4-Vinylcyclohexene diepoxide

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 diepoxide*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-5140/JR/pg/246-M12
Bijlagen : 1
Datum : 1 april 2008

Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van 4-vinylcyclohexene diepoxide. 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 diepoxide

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. 2008/03OSH, 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.

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.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

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INAHTA

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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 diepoxide onder de loep. De stof wordt gebruikt als verdunner voor andere diepoxiden en voor epoxyharsen.

Op basis van de beschikbare gegevens leidt de commissie af dat 4-vinylcyclohexene diepoxide *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 een stochastisch genotoxisch werkingsmechanisme heeft.

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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 diepoxide. The agent is used as a diluent for other diepoxides and for epoxy resins

Based on the available information, the committee is of the opinion that 4-vinylcyclohexene diepoxide *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 diepoxide acts by a stochastic genotoxic 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 diepoxide.

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 diepoxide, 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.

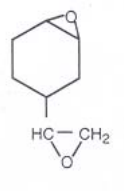
Chapter 2

General information

2.1 Identity and physico-chemical properties

4-Vinylcyclohexene diepoxide is used as a diluent for other diepoxides and for epoxy resins derived from bisphenol A and epichlorohydrin.[1-3]

Below is given the identity and some of its physical and chemical properties.[1-3]

Chemical name	:	4-vinylcyclohexene diepoxide
CAS registry number	:	106-87-6
EINECS number	:	203-437-7
IUPAC name	:	3-(Epoxyethyl)-7-oxabicyclo[4.1.0]heptane
Synonyms	:	1,2-Epoxy-4-(epoxyethyl)cyclohexane; 1-(epoxyethyl)-3,4-epoxycyclohexane; 3-(1,2-epoxyethyl)-7-oxabicyclo[4.1.0]heptane; vinylcyclohexene diepoxide; 4-vinyl-1-cyclohexene diepoxide; 4-vinyl-1,2-cyclohexene diepoxide; 4-vinylcyclohexene dioxide; 1-vinyl-3-cyclohexene dioxide; 4-vinyl-1-cyclohexene dioxide
Description	:	Clear, colourless or pale yellow liquid
Molecular formula	:	$C_8H_{12}O_2$
Structure	:	

Molecular weight	:	140.18
Melting point	:	-55 °C
Boiling point	:	227 °C
Relative vapour density	:	1.0986 at 20 °C / 4 °C
Vapour pressure	:	13 Pa at 20 °C
Log P_{ow}	:	3.38
Solubility	:	Very soluble in water
Stability	:	Flash-point, 110 °C in open cup
Conversion factors (20 °C)	:	1 ppm = 5.82 mg/m^3 air 1 mg/m^3 = 0.17 ppm
EU Risk phrases	:	R23/24/25: toxic by inhalation, in contact with skin and if swallowed R68: possible risk for irreversible effects T: toxic

2.2 IARC classification

In 1994, IARC summarized that there is inadequate evidence in humans for the carcinogenicity of 4-vinylcyclohexene diepoxide, 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 diepoxide in humans.

3.2 Carcinogenicity studies in animals

The National Toxicology Program performed a carcinogenicity study in rats and mice.[3,4] Groups of 60 male and 60 female F344/N rats and B6C3F$_1$ mice received the agent by topical application at doses of 0 (vehicle), 15, or 30 mg/animal (rats), and 0 (vehicle), 2.5, 5, or 10 mg/animal (mice), five days per week for 105 weeks. At month 15, ten animals from each group were sacrificed for interim histopathological examination.

The survival in rats at the end of the study was very low for all groups, including the vehicle controls. No significant differences in survival were observed among any groups of males. However, survival in the high-dose female group was significantly lower than in vehicle female group after day 648; the low-dose female group had significantly lower survival between days 637 and 715.

