4-Vinylcyclohexene

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *4-Vinylcyclohexene*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-5139/JR/pg/246-L12
Bijlagen : 1
Datum : 1 april 2008

Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van 4-vinylcyclohexene. Het maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

Het advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Het advies is voorgelegd aan de Commissie GBBS en vervolgens getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

prof. dr. J.A. Knottnerus

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4-Vinylcyclohexene

Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the classification of carcinogenic substances of the
Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2008/04OSH, The Hague, April 1, 2008

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 22, 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.

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INAHTA

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Preferred citation:
Health Council of the Netherlands. 4-Vinylcyclohexene; Evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2008; publication no. 2008/04OSH.

ISBN: 978-90-5549-689-1

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens het uitoefenen van hun beroep kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie 4-vinylcyclohexeen onder de loep. De stof wordt voor diverse industriële doeleinden gebruikt.

Op basis van de beschikbare gegevens leidt de commissie af dat 4-vinylcyclohexeen *beschouwd moet worden als kankerverwekkend voor de mens*. Dit is vergelijkbaar met een classificatie in categorie 2 volgens de richtlijnen van de Europese Unie. De commissie is verder van mening dat de stof als genotoxisch dient te worden beschouwd met een stochastisch werkingsmechanisme.

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Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. In this report, the committee evaluated 4-vinylcyclohexene. The agent is used for various industrial purposes.

Based on the available information, the committee is of the opinion that 4-vinylcyclohexene *should be considered as carcinogenic to humans*. This recommendation is comparable to the EU classification in category 2. The committee is furthermore of the opinion that 4-vinylcyclohexene should be considered a genotoxic agent that acts by a stochastic mechanism.

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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. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification (see Annex A). The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex E). The criteria used for classification are partly based on an EU-directive (see Annex F). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

This report contains the evaluation of the carcinogenicity of 4-vinylcyclohexene.

1.2 Committee and procedures

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. The members of the committee are listed in Annex B. The first draft was prepared by I.A. van de Gevel and M.I. Willems, from the Department of Occupational Toxicology of the TNO Nutrition

and Food Research, by contract with the Ministry of Social Affairs and Employment.

In 2007 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 and recommendation of the committee is standardly based on scientific data, which are publicly available. The starting points of the committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of 4-vinylcyclohexene, such an IARC-monograph is available, of which the summary and conclusion of IARC is inserted in Annex D.

More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in June 2007. The new relevant data were included in this report.

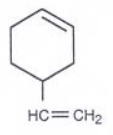
Chapter 2

General information

2.1 Identity and physico-chemical properties

4-Vinylcyclohexene is formed from the dimerization of 1,3-butadiene, a reaction that occurs in the manufacture of synthetic rubber.[1] The agent has been used for various purposes, such as: an intermediate for producing flame retardants, flavours and fragrances; in the manufacture of polyolefins; a solvent; and, in the manufacture of special chemicals such as the diepoxide.[2]

Below is given the identity and some of its physical and chemical properties.[2]

Chemical name	:	4-vinylcyclohexene
CAS registry number	:	100-40-3
EINECS number	:	202-848-9
IUPAC name	:	4-vinylcyclohexene
Synonyms	:	1-vinyl-3-cyclohexene; 4-vinyl-1-cyclohexene; 4-ethenylcyclohexene
Description	:	colourless liquid
Molecular formula	:	C_8H_{12}
Structure	:	

Molecular weight	:	108.18
Melting point	:	-109 °C
Boiling point	:	128.9 °C
Relative vapour density	:	3.76
Vapour pressure	:	2 kPa at 25 °C
Log P_{ow}	:	3.93
Solubility	:	soluble diethyl ether, benzene and petroleum ether; slightly soluble in water (50 mg/L)
Stability	:	flash-point, 21.2 °C (open cup); temperatures above 26.6 °C and prolonged exposure to oxygen lead to discolouration and gum formation
Conversion factors (20 °C)	:	1 ppm = 4.42 mg/m³ 1 mg/m³ = 0.23 ppm

2.2 IARC classification

In 1994, IARC summarized that there is inadequate evidence in humans for the carcinogenicity of 4-vinylcyclohexene, but that there is sufficient evidence in experimental animals.[2] Therefore, IARC concluded that the agent is possibly carcinogenic to humans (Group 2B).

Chapter 3

Carcinogenicity

3.1 Observations in humans

No data were available on the carcinogenicity of 4-vinylcyclohexene in humans.

