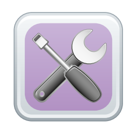
Health Council of the Netherlands

4-Vinylcyclohexene diepoxide

Evaluation of the carcinogenicity and genotoxicity

2016/02



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4-Vinylcyclohexene diepoxide

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *4-Vinylcyclohexene diepoxide*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-915764/DC/fs/246-D24
Bijlagen : 1
Datum : 29 februari 2016

Geachte minister,

Graag bied ik u hierbij het advies *4-Vinylcyclohexene diepoxide* aan.

Dit advies is een herevaluatie van een eerder door de Gezondheidsraad uitgebracht advies voor classificatie als kankerverwekkende stof. De raad is gevraagd om deze herevaluatie omdat de voorgestelde classificatie uit het eerdere advies afwijkt van de classificatie die op dit moment in de Europese Unie wordt gehanteerd. Tevens is de raad gevraagd de stof te classificeren voor mutageniteit. De classificaties in het voorliggende advies zijn gebaseerd op het Europese classificatiesysteem.

Dit advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. De subcommissie heeft daarbij gebruik gemaakt van commentaren die zijn ontvangen op het openbare concept van dit advies. Het advies is getoetst door de Beraadsgroep Volksgezondheid van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. J.L. Severens,
vicevoorzitter

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
E-mail: d.coenen@gr.nl
Telefoon (070) 340 74 73

Postadres
Postbus 16052
2500 BB Den Haag
www.gr.nl

4-Vinylcyclohexene diepoxide

Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the Classification of Carcinogenic Substances of the
Dutch Expert Committee on Occupational Safety
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2016/02, The Hague, February 29, 2016

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 and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare and Sport, Infrastructure and the Environment, Social Affairs and Employment, and Economic Affairs. 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.

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

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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 de beroepsmatige uitoefening 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. Verder heeft het ministerie aan de Gezondheidsraad gevraagd om een aantal stoffen te herevalueren en daarbij ook een voorstel voor classificatie voor mutageniteit in geslachtscellen te doen. In het voorliggende advies herevalueert de commissie 4-vinylcyclohexeen diepoxide. 4-Vinylcyclohexeen diepoxide wordt gebruikt als verdunner voor andere diepoxiden en voor epoxyharsen.

De commissie concludeert dat 4-vinylcyclohexeen diepoxide beschouwd moet worden als kankerverwekkend voor de mens, en beveelt aan de stof te classificeren in categorie 1B*. Op basis van de beschikbare gegevens beveelt de commissie geen classificatie aan voor mutageniteit voor geslachtscellen. De stof kan kanker verwekken via een stochastisch genotoxisch werkingsmechanisme.

* Zie Annex F (carcinogeniteit) en G (mutageniteit) voor classificatiesysteem.

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 Safety of the Health Council, hereafter called the committee. In addition, the ministry asked the Health Council to re-evaluate a series of substances, and to include in the re-evaluation a proposal for classification on germ cell mutagenicity In this report, the committee re-evaluated 4-vinylcyclohexene diepoxide. 4-Vinylcyclohexene diepoxide is used as a diluent for other diepoxides and for epoxy resins.

The committee concludes that 4-vinylcyclohexene diepoxide is presumed to be carcinogenic to man, and recommends classifying this substance in category 1B*. Based on the available data, the committee does not recommend a classification as a germ cell mutagen. The substance acts by a stochastic genotoxic mechanism.

* See Annex F (carcinogenicity) and G (mutagenicity) for the classification system.

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 F). In addition to classifying substances on carcinogenicity, the Health Council also assesses the genotoxic properties of the substance in question.

Recently, with reference to the EU Regulation 1272/2008 on classification, labelling and packaging of substances (see Annex G), the ministry of Social Affairs and Employment asked the Health Council to update the evaluations and classification on carcinogenicity of a series of substances, and to propose for these substances a classification on germ cell mutagenicity as well.

In this report, such an update was performed for 4-vinylcyclohexene diepoxide. An earlier evaluation of this substance was published in 2008.[1] The re-evaluation now includes a proposal for classification on germ cell mutagenicity.

1.2 Committee and procedure

The evaluation is performed by the Subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the committee. The members of the committee are listed in Annex B. A submission letter (in English) to the Minister can be found in Annex C.

In 2015 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 D. The committee has taken these comments into account in deciding on the final version of the report. The received comments, and the replies by the committee, can be found on the website of the Health Council.

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 (1994) are inserted in Annex E.

Furthermore, if available, relevant data of the European Chemicals Agency (ECHA) were retrieved and included in this advisory report.

Additional data were obtained form the online databases Medline, Toxline, Chemical Abstracts, and RTECS covering the period to January 2016 using 4-vinylcyclohexene diepoxide as keywords in combination with keywords representative for carcinogenesis and mutagenesis.

Chapter 2

Identity of the substance

2.1 Name and other identifiers of the substance

Table 1 Substance identity.

EU name	:	4-vinylcyclohexene diepoxide
CAS number	:	106-87-6
Replaced CAS Reg. No.:		25550-49-6
EC number	:	203-437-7
Index number	:	603-066-00-4
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
Physical description and colour	:	Clear, colourless or pale yellow liquid
Molecular formula	:	$C_8H_{12}O_2$
Structure	:	
Molecular weight	:	140.18 g/mol

2.2 Composition of the substance

Not applicable.

2.3 Physico-chemical properties

Table 2 Summary of physico- chemical properties.

Properties		Value	Reference	Comment
State of substance	:	Liquid	ACGIH 2001[2]	
Melting/freezing point	:	-55 °C	ACGIH 2001[2]	
Boiling point (101.3 kPa)	:	227 °C	ACGIH 2001[2]	
Relative density	:	-		
Vapour pressure (25 °C)	:	< 0.13 KPa (20 °C)	INCHEM 1998	
Surface tension	:	-		
Specific gravity	:	1.0986 at 20 °C / 20 °C	ACGIH 2001[2]	
Water solubility	:	35.2 g/L, 25 °C	ACGIH 2001[2]	
Partition coefficient n-octanol/water	:	0.44 Log P_{ow}		
Flammability	:			
Flash point	:	110 °C	ACGIH 2001[2]	
Oxidising properties	:	-		
Granulometry	:	-		
Stability in organic solvents	:	-		
Dissociation constant (pKa)	:	-		
Viscosity	:	-		
Conversion factor (25 °C, 101.3 kPa)	:	1 mg/m^3 = 0.174 ppm 1 ppm = 5.733 mg/m^3		

2.4 International classifications

2.4.1 *European Commission*

4-Vinylcyclohexene diepoxide is classified for carcinogenicity in Annex VI of regulation (EC) No 1272/2008 of the European Parliament as follows: Carc 2 (suspected human carcinogen: H351 suspected of causing cancer), according to the Globally Harmonised System of Classification and Labelling of Chemicals. The classification by the European Commission dates from 1991.