Regarding tumour development, increased incidences of skin tumours at the site of application were observed in male and female rats in all dose groups. Details are shown in Table 3.1. No other treatment related tumours were

Table 3.1 Survival and skin tumour incidences of F344 rats receiving 4-vinylcyclohexene diepoxide by topical application for two years.[3,4]

	Overall rates			Terminal rates[a]		
Dose (mg/rat)	0	15	30	0	15	30
Survival						
males	7/50	8/50	4/50			
females	27/50	23/50	15/50			
Tumour incidences						
males						
skin: basal cell adenoma or carcinoma	0/50	1/50	6/50	0/7	0/8	1/4
skin: squamous cell papilloma	0/50	3/50	6/50*	0/7	1/8	0/4
skin: squamous cell carcinoma	0/50	33/50**	36/50**	0/7	8/8	4/4
females						
skin: basal cell carcinoma	0/50	3/50	4/50*	0/27	2/23	2/15
skin: squamous cell carcinoma	0/50	16/50**	34/50**	0/27	14/23	15/15

[a] Terminal rates are tumour incidence rates in animals, which were still alive at 105 weeks.
* $p<0.05$ versus vehicle control; ** $p<0.01$ versus vehicle control.

observed. At the site of application, the treated animals had also significantly increased nonneoplastic skin lesions, such as acanthosis and sebaceous gland hypertrophy.

In male and female mice, survival of the mid- and the high-dose groups was significantly lowered compared to vehicle controls. All females in the high-dose group were killed at week 85.

As in rats, 4-vinylcyclohexene diepoxide induced squamous cell carcinomas at the site of application in male and female mice, as shown in Table 3.2. No other treatment related skin tumours were observed. However, nonneoplastic skin lesions, such as acanthosis, hyperkeratosis and necrotizing inflammation (mid and high dose groups), were found to be significantly increased in both male and female mice at all dose groups. Furthermore, in treated female mice a significant increase of number of animals with ovarian tumours were observed compared to vehicle controls (see table 3.2). Also in female mice, an increased incidence of lung tumours in the mid-dose group was found, but not in the high-dose group. No other treatment related tumours were found in any of the exposed groups.

Table 3.2 Survival and tumour incidences of B6C3F$_1$ mice receiving 4-vinylcyclohexene diepoxide by topical application for two years.[3,4]

	Overall rates				Terminal rates[a]			
Dose (mg/mouse)	0	2.5	5	10	0	2.5	5	10
Survival								
males	38/50	35/50	4/50	0/50				
females	30/50	31/50	15/50	12/50§				
Tumour incidences								
males								
skin: squamous cell carcinoma	0/50	15/50**	39/50**	42/50**	0/38	10/35	4/4	0/0
females								
skin: squamous cell carcinoma	0/50	6/50*	37/50**	41/50**	0/30	3/31	15/15	0/0
ovary: luteoma, granulosa cell tumour, benign mixed tumour, or malignant cell tumour	1/50	0/49	17/49*	18/50*	1/30	0/31	7/14	0/0
lung: alveolar/bronchiolar adenoma or carcinoma	4/50	9/50	11/50*	7/50	3/30	7/31	4/15	0/0

[a] Terminal rates are tumour incidence rates in animals, which were still alive at 105 weeks.
§ Number of animals alive at week 85. * $p<0.05$ versus vehicle control; ** $p<0.01$ versus vehicle control.

Tennant *et al*. (1996) evaluated the carcinogenicity of 4-vinylcyclohexene diepoxide in a transgenic mouse bioassay.[5] In that assay, male and female heterozygous p53-deficient C57BL/6 mice were used. These knock-out mice are susceptible for tumour development, due to a loss of expression of the p53 tumour suppressor gene. The agent was topically applied at doses of 0, 12.5, or 25 mg/animal, five times per week for 24 weeks. The same type of squamous cell tumours were observed in the treated transgenic mice as in normal mice of the two-year study of the National Toxicology Program[3], whereas in non-treated transgenic animal no such tumours were observed (tumour incidence: nontreated, 0%; 12.5 mg/mouse, 20-28%; 25 mg/mouse, 30-40%). Further details are not given.