3.2 Carcinogenicity studies in animals

The National Toxicology Program performed a long-term animal study to assess toxicity and carcinogenicity of 4-vinylcyclohexene.[3] In summary, groups of 50 F344 rats and B6C3F_1 mice of each sex were administered the agent by gavage at a dose of 0, 200, or 400 mg/kg bw, five days per week for 103 weeks.

In treated rats, the survival was statistically significantly lower compared to the vehicle controls (see Table 3.1). The animals developed various types of tumours at different sites of the body, of which a summary of the most relevant ones are summarized in Table 3.1. In males, exposure to 4-vinylcyclohexene resulted in increased number of animals with skin and preputial gland tumours in the highest dose group. In females, the number of animals with anterior pituitary gland and clitoral gland tumours was significantly increased in the low-dose group, but not in the high-dose group, compared to controls.

Table 3.2 shows the results of the study in mice. In the high-dose group the survival was significantly lowered compared to vehicle controls. Also in mice various types of tumours were found in various parts of the body. More specifically, in males the number of animals with lung tumours and lymphomas of the

Table 3.1 Survival and tumour incidences of F344 rats receiving 4-vinylcyclohexene by gavage.[3]

	Overall rates			Adjusted rates		
Dose (mg/kg bw)	0	200	400	0	200	400
Survival (percentage)						
males	66	26*	10*			
females	80	56*	26*			
tumour incidences (percentages)						
males						
Skin: squamous cell papilloma or carcinoma	0	2	8*	0	3.6	37.5*§
Preputial gland: adenoma or carcinoma	2	2	6	2.4	5.3	20.9§
females						
Anterior pituitary gland: adenoma or carcinoma	38	50*	16	44.9	66.0*	39.9
Clitoral gland: adenoma or squamous cell carcinoma	2	10*	0	2.5	17.9*	0

Adjusted rates are tumour incidence rates adjusted for intercurrent mortality.
* $p<0.05$ versus vehicle control; § significant positive trend.

Table 3.2 Survival and tumour incidences of B6C3F$_1$ mice receiving 4-vinylcyclohexene by gavage.[3]

	Overall rates			Adjusted rates		
Dose (mg/kg bw)	0	200	400	0	200	400
Survival (percentage)						
males	74	78	14*			
females	80	78	34*			
tumour incidences (percentages)						
males						
lung: alveolar/bronchiolar adenoma or carcinoma	8	22	8	10.4	26.5	44.7*§
hematopoietic system: malignant lymphomas	8	14	10	10.5	17.7	62.5*§
liver: hepatocellular adenoma or carcinoma	37	40	12	43.5	43.2	54.5
females						
lung: alveolar/bronchiolar adenoma or carcinoma	12	2	8	14.1	2.6	19.3
hematopoietic system: malignant lymphomas	32	30	23	34.3	33.0	48.3
liver: hepatocellular adenoma or carcinoma	2	6	6	2.5	7.7	15.8
pituitary gland: adenoma or carcinoma	18	16	7	21.4	19.4	20.0
adrenal gland capsule or cortex: adenoma	0	6	8*	0	7.7	18.3*§
ovary: granulosa cell tumour or carcinoma	2	21*	28*	2.6	25.5*	54.9*§

Adjusted rates are tumour incidence rates adjusted for intercurrent mortality.
* $p<0.05$ versus vehicle control; § significant positive trend.

hematopoietic system was significantly increased of the high-dose group compared to controls, whereas in females in the high-dose group significant increases were found of animals with tumours in the adrenal gland and ovary. In addition, in female mice of the low-dose group the incidence in ovary tumours was also significantly increased.

Although the high mortality among male and female rats (low and high dose groups) and male mice (high dose group only) confounded the interpretation of the results, making evidence inconclusive for these groups, the committee considers the data strongly suggestive for carcinogenicity of 4-vinylcyclohexene.

The Working Group of IARC also reported on two studies in mice, which received 4-vinylcyclohexene by skin applications for life.[2] However, the Working Group noted that the agent was applied in benzene-solution, which has carcinogenic potential by itself. Therefore, and although no treatment related skin tumours were observed, IARC concluded that these studies were inadequate for evaluation. The committee agrees with this conclusion.