2.4.2 *The Health Council of the Netherlands*

In 2008, the Dutch Expert Committee on Occupational Standards, a committee of the Health Council of the Netherlands concluded that 4-vinylcyclohexene

diepoxide should be regarded as carcinogenic to humans (comparable to EU category 1B) and that it acts by a stochastic genotoxic mechanism.[1]

2.4.3 *IARC*

In 1994, IARC concluded that there is inadequate evidence in humans for the carcinogenicity of 4-vinylcyclohexene diepoxide, but that there is sufficient evidence in experimental animals for the carcinogenicity of 4-vinylcyclohexene diepoxide (Annex D). IARC classified 4-vinylcyclohexene diepoxide as possibly carcinogenic to humans (Group 2B).[3]

Chapter 3

Manufacture and uses

3.1 Manufacture

Not relevant for classification.

3.2 Identified uses

4-Vinylcyclohexene diepoxide is used as a chemical intermediate and a diluent for other diepoxides and for epoxy resins derived from bisphenol A and epichlorohydrin.[1,3,4] One of the applications is preparation of epoxy resin tissue-embedding agents for electron microscopy.[1,3-5]

4-Vinylcyclohexene diepoxide is a metabolite of the occupational chemical, 4-vinylcyclohexene. 4-Vinylcyclohexene is a source of exposure in the manufacture of the rubber tires, flame tetardants, insecticides, plasticizers and antioxiddants. Hence, by using 4-vinylcyclohexene workers may also be exposed to 4-vinylcyclohexene diepoxide.

Chapter 4

Summary of toxicokinetics

The data presented below is a summary based on evaluations and reviews by others, such as NTP, IARC, DECOS.[1,3,4]

4.1 Absorption, distribution, metabolism and elimination

4-Vinylcyclohexene diepoxide is absorbed by rodents exposed dermally, orally, or by inhalation (Weil et al., 1963 in NTP).[4,6] The National Toxicology Program (NTP) has studied the fate of a single dermal application of [^{14}C] 4-vinylcyclohexene diepoxide in female F344/N rats and B6C3F1 mice. These studies were conducted to determine if there were differences in disposition which could explain the differences in toxicity observed in rats and mice. Rats and mice received 0.1 ml and 0.001 ml, respectively, of solutions containing 500 mg/ml (200 pC/ml) [ethylene-^{14}C]4-vinylcyclohexene diepoxide in acetone. The preliminary results indicate that 30% of the dose applied to the skin is absorbed over a 24-hour period for both rats and mice; only 1%-3% of the dose remained on the skin at the site of application. By 24 hours, 70%-80% of the absorbed dose had been eliminated from the body, virtually all in the urine. The radioactivity remaining in the body was distributed over a number of tissues, with no tissue containing more than 1% of the applied dose.[4] The liver, muscle, and adipose tissue, however, contained 0.5%-1.6% and 1.2%-2.9% of the absorbed dose in rat and mouse tissue, respectively. Tissue to blood ratios ranged from 0.3 to 1.5 in rats and from 0.8 to 2.8 in mice (NTP unpublished data in NTP 1998).[4]

In vitro studies with rabbit liver microsomal preparations showed that 4-vinylcyclohexene diepoxide can be metabolized to monoepoxymono-glycols: 1,2-hydroxy-4-vinylcyclohexane oxide, and 4-(1',2'-dihydroxyethyl)-1-cyclohexane oxide (Watabe and Sawahata, 1976 in NTP 1998)[4]. Formation of these products is catalyzed by epoxide hydrolase. Conjugation with glutathione is another pathway for metabolism of 4-vinylcyclohexene diepoxide, proposed by Giannarini et al. (1981 in NTP 1998), who reported depletion of reduced glutathione in the liver of mice given an intraperitoneal injection of 500 mg/kg 4-vinylcyclohexene diepoxide.[4]

4.2 General toxicity with focus on germ cell toxicity

4-Vinylcyclohexene diepoxide is a metabolite of 4-vinylcyclohexene. The latter substance, like the diepoxide, exhibits selective toxicity in primordial and primary ovarian follicles and ovarian carcinogenicity in mice, but not in rats.[7] Studies on the disposition and metabolism of 4-vinylcyclohexene and extensive structure-activity studies led to the identification of 4-vinylcyclohexene diepoxide as the ultimate ovotoxic metabolite of 4-vinylcyclohexene, being more reactive than the parent compound.[8-10] The diepoxide was subsequently used in mechanistic studies on ovarian toxicity. Hoyer and colleagues found that the types of morphological lesions in destroyed follicles are consistent with (accelerated) programmed cell death (apoptosis) rather than cytotoxicity or necrosis.[10-12] Hoyer et al. also showed that altered expression of the cell death enhancer gene *bax* was involved in 4-vinylcyclohexene diepoxide-induced ovotoxicity.[11] Protection against 4-vinylcyclohexene diepoxide-induced ovotoxicity by concurrent treatment with 17β-estradiol or genistein (an estrogen receptor agonist) provided support for an estrogen receptor-mediated mechanism.[11]

Appt (2006) studied the effect of 4-vinylcyclohexene diepoxide in nonhuman primates that received once-daily intramuscular injections for 15 days of 250, 160 or 80 mg/kg. At 250 mg/kg nearly complete elimination of primordial, intermediate, primary and secondary follicles was achieved, at 160 mg/kg a 50% elimination and at 80 mg/kg no elimination was achieved. No gross of histological lesions in the organs studied were found at postmortum evaluations after 9 months.[13]

Chapter 5

Genotoxicity

5.1 Non-human information

5.1.1 In vitro data

Data on in vitro mutagenicity testing are presented in Table 3.

Table 3 Summary of in vitro mutagenicity studies.

