N-Methylhydrazine

Evaluation of the carcinogenicity and genotoxicity

Dutch Expert Committee on Occupational Standards,

a committee of the Health Council of the Netherlands

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies over ***N***-methylhydrazine
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-451/JR/tvdk/459-N36
Bijlagen : 1
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Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/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. In dat kader bied ik u hierbij een advies aan over de kankerverwekkende eigenschappen van ***N***-methylhydrazine. Het 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, de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer en de Minister van Sociale Zaken en Werkgelegenheid.

Hoogachtend,

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Dutch Expert Committee on Occupational Standards,
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to

the Minister and State Secretary of Social Affairs and Employment

Nr 2002/07OSH, The Hague, 16 April 2002

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 Preservation & Fisheries. 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.

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. In het voorliggende rapport neemt de Commissie WGD van de Raad, die deze beoordelingen verricht, *N*-methylhydrazine onder de loep. De commissie heeft haar oordeel gegoten in door de Europese Unie aangegeven termen.

De commissie concludeert dat *N*-methylhydrazine beschouwd moet worden als kankerverwekkend voor de mens (vergelijkbaar met EU-categorie 2). *N*-methylhydrazine is een genotoxisch carcinogeen.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the carcinogenic properties of substances at the workplace and proposes a classification with reference to the EU-directive. This evaluation is performed by the Dutch Expert Committee on Occupational Standards. The present report contains an evaluation by the committee on the carcinogenicity of *N*-methylhydrazine.

The committee concludes that *N*-methylhydrazine should be considered as carcinogenic to humans (comparable with EU-category 2). *N*-methylhydrazine is genotoxic.

Chapter **1**

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. The Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to study the carcinogenic properties of substances and to propose a classification with reference to an EU-directive (annex A and F). This task is carried out by the Council's Dutch Expert Committee on Occupational Standards, hereafter called the committee.

The evaluation of the carcinogenicity of a substance is based on IARC* evaluations. The original publications are not reviewed and evaluated in the text of the report, but the overall conclusion of the IARC on the carcinogenic properties is included (annex D).

In addition to classifying substances with respect to their possible carcinogenicity according to the EU Guidelines, the committee also assesses the genotoxic properties of the substances in question. The committee expresses its conclusions in the form of standard sentences (annex E).

* International Agency for Research on Cancer

1.2 Committee and procedures

The present report contains evaluations by the committee of the carcinogenicity of *N*-methylhydrazine. 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 2000 the President of the Health Council released a draft of the report for public review. The individuals and organisations 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 *N*-methylhydrazine has been based on a brief summary by IARC (IARC83). Where relevant, the original publications were reviewed and evaluated in the text.

In addition, information was obtained from reviews by the American Conference of Governmental Industrial Hygienists (ACGIH) (ACG91), the Swedish Criteria Group on Occupational Standards (SCG) (Lun92), and the American Environmental Protection Agency (USEPA) (EPA84). Furthermore, literature has been retrieved form the online data bases Toxline, Cancerlit, and Medline, covering the period 1965 to March 1997, 1963 to April 1997, and 1966 to May 1997, respectively. An additional search up to May 2001 did not reveal new relevant literature.

Chapter **2**

N-Methylhydrazine

2.1 Introduction*

Name	:	*N*-methylhydrazine
CAS no	:	60-34-4
EINECS no	:	200-471-4
EEC no	:	not available
CAS name	:	methylhydrazine
IUPAC name	:	methylhydrazine
Synonyms	:	monomethylhydrazine
Description	:	clear colourless liquid with an ammonia-like odour
Occurrence		no data
Use	:	as an altitude control fuel in missile propellants, as a solvent, as a chemical intermediate
Chem formula	:	CH_6N_2
Chem structure	:	CH_3-NH-NH_2
Molecular weight	:	46.1
Boiling point (101.3 kPa)	:	87.5 °C
Melting point (101.3 kPa)	:	-52.4 °C

* Data from ACG91, Stu96

Relative density	:	0.9
Vapour pressure (20 °C)	:	4.8 kPa
Relative vapour density (air=1)	:	1.6
Relative density of saturated vapour/air mixture (air=1; 20 °C)	:	1.03
Solubility in water	:	miscible
Solubility in organic solvents	:	soluble in hydrocarbons, diethyl ether; miscible with hydrazine and low molecular weight alcohols
Conversion factors (101.3 kPa; 20°C)	:	1 ppm = 1.92 mg/m^3 1 mg/m^3 = 0.52 ppm
EC classification	:	-

2.2 IARC conclusion

IARC has not presented an evaluation of the carcinogenicity of *N*-methylhydrazine. Being a metabolite of gyromitrin (acetaldehyde formylmethylhydrazone), some relevant data on *N*-methylhydrazine were presented in the evaluation of the carcinogenicity of gyromitrin (see IARC83).

