Pharynx (R)
N-Nitrosopyrrolidine **(NPYR)** (P65, IARC, NTP)				+			+					+		+						+						

[1] Species: Rat (R), Mouse (M), Hamster (H), Guinea Pig (G), Trout (T). [2] Cancer classification: "P65": Proposition 65 carcinogen; "IARC": IARC Group 2B carcinogen; "NTP": NTP Report on Carcinogens *reasonably anticipated to be a human carcinogen.* [3]HC=hepatocellular

As shown in Table 14, all of the comparison chemicals tested positive for mutagenicity in bacteria, as did NHEX. Three of the comparison chemicals, namely NM, NP, and NPYR, were tested for mutagenicity in mammalian cells *in vitro* and all three tested positive, as did NHEX. All five of the comparison chemicals have been shown to induce DNA or chromosomal damage or DNA binding in mammalian cells *in vitro*; however, NHEX has not been tested for these endpoints in mammalian *in vitro* assays. Four of the comparison chemicals, namely DMNM, NM, NP, and NPYR, were tested in an X-linked recessive-lethal mutation assay in *D. melanogaster in vivo* and all four tested positive, as did NHEX. The three chemicals that were tested for DNA or RNA binding in rats *in vivo*, namely DMNM, NM, and NPYR, each tested positive, as did NHEX.

Table 14. Comparison of genotoxicity for NHEX and structurally related cyclic nitrosamines

Test system/ endpoint / 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	+	+	+ Chinese hamster V79 lung cells	NT	+	+ Rat
2,6-Dimethyl-nitrosomorpholine (DMNM)	+	NT	NT	+ UDS in rat hepatocytes	+[1]	+ Rat Hamster
N-Nitroso-heptamethyleneimine (NHMI)	+	+	NT	+ DNA damage & UDS in rabbit lung Clara cells	NT	NT
Nitrosomorpholine (NM)	+	+	+ Chinese hamster V79 lung & BHK-21 cells	+ DNA damage in CHO cells, CA in Chinese hamster V79 lung & HepG2 cells, chromatid breaks & rearrangements in BHK-21 cells, UDS in rat hepatocytes	+	+ Rat
N-Nitrosopiperidine (NP)	+	+[2]	+ Chinese hamster V79 lung & mouse lymphoma cells	+[3] UDS in Syrian hamster embryo cells, DNA adducts in human Caco-2 cells	+	NT
N-Nitrosopyrrolidine (NPYR)	+	+	+ Chinese hamster V79 lung & CHO cells	+[3] UDS in rat hepatocytes, DNA adducts in human Caco-2 cells	+	+ Rat

NT: Not tested; UDS: unscheduled DNA synthesis; CA: chromosomal aberrations

[1] Also induces DNA single strand breaks in pancreatic acinar cells in hamsters

[2] Also mutagenic in *S. cerevisiae*

[3] Also binds to calf thymus DNA in cell-free systems

QSAR predictions for NHEX

Quantitative structure activity relationship (QSAR) predictions for NHEX have been published by the European Chemicals Agency (ECHA 2018a). QSAR models predict the toxicity of chemicals by correlating physiochemical properties of related compounds to their biological activity quantitatively. Numerous QSAR models have been developed. ECHA used several QSAR models to predict the carcinogenicity and mutagenicity of NHEX.

Specifically, ECHA found that NHEX is predicted to be a suspected carcinogen based on several models:

- QSAR Toolbox (OECD 2018): the predictions are based on several databases and sources of information, including IARC Monographs on the Evaluation of Carcinogenic Risks to Humans and NTP Reports on Carcinogens.
- VEGA QSAR platform (VEGA 2018):
    - The CAESAR model, which provides a prediction of carcinogenic potency in male and female rats.
    - The ISS (Istituto Superiore di Sanità) model, which provides a prediction of carcinogenic potency according to specific requirements of chemical structure alerts. The model was built on a set of rules from Benigni et al. (2008).

In addition, NHEX is predicted to be a suspected mutagen based on several models:

- QSAR Toolbox (OECD 2018): the ISS model provides a prediction of mutagenicity in *S. typhimurium* (Ames test).
- VEGA QSAR platform:
    - The CAESAR model, which provides a prediction of mutagenicity based on experimental data, including results of the Ames test.
    - The ISS model, which provides a prediction of mutagenicity based on chemicals tested with the Ames test.
    - The SarPy model, which provides a prediction of mutagenicity from the Ames test. The model is a statistical-based model and commonly defined the applicability domain using structural rules.
    - The KNN (k-nearest neighbor) model, which provides a prediction of mutagenicity from the Ames test. The model uses the value of the closest neighbors based on chemical structure, and then assesses a dataset of 5770 chemicals to make predictions [Istituto di Ricerche Farmacologiche Mario Negri (IFRMN)] (IFRMN 2016).