Method	Cell type	Concentration range	Results - negative + postitive	Klimisch score	References
Micro-organisms					
Reverse mutation	*Salmonella typhimurium*	*Strains*: TA100, TA1535, TA98, TA1537 *Method*: 5 doses, triplicate plates, two separate experiments *Solvent*: distilled water *Concentrations*: 100, 333; 1,000; 3,333; 10,000 µg/plate *Metabolic system*: Liver S9 mix from Aroclor 1254-induced male Sprague-Dawley and male Syrian hamsters *Controls*: Negative: solvent; Positive: -S9 mix: sodium azide (TA1535, TA100), 4-nitro-o-phenylene-diamine (TA98) and 9-aminoacridine (TA97, TA1537);	*Outcome*: TA100, TA1535, TA98 positive with and without metabolic activation; TA1537 without metabolic activation equivocal, with activation positive in first trial, equivocal in second trial. *Cytotoxicity*: Nontoxic up to highest concentration tested	2	NTP 1989[4]; Mortelmans et al., 1986[14]

		+S9 mix: 2-amino-anthracene (all strains) *Purity*: 97% *Statistical analysis*: not performed			
Reverse mutation	*Salmonella typhimurium*	*Strains*: TA1535, TA98, TA100 *Method*: 4 doses, triplicate plates; bacteria exposed to vapour for 7 hr in sealed desiccator *Concentrations*: 0.01, 0.05, 0.1 and 0.5 ml/9 litre desiccator *Metabolic system*: Liver S9 mix from Aroclor-1254 induced male Sprague-Dawley rats *Control*: negative and positive controls were used but not specified. *Purity*: >98% *Statistical analysis*: not reported	*Outcome*: TA1535, TA98 and TA100 positive with and without S9 *Cytotoxicity*: no cytotoxicity at the concentrations tested	2	Simmon and Baden 1980[15]
Reverse mutation	*Salmonella typhimurium*	*Strains*: TA100 and TA1535 *Method*: 4 doses, triplicate plates, two separate experiments *Solvent*: DMSO *Concentrations*: 15, 30, 45, 60 µmoles *Metabolic system*: not used *Control*: Negative: DMSO, Positive: sodium azide *Purity*: 99% *Statistical analysis*: not reported	*Outcome*: Positive TA1535 and TA100 *Cytotoxicity*: 6% and 12% growth inhibition in TA100, 8% and 15% growth inhibition in TA1535 at 48 and 60 µmoles/plate, resp.	2	Frantz and Sinsheimer 1981[16]
Reverse mutation	*Salmonella typhimurium*	*Strains*: TA1535, TA100, TA1537, TA98 *Method*: at least 3 replicates in 3 separate experiments *Solvent*: DMSO *Concentrations*: (µg/plate): 62.5; 125; 250; 500; 1,000; 2,000 *Metabolic system*: not used *Control*: Negative: solvent; Positive: not reported *Purity*: no data *Statistical analysis*: not reported	*Outcome*: Positive in TA1535 and TA100 Negative in TA1537 and TA98 *Cytotoxicity*: 2,000 µg/plate was toxic	2	El Tantawy and Hammock 1980[17]

Reverse mutation	*Salmonella typhimurium*	Strains: TA100 *Method*: 3 doses, using duplicate plates *Solvent*: DMSO *Concentrations* (µl/plate): 1 and 10 (-S9,+S9) 100 (no info on S9) *Metabolic system*: Liver S9 mix from phenobarbital-induced pregnant female Sprague-Dawley rats *Control*: Negative: solvent; Positive: 2-aminoanthracene *Purity*: no data *Statistical analysis*: not reported	*Outcome*: positive with and without S9; S9 enhanced activity *Cytotoxicity*: no data	3 (only one strain used, only 3 concentrations used, duplicate plating, no data on cytotoxicity and compound purity)	Murray et al., 1979[18]
Reverse mutation	*Salmonella typhimurium*	*Strains*: TA100 *Method*: 2 doses, at least 3 experiments *Solvent*: DMSO Concentrations (µmoles/plate): 1, 10 *Metabolic system*: not used *Control*: Negative: solvent; Positive: not reported *Purity*: no data *Statistical analysis*: not reported	*Outcome*: positive *Cytotoxicity*: no data	3 (only one strain used, only 2 concentrations used, not tested with metabolic activation, no data on cytotoxicity and compound purity, no positive control)	Watabe et al., 1980[19]
Reverse mutation	*Salmonella typhimurium*	*Strains*: TA100 *Method*: Several concentrations tested, number of replicates and trials not reported *Solvent*: DMSO *Concentrations*: not specified; 100 µl diluted compound/plate, samples tested over a dilution range of at least 1,000-fold *Metabolic system*: not used *Control*: Negative: solvent; Positive: no data *Purity*: no data *Statistical analysis*: not reported	*Outcome*: positive *Cytotoxicity*: no data	3 (limited information on design and results; purity compound unknown; no metabolic activation used, only one strain used, concentrations tested not specified, no data on positive control, number of replicates and trials not known, no information on cytotoxicity)	Ringo et al., 1982[20]

Reverse mutation	*Salmonella typhimurium*	*Strains*: TA98, TA100 *Concentrations*: 0.05 and 10 mg *Solvent*: unknown *Method*: Spot test, 2-5 determinations (not specified per concentration), number of trials not known *Metabolic system*: Rat liver S9 mix; no information on chemical treatment of the rats *Control*: Negative: DMSO; Positive: -S9: N-methyl-N'-nitrosoguanidine (TA100), 4-nitroquino-line-N-oxide (TA98); +S9: no information on positive controls *Purity*: no data *Statistical analysis*: not reported	*Outcome*: T98 and TA100 positive without and with metabolic activation (NB: numbers of revertants were reported only for tests without S9; authors stated that addition of S9 did not alter the mutagenicity) *Cytotoxicity*: no cytotoxicity observed	3 (limited information on design and results, no information on compound purity and potential solvent used, only two concentrations tested, no information on number of trials, no standard deviations reported for results without S9, numbers of revertants with S9 not reported)	Wade et al., 1979[21]
Point mutation	*Salmonella typhimurium*	*Strains*: TA100 *Method*: 4 concentrations, number of replicates and trials not reported *Solvent*: used but not specified *Concentrations*: 0.33, 1, 3.3, 100 mM *Metabolic system*: not used *Control*: Negative: solvent; Positive: no data *Purity*: no data *Statistical analysis*: regression analysis	*Outcome*: positive *Cytotoxicity*: about 20% and 60% growth inhibition at 3.3 and 100 mM, resp.	3 (limited information on design and results; purity compound unknown; no metabolic activation used, only one strain used, no information on what has been used as negative control, no data on positive control, number of replicates and trials not known)	Turchi et al., 1981[22]
Mammalian cells					
Gene mutation	Mouse lymphoma: L5178Y cells *tk* locus	*Method*: 5 concentrations at least in duplicate, two separate experiments *Solvent*: distilled water *Concentrations*: 25, 50, 100, 200, 400 µg/ml *Metabolic system*: not used *Controls*: Negative: solvent; Positive: methyl methanesulphonate *Purity*: 97% *Statistical analysis*: dose-trend test and variance analysis of pair-wise comparisons of each dose against vehicle control	*Outcome*: Positive. Mean mutant frequency (mutants/10E6 clonable cells): at 0 through 200 µg/ml, resp. 48, 157, 273, 895 and 804 (test 1); 96, 175, 274, 590 and 1,595 (test 2). *Cytotoxicity*: Relative total growth at 0 through 400 µg/ml, resp. 100, 61, 43, 15, 9 and 0% in test 1; 100, 95, 69, 50, 7 and 0% in test 2	2	NTP 1989[4]; McGregor et al., 1988[23]