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Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

4-Vinlycyclohexene did not induce reverse mutations in the conventional Ames test using *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA 98, in the presence and absence of different exogenous metabolic systems.[2-4]

4.2 *In vivo* assays

Bentley *et al*. (1995; abstract only) did not find treatment-related increases in numbers of micronuclei in polychromatic erythrocytes of B6C3F_1 mice and Crl:CD®BR rats.[5] The animals inhaled 4-vinylcyclohexene for two days (concentration range, 0-1,000 ppm (mice), 0-2,000 ppm (rats); 6 hours per day), or for 13 weeks (concentrations range, 0-1,000 ppm (mice), 0-1,500 ppm (rats); 6 hours per day, five days per week).

4.3 Additional information and carcinogenic mechanism

Due to the limited set of data, the mechanism through which 4-vinylcyclohexene may exert its carcinogenic and genotoxic potential is not completely clarified yet, but some ideas are put forward. For instance, its diepoxide metabolite is genotoxic and acts by a stochastic mechanism.[6] It is, therefore, plausible that 4-vinyl-

cyclohexene has genotoxic potential as well. Furthermore, some ideas are given that might explain induction of ovary tumours in mice.

Firstly, in animal studies it has been repeatedly shown that exposure to 4-vinylcyclohexene destroys primary ovarian oocytes in mice, but not in rats.[1,4,7] The findings of the ovary tumours are in line with this observation, in that these are found in mice, but not in rats. Regarding ovotoxicity, the different outcomes between the species are explained by differences in metabolism. In the first place, mice are more efficient in bioactivating 4-vinylcyclohexene by certain mixed function oxidase isoenzymes into epoxide metabolites, such as 4-vinylcyclohexene diepoxide, 1,2-vinylcyclohexene epoxide, and 7,8-vinylcyclohexene epoxide, than rats.[1,8,9] Regarding diepoxide metabolites, based on structure-activity studies it is even suggested that the diepoxide metabolites are the ultimate ovotoxic compound, being more reactive than 4-vinylcyclohexene itself.[1,8,10-12] In the second place, it has been shown that mice had reduced capacity to convert a diepoxide metabolite to its inactive tetrol derivate, as compared to rats.[11] As a result of the better bioactivation of 4-vinylcyclohexene and the reduced capacity to detoxify diepoxide metabolites, female mice easier exhibit ovarian toxicity and carcinogenicity than female rats.

Secondly, although it is unknown how ovary tumours develop, it has been proposed to start with destruction of primary oocytes. Whatever the cause is of the destruction (direct effect by cytotoxicity or apoptosis, or indirect effect by affecting surrounding granulosa cells) depletion of ovarian follicles may persistently elevate levels of circulating gonadotropines due to a loss of negative feedback on the hypothalamic-pituitary axis that is usually confirmed by ovarian hormones.[1] It has been proposed that high levels of gonadotropines act as a promotor of ovarian tumour development. However, evidence to support this view is conflicting, and other yet unknown mechanisms may play a role.[1]

Regarding oocyte destruction, consistent data are presented suggesting that its diepoxide metabolite, 4-vinylcyclohexene diepoxide, induces or accelerates the overall rate of programmed cell death rather than cytotoxicity or necrosis.[1,13] Whether 4-vinylcyclohexene itself can induce apoptosis is not known yet.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity of 4-vinylcyclohexene in humans were available, nor were there any carcinogenicity data available on inhalation exposure in animals.

In rats and mice given 4-vinylcyclohexene by gavage for 2 years several treatment-related tumours were observed in various parts of the body, including: skin and preputial gland tumours (male rats); anterior pituitary gland and clitoral gland tumours (female rats); lung tumours and lymphomas of the hematopoietic system (male rats); and, tumours in the adrenal gland and ovary (female mice). Despite the high mortality among the animals (except in female mice), the committee considers the findings on the whole sufficient to consider 4-vinylcyclohexene as a carcinogenic agent.

4-Vinylcyclohexene did not show mutagenic activity in conventional mutagenicity tests, nor did it show clastogenic activity *in vivo*. Overall, the number of data available on genotoxicity was limited. However, since its diepoxide metabolite (4-vinylcyclohexene diepoxide) is a genotoxic compound that acts by a stochastic mechanism, it is plausible that 4-vinylcyclohexene is genotoxic as well.

The committee did not find indications that the observations in animals, and the proposed carcinogenic mechanism would not occur in humans.

5.2 Recommendation for classification

Based on the available information, the committee is of the opinion that 4-vinylcyclohexene should be considered as carcinogenic to humans. This recommendation is comparable to the EU classification in category 2. The committee is furthermore of the opinion that 4-vinylcyclohexene should be considered a genotoxic agent that acts by a stochastic mechanism.