3.3.5 ToxCast high-throughput in vitro assays

OEHHA has searched the US Environmental Protection Agency's (US EPA) ToxCast database (Dix et al. 2007) via the Interactive Chemical Safety for Sustainability (iCSS) Dashboard (http://actor.epa.gov/dashboard/, accessed on 7/03/2018), and identified the available *in vitro* high-throughput assay data on NHEX and its metabolites. Few NHEX metabolites have been tested in ToxCast assays: hexamethyleneimine (a proposed metabolite), adipic acid, and ϵ-caprolactam (an IARC Group 4 chemical, "*probably not carcinogenic to humans",* IARC (1999a)).

ToxCast data on NHEX and its metabolites are limited, since NHEX is not metabolically activated in most of the assays, and some of the known or proposed metabolites most likely to be of concern for carcinogenicity, such as 1,6-hexanediol, the electrophilic NHEX radical, and the electrophilic carbonium ion metabolite have not been tested.

As shown in Table 15, NHEX was active in two of 276 ToxCast assays, hexamethyleneimine was active in five of 334 assays, adipic acid was active in three of 881 assays, and ϵ-caprolactam was active in seven of 882 assays.

Table 15. Overview of ToxCast HTS assay activity for NHEX and its metabolites, hexamethyleneimine, adipic acid, and ϵ-caprolactam

Chemical	NHEX	Hexa-methyleneimine[1]	Adipic acid	ϵ-Caprolactam
Number of active assays / tested assays	2/276	5/334	3/881	7/882
Range of AC_{50} values (µM) in active assays	51.1-73.2	0.00001-86.2	12.4-91.1	0.855-62.8

[1] Proposed metabolite of NHEX

OEHHA mapped the active ToxCast assays for NHEX and its tested metabolites to the key characteristics of carcinogens (Smith et al. 2016; see Table 16 in Section 4 Mechanism), as follows:

NHEX

- *Modulates receptor-mediated effects (1 assay)* – Upregulates pregnane X receptor (PXR) transcription in human liver carcinoma (HepG2) cells.
- *Alters cell proliferation, cell death or nutrient supply (1 assay)* – Increases cell proliferation in HepG2 cells in a dose-dependent manner at concentrations above 10 µM.

Hexamethyleneimine

- *Is genotoxic (1 assay)* – Active in an assay related to DNA binding. Specifically, it upregulates TP53 transcriptional activity in a human intestinal cancer (HCT116) cell line, with AC_{50} equal to 0.00001 µM.
- *Modulates receptor-mediated effects (4 assays)* – In three assays, hexamethyleneimine downregulates the transcriptional activity of PXR, estrogen receptor 1 (ESR1) and aryl hydrocarbon receptor (AhR) in HepG2 cells. In the fourth assay, at concentrations above 50 µM, the chemical increases cell proliferation in an ESR1-positive human breast (T47D) cancer cell line in a dose-response manner.

Adipic acid

- *Modulates receptor-mediated effects (2 assays)* – Downregulates the transcriptional activity of PXR and AHR in HepG2 cells.
- *Alters cell proliferation, cell death or nutrient supply (1 assay)* – Increases human caspase activity in a cell-free assay. The caspases are responsible for the modulation of apoptotic changes observed in mammalian cells undergoing programmed cell death.

ϵ-Caprolactam

- *Modulates receptor-mediated effects (6 assays)* – Upregulates the transcriptional activity of PXR, ESR1, and peroxisome proliferator-activated receptor alpha (PPARα), and downregulates the transcriptional activity of glucocorticoid receptor and Farnesoid X receptor (also known as bile acid receptor) in HepG2 cells. In addition, in a human breast carcinoma (MDA-kb2) cell line that overexpresses the androgen receptor (AR), ϵ-caprolactam acts as an AR antagonist to block the transcriptional activity of AR-regulated downstream target genes.
- *Alters cell proliferation, cell death or nutrient supply (1 assay)* – Activates human non-receptor protein tyrosine phosphatase (PTP) activity in a cell-free assay. In general, PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, cell cycle progression, and oncogenic transformation.

4. MECHANISMS

NHEX may act via multiple mechanisms, which can be grouped according to the 10 key characteristics of carcinogens described by Smith *et al*. (2016). As discussed below, there is considerable evidence for two of the key characteristics, namely the formation of electrophilic metabolites, and genotoxicity (Table 16).