2.3 Human data

2.3.1 IARC data

No human data were presented by IARC.

2.3.2 Additional data

No additional data were found.

2.4 Animal data

2.4.1 IARC data

In random-bred Swiss mice (n=50/sex; untreated controls, 110/sex) given *N*-methylhydrazine at a concentration of 100 mg/L in drinking water, lung adenomas were found in 12/50 (17 tumours) female and 11/50 (12 tumours) male animals. According to IARC, no information on the lung tumour incidence in control animals was available. Treated animals had died by 70 (female) and 80 (male) weeks of age, while

controls were killed at 120 weeks of age. Treatment clearly reduced the survival rates at 50 weeks of age, being 13/50 and 6/50 for female and male treated mice compared with 96/110 and 67/110 for female and male untreated controls (IARC83). According to USEPA, in the study summarised above, both *N*-methylhydrazine and *N*-methylhydrazine sulphate were administered at concentrations of 100 and 10 mg/L, respectively. These ingested amounts were approximately 0.7 and 0.09 mg/day, respectively*. The lung adenoma incidences were 23/100 and 46/100, respectively and for control animals 23/100. The average latency periods of lung tumours were 51 and 91 weeks, respectively, and for controls 82 weeks (EPA84).

In random-bred Syrian golden hamsters (n=50/sex; untreated controls, 110/sex), treatment of *N*-methylhydrazine at a concentration of 100 mg/L in drinking water for life, induced malignant histiocytomas (Kupffer-cel sarcomas) in the liver in 16/49 and 27/50 females and male hamsters and none in control animals. Tumours in the ceacum were observed in 9/49 and 7/50 in female and male treated animals, whereas in control animals it was respectively 1/99 and 1/97. Treated animals had died by 110 weeks of age, while controls were killed at 120 weeks of age. Treatment reduced survival rates in animals, being 4/50 and 18/50 for female and males respectively at 80 weeks of age compared with 31/100 and 42/100 for female and male controls (IARC83). According to USEPA, the ingested amount was approximately 1.2 mg/day (EPA84).

2.4.2 Additional data

In reviews by ACGIH and SCG, data are presented on the carcinogenicity of *N*-methylhydrazine after inhalatory exposure of rats, mice, hamsters and dogs to 0, 0.04 (rats and mice only), 0.38, 3.8, and 9.6 (rats and hamsters only) mg/m^3 (0.02, 0.2, 2.0, 5.0 ppm) for one year (6 hours a day, 5 days a week), with observation times of 1 and 5 years for rodents and dogs, respectively. No increased tumour incidences were found in rats or dogs. Signs of toxicity observed were dose-related growth reductions in rats, and elevated serum liver enzyme and methaemoglobin levels in dogs, both of the high concentration group. In mice exposed to 3.8 mg/m^3, significantly higher incidences of lung tumours, nasal adenomas, nasal polyps, nasal osteomas, haemangiomas, and liver adenomas and carcinomas were observed. In hamsters, treatment induced nasal polyps and adrenal adenomas at 3.8 and 9.6 mg/m^3 and nasal cavity adenomas at 9.6 mg/m^3 (ACG91, Lun92).

EPA discusses studies in which oral or intraperitoneal treatment with *N*-methylhydrazine did not result in increased incidences of lung tumours in mice

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* It is not clear whether these amounts are per animal or per unit body weight.

(EPA84), but treatment times are considered to be too short (8 or 40 weeks) to be relevant for the assessment of the carcinogenic potential of *N*-methylhydrazine.

In hamsters treated with 100 mg *N*-methylhydrazine/L drinking water (both buffered, pH 3.5 and aqueous solutions), no evidence for carcinogenicity was observed (EPA84).

2.5 Mutagenicity and genotoxicity

2.5.1 IARC data

Data presented by IARC included a positive response in the *Escherichia coli* pol A assay and weakly positive responses in *Escherichia coli* WP2 *hcr*$^-$ and in a spot test with *Salmonella typhimurium* TA100. No mutagenic effects were obtained in the plate test, with or without adding a metabolic activation system, or in the host-mediated assay (IARC83).

2.5.2 Additional information

Additional information presented in the review by ACGIH, SCG, and USEPA include the lack of effects on lambda prophages and DNA damage in *Escherichia coli*. Furthermore, negative results were reported in L5178Y mouse lymphoma cells, in V79 liver cells, and in W1-38 cells. Chromosome damage was found *in vitro* in human leucocytes (sister chromatid exchanges) and in rats cells (not specified). *N*-methylhydrazine caused DNA single strand breaks in Ehrlich ascites tumour cells but not in rat liver cells. *In vivo*, no dominant lethal mutations were induced in rats and mice given five daily intraperitoneal injections of up to 2.6 and 2.5 mg/kg bw, respectively. No increase in micronuclei was found in dogs exposed by inhalation. Both positive and negative results were obtained regarding DNA damage in the liver using DNA alkaline elution techniques (ACG91, EPA84, Lun92).