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A	Request for advice
B	The committee
C	Comments on the public review draft
D	IARC Monograph
E	Carcinogenic classification of substances by the committee
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.

Annex **B**

The committee

- G.J. Mulder, *chairman*
  emeritus professor of toxicology, Leiden University, Leiden
- P.J. Boogaard
  toxicologist, SHELL International BV, The Hague
- Ms. M.J.M. Nivard
  molecular biologist and genetic toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  epidemiologist, Dow Chemicals NV, Terneuzen
- R.A. Woutersen
  toxicologic pathologist, TNO Quality of Life, Zeist
- A.A. van Zeeland
  professor of molecular radiation dosimetry and radiation mutagenesis, University Medical Center, Leiden
- E.J.J. van Zoelen
  professor of cell biology, Radboud University Nijmegen, Nijmegen
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The committee consulted an additional expert, Prof. dr. G. Mohn, working at Department of Radiation Genetics and Chemical Mutagenesis of the University of Leiden, with respect to the genotoxic data.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2007 for public review. The following organisations and persons have commented on the draft document:

- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain;
- R.D. Zumwalde, National Institute for Occupational Safety and Health, the USA.

Annex **D**

IARC Monograph

Vol.: 60 (1994) (p. 347)[2]
CAS No.: 100-40-3
Chem. Abstr. Name: 4-Ethenylcyclohexene

Summary of Data Reported and Evaluation

Exposure data

4-Vinylcyclohexene is produced by catalytic dimerization of 1,3-butadiene. 4-Vinylcyclohexene has been used as a chemical intermediate for production of flame retardants, flavours and fragrances, in the manufacture of polyolefins, as a solvent and in the manufacture of its diepoxide. Low levels of occupational exposure have been measured during the production and use of 1,3-butadiene.

Human carcinogenicity data

No data were available to the Working Group.

Animal carcinogenicity data

4-Vinylcyclohexene was tested for carcinogenicity in one experiment in mice and in one experiment in rats by gastric intubation and in two skin application

studies in mice. Administration of 4-vinylcyclohexene by gastric intubation produced granulosa-cell and mixed tumours of the ovary and adrenal subcapsular tumours in female mice. In male mice, there was an increase in the incidence of lymphoma and of lung tumours. Following gastric intubation in rats, increased incidences of squamous-cell tumours of the skin in males and of clitoral gland tumours in females were observed. The studies by skin application were inadequate for evaluation.

Other relevant data

4-Vinylcyclohexene is distributed mainly to adipose tissue in rodents. The ethylene carbons are eliminated mainly in urine and expired air. Metabolism primarily involves oxidation to 4-vinylcyclohexane-1,2-epoxide, which is formed 13 times faster by liver microsomes from mice and twice as fast by those from rats than by human microsomes. 4-Vinyl-1,2-epoxycyclohexane, 4-epoxyethylcyclohexene and, particularly, the diepoxide are more toxic to mouse oocytes than 4-vinylcyclohexene itself. Treatment with 4-vinylcyclo-hexene decreased the number of oocytes in mice but not in rats. The difference seemed to be due to the reduced ability of the rat to metabolize 4-vinylcyclohexene to epoxides.

No data were available on the genetic and related effects of 4-vinylcyclohexene in humans.

4-Vinylcyclohexene and its mono-epoxide metabolites were not mutagenic to *Salmonella typhimurium*. 4-Vinyl-1,2-epoxycyclohexane induced micronuclei but not hprt mutations in cultured Chinese hamster cells.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 4-vinylcyclohexene.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-vinylcyclohexene.

Overall evaluation

4-Vinylcyclohexene is *possibly carcinogenic to humans (Group 2B)*.

Previous evaluation: Suppl. 7 (1987) (p. 73)

Annex **E**

Carcinogenic classification of substances by the committee

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

Judgment of the committee	*Comparable with EU class*
This compound is known to be carcinogenic to humans • It is stochastic or non-stochastic 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 stochastic or non-stochastic 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. • This compound has been extensively investigated. Although there is insufficient evidence for 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. • 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.	3 (A) (B)
This compound cannot be classified • There is a lack of carcinogenicity and genotoxicity data. • Its carcinogenicity is extensively investigated. The data indicate sufficient evidence suggesting lack of carcinogenicity.	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 Possible risk of irreversible effects

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.