Table 16. Ten key characteristics of carcinogens (Smith et al. 2016)

Characteristic	Example of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (*e.g.,* epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, UDS), intercalation, gene mutations, cytogenetic changes (*e.g.,* CAs, MN)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (*e.g.,* topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (*e.g.,* DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor inactivation/activation (*e.g.,* ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics, and signaling pathways related to cellular replication or cell cycle control, angiogenesis

AhR, aryl hydrocarbon receptor; CA, chromosome aberrations; ER, estrogen receptor; MN, micronucleus; PPAR, peroxisome proliferator–activated receptor; UDS: unscheduled DNA synthesis.

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NHEX forms electrophilic metabolites. These include 1,6-hexanediol, and several proposed reactive intermediates, including a NHEX radical, a NHEX imminium ion, a carbonium ion metabolite, and NO^+. The ability to form adducts to nucleic acids and proteins is a common property of electrophilic and/or metabolically activated human carcinogens (Obach and Kalgutkar 2010). NHEX binds to liver RNA and DNA in rats exposed via gavage, and the metabolite 1,6-hexanediol has been identified as one of

the alkylating species (Lijinsky and Ross 1969; Ross and Mirvish 1977; Ross and Lawson 1982).

As discussed in Section 3.3.2, NHEX is genotoxic. NHEX has demonstrated mutagenic activity in *S. typhimurium* and *E. coli* in the presence of S-9, in primary rat hepatocyte-mediated assays in Chinese hamster V79 cells, and in *D. melanogaster* in the X-linked recessive-lethal mutation assay. NHEX has been shown to bind to DNA and RNA in rat liver *in vivo*. In addition, the NHEX metabolites β-hydroxy NHEX and γ-hydroxy NHEX are mutagenic in *S. typhimurium*, and, as noted above, 1,6-hexanediol covalently bound to RNA and DNA has been detected in the liver of rats administered NHEX.

5. REVIEWS BY OTHER AGENCIES

NHEX has not been classified as to its potential carcinogenicity by US EPA, the US Food and Drug Administration, the National Toxicology Program, the National Institute for Occupational Safety and Health, or IARC.

The European Chemicals Agency (ECHA) has classified NHEX as a carcinogen in Category 1B (presumed to have carcinogenic potential for humans, classification is largely based on animal evidence) (ECHA 2018b).

6. SUMMARY AND CONCLUSIONS

6.1 Summary of Evidence

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

Carcinogenicity studies of NHEX have been conducted in mice (15 bioassays in 8 strains by drinking water, gavage, or *s.c.* injection), rats (7 bioassays in four strains by drinking water or *s.c.* injection) and hamsters (11 bioassays in Syrian hamsters by *s.c.* injection or transplacental exposure), often in both sexes. Evidence on the carcinogenicity of NHEX comes from bioassays showing statistically significant increases in benign and/or malignant tumors (including several rare tumor types) that have been observed at multiple sites in multiple species (mice, rats, and hamsters), often in multiple strains and both sexes, and by various routes of exposure. Additional tumor findings include increases in rare tongue, lung, forestomach, and glandular stomach tumors in rats, and marginally statistically significant increases ($p = 0.053$) in forestomach tumors in hamsters.

Findings from the carcinogenicity studies of NHEX are summarized below, organized by tumor site and type:

Nasal cavity tumors (rare in mice, rats and hamsters)

- Combined adenomas or mucosa-carcinomas
  - Female BALB/c, CD-1 and SENCAR mice; increase (gavage: 3 studies)
- Adenocarcinomas
  - Male S-D rats; statistically significant increase (drinking water: 1 study)[4]
  - Male and female Syrian golden hamsters; statistically significant increase (*s.c.* injection: 2 studies)
  - Female pregnant Syrian golden hamsters; increase (*s.c.* injection: 1 study)
- Squamous cell carcinomas, undifferentiated carcinomas or neuroepithelial tumors
  - Male and female Wistar rats; increase (no controls) (drinking water: 3 studies)

[4] The studies did not include a specific concurrent control group; however, a continuous series of unexposed rats from the same animal colony was maintained in the same facility.

Oropharynx tumors (including nasal cavity, tongue, and larynx; rare in rats and mice)

- Squamous cell papilloma and carcinoma and other tumors
  - Male and female NZO, female NZB, and male NZC mice; statistically significant increase (drinking water: 6 studies)
  - Female NZC and male NZY mice; increase (drinking water: 2 studies)
- Squamous cell papillomas or carcinomas of the tongue
  - Male and female Wistar rats; increase (no controls) (drinking water: 3 studies)
  - Male S-D rats; one papilloma observed (drinking water: 1 study)

Esophageal tumors (rare in mice and rats)

