Nitrogen Mustard (hydrochloride)

Health-based calculated occupational cancer risk values

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies 'Nitrogen Mustard (Hydrochloride)'
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U 1042/AvdB/459-R43
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Mijnheer de staatssecretaris,

Bij brief van 3 december 1993, nr DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. Tevens vroeg de Staatssecretaris om in het geval van beroepsmatige blootstelling aan genotoxisch carcinogene stoffen de risico's op sterfte als gevolg van kanker te berekenen.

In dat kader bied ik u hierbij een advies aan over stikstofmosterd. Dit advies is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieu.

Hoogachtend,

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Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

No. 2004/06OSH, The Hague, September 2, 2004

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature & Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

This report can be downloaded from www.healthcouncil.nl.

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Samenvatting

Op verzoek van de Minister van Sociale zaken en Werkgelegenheid, schat de Commissie WGD van de Gezondheidsraad het extra kankerrisico bij beroepsmatige blootstelling aan stoffen die door de Europese Unie of door de Commissie WGD als genotoxisch kankerverwekkend zijn aangemerkt. In dit rapport maakt zij zo'n schatting voor stikstofmosterd (hydrochloride). Zij heeft daarbij gebruik gemaakt van de methode die beschreven is in het rapport 'Berekening van het risico op kanker' (1995/06WGD) (Hea95).

De commissie is echter van mening dat wegens een gebrek aan voldoende gegevens het niet mogelijk is om het extra kankerrisico voor stikstofmosterd (hydrochloride) te berekenen.

Executive summary

On request of the Minister of Social Affairs and Employment the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, estimates the additional cancer risk associated with occupational exposure to substances that have been classified by the European Union or the DECOS as genotoxic carcinogen. In this report the committee presents such estimates for nitrogen mustard (hydrochloride). It has used the method described in the report 'Calculating cancer risks due to occupational exposure to genotoxic carcinogens' (1995/06WGD) (Hea95).

The committee is of the opinion that due to a lack of sufficient data, it is not possible to estimate the additional lifetime cancer risk for nitrogen mustard (hydrochloride).

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the Minister of Social Affairs and Employment (annex A). This evaluation should lead to a health-based recommended occupational exposure limit for the concentration of the substance in air. Such an exposure limit cannot be derived if the toxic action cannot be evaluated using a threshold model, as is the case for substances with genotoxic carcinogenic properties.

In this case an exposure-response relationship is recommended for use in regulatory standard setting, *i.e*. the calculation of so-called health-based calculated occupational cancer risk values (HBC-OCRVs). The committee calculates HBC-OCRVs for compounds, which are classified as genotoxic carcinogens by the European Union or by the present committee.

For the establishment of the HBC-OCRV's the committee generally uses a linear extrapolation method, as described in the committee's report 'Calculating cancer risk due to occupational exposure to genotoxic carcinogens' (1995/06WGD). The linear model to calculate occupational cancer risk is used as a default method, unless scientific data would indicate that using this model is not appropriate.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister of Social Affairs and Employment on the feasibility of using the HBC-OCRVs as regulatory occupational exposure limits. In the final step of the procedure the Minister sets the official occupational exposure limits.

1.2 Committee and procedure

The present document contains the derivation of HBC-OCRV's by the committee for nitrogen mustard (hydrodichloride). The members of the committee are listed in Annex B. The first draft of this report was prepared by MI Willems, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

In 2004, the President of the Health Council released a draft of this report for public review. The Individuals and organizations that commented on the draft are listed in Annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation of the carcinogenicity of nitrogen mustard has been based on reviews by IARC (IARC75, IARC87). Where relevant, the original publications were reviewed and evaluated as indicated in the text. In addition, literature has been retrieved from the online databases Chemical Abstracts, Toxline, and Medline, covering the period 1987 to April 2004. Toxicity and carcinogenicity data are mainly available from studies performed with nitrogen mustard hydrochloride.

Chapter 2

Nitrogen Mustard (hydrochloride)

2.1 Introduction

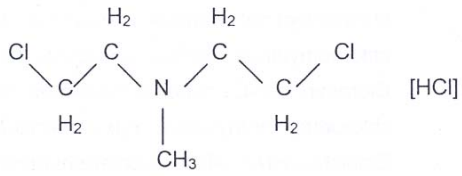
Evidence for nitrogen mustard (hydrochloride) induced carcinogenicity is considered sufficient to animals, but limited to humans (IARC87). Nitrogen mustard and its hydrochloride are classified by the IARC as probably carcinogenic to humans (group 2A) (IARC75, IARC87).

