Tetranitromethane

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 tetranitromethaan
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-451/JR/tvdk/459-N36
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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 tetranitromethaan. 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,

prof. dr JA Knottnerus

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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 2002/09OSH, 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, tetranitromethaan onder de loep. De commissie heeft haar oordeel gegoten in door de Europese Unie aangegeven termen.

De commissie concludeert dat tetranitromethaan beschouwd moet worden als kankerverwekkend voor de mens (vergelijkbaar met EU categorie 2). De genotoxische eigenschappen zijn echter onvoldoende onderzocht. Het is daarom niet bekend of de stof genotoxisch is. De commissie raadt voorzichtigheidshalve aan om tetranitromethaan voorlopig als een genotoxische stof te beschouwen.

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 tetranitromethane.

The committee concludes that tetranitromethane should be considered as carcinogenic to humans (comparable with EU category 2). Its potential genotoxicity has been insufficiently investigated. It, therefore, is unclear whether tetranitromethane is genotoxic. As a way of precaution, the committee considers, for the time being, tetranitromethane as a genotoxic carcinogen.

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 tetranitromethane. 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

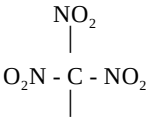
The evaluation of the carcinogenicity of tetranitromethane has been based on an IARC evaluation (IARC96). The conclusion of the IARC on mutagenic or carcinogenic properties of tetranitromethane is included in this report. Where relevant, the original publications were reviewed and evaluated as shown in the text.

In addition, literature has been retrieved from the on-line data bases Cancerlit, Toxline, and Medline, covering the period 1991 to May 2001.

Chapter **2**

Tetranitromethane

2.1 Introduction*

Name	:	tetranitromethane (TNM)
EINECS no	:	208-094-7
CAS-no	:	509-14-9
IUPAC name	:	tetranitromethane
CAS name	:	tetranitromethane
Description	:	pale yellow liquid with a pungent odour
Occurrence	:	not known to occur naturally
Use	:	as an oxidiser in rocket propellants, as an explosive in a mixture with toluene, to increase the cetane number of diesel fuels, as a reagent for detecting the presence of double bonds in organic compounds and for nitration of tyrosine in proteins and peptides
Chem formula	:	CN_4O_8
Chem structure:	:	

* data from ACG91, IARC96, Ver83

Molecular weight	:	196.0
Boiling point	:	126°C
Melting point	:	14°C
Relative density (20 °C/4 °C)	:	1.64
Saturated vapour concentration (20 °C)	:	90 g/m^3 (10,800 ppm)
Vapour pressure (20 °C)	:	1.1 kPa
Relative vapour density (air=1)	:	0.8
Solubility in water (20 °C)	:	insoluble
in organic solvents		soluble in ethanol and diethyl ether
Conversion factors (20°C)	:	1 ppm = 8.17 mg/m^3 1 mg/m^3 = 0.12 ppm
EC classification	:	-

2.2 IARC conclusion

In 1996, IARC concluded that there was inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of tetranitromethane. It was classified as possibly carcinogenic to humans (Group 2B) (IARC96).

2.3 Human data

2.3.1 IARC data

No data were presented.

2.3.2 Additional data

No additional data were found.

2.4 Animal data

2.4.1 IARC data

In rats exposed to 0, 16, 40 mg/m^3 (0, 2 or 5 ppm) for 103 weeks (6 hours a day, 5 days a week), tetranitromethane increased the incidence of alveolar adenomas and carcinomas (male: low concentration: 33/50, high concentration: 46/50, controls: 1/50; female: low concentration: 22/50, high concentration: 50/50, controls: 0/50) and of squamous cell carcinomas of the lung (male: low concentration: 1/50, high

concentration: 19/50, controls: 1/50; female: low concentration: 1/50, high concentration: 12/50, controls: 0/50).

In mice exposed to 0, 4, 16 mg/m^3 (0, 0.5, or 2 ppm) for 103 weeks (6 hours a day, 5 days a week), the incidences of alveolar-bronchiolar adenomas and carcinomas were increased (male: low concentration: 27/50, high concentration: 47/50, controls: 12/50; female: low concentration: 24/50, high concentration: 49/50, controls: 4/49).

Both in rats and mice, the incidences of hyperplasia of the alveolar epithelium and of the bronchioles were significantly increased in all exposure groups. Treatment affected body weight of the rats and mice and survival of the male animals of the high exposure group (IARC96).

2.4.2 Additional data

No additional data were found.

2.5 Mutagenicity and genotoxicity

2.5.1 IARC data

According to the data presented by IARC, tetranitromethane was (weakly) positive when tested with and without metabolic activation in *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535 (reverse mutations), and negative in strain TA1537 (reverse mutations) and LT2 (deletion mutations). In strain TA102, a positive result was obtained when tested with metabolic activation (S9), whereas without a metabolic activation system the test was negative. In Chinese hamster ovary cells, sister chromatid exchanges were induced when tested without or with a metabolic activation system (S9) and chromosome aberrations were induced when tested with a metabolic activation system. Tumours from tetranitromethane-treated rats and mice had a GC → AT transition in the second base of codon 12 of the K-*ras* oncogene (IARC96).

2.5.2 Additional information

Tetranitromethane induced DNA single-strand breaks in the *in vitro* alkaline elution assay using primary rat hepatocytes after a 3 hour lasting treatment with tetranitromethane at concentrations of between 0.03 and 0.10 mM (Sto96).

2.6 Evaluation

No data on the effects of tetranitromethane on humans were available.

There is sufficient evidence for the carcinogenicity of tetranitromethane in experimental animals. Following inhalatory exposure, it increased the incidence of alveolar-bronchiolar adenomas and carcinomas in rats and mice and of squamous-cell carcinomas of the lungs in rats.

Tumours from tetranitromethane-treated rats and mice had a GC → AT transition in the second base of codon 12 of the K-*ras* oncogene.

Tetranitromethane is a bacterial mutagen and induces chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells, and single-strand breaks in rat hepatocytes *in vitro*. No genotoxicity tests in mammals *in vivo* were available.

2.7 Recommendation for classification

The committee concludes that tetranitromethane should be regarded as carcinogenic to humans (classification comparable with EU class 2). Its potential genotoxicity has been insufficiently investigated. It, therefore, is unclear whether it is genotoxic.

Additional consideration

The committee considers tetranitromethane, as a way of precaution, as a genotoxic carcinogen as long as the available data do not allow an evaluation of the potential genotoxicity and the assessment of the mode of action underlying the carcinogenicity.

References

ACG91 American Conference of Governmental Industrial Hygienists (ACGIH). Tetranitromethane. In: Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Cincinnati OH, USA: ACGIH, 1991: 1526-8.

IARC96 International Agency for Research on Cancer (IARC). Tetranitromethane. In: Printing processes and printing inks, carbon black and some nitro compounds. Lyon, France: IARC, 1996: 437-48 (IARC monographs on the evaluation of carcinogenic risks to humans; Vol 65).

Sto96 Storer RD, McKelvey TW, Kraynak AR, *et al.* Revalidation of the *in vitro* alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for the assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat Res 1996; 368: 59-101.

Ver83 Verschueren K. Tetranitromethane. In: Handbook of environmental data and organic chemicals. 2nd ed. New York, USA: Van Nostrand Reinhold Company, 1983: 1093.

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.

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.