- Squamous cell papillomas
  - Female BALB/c, CD-1 and SENCAR mice; increase (gavage: 3 studies)
  - Male S-D rats; statistically significant increase (drinking water: 1 study)[5]
  - Female Fischer 344 rats; increase (drinking water: 1 study)
- Carcinomas
  - Female Fischer 344 rats; statistically significant increases for carcinomas and papillomas and carcinomas combined (drinking water: 1 study)
  - Male S-D rats; increase (drinking water: 1 study)[6]
- Squamous papillomas and carcinomas combined
  - Male and female NZO, NZB, NZC, and NZY mice; statistically significant increase (drinking water: 10 studies)
  - Male and female Wistar rats; increase (no controls) (drinking water: 4 studies)

Laryngeal tumors (rare in hamsters)

- Papillary polyps
  - Pregnant female Syrian golden hamsters; statistically significant increase (*s.c.* injection: 1 study)
  - Male and female Syrian golden hamster offspring; statistically significant increase (transplacental exposure: 1 study)
- Unspecified neoplasms, included squamous cell papillomas
  - Male and female Syrian golden hamsters; increase (*s.c.* injection: 2 studies)

Tracheal tumors (rare in hamsters)

- Papillary polyps/tumors
  - Male and female Syrian golden hamsters; statistically significant increase (*s.c.* injection: 4 studies; 3 of these studies also have positive dose-response trend)
  - Pregnant female Syrian golden hamsters; statistically significant increase (*s.c.* injection: 1 study)

[5] The studies did not include a specific concurrent control group; however, a continuous series of unexposed rats from the same animal colony was maintained in the same facility.
[6] *Ibid*

- Male and female Syrian golden hamster offspring; statistically significant increase (transplacental exposure: 1 study)

Lung tumors (rare in rats and hamsters)

- Papillary polyps/adenomas
  - Male S-D rats; increase (drinking water: 1 study)[7]
  - Pregnant female Syrian golden hamsters; one tumor observed (*s.c.* injection: 1 study)
  - Male and female Syrian golden hamster offspring; one tumor observed (transplacental exposure: 1 study)
- Combined adenomas and adenocarcinomas/carcinomas
  - Male and female NZB and male NZC mice; statistically significant increase (drinking water: 3 studies)
  - Female CD-1 mice; statistically significant increase for adenomas and adenocarcinomas combined (gavage: 1 study)
  - Female SENCAR mice; statistically significant increase for adenomas, adenocarcinomas, and adenomas and adenocarcinomas combined (gavage: 1 study)
- Unspecified lung bronchi neoplasms, included papillary tumors, adenocarcinomas, and other carcinomas
  - Male Syrian golden hamsters; statistically significant increase (*s.c.* injection: 1 study)
  - Female Syrian golden hamsters; one tumor observed (*s.c.* injection: 1 study)

Liver tumors

- Hepatocellular tumors (rare in rats)
  - Hepatocellular adenomas
    - Female BALB/c and CD-1 mice; statistically significant increase (gavage: 2 studies)
  - Hepatocellular carcinomas
    - Male and female NZO, male and female NZB, male and female NZC, and male NZY mice; statistically significant increase (drinking water: 8 studies)
    - Male S-D rats; statistically significant increase (drinking water: 1 study)[8]
    - Female Fischer 344 rats; statistically significant increase (drinking water: 1 study)
    - Male and female Wistar rats; increase (no controls) (drinking water: 4 studies)
    - Male BR46 rats; one tumor observed (no control) (*s.c.* injection: 1 study)

[7] The studies did not include a specific concurrent control group; however, a continuous series of unexposed rats from the same animal colony was maintained in the same facility.
[8] *Ibid*.

- Mesenchymal tumors (uncommon in mice, rare in rats)
  - Hemangiomas
    - Male NZO mice; statistically significant increase (drinking water: 1 study)
    - Male BR46 rats; increase (no control) (*s.c.* injection: 1 study)
  - Hemangiosarcomas
    - Female BALB/c and CD-1 mice; statistically significant increase (gavage: 2 studies)
    - Male NZC and NZY mice; statistically significant increase (drinking water: 2 studies)
    - Female SENCAR mice; increase (gavage: 1 study)
    - Male S-D rats; statistically significant increase (drinking water: 1 study)[9]
    - Female Fischer 344 rats; statistically significant increase (drinking water: 1 study)
    - Male and female Wistar rats; increase (no controls) (drinking water: 4 studies)
- Bile duct tumors (rare in mice and rats)
  - Cholangiomas
    - Female BALB/c, CD-1 and SENCAR mice; increase (gavage: 3 studies).
  - Cholangiomas and cholangiocarcinomas
    - Male and female NZO, male NZC, and male NZY mice; statistically significant increase (drinking water: 5 studies)
    - Female NZC mice; increase (drinking water: 1 study)

Forestomach tumors (rare in rats; only forestomach carcinoma is rare in mice)

- Squamous cell papillomas
  - Male S-D rats; one tumor observed (drinking water: 1 study)
  - Female F344 rats; one tumor observed (drinking water: 1 study)
- Squamous cell carcinomas
  - Male Wistar rats; increase (no control) (drinking water: 1 study)
- Combined squamous cell papillomas and carcinomas
  - Male and female NZO, NZB, NZC, and NZY mice; statistically significant increase (drinking water: 10 studies)
  - Female SENCAR mice; statistically significant increase in carcinomas, papillomas and carcinomas combined (gavage: 1 study)
  - Female CD-1 mice; increase in carcinomas, papillomas and carcinomas combined (gavage: 1 study)

[9] The studies did not include a specific concurrent control group; however, a continuous series of unexposed rats from the same animal colony was maintained in the same facility.