DECOS classified nitrogen mustard in 1995 as genotoxic carcinogen (comparable with EU categroy 2) (DEC95).

2.2 Identity, and physical and chemical properties

As nitrogen mustard is mainly tested as hydrochloride, specific information on nitrogen mustard hydrochloride is placed between [ ].

Chemical name	: N,N-bis(2-chloroethyl)methylamine [hydrochloride]
Chem. Abstr. Name	: 2-chloro-N-(2-chloroethyl)-N-methylethanamine [hydrochloride]
CAS registry number	: 51-75-2 [55-86-7]
EU number	: not available
EINECS number	: 200-120-5 [not available]

Synonyms nitrogen mustard	:	*N*,*N*-Bis(2-chloroethyl)-*N*-methylamine; *N*,*N*-Bis(2-chloroethyl)methylamine; Bis(2-chloroethyl)methylamine; Bis(b-chloroethyl)methylamine; Caryolysin; Chloramine; Chlormethine; Cloramin; b,b'-Dichlorodiethyl-*N*-methylamine; Di(2-chloroethyl)methylamine; b,b'-Dichloro-*N*-methyldiethylamine; Embichin; HN2; MBA; Mechlorethamine; *N*-Methyl-bis(2-chloroethyl)amine; *N*-Methyl-bis(b-chloroethyl)amine; Methylbis(b-chloroethyl)amine; Methylbis(chloroethylamine); *N*-Methyl-2,2'-dichlorodiethylamine; Methyldi(2-chloroethyl)amine; Mustargen; Mustine; Mutagen; Nitrogen mustard
Synonyms hydrochloride salt	:	Azotoperite; *N*,*N*-Bis(2-chloroethyl)methylamine hydrochloride; Bis(2-chloroethyl)methylamine hydrochloride; Caryolysine; Chloramin; Chloramine; Chlorethamine; Chlorethazine; Dichloren; b1-Dichlorodiethyl-*N*-methylamine hydrochloride; Di(2-chloroethyl)methylamine hydrochloride; Di(chloroethyl)methylamine chloride; b,b'-Dichloro-*N*-methyl-diethylamine hydrochloride; Dimitan; Embichin; Embikhine; Erasol; HN2; HN2 hydrochloride; MBA hydrochloride; Mebichloramine; Mechlorethamine; Mechlorethamine hydrochloride; Metagen; *N*-Methyl-bis(2-chloroethyl)amine hydrochloride; Methylbis(2-chloroethyl)amine hydrochloride; *N*-Methylbis(b-chloroethyl)amine hydrochloride; Methyl-bis(b-chloroethyl)amine hydrochloride; *N*-Methyl-2,2'-dichlorodiethylamin hydrochloride; Methyldi(2-chloroethyl)amine hydrochloride; Methyldi(b-chloroethyl)amine hydrochloride; Mitoxine; Mustargen; Mustargen hydrochloride; Mustine; Mustine Hydrochlor; Mustine hydrochloride; Nitrogen mustard; Nitrogranulogen; N-Lost; NSC-762 hydrochloride
Trade names:	:	Caryolysine®; Mustargen® (hydrochloride salt)
Description:	:	clear to yellow liquid, faint odour of herring [white, hygroscopic crystals]
Uses:	:	chemical warfare agent [antineoplastic agent]
Uses:	:	chemical warfare agent [antineoplastic agent]
Molecular weight:	:	156.1 [192.5]
Molecular formula:	:	$C_5H_{11}C_{12}N$ [$C_5H_{12}C_{13}N$]
Structure	:	
Melting point	:	- 60 °C [109-111 °C]
Boiling point	:	87°C at 18 mm Hg; 75°C at 10 mm Hg; 64°C at 5 mm Hg [no data]
Water solubility	:	very slightly soluble [soluble in water; 0.1 g/L]
Stability	:	undiluted liquid decomposes slowly on standing, reacts rapidly with water [dry crystals are stable up to 40°C, unstable in aqueous solution]
EC classification	:	not classified or labeled according to the 23rd Amendment to Annex I of Directive 67/548/EEC (dated December 5, 1997)

2.3 Carcinogenicity studies and selection of study suitable for risk estimation in the occupational situation

2.3.1 Human data

IARC data

IARC considered the evidence for carcinogenicity to humans as limited. No epidemiological study concerning the effects of exposure to nitrogen mustard as a single agent was available to the Working Group (IARC87). However, it is the principal alkylating agent in leukaemogenic combination chemotherapy given for Hodgkin's disease, and other alkylating agents are clearly leukaemogenic. The many case reports of cancer following topical application of nitrogen mustard cannot be interpreted with certainty because concurrent treatment with radiation and other potent drugs has been the rule rather than the exception, and occasionally such associations would be expected by chance (IARC87).