Gene mutation	V79 Chinese hamster cells	*Method*: 4 concentrations, 2-3 independent experiments *Solvent*: DMSO *Concentrations*: Up to 10 mM. *Metabolic system*: not used *Control*: Negative: solvent; Positive: no data *Purity*: no data *Statistical analysis*: regression analysis	*Outcome*: positive *Cytotoxicity*: LD50 of 2.3 mM was calculated from survival curve	3 (limited information on design and results; purity compound unknown; no metabolic activation used, no data on positive control, number of replicates per concentration not known, means and standard deviations of mutants not tabulated (results shown only in dose-effect curve), no purity data	Turchi et al., 1981[22]
Chromo-some aberration	Chinese Hamster Ovary cells	*Method*: 3 concentrations, no information on number of trials *Solvent*: DMSO *Concentrations*: (μg/ml) -S9: 37.8, 50.3, 62.9 +S9: 447, 503, 548 *Metabolic system*: Liver S9 mix from Aroclor 1254-induced male Sprague Dawley rats *Controls*: Negative: solvent; Positive: -S9: mitomycin C, +S9:cyclophosphamide *Purity*: 97% *Statistical analysis*: conducted on slopes of the dose-response curves and on individual dose points	*Outcome*: Positive with and without metabolic activation; % of cells with aberrations (* indicates statistical significance): -S9: 3, 43*, 82*, 100* +S9: 5, 33*, 45*, 60* for control through highest concentration, resp. *Cytotoxicity*: Cell cycle delay (-S9)	2	NTP 1989[4]
Chromo-some aberration and micro-nucleus test	V79 Chinese hamster cells	*Method*: No information on numbers of concentrations, replicates and experiments; endpoints micronuclei and chromosome aberrations (bridges) in anaphase *Solvent*: DMSO *Concentrations*: 2 mM (no data on possible other concentrations) *Metabolic system*: not used *Control*: Negative: solvent; Positive: no data *Purity*: no data *Statistical analysis*: not reported	*Outcome*: Chromosome aberrations: positive Micronuclei: negative *Cytotoxicity*: Cloning efficiency decreased from 85% in control to 39% at 2 mM	3 (limited information on design and results; purity compound unknown; no metabolic activation used, no data on positive control, number of replicates per concentration not known, results shown for only one concentration, no standard deviations reported)	Turchi et al., 1981[22]

Other tests					
Reverse mutation	*Saccharomyces cerevisiae*	*Strain*: diploid D_7 strain *Method*: each concentration was tested in 5-fold *Solvent*: no data *Concentrations*: 25, 50, 75 mM *Metabolic system*: not used *Control*: Negative: used but not specified; Positive: no data *Purity*: no data *Statistical analysis*: not reported	*Outcome*: positive *Cytotoxicity*: survival 100, 80, 65, 55% at 0 through 75mM, resp.	3 (limited information on design and results, no metabolic activation used, no information on compound purity, no information on potential solvent used, no information on what has been used as negative control, no data on positive control)	Bronzetti et al., 1980[24]
Mitotic gene conversion and mitotic cross over	*Saccharomyces cerevisiae*	*Strain*: diploid D_7 strain *Method*: each concentration was tested in 5-fold *Solvent*: no data *Concentrations*: 25, 50, 75 mM *Metabolic system*: not used *Control*: Negative: used but not specified; Positive: no data *Purity*: no data *Statistical analysis*: not reported	*Outcome*: positive for mitotic gene conversion and mitotic cross over *Cytotoxicity*: survival 100, 80, 65, 55% at 0 through 75mM, resp.	3 (limited information on design and results, no metabolic activation used, no information on compound purity, no information on potential solvent used, no information on what has been used as negative control, no data on positive control)	Bronzetti et al., 1980[24]
Sister chromatid exchange	Chinese Hamster Ovary cells	*Method*: 3 concentrations, no information on number of trials *Solvent*: DMSO *Concentrations*: (µg/ml) -S9: 1.12, 3.73, 11.2 +S9: 37.3, 112, 373 *Metabolic system*: Liver S9 mix from Aroclor 1254-induced male Sprague Dawley rats *Controls*: Negative: solvent; Positive: -S9: mitomycin C, +S9:cyclophosphamide *Purity*: 97% *Statistical analysis*: conducted on slopes of the dose-response curves and on individual dose points	*Outcome*: Positive with and without metabolic activation; Number of SCE/cell (* indicates statistical significance): -S9: 12.0, 16.2*, 32.9*, 37.8* +S9: 11.6, 29.4*, 38.6*, 119.2* for control through highest concentration, resp. *Cytotoxicity*: most of the increases in SCEs occurred in the absence of overt toxicity	2	NTP 1989[4]

Comet assay	Human skin biopt	*Method*: Ex vivo validation study with human skin tissue *Concentrations*: 0, 50, 160; 500; 1,600 µl/cm² applied directly on skin membrane of two different donorsin triplicate for 3 hour period *Metabolic system*: not used *Controls*: Negative: solvent Positive: methyl methane sulphonate and TX-100 *Purity*: unknown *Statistical analysis*: Dunnett's t-test (one-sided $p<0.05$)	*Outcome*: positive *Cytotoxicity*: viability test; not cytotoxic	3 (no validated study)	Rues et al., 2012[25]

Conclusion

The available in vitro mutagenicity studies are summarized in Table 3.