Glandular stomach tumors (rare in mice and rats)

- "Mostly benign"
  - Male and female NZO and female NZB mice; statistically significant increase (drinking water: 5 studies)
  - Female NZC and NZY mice; increase (drinking water: 2 studies)
- Carcinomas
  - Female Wistar rats; one tumor observed (no control) (drinking water: 1 study)

Reticuloendothelium tumors

- Lymphomas
  - Male and female NZO and female NZB mice; statistically significant increase (drinking water: 3 studies)

Pharmacokinetics and metabolism studies in animals indicate that NHEX is rapidly absorbed and distributed, completely metabolized, and excreted in the urine and as expired CO_2. NHEX can be metabolized by cytochrome P450 enzymes to form a number of metabolic products, some of which have not been identified. Reactive or genotoxic metabolites of NHEX include 1,6-hexanediol, β- hydroxyl NHEX, and γ-hydroxy NHEX. Additionally, several reactive electrophilic intermediates and products of NHEX metabolism have been proposed, including a NHEX radical, a NHEX imminium ion, a carbonium ion metabolite, and NO^+.

Evidence of NHEX genotoxicity comes from multiple *in vivo* and *in vitro* test systems. NHEX induces base-pair substitution reverse mutations in *S. typhimurium* and reverse mutations in *E. coli* in the presence of metabolic activation. NHEX induces 6-thioguanine and ouabain resistant mutations in a primary rat hepatocyte-mediated mutagenesis assay in Chinese hamster V79 cells *in vitro*. NHEX was mutagenic in *D. melanogaster*, inducing X-linked recessive-lethal mutations. NHEX binds to RNA and DNA in rat liver following *in vivo* exposure. NHEX is postulated to interact with oxygen atoms present in guanine, thymidine, and phosphate subunits, and to form crosslinks within the RNA and DNA. As noted above, the NHEX metabolites β-hydroxy NHEX and γ-hydroxy NHEX induce base-pair substitution mutations in *S. typhimurium* in the presence of metabolic activation, and the NHEX metabolite 1,6-hexanediol, has been identified as being covalently bound to liver RNA and DNA in rats following administration of NHEX.

NHEX shares several common target tumor sites with five structurally similar heterocyclic nitrosamines, DMNM, NHMI, NM, NP, and NPYR. Tumor types that have been observed in NHEX and at least one of the comparison compounds include nasal cavity tumors (rat: in four comparison compounds, hamster: in five), larynx and/or

trachea tumors (hamster: in five), esophagus tumors (rat: in four), lung tumors (rat: in two, mouse: in three, hamster: in three), hepatocellular adenomas/carcinomas (rat: in three, mouse: in two), liver hemangiomas/hemangiosarcomas (rat: in two, mouse: in two), and forestomach tumors (rat: in one, hamster: in four). NHEX and all five comparison compounds also induced mutations in bacteria; three comparison compounds were tested and induced mutations in Chinese hamster V79 lung cells; four comparison compounds were tested and induced X-linked recessive lethal mutations in *D. melanogaster;* and three were tested and bound to DNA or RNA in rats *in vivo*. DMNM, NM, NP, and NPYR are each listed as Proposition 65 carcinogens. Additionally, several QSAR models predict that NHEX is both mutagenic and carcinogenic based on the experimental data of similar compounds.

ToxCast data on NHEX and its metabolites are limited, since NHEX is not metabolically activated in most of the assays, and some of the known or proposed metabolites most likely to be of concern for carcinogenicity, such as 1,6-hexanediol, the electrophilic NHEX radical, and the electrophilic carbonium ion metabolite have not been tested. NHEX was active in two of 276 ToxCast assays, hexamethyleneimine (a proposed metabolite) was active in five of 334 assays, adipic acid was active in three of 881 assays, and ϵ-caprolactam (an IARC Group 4 chemical) was active in seven of 882 assays.

NHEX may act via multiple mechanisms, including formation of electrophilic metabolites, and genotoxicity (as summarized above).