Squamous-cell carcinomas of the skin following long-term topical application of nitrogen mustard alone or in combination with systemic therapy for mycosis fungoides and psoriasis have been observed to appear on skin surfaces not exposed to the sun (IARC87).

Additional studies

The literature search did not reveal relevant additional human data.

2.3.2 Animal data

IARC data

Animal carcinogenicity data are summarised in annex D. Nitrogen mustard, administered mainly as the hydrochloride, has been tested for carcinogenicity in mice and rats by subcutaneous, intravenous and intraperitoneal administration and by skin painting. It produced mainly lung tumours and lymphomas in mice after subcutaneous, intravenous and intraperitoneal administration. Intravenous injection of nitrogen mustard to rats induced tumours in different organs. Application by skin painting produced local tumours in mice in a dose-dependent manner (IARC87).

None of the experiments summarized in annex D fulfilled the criteria for calculating the additional lifetime cancer risk. The only experiments with a sufficiently long expo-

sure time were those of Boyland and Horning and Schmähl and Osswald (Boy49, Sch70). In all other experiments listed in annex D the exposure times were less than 25% of the standard lifespan.

The study of Schmähl and Osswald was considered less suitable for calculating an additional lifetime cancer risk, since only one dose of nitrogen mustard was examined. This dose, the maximally tolerated dose, that represented 7% of the lethal dose, was reported to cause a decrease in the mean survival time of approximately 7 months (Sch70). Moreover, an intravenous study seems less appropriate for establishing a health-based occupational cancer risk level for nitrogen mustard in air, because it cannot be excluded that nitrogen mustard may induce local effects in the respiratory tract upon exposure by inhalation. It has been shown that dermal application of nitrogen mustard causes skin tumours at dose levels that are also severely irritating for the skin (Zac80, annex D, remarks).

In addition, in the experiment of Boyland and Horning no concurrent control group was included (Boy49).

There were no inhalatory or oral studies (see annex D).

Additional data

The literature search did not reveal relevant additional animal carcinogenicity data.

2.3.3 Conclusion

From the results of the studies summarised, the committee concludes that nitrogen mustard is an animal carcinogen. Moreover, according to the committee dermal exposure to nitrogen mustard may cause cancer. None of these experiments, however, did meet the criteria for calculating a health-based calculated occupational cancer risk value for nitrogen mustard in air.

2.4 Existing occupational exposure limits

No occupational exposure limits are available for nitrogen mustard in European countries and in the USA. IARC has classified the nitrogen mustard as probably carcinogenic to humans (Group 2A) (IARC87).

2.5 Toxicity profile of nitrogen mustard (hydrochloride)

Nitrogen mustard is a bifunctional alkylating agent.

No information is available on uptake, distribution, and excretion after oral, dermal, or inhalatory exposure. An intravenous dose of 3 mg/kg bw nitrogen mustard administered to dogs rapidly disappeared from the blood: 0.01% was found in the urine, and low levels were found in the tissues, the highest concentration being in the bone marrow. An almost immediate disappearance of ^{14}C-nitrogen mustard from the blood and a low urinary excretion of nitrogen mustard was observed in dogs given 0.5 mg/kg bw intravenous over 5 seconds or 60 minutes. Mice, given 35 mg/kg bw nitrogen mustard hydrochloride intravenous and examined by autoradiography, had significant levels of the compound in brain, spinal cord, lungs and submaxillary glands. In rats, 16% of an injected dose of nitrogen mustard was found in the spleen, lungs, kidneys, liver and blood, and 17% was excreted in the urine.

Following its *in vivo* administration, nitrogen mustard or its hydrochloride is probably converted into an ethylene-immonium ion, which reacts with the guanine residues in adjacent strands of DNA as well as with SH groups.