4-Vinylcyclohexene diepoxide was found to be mutagenic in various strains of *Salmonella typhimurium*, in the presence and absence of an exogenous metabolic system.[4,14-22] *Salmonella typhimurium* strain TA100 was used most frequently and consistently showed positive results. Strains TA1535 and TA98, used in four studies, showed positive results in all (TA1535) or three (TA98) studies. Strain TA1537, used in only two studies, was positive with metabolic activation but equivocal or negative without activation.

Furthermore, exposure resulted in an increased mutant frequency in L5175Y mouse cells at the heterozygous *tk* locus in the absence of metabolic activation.[4,23]

4-Vinylcyclohexene diepoxide caused an increase in the number of Chinese hamster ovary cells with chromosome aberrations in the presence and absence of metabolic activation.[4] Moreover, 4-vinylcyclohexene diepoxide induced sister chromatid exchanges in Chinese hamster ovary cells in the presence and absence of metabolic activation.

The studies with *Saccharomyces cerevisiae* were considered not adequate for genotoxicity assessment because of deficiencies in design and reporting. The committee further identified two publications of Mabon and Randerath in 1996 on the formation of DNA adducts by 4-vinylcyclohexene diepoxide (not summarized in Table 3).[26,27] The authors showed that 4-vinylcyclohexene diepoxide is able to produce DNA-adducts in vitro (calf thymus DNA), using the ^{32}P-postlabelling technique.[26,27] The adduct levels were, however, far below those generally found for highly potent carcinogens (such as benzo[a]pyrene) at comparable doses. Overall the committee concluded that 4-vinylcyclohexene

diepoxide is mutagenic in vitro causing gene mutations and chromosomal aberrations.

5.1.2 *In vivo data*

Mabon and Randerath (1996) also showed that 4-vinylcyclohexene diepoxide is able to produce DNA-adducts in female ICR mice (topical skin application; 17-225 µmol/mouse; once a day for three days), using the ^{32}P-postlabelling technique.[26,27] The adduct levels were, however, far below those generally found for highly potent carcinogens (such as benzo[a]pyrene) at comparable doses. No other in vivo mutagenicity studies were retrieved.

5.2 Human information

No studies on humans were retrieved.

5.3 Summary and discussion on mutagenicity

Below, only data are summarized of a reliable experimental design according to the Klimisch criteria 1 and 2 (See Annex H).[28]

Germ cell genotoxicity

As no relevant genotoxicity studies of 4-vinylcyclohexene diepoxide in germ cells were found, the committee is not able to make a conclusion whether 4-vinylcyclohexene diepoxide is mutagenic in germ cells.

Somatic cell genotoxicity

Vinylcyclohexene diepoxide was investigated predominantly in in vitro genotoxicity tests only for the 3 endpoints of genotoxicity: gene mutations, structural and numerical chromosome aberrations.

Both in vitro (calf thymus DNA) and in vivo (skin of mice treated topically) 4-vinylcyclohexene diepoxide produced DNA-adducts. 4-Vinylcyclohexene diepoxide induced gene mutations in *Salmonella typhimurium* strains in the presence and absence of metabolic activation and in mammalian cells (mouse lymphoma study, *tk* locus) in the absence of metabolic activation.[4,15-17]

Exposure to vinylcyclohexene diepoxide did also result in an increase in cells with chromosome aberrations with and without metabolic activation[4]. The supporting genotoxicity tests confirmed the positive findings in in vitro tests (Table 3). In vivo, no other mutagenicity studies were retrieved.[26,27]

Overall the committee concludes that 4-vinylcyclohexene diepoxide is mutagenic in vitro and acts by a stochastic genotoxic mechanism.

5.4 Comparison with criteria

According to the criteria in Annex VI of the European regulation No. 1272/2008 (see Annex G), classification as a mutagen in category 1 is warranted when positive evidence for in vivo heritable germ cell mutagenicity in humans (1A) or mammals (1B) has been reported. No data have been presented on human germ cell mutagenicity. Overall, due to a lack of data the committee concludes that there is no evidence for in vivo heritable germ cell mutagenicity of 4-vinylcyclohexene diepoxide.

In addition, substances may be categorized in 1B if there are “positive results from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells”. The latter may be based on a) “supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo”, or b) “by demonstrating the ability of the substance or its metabolites to interact with the genetic material of germ cells” (see Annex G). No evidence has been found for in vivo mutagenicity testing in mammals. Regarding the second part of the criterion, there is no evidence that 4-vinylcyclohexene diepoxide is genotoxic in germ cells. Overall, due to lack of data on germ cell mutagenicity, the committee is of the opinion that no evidence exists that 4-vinylcyclohexene diepoxide has the potential to cause mutations to germ cells.

If substances do not meet the criteria for classification in category 1, they may be classified in category 2 if there is “positive evidence from experiments in mammals and/or in some cases from in vitro experiments obtained from a) somatic cell mutagenicity tests in vivo, in mammals” or b) “other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays” (see Annex G).

The committee concludes that there is no relevant data from in vivo experiments in mammals, only from in vitro experiments.[4,15-17] Therefore, the committee does not recommend a classification as a germ cell mutagen.

5.5 Conclusions on classification and labelling

Based on the available data, the committee does not recommend to classify 4-vinylcyclohexene diepoxide as a germ cell mutagen. The substance acts by a stochastic genotoxic mechanism.

Chapter 6

Carcinogenicity

6.1 Non-human information

Data on carcinogenicity are summarised in Table 4.

Table 4 Summary of animal carcinogenicity studies on 4-vinylcyclohexene diepoxide exposure.