- The electrophilic metabolites of NHEX include 1,6-hexanediol, and several proposed reactive intermediates, including a NHEX radical, a NHEX imminium ion, a carbonium ion metabolite, and NO^+.

6.2 Conclusions

The evidence for the carcinogenicity of NHEX comes from:

- Studies in mice
  - Nasal cavity tumors (rare)
  - Oropharynx (including nasal cavity, tongue and larynx) tumors (rare)
  - Esophageal tumors (rare)
  - Lung tumors
  - Liver hepatocellular adenomas/carcinomas
  - Liver hemangiomas/hemangiosarcomas (uncommon)
  - Liver cholangiomas/cholangiocarcinomas (rare)
  - Forestomach tumors (carcinomas, rare)
  - Glandular stomach tumors (rare)

- Reticuloendothelial lymphomas

- Studies in rats
  - Nasal cavity tumors (rare)
  - Tongue tumors (rare)
  - Esophageal tumors (rare)
  - Lung tumors (rare)
  - Liver hepatocellular adenomas/carcinomas (rare)
  - Liver hemangiomas/hemangiosarcomas (rare)
  - Forestomach tumors (rare)
  - Glandular stomach tumors (rare)

- Studies in Syrian golden hamsters
  - Nasal cavity tumors (rare)
  - Laryngeal tumors (rare)
  - Tracheal tumors (rare)
  - Lung tumors (rare)

- Studies of metabolism
  - NHEX, like many other nitrosamines, requires metabolic activation via cytochrome P450 enzymes in order to express genotoxic activity.
  - Several NHEX metabolites have been identified, some of which have been found to react with nucleic acids, or be genotoxic:
    - DNA and RNA alkylation *in vivo*, with NHEX exposure: 1,6-hexanediol
    - Mutagenic metabolites: β- hydroxy NHEX and γ-hydroxy NHEX
  - Additional electrophilic reactive metabolites have been proposed, including:
    - NHEX radical
    - NHEX imminium ion
    - Carbonium ion metabolite
    - NO^+

- Observations from genotoxicity studies
  - NHEX
    - Mutations in *S. typhimurium* and *E. coli* in the presence of S-9
    - Mutations in primary rat hepatocyte-mediated assays in Chinese hamster V79 cells *in vitro*
    - X-linked recessive lethal mutations in *D. melanogaster*
    - Covalent binding to RNA and DNA in rat liver following *in vivo* exposure
  - NHEX metabolites
    - 1,6-hexanediol alkylates liver RNA and DNA in NHEX-treated rats
    - β- and γ-hydroxy NHEX induce mutations in *S. typhimurium* in the presence of S-9

- There are strong structure-activity similarities between NHEX and the five comparison nitrosamines, four of which are listed as carcinogens under Proposition 65. In addition, several QSAR models predict that NHEX is both mutagenic and carcinogenic.

- Mechanistic findings for NHEX are associated with the following key characteristics of carcinogens:
    - Is electrophilic or can form electrophilic metabolites
    - Is genotoxic

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Appendix A Literature Search Strategies on the Carcinogenicity of N-Nitrosohexamethyleneimine (NHEX)

General searches of the literature on the carcinogenicity of NHEX were conducted 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 this chemical. As described below, we used a similar approach as that recommended by the National Toxicology Program (NTP) Handbook for Preparing Report on Carcinogens (RoC) monographs (NTP, 2015; https://ntp.niehs.nih.gov/ntp/roc/handbook/roc_handbook_508.pdf), with the goal of systematically identifying and reviewing all literature relevant to the assessment of evidence on NHEX carcinogenicity. Similar to the approach recommended by the NTP, the web-based platform called Health Assessment Workspace Collaborative (HAWC) (https://hawcproject.org/about/) was used to organize the literature search results.

Search strategies

OEHHA used the following key words to search all databases listed below: N-nitrosohexamethyleneimine [All Fields], CAS Number: 932-83-2, N-nitrosoperhydroazepine. As there were not many publications on NHEX, we did not limit the search results to carcinogenicity or cancer.

Data sources

The following is a list of the data sources that were searched to find information on NHEX. This list consists of the data sources recommended by the NTP RoC handbook (NTP, 2015; https://ntp.niehs.nih.gov/ntp/roc/handbook/roc_handbook_508.pdf), with additions by OEHHA of other authoritative reviews, study reports, and web-based resources and/or databases.