Yamamoto *et al.* (1998) reported of a transgenic mouse bioassay for rapid carcinogenicity testing.[6] This time *ras*H2 (CB6F$_1$) mice were used carrying the human prototype c-Ha-*ras* gene. In various human and animal tumours *ras* genes are activated by point mutations. Therefore, this transgenic mouse line should be vulnerable to developing tumours. To the dorsal skin of the transgenic and non-transgenic mice the agent was applied at doses of 5 or 10 mg/kg bw, five times per week for 24 weeks. 4-Vinylcyclohexene diepoxide induced skin papillomas around the site of application 26 weeks after the first administration of the agent; in the high-dose groups, the incidence of skin papillomas was significantly

higher in female transgenic mice than in the vehicle treated transgenic mice. In both male and female transgenic mice of the high-dose group, the incidence of skin papillomas was significantly higher than in the corresponding non-transgenic mice. Furthermore, non-significant increases in incidences of forestomach papilloma, thymic lymphoma, and lung adenoma were observed in transgenic mice, but not in the corresponding non-transgenic groups. No skin squamous cell carcinomas, or spleen hemangiosarcomes were found in non-transgenic groups, whereas in transgenic groups these were observed. Further details on study design and results are not given.

The Working Group of IARC also reported of three skin application carcinogenicity studies, which had serious shortcomings in study design and reporting of the results.[2] For this reason the committee did not evaluate them.

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

4-Vinylcyclohexene diepoxide was mutagenic in the conventional mutagenicity assay using various strains of *Salmonella typhimurium*, in the presence and absence of an exogenous metabolic system.[2] It, furthermore, induced mutations: in *Saccharomyces cerevisiae* (gene conversion, mitotic cross-over, and reverse mutations); at the *hprt* locus of Chinese hamster V79 cells; and, at the *tk* locus of mouse lymphoma L5178 cells, all in the absence of an exogenous metabolic system.[2]

4-Epoxyethylcyclohexene-1,2-diol, a metabolite of 4-vinylcyclohexene diepoxide, did not induce reverse mutations in *Salmonella typhimurium* TA100, nor did it cause gene mutations at the *hprt* locus of Chinese hamster V79 cells.[2]

The agent also caused clastogenic effects in Chinese hamster ovary cells (sister chromatid exchanges, and chromosomal aberrations).[2] Furthermore, it did increase the frequency of micronuclei in plant systems (*Allium cepa, Vicia faba*), but not in Chinese hamster V79 lung cells.[2] Tests on micronucleus formation were performed without an exogenous metabolic system. Finally, the metabolite 4-epoxyethylcyclohexene-1,2-diol increased the frequency of micronuclei in Chinese hamster V79 lung cells.[2]

4.2 *In vivo* assays

Mabon *et al*. (1996) and Randerath *et al*. (1996), both of the same research group, showed that 4-vinylcyclohexene diepoxide is able to produce DNA adducts *in vivo* (female ICR mice; topical application; 17-225 µmol/ mouse; once a day for three days).[7,8] The adduct levels were, however, far below the levels generally found for highly potent carcinogens at comparable doses, such as benzo[a]pyrene. No human or other animal data were available on the genotoxicity of 4-vinylcyclohexene diepoxide using *in vivo* bioassays.

4.3 Additional information and carcinogenic mechanism

4-Vinylcyclohexene diepoxide is a potent electrophilic agent with alkylating properties.[7,8] Regarding this property, Mabon *et al*. (1996) and Randerath *et al*. (1996) not only showed induction of DNA-adducts in mice, but also in isolated calf thymus DNA. Although the adduct levels were far below the levels generally found for highly potent carcinogens at comparable doses, such as benzo[a]pyrene, these results provide further evidence for a direct-acting carcinogenic mechanism.

Furthermore, 4-vinylcyclohexene diepoxide is a metabolite of 4-vinylcyclohexene, which exhibits selective toxicity in primordial and primary ovarian follicles and ovarian carcinogenicity in mice, but not in rats.[9] Various animal studies showed that the diepoxide is also able to induce ovarian toxicity. Based on structure-activity studies it is even suggested that the diepoxide metabolite is the ultimate ovotoxic compound, being more reactive than 4-vinylcyclohexene itself.[10-12]

Another subject is the difference in susceptibility of 4-vinylcyclohexene diepoxide of ovarian carcinogenicity between mice and rats. Hoyer and Sipes (1996) reported that it has been shown that mice had reduced capacity to convert the diepoxide to its inactive tetrol derivate, as compared to rats.[13] Thus, mice appear to be deficient in detoxifying the diepoxide, resulting in a greater susceptibility in ovarian toxicity than rats.

The carcinogenic mechanism through which 4-vinylcyclohexene diepoxide exerts its effect on ovarian follicles is not completely understood, although studies point to disruption in hormonal feedback due to destruction of primary oocytes.[13] This would suggest a role as a tumour promotor. At least, the types of morphological lesions observed in destroyed follicles are consistent with (accelerated) programmed cell death (apoptosis) rather than cytotoxicity or necrosis.[12-14]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity of 4-vinylcyclohexene diepoxide in humans were available, nor were there any carcinogenicity data available on inhalation exposure in animals. However, after chronic dermal application the agent did induce benign and malignant skin tumours in male and female rats and mice. It furthermore induced ovarian and lung tumours in female mice. Based on these findings, the committee is of the opinion that there is sufficient evidence for carcinogenicity in animals.

4-Vinylcyclohexene diepoxide is in fact an electrophilic compound that was shown to be mutagenic *in vitro* in various test systems, and clastogenic in mammalian cells. It, furthermore, increased the level of DNA adducts *in vitro* and *in vivo*. Based on these data, the committee considers the diepoxide as a genotoxic compound that acts by a stochastic mechanism. Regarding ovarian carcinogenicity, it should be taken into account that 4-vinylcyclohexene diepoxide might exert its effect also by acting as a promotor.

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

The committee concludes that 4-vinylcyclohexene diepoxide 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 diepoxide should be considered as 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. 361)[2]
CAS No.: 106-87-6
Chem. Abstr. Name: 3-Oxiranyl-7-oxabicyclo[4.1.0]heptane

Summary of Data Reported and Evaluation

Exposure data

4-Vinylcyclohexene diepoxide is produced by epoxidation of 4-vinylcyclohexene with peroxyacetic acid. It is used as a reactive diluent for other diepoxides and for epoxy resins. No data are available on levels of occupational exposure to 4-vinylcyclohexene diepoxide.

Human carcinogenicity data

No data were available to the Working Group.

Animal carcinogenicity data

4-Vinylcyclohexene diepoxide was tested for carcinogenicity by skin application in three studies in mice and in one study in rats. Skin application of 4-vinylcyclohexene diepoxide produced benign and malignant skin tumours in all studies in

mice and in the study in rats. In one study in mice, it also increased the incidences of ovarian and lung tumours in females.

Other relevant data

4-Vinylcyclohexene diepoxide can be absorbed through the skin of rodents. Higher concentrations tend to be found in the ovary rather than in other organs, and virtually all elimination occurs via the urine. Its metabolism involves hydration to a mixture of glycols and conjugation with glutathione. 4-Vinylcyclohexene diepoxide is locally toxic and, when given orally, causes ovarian degeneration in both mice and rats and testicular degeneration in mice, as well as lesser effects in other organs. No data were available on the genetic and related effects of 4-vinylcyclohexene diepoxide in humans. 4-Vinylcyclohexene diepoxide induced gene mutation, sister chromatid exchange and chromosomal aberrations but not micronuclei in mammalian cells *in vitro*. It was mutagenic in bacteria and caused gene conversion and mitotic crossing-over in *Saccharomyces cerevisiae*. A metabolite of 4-vinylcyclohexene diepoxide, 4-epoxyethylcyclohexane-1,2-diol, was not mutagenic to *Salmonella typhimurium*.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 4-vinylcylcohexene diepoxide. There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-vinyl-cyclohexene diepoxide.

Overall evaluation

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

Previous evaluation: Suppl. 7 (1987) (p. 63)

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.