Species	Design	Exposure levels	Observations and remarks (klimisch score)[a]	References
Dermal application				
344/N rats	60/sex/dose	Dermal application, 5 days/week, vehicle acetone (3 consecutive 0.1 ml applications) Doses applied uniformely at site of clipped dosal interscapular region; surface area not given) Dose: 0, 15, 30 mg/rat Purity: about 97% Xpo= 105 weeks Xpe= 106-107 weeks (10 animals of each sex were killed after 15 months for interim evaluation). Statistical analysis tumour incidences: Life table tests, logistic regression tests (with adjustment for intercurrent mortality), Cochran-Armitage trend test, and Fisher exact test	Klimisch score: 2 *Survival*: males 7/50, 8/50, 4/50; females 27/50, 23/50, 15/50 at 0, 15 and 30 mg resp. (significantly lower than control between day 637-715 at 15 mg, from day 648 at 30 mg) *Adverse effects*: Body weight about 10% lower than control at 30 mg after week 49 in males and after week 57 in females. *Non-neoplastic lesions*: significantly increased incidence of acanthosis and sebaceous gland hypertrophy at application site at 15 and 30 mg. *Tumours*: at 0, 15 and 30 mg, resp. Skin tumours listed below occurred at application site. Skin squamous cell carcinoma 0/50, 33/50 ($p<0.001$), 36/50 ($p<0.001$) in males, 0/50, 16/50 ($p<0.001$), 34/50 ($p<0.001$) in females. Skin squamous cell papilloma 0/50, 3/50, 6/50 ($p<0.05$) in males, 0/50, 0/50, 1/50 in females; animals with this tumour also had a squamous cell carcinoma.	NTP 1989[4]; Chhabra et al., 1990[29]; Maronpot 1987[30]

			Skin basal cell adenoma 0/50, 0/50, 4/50 ($p<0.05$) in males, none in females. Skin basal cell carcinoma 0/50, 1/50, 3/50 in males, 0/50, 3/50, 4/50 ($p<0.05$) in females. Skin sebaceous gland adenoma 0/50, 2/50, 1/50 in males, 1/50, 1/50, 1/50 in females. (see also Table 5)	
B6C3F$_1$ mice	60/sex/dose	Dermal application, 5 days/week Vehicle: acetone (0.1 ml application) Dose: 0, 2.5, 5 and 10 mg/ mouse Purity: about 97% X_{po} = 103 weeks X_{pe} = 105 weeks (10 animals of each sex were killed after 15 months for interim evaluation). Statistical analysis tumour incidences: Life table tests, logistic regression tests (with adjustment for intercurrent mortality), Cochran-Armitage trend test, and Fisher exact test	Klimisch score: 2 *Survival*: males 38/50, 35/50, 4/50, 0/50, females 30/50, 31/50, 15/50, 10/50 at 0, 2.5, 5 and 10 mg, resp. *Adverse effects*: Body weight lower than control, dose-dependently, at 5 and 10 mg in both sexes (after week 29). Clinical signs: crusts, scales and ulcers at application site. *Non-neoplastic lesions:* (increased incidences of): • Skin: acanthosis, hyper-keratosis and necrotizing inflammation at application site in both sexes at all doses (statistically significant except for inflammation at 2.5 mg); • Ovaries: follicular atrophy and tubular hyperplasia at all doses; • Spleen: hematopoietic cell proliferation, primarily due to hyperplasia of myeloid elements (in response to skin inflammation and neoplasms) in both sexes, most markedly at 5 and 10 mg; • Epididymis: subacute inflammation at 5 and 10 mg. *Tumours*: at 0, 2.5, 5 and 10 mg, resp. Skin squamous cell carcinoma (application site): 0/50, 14/50 ($p<0.001$), 39/50 ($p<0.001$), 42/50 ($p<0.001$) in males, 0/50, 6/50 ($p<0.05$), 37/50 ($p<0.001$), 41/50 ($p<0.001$) in females. Ovary: granulosa cell tumour benign or malignant: 0/50, 0/49, 7/49 ($p<0.01$), 12/50 ($p<0.01$). Ovary: benign mixed tumour: 0/50, 0/49, 11/49 ($p<0.001$), 6/50 ($p<0.01$). Lungs: alveolar/bronchiolar adenoma or carcinoma: 4/50, 9/50, 11/50 ($p<0.05$), 7/50 in females (see also Table 6)	NTP 1989[4]; Chhabra et al., 1990[29]; Maronpot 1987[30]

C57BL/6 mice $p53^{+/-}$	Vehicle control: • p53: 7 male, 8 female • wild-type: 5/sex Treated: • p53: 7 male, 8 female (low-dose) or 10/sex (high-dose) • wild type: 5/sex (high-dose)	Dermal application, 2 days/week Vehicle: acetone (0.1 ml application) Dose: 0, 12.5 (p53 only), 25 mg/ mouse Purity: no data X_{po} = 24 weeks X_{pe} = 28 weeks	Klimisch score: 2 *Mortality*: 2/10 p53 males at 25 mg and 2/8 p53 females at 12.5 mg; no deaths in the other groups. *Adverse effects*: In p53 and wild-type slight, dose-related decrease in weight gain throughout Xpo, reversed after cessation of treatment Skin: Nodular epidermal hyperplasia which appeared to be a continuum with the development of squamous cell carcinomas (no further details). *Tumours*: Skin tumours (squamous cell or basal cell carcinoma or fibrosacroma): p53: 0/7, 2/7, 3/10 in males, 0/8, 0/8, 3/8 in females; none in wild-type mice	Tennant et al., 1995[31]; Tennant et al., 1996[32]
Swiss-Millerton mice	Treated: 30 males Controls: • vehicle: 150 males (3 x 30; 1 x 60) • untreated: 207 males (4 x 27-30; 1 x 60) • benzo(a)-pyrene in benzene: 90 (3 x 30)	Dermal application, 3 days/week Vehicle: benzene (10% solution) Dose: ca. 100 mg of solution/ application Purity: not specified (commercial quality material purified by vacuum distillation, 2 minor, unspecified impurities could not be removed) X_{po} = Life span X_{pe} = Life-span Statistical analysis: life-table analysis	Klimisch score: 3 (limited information on study design and results) *Mortality*: median survival time: 326 days for treated mice, 262-412 for vehicle controls, 112-345 for untreated controls, 348-370 for positive controls *Adverse effects*: no data *Skin tumours*: numbers of mice with tumour (total = papillomas or squamous cell carcinoma [scc]): • treated: total 14, 9 of these scc • vehicle: total 11 (2-5/group), 1 of these scc • untreated: total 13 (0-5/group), 1 of these scc • benzo(a)pyrene: total 49 (10-23/group), 26 of these scc (6-13/group)	Van Duuren et al., 1963[33]
C3H mice (sex not specified)	Treated: 30-40 Control: no data	Dermal application, 3 days/week Vehicle: acetone (10% solution) Dose: no quantitative data (one brushful) Purity: no data Xpo= Life-span (max. 21 months) Xpe= Life-span Tumour observations: for papillomas and carcinomas during each painting period. Statistical analysis: no data	Klimisch score: 3 (very limited information on study design and results) *Mortality*: 18, 6 and 0 survivors at 12, 17 and 24 months, resp. *Adverse effects*: no data. *Tumours*: Skin, application site: • papillomas: in 3 mice • carcinomas: in 1 mouse First tumour appeared at 17 months	Weil et al., 1963[6]