Biomedical literature databases

- PubMed (National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pubmed)
- TOXNET (National Library of Medicine) (https://toxnet.nlm.nih.gov): Toxicology Literature Online (TOXLINE)
- Scopus (https://www.scopus.com/search/form.uri?display=basic)
- Embase (https://www.elsevier.com/solutions/embase-biomedical-research)
- Web of Science® (Thomson-Reuters, Inc.) (https://clarivate.com/products/web-of-science/)

Authoritative reviews and reports

- International Agency for Research on Cancer (IARC) Monographs (https://monographs.iarc.fr/ENG/Monographs/PDFs/index.php)
- National Toxicology Program (NTP) publications, including, but not limited to, technical reports, nominations for toxicological evaluation documents, Report on Carcinogens (RoC) monographs, RoC background documents or monographs, and NTP Office of Health Assessment and Translation (OHAT) monographs (https://ntp.niehs.nih.gov/)
- US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) (https://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList)
- Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles (https://www.atsdr.cdc.gov/toxprofiles/index.asp)
- European Chemicals Agency Risk Assessments (https://echa.europa.eu)
- Health Canada Environmental Health Assessments (https://www.hc-sc.gc.ca/index- eng.php)
- New York State Department of Health — Health Topics A to Z (https://www.health.ny.gov/healthaz/)
- National Academy of Sciences reports and publications (https://www.nationalacademies.org/publications/)
- World Health Organization (WHO)/United Nations Environment Programme (UNEP) International Programme on Chemical Safety (IPCS) INCHEM-related documents (https://www.inchem.org/)

Other Databases or web resources

- TOXNET: Genetic Toxicology Data Bank (GENE-TOX), Carcinogenic Potency Database (https://toxnet.nlm.nih.gov/cpdb/)
- Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/)
- iCSS Dashboard v2 (US EPA ToxCast Phase II data, (https://actor.epa.gov/dashboard2/)
- European Chemicals Agency (https://echa.europa.eu/)
- European Food Safety Authority (https://www.efsa.europa.eu/en/publications.htm)

- International Labour Organization (https://www.ilo.org/global/publications/lang--en/index.htm)
- International Uniform Chemical Information Database (https://iuclid.eu/)
- National Institute for Occupational Safety and Health (NIOSH) Publications (https://www2.cdc.gov/nioshtic-2/)
- United Nations Environment Programme (www.unep.org)
- PubChem BioAssay (National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pcassay)
- Google search engine (www.google.com)
- Relevant literature was also identified from citations in individual articles and reports.

Use of Health Assessment Workspace Collaborative (HAWC)

HAWC (https://hawcproject.org/about/) was used as a tool in the systematic review of the literature on the carcinogenicity of NHEX following the guidance provided in the NTP RoC handbook (NTP, 2015). Specifically:

Citations retrieved from literature searches were uploaded to EndNote libraries, and duplicates were removed. Next, the EndNote libraries were uploaded to HAWC for multi-level screening using specific inclusion and exclusion criteria.