The LD50 of nitrogen mustard in mice and rats by subcutaneous and intravenous injection is approximately 1-4 mg/kg bw. The intravenous LD50 of nitrogen mustard hydrochloride in rats is 1.1 mg/kg bw. The oral LD50 in mice is 10-20 mg/kg bw, depending upon whether the animals receive food prior to dosing or not.

Nitrogen mustard is a strong irritating agent and can cause sensitisation (DFG98). When injected intradermally into coloured mice, nitrogen mustard causes local greying of hair.

Nitrogen mustard is embryotoxic in rats when given by intravenous injection on the 4th day of pregnancy and also causes fetal abnormalities in rats. Four women with Hodgkin's disease who were treated with nitrogen mustard during the first and third (1 case) or second and third (3 cases) trimesters of pregnancy. No abnormalities were recorded in the offspring at 2 months, 7½, 8½ and 9½ years.

Nitrogen mustard is genotoxic. It has been reported to induce chromosome aberrations and primary DNA damage in mammalian cells in vitro, micronuclei and dominant lethal mutations in laboratory animals, and chromosome aberrations in lymphocytes of patients treated with nitrogen mustard.

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A Request for advice

B The Committee

C Comments on the public review draft

D Animal studies

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of

genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- LJNGM Bloemen
  epidemiologist; Dow Benelux NV, Terneuzen
- PJ Boogaard
  toxicologist; Shell International BV, The Hague
- PJ Borm
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- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
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  Health Council of the Netherlands, The Hague

The first draft of the present advisory report was prepared drs MI Willems, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance was provided by F Smith.
Lay-out: J van Kan.

Annex C

Comments on the public review draft

A draft of the present report was released in 2004 for public review. The following organizations and persons have commented on the draft document:

- JA Wess, National Institute of Occupational Safety and Health, USA.

Annex **D**

Animal data

The following abbreviations were used:

Xpo	exposure period
Xpe	total experimental/observation period
MST	mean survival time
MTD	maximally tolerated dose
TBA	tumour bearing animals

Ref.	Species/route	Experimental	Findings, tumours	Remark(s)
dermal administration				
Zac80	female Swiss mice	Exp. series 1) n=24, 1 x 0.3 mg/ wk for <= 33 wks; 2) n=30, 1 x 0.1 mg/ wk for 33 wks; 3) n=30, 3 x 0.1 mg/ wk for <= 33 wks; 4) n=36, 3 x 0.1 mg/ wk for 23 wks.	Skin tumour incidence: 1) squamous cell carcinoma 1/24, papillomas 7/24 2) no skin tumours 3) papillomas 6/29 4) squamous cell carcinoma 9/33, papillomas 1/33 Controls: no skin tumours	MST in days for experiments 1, 2, 3, 4 was 140, ?, 177, and 214 days respectively. In all series in which HN_2 caused tumours there was a high incidence of dermatitis, alopecia, and scarring. A non-irritating carcinogenic dose for HN_2 was not established.