Albino mice (no further information)	Treated: 20 males Control: no data	Dermal application, 5 days/week Vehicle: no data Dose: ca. 16 mg/mouse Purity: commercial product, contaminated with water-insoluble material Xpo= 12 months Xpe= Life-span Method of tumour detection: no data Statistical analysis: no data	Klimisch score: 3 (contaminated test material of unknown purity used, very limited information on study design and results) *Mortality*: Last mouse died at 21 months after initiating treatment. 9 mice died without tumours and 2 died with papillomata that regressed after treatment cessation. *Tumours (in survivors)*: Skin, application site: • Squamous cell carcinoma: 4/9 • Mixed cell sarcoma: 3/9 • Both of above tumours: 2/9 Lung: • adenoma, probably malignant: 1/9 (in mouse with both skin tumours) • adenomata showing no signs of malignancy: 2/9 (in mice with skin carcinomas)	Hendry et al., 1951[34]
$CB6F_1$-TgH*ras*2 and wild type $CB6F_1$ mice.	Vehicle control: 10/sex/strain Treated: generally 15/sex/strain/dose	Dermal application, 5 days/week Vehicle: not known Dose: 0, 5, 10 mg/mouse Purity: no data X_{po} = 24 weeks X_{pe} = 26 weeks Statistical analysis tumour incidences: Fisher exact test	Klimisch score: 4 (*not a representative 2-year study, only supportive*) *Mortality*: no data. *Adverse effects*: no data. *Tumours*: Skin papilloma ($p<0.05$ for Tg females dosed with 10 mg), forestomach papilloma, thymic lymphoma, lung adenoma: increased incidences in treated Tg and non-Tg mice compared to vehicle controls (incidences in Tg mice were higher than in non-Tg mice). Skin squamous cell carcinomas and spleen hemangiosarcomas in treated Tg mice (not in treated non-Tg mice).	Yamamoto et al., 1998[35]
Studies below: Administration route intraperitoneal or unknown				
Female Sprague-Dawley rats	Young (1 month old): • treated: 12 and 21 rats at low- and high-dose, resp. • vehicle control: 17 rats (interim kill after 15 doses: 10 high-dose rats, 7 controls); Mature (3 months old):	Intraperitoneal administration, 25 doses between post-natal days (PND) 35-68 (young rats) or PND 94-119 (mature rats) Vehicle: DMSO Dose: 80 (low) and 160 (high) mg/kg body weight/day Purity: at least 96% X_{po} = 25 days (young and mature rats) X_{pe} = 570 days (young rats), 261 days (mature rats) Tumour detection (mammary tumours only): visual inspection and palpation. Statistical analysis tumours: Kaplan-Meyer survival plots analysed by Logrank test with	Klimisch score: 3 (no individual animal data reported, low number of animals used, route of exposure not relevant)_ *Mortality*: no data. *Adverse effects*: acceleration of onset of persistent estrus and of transition from persistent estrus to ovarian failure at 160 mg/kg in young and mature rats; decrease in number of alveolar buds in mammary glands at 160 mg/kg in young rats (examined at interim kill); β-casein gene expression [biomarker for differentiation and maturation of mammary epithelium] down-regulated (examined at interim kill). No effect of treatment on serum hormone levels (17β-estradiol, androstenedione, prolactin) *Tumours mammary gland*: • Young rats: dose-related acceleration of onset and increase of incidence of fibroadenoma at	Wright et al., 2011[36]

	• treated: 7 and 12 rats at low- and high-dose, resp. • vehicle control: 17 rats	post-hoc Logrank test for trend to confirm dose-dependency; Chi-square analysis to compare tumour incidence between treated groups and controls at individual time points.	low- and high-dose (from 38% to 84%); • Mature rats: tumour onset and incidence (0% in all groups) not affected.	
Albino rats (no further information)	Treated: 10 males and 4 females Control: no data	Intraperitoneal administration, 2 days/week Vehicle: arachis oil Dose: 25 mg/100 g body weight Purity: commercial product, contaminated with water-insoluble material Xpo= 10 weeks Xpe= Life-span Method of tumour detection: no data Statistical analysis: no data	Klimisch score: 3 (Not adequate for carcinogenicity assessment. Deficiencies: contaminated test material of unknown purity used, very limited information on study design and results, insufficient number of animals used, no controls, short exposure period, route of exposure not relevant, limited information on non-cancer effects.) *Mortality*: 6 survivors (sex not specified) at 21 months *Adverse effects*: Loss of spermatogenesis in decedents *Tumours*: Mixed-cell sarcoma tissue in peritoneal cavity and large area of lung infiltrated with tumour tissue: in one male at 7 months	Hendry et al., 1951[34]
C57 Black mice	Treated: 20 (sex not specified) Control: no data	Exposure route, frequency and duration, vehicle, purity test material, observation period, method of tumour detection: no data Concentration: 0.5 mM Statistical analysis: no data	Klimisch score: 3 (Not adequate for carcinogenicity assessment. Deficiencies: very limited information on study design and results, sex animals not specified, low number of animals used, no data on purity of test material and exposure conditions, no data on non-cancer effects.) *Mortality*: 16/20 survivors (no further information) *Adverse effects*: no data. *Tumours* (in survivors): Skin tumours: 1/16 Malignant lymphomas: 4/16 First tumour (type not specified) appeared at 14 months	Kotin and Falk 1963[37]

[a] See Annex H.

The carcinogenicity studies in experimental animals are summarized in Table 4. The summarized studies comprise seven dermal studies (six in mice and one in rats), two studies in intraperitoneally exposed rats and one study in mice using an unspecified administration route. No long-term oral and inhalation studies were identified.

The National Toxicology Program (NTP) performed carcinogenicity studies in rats and mice.[4,29,30] Groups of 60 male and 60 female F344/N rats and B6C3F_1 mice received 4-vinylcyclohexene diepoxide by topical application at doses of

0 (vehicle), 15 or 30 mg/animal (rats) five days per week for 105 weeks, and 0 (vehicle), 2.5, 5 or 10 mg/animal (mice), five days per week up to 103 weeks. At month 15, ten animals from each group were sacrificed for interim histopathological examination.

Table 5 Tumour incidences in rats, which were given 4-vinylcyclohexene diepoxide by dermal application for 2 years[4].

Exposure level (mg/kg bw)	0	15	30
Male rats			
Skin: squamous cell carcinoma	0/50	33/50**	36/50**
• squamous cell papilloma	0/50	3/50	6/50*
• basal cell adenoma	0/50	0/50	4/50*
• basal cell carcinoma	0/50	1/50	3/50
• sebaceous gland adenoma	0/50	2/50	1/50
Female rats			
Skin: squamous cell carcinoma	0/50	16/50**	34/50**
• squamous cell papilloma	0/50	0/50	1/50
• basal cell adenoma	0/50	0/50	0/50
• basal cell carcinoma	0/50	3/50	4/50*
• sebaceous gland adenoma	1/50	1/50	1/50

Fischer exact test: * $p<0.05$, ** $p<0.001$.

Table 6 Tumour incidences in mice, which were given 4-vinylcyclohexene diepoxide by dermal application for 2 years[4].

Exposure level (mg/kg bw)	0	2,5	5	10
Male mice				
Skin: squamous cell carcinoma	0/50	14/50**	39/50**	42/50**
Female mice				
Skin: squamous cell carcinoma	0/50	6/50*	37/50**	41/50**
Ovary: granulosa cell tumour benign or malignant:	0/50	0/49	7/49*	12/50*
Ovary: benign mixed tumour	0/50	0/49	11/49**	6/50*
Lungs: alveolar/bronchiolar adenoma or carcinoma	4/50	9/50	11/50*	7/50

Fischer exact test: * $p<0.05$, ** $p<0.001$.

Survival in males rats was very low for all groups, controls included, but showed no significant differences between dosed males and controls. Survival of high-dose females was significantly lower compared to controls after day 648 and survival of low-dose females was significantly lower between days 637 and 715. In the second year of the study, male and female rats of the high-dose group had slightly lower body weights than controls. Treatment-related non-neoplastic

lesions were observed in treated males and females, at both dose levels, and consisted of acanthosis and sebaceous gland hypertrophy.

Regarding tumour development, increased incidences of skin tumours, predominantly squamous cell carcinomas, were observed at the site of application in male and female rats of both doses groups. Details are shown in Table 5. No other treatment-related tumours were observed.

In male and female mice, survival at 5 mg/mouse (males after day 543, females after day 666) and 10 mg/mouse (males after day 451, females after day 474) was significantly lowered compared to vehicle controls. All male mice of the 10 mg group were dead by week 82; the surviving females of this group were killed at week 85 because of ulcerated tumour sites. In the course of the study, body weights of male and female mice dosed with 5 or 10 mg became lower, dose-dependently, than those of controls. At the site of application increased incidences of acanthosis, hyperkeratosis and, to a lesser extent, necrotizing inflammation were observed at all dose levels in both sexes. Other treatment-related non-neoplastic changes were observed in the ovaries (follicular atrophy and tubular hyperplasia), spleen (hematopoietic cell proliferation, primarily due to hyperplasia of the myeloid elements; considered a response to the necrotizing inflammation and neoplasms of the skin) and epididymides (subacute inflammation).

As in rats, 4-vinylcyclohexene diepoxide induced squamous cell carcinomas at the site of application in male and female mice (incidences are shown in Table 8). No other treatment-related skin tumours were observed. Furthermore, the incidences of ovarian tumours (benign mixed tumours, granulosa cell tumours) were significantly increased in treated female mice. Also in female mice, the incidence of lung tumours (alveolar/bronchiolar adenomas or carcinomas combined) was statistically significantly increased at 5 mg/mouse. The incidence of these lung tumours in females dosed with 10 mg did not reach statistical significance, probably because these animals were not at risk long enough for these tumours to develop. The incidence of the lung tumours in treated females exceeded the historical control incidence and, therefore, these tumours may have been related to treatment. No other treatment related tumours were found in any of the exposed groups.

The NTP studies showed that mice were more susceptible to 4-vinylcyclohexene diepoxide-induced ovarian toxicity and carcinogenicity than rats. A plausible explanation for this observation is a difference in detoxification capacity. Hoyer and Sipes (1996) referred to a study which showed that the mouse, as compared with the rat, has a reduced capacity to convert 4-vinylcyclohexene diepoxide to its inactive tetrol derivate.[10]

Tennant et al. (1995, 1996) used 4-vinylcyclohexene diepoxide as model compound to examine the potential of transgenic mouse models to identify carcinogens and non-carcinogens.[31,32] He used p53-deficient C57BL/6 mice which are susceptible to tumour development due to reduced expression of the p53 tumour suppressor gene. After dermal application of 4-vinylcyclohexene diepoxide at 12.5 or 25 mg/animal, two times per week for 24 weeks, treated transgenic mice developed the same type of squamous cell tumours at the application site as did normal mice in the two-year dermal carcinogenicity study of the NTP[2] (see Table 6 for study details).

Yamamoto et al. used 4-vinylcyclohexene diepoxide as model carcinogen to validate a transgenic mouse bioassay, using *ras*H2 ($CB6F_1$) mice carrying the human prototype c-Ha-*ras* gene, for rapid carcinogenicity testing.[35] In various human and animal tumours *ras* genes are activated by point mutations. Therefore, this transgenic mouse line should be vulnerable to developing tumours. 4-Vinylcyclohexene diepoxide was applied to the dorsal skin of the transgenic (Tg) and non-transgenic mice (non-Tg mice) at 5 or 10 mg/kg body weight, five times per week for 24 weeks. 4-Vinylcyclohexene diepoxide induced skin papillomas around the site of application 26 weeks after initiation of treatment; the incidence of skin papillomas was statistically significantly increased in high-dose female Tg mice compared with vehicle control Tg mice. At the high-dose the incidence of skin papillomas was significantly higher in Tg mice (both sexes) than in non-Tg mice. Furthermore, forestomach papilloma, thymic lymphoma and lung adenoma were induced in treated Tg mice and, to a lesser extent, in treated non-Tg mice. Additionally, skin squamous cell carcinomas and spleen hemangiosarcomas were observed in Tg mice but not in non-Tg mice. The review of Yamamoto et al. does not present further details on study design and results.

Although the design of the above studies in transgenic mice differs considerably from that of a conventional two-year rodent carcinogenicity bioassay, these studies provide supportive evidence for the carcinogenicity of 4-vinylcyclohexene diepoxide in mice.

The studies of the NTP were well performed and reported and, therefore, considered suitable for assessing