N-methylhydrazine was mutagenic for the *Salmonella typhimurium* strain TA102 when tested without metabolic activation at concentrations of between 0.5 and 2.0 µg/plate, and caused DNA lesions in the *Escherichia coli* DNA repair-assay (Pos95).

2.6 Evaluation

No data on humans are available.

There is sufficient evidence for the carcinogenicity of *N*-methylhydrazine in experimental animals. Inhalation of *N*-methylhydrazine induced benign and malignant tumours in mice and hamsters and oral (drinking water) exposure caused benign

tumours in mice and malignant tumours in hamsters in one experiment. No tumours were found in rats and dogs following inhalation, but the exposure time in rats may have been too short, that is 1 year instead of 2 years as recommended in OECD guideline 451.

There is some evidence for mutagenic activity in *in vitro* bacterial systems. No mutations were induced in mammalian cell systems, but chromosome and DNA damage have been found. *In vivo*, *N*-methylhydrazine was negative in a dominant lethal assay in rats and mice and in a micronucleus test in dogs. Conflicting results were obtained with respect to DNA damage in liver *in vivo* assessed with the alkaline elution technique.

2.7 Recommendation for classification

The committee is of the opinion that *N*-methylhydrazine should be considered as carcinogenic to humans. It is classified as a genotoxic carcinogen (classification comparable with EU category 2).

References

ACG91 American Conference of Governmental Industrial Hygienists (ACGIH). Methyl hydrazine. In: Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed., suppl 1996, Cincinnati OH, USA: ACGIH, 1991.

EPA84 US Environmental Protection Agency (EPA). Health and environmental effects profile for methylhydrazine. Cincinnati OH, USA: USEPA, 1984; rep no EPA/600/X-84/142 (available from NTIS,

IARC83 International Agency for Research on Cancer (IARC). Gyromitrin (acetaldehyde formylmethylhydrazone). In: Some food additives, feed additives and naturally occurring substances. Lyon, France: IARC, 1983: 160-70 (IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans; Vol 31).

Lun92 Lundberg P (ed). Consensus report for monomethylhydrazine. Arbete och Hälsa 1992; 1992/47: 50-6.

Pos95 Poso A, von Wright A, Gynther J. An empirical and theoretical study on the mechanisms of mutagenic activity of hydrazine compounds. Mutat Res 1995; 332: 63-71.

Stu96 Studiegroep Chemiekaarten, eds. Methylhydrazine. In: Chemiekaarten: gegevens voor het veilig werken met chemicaliën. 11th ed. Alphen a/d Rijn, The Netherlands: Samson HD Tjeenk Willink bv, 1996: 773.

A Request for advice

B The committee

C Comments on the public review draft

D IARC Monograph

E Classification of substances with respect to carcinogenicity

F Guideline 93/21/EEG of the European Union

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
- P Boogaard
  toxicologist; Shell International Petroleum Company, The Hague
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; Utrecht University, Utrecht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- TM Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of toxicology; Wageningen University, Wageningen.
- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague

- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

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

Secretarial assistance was provided by mrs A van der Klugt.
Lay-out: J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2000 for public review. No organisations and persons have commented on the draft document.

Annex **D**

IARC Monograph

See next page.

IARC Monograph, gyromitrin (1983)

Annex **E**

Classification of substances with respect to carcinogenicity

See next page.

The committee expresses its conclusions in the form of standard phrases:

Judgement of the committee	Comparable with EU class
This compound is known to be carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	1
This compound should be regarded as carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	2
This compound is a suspected human carcinogen.	3
This compound has been extensively investigated. Although there is insufficient evidence of a carcinogenic effect to warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern.	(A)
This compound has been insufficiently investigated. While the available data do not warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is a cause for concern.	(B)
This compound cannot be classified	not classifiable

Annex **F**

Guideline 93/21/EEG of the European Union

4.2 Criteria for classification, indication of danger, choice of risk phrases

4.2.1 Carcinogenic substances

For the purpose of classification and labelling, and having regard to the current state of knowledge, such substances are divided into three categories:

Category 1:

Substances known to be carcinogenic to man.

There is sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer.

Category 2:

Substances which should be regarded as if they are carcinogenic to man.

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of:

- appropriate long-term animal studies
- other relevant information.

Category 3:

Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

However for substances and preparations which present a carcinogenic risk only when inhaled, for example, as dust, vapour or fumes, (other routes of exposure e.g. by swallowing or in contact with skin do not present any carcinogenic risk), the following symbol and specific risk phrase should be used:

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Limited evidence of a carcinogenic effect

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3 actually comprises 2 sub-categories:

a substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

b substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds); if the particular target is not relevant to man;
- lack of genotoxicity in short-term tests *in vivo* and *in vitro*;
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation;
- existence of a species - specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man;
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories;
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.