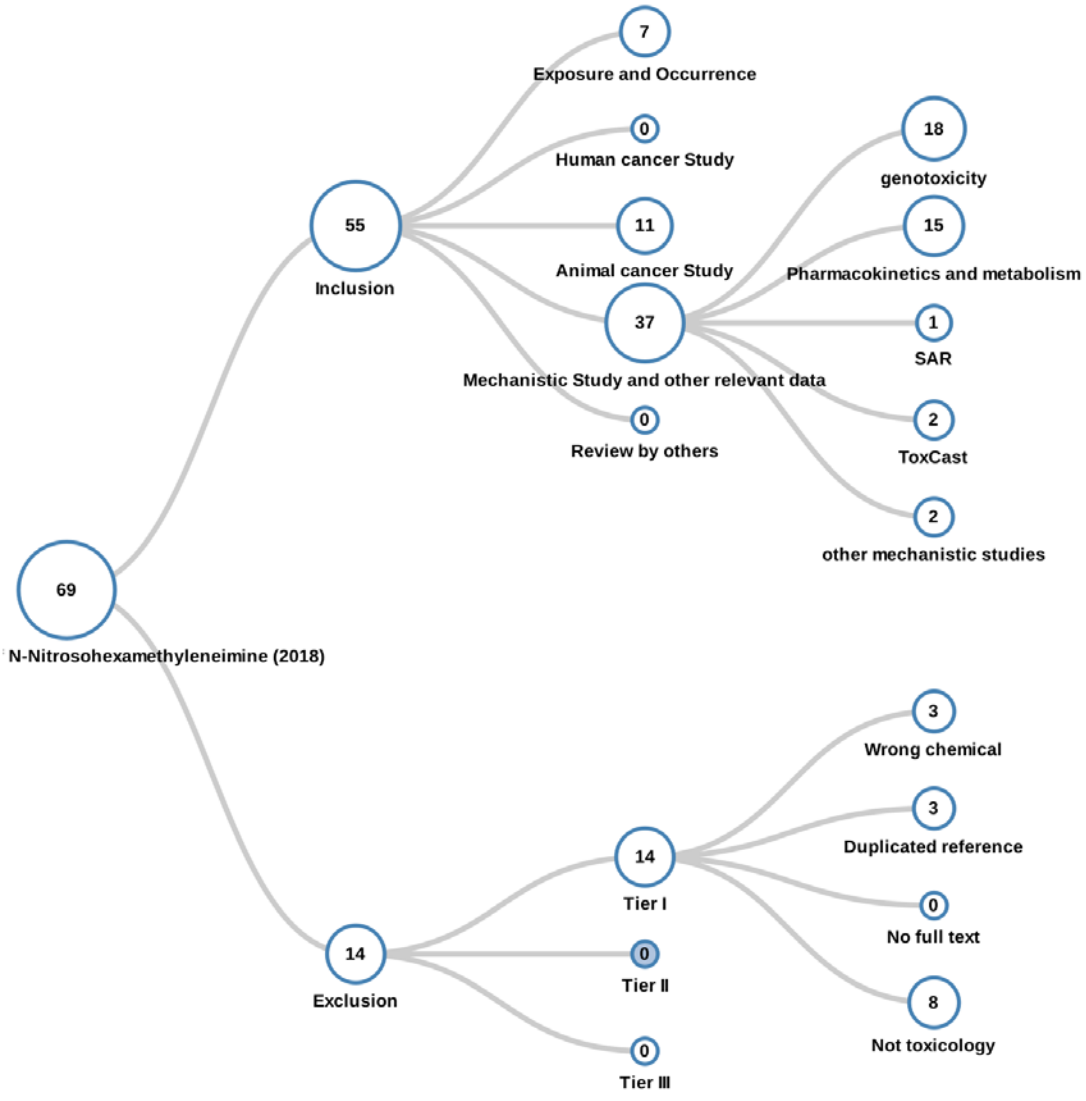
In Level 1 screening, the citations were screened independently by two OEHHA staff, based solely on titles and abstracts, to eliminate studies or articles that do not contain information on NHEX or on any of the key topics such as exposure, cancer studies in humans and animals, toxicokinetics, metabolism, genotoxicity, toxicity, or cancer-associated mechanisms. The initial screen was intended to retrieve all studies deemed to have a reasonable possibility of containing information that could be useful for the review process. A positive response by only one of the reviewers was sufficient to pass a publication on to the next review level. The initial reviewers assigned (or tagged) the citation to one or more of the key topic(s) (see Figure A-1).

In Level 2 screening, the full papers were obtained for all citations that passed the Level 1 screen. These full papers were screened independently by two OEHHA staff, using similar inclusion/exclusion criteria as was used in the Level 1 screening. However, Level 2 reviewers could make more accurate judgments about the relevance of the citations because they were reviewing the full text of the articles, in addition to the title and abstract. Following Level 2 screening, the tagging of articles according to key topics was updated in HAWC.

Level 1 and 2 screenings were repeated and HAWC search results were updated if additional relevant studies cited in the original set of publications ("secondary citations") were identified.

Figure A-1 presents the results of OEHHA's systematic review of the literature on the carcinogenicity of NHEX. In summary, 69 references were identified through OEHHA's literature search strategies. Among these, 55 were identified as having information relevant to the carcinogenicity of NHEX and 14 were excluded.

Figure A-1. Overview of the systematic review of the literature on the carcinogenicity of NHEX



Additional focused literature searches

In addition to the literature searches described above, additional focused literature searches were performed by OEHHA for certain topics covered in various sections of the document. The specific additional search strategies used for each of these sections are briefly described as follows:

Section 2. Introduction

A focused search was conducted for NHEX using the US EPA National Service Center for Environmental Publications (https://www.epa.gov/nscep/). Additional relevant literature was identified from citations in the US EPA publications.

Section 3.3.2. Genotoxicity

Focused searches were conducted for NHEX and its metabolites, using PubMed, TOXNET, Scopus and GENE-TOX. Additional relevant literature was identified from citations in individual articles.

Section 3.3.3. Animal Tumor Pathology

Focused searches were conducted using three pathology books published by IARC [Pathology of Tumors in Laboratory Animals: Volume 1-3, 1991 (Vol. 1), 1994 (Vol. 2), 1996 (Vol. 3)], and searching for the information of species-specific tumor sites. Additional relevant literature was identified from citations in individual books or articles.

Section 3.3.4. Structure Activity Considerations

Focused searches were conducted for the five comparison chemicals using the TOXNET databases, PubMed, Scopus, IARC monographs, and the NTP Report on Carcinogens. PubMed and Google were used to identify QSAR models and their instruction manuals. Additional relevant literature was identified from citations in individual articles.

In summary, around 200 references, including government reports, peer-reviewed journal articles, QSAR model-instruction manuals and books, were identified through these search strategies. Among these, 107 were cited in this document.