Eps84	Mice, Uscd (Hr) strain albino hairless mice (sex not reported)	n=46, 2x 0.1 mg/wk n=60, controls Xpo 52 wks, Xpe 52 wks	Skin tumour incidence: 34% of HN_2 group had tumours, 0% in solvent controls. Skin tumours included papillomas and squamous cell carcinomas.	Pathological examinations restricted to skin.
Subcutaneous and/or intramuscular administration				
Boy49	Mice, stock s.c n = 20/group (sex not reported)	HN_2.HCl , 1x1 mg/kg bw a week for 50 wks, Xpo 50 wks, Xpe lifetime	Tumour incidence in HN_2-treated mice: TBA total, 6/10; lung carcinoma, 3/10; lung adenoma, 1/10; lymphosarcoma in liver, 1/10; uterine fibromyoma, 1/10. A group of 40 untreated mice from the same source as the experimental mice were killed when between 14 and 18 months of age. In this series 6 mice had lung adenomas and 2 hepatomas.	The experiment did not include a concurrent control group, see tumour findings. MST in HN2-treated mice was 148-580 days. Reported tumour incidence regards mice surviving 284-580 days
Hes53 (cited from IARC75)	Mice, C3H and C3Hf, s.c. males and females	0.025 mg HN_2.HCl weekly, Xpo 8 wks, Xpe not reported.	Tumour incidence. **HN_2-treated animals**, at injection site: sarcoma, skin papilloma and squamous-cell carcinoma: 3/15 (♂♂, ♀♀,C3H), 5/21 (♂♂, C3H) and 3/37 (♂♂, C3Hf), remote: pulmonary tumours: 5/15 (♂♂, ♀♀,C3H), 8/21 (♂♂, C3H) and 21/37 (♂♂, C3Hf). **Controls**: no tumours at injection site, pulmonary tumours: 3/14 (♂♂, ♀♀, C3H), 5/21 (♂♂, C3H) and 6/39 (♂♂, C3Hf).	
Intraperitoneal administration				
Shi66 (cited from IARC75)	Mice, A/J30 n=60/ group/ sex	1)12 times in 4 wks HN_2.HCl dissolved in water. Cumulative doses 3.85, 212, 866 and 3369 µg/kg bw; Xpo 4 wks, Xpe 39 wks	Lung tumour incidence: 1) HN_2/water-treatment: 30%, 40%, 69% and 95% in 3.85, 212, 866, and 3369 µg/kg bw groups, respectively. Control/water: ♂♂: 37%, ♀♀: 27%	Lung tumour incidence in HN_2-treated animals was not reported separately for males and females
	n=15/group/ sex	2) 12 times in 4 wks HN_2.HCl dissolved in tricaprylin. Cumulative dose 46, 206, 828 and 3311 µg/kg bw; Xpo 4 wks, Xpe 39 wks.	2) HN_2/tricaprylin-treatment: 46%, 50%, 29%, and 63% in 46, 206, 828, and 3311 µg/kg bw groups, respectively. Control/tricaprylin: ♂♂: 36%, ♀♀: 32%	

Intravenous administration				
Hes49 (cited from IARC75)	Mouse, ATest: n=37 (♂♂+♀♀) Control n=38 (♂♂+♀♀)	2-4 times at 2 day intervals HN_2.HCl dissolved in water. Dose 1 mg/kg bw. Xpo 8 day, Xpe 16 wks	Lung tumour incidence: Test animals: 29/29 (3.5 tumours/mouse). Control group: 4/30 (0.17 tumours/mouse)	
Hes50 (cited from IARC75)	Mouse, ATest: n=20 ♂♂,♀♀ control n=32 ♂♂,♀♀	1) 4 injections of HN2.HCl; total dose 0.1 mg. Xpo not reported, Xpe 10 months.	Lung tumour incidence 1) test animals: 100% (20/20) (9.6 tumours/mouse); control group: 20/32 (0.81 tumours/mouse)	Time between injections not reported
		2) single injection of 0.4 mg/kg HN2.HCl Xpo 1 day, Xpe 10 months	2) test animals: 100% (9/9) (7.5 tumours/mouse); control group: 18/31 (0.94 tumours/mouse)	
Intravenous administration				
Con65 (cited from IARC75)	Mouse, RFTest: n=104 ♀♀ Control n=112 ♀♀	HN_2, 1 x 2.4 mg each 2 wks for 8 wks. Total dose 9.6 mg/kg bw. Xpo 8 wks, Xpe 2 years.	Tumour incidence **HN_2-treated mice**: thymic: lymphomas 21%, pulmonary adenomas 68%; **control group**: thymic lymphomas 10%, pulmonary adenomas 15%	MST, control animals 632 days; test animals 490 days
Sch70 (cited from IARC75)	Rat, BR 46Test: n=48 ♂♂ Control n=89 ♂♂	0.11 mg/kg bw/ weekly for 52 weeks; total dose 5.72 mg/kg bw HN_2.HCl Xpo 52 weeks, Xpe lifespan.	Tumour incidence **HN_2-treated rats**: TBA, total, malignant 7/27: lymphatic (1), myeloid leukaemia (1), reticulum-cell sarcoma (1), liposarcoma, (1), adenocarcinoma of large intestine (1), sarcoma of meninges (1) and haemangioendothelioma of salivary glands (1); TBA, total, benign 5/27: thymoma (3), mammary fibroma (1), lung angiofibroma (1) **Controls**: TBA, total, malignant 4/65: mammary sarcoma (3), phaechromocytoma (1). TBA, total, benign 3/65: thymomas (2), mammary fibroma (1)	Dose applied was 7% of lethal dose. MST, controls 25±6 months, in animals treated with alkylating agents incl. HN_2 7 months less. Tumour latency time, controls 23±5, HN_2-treated animals 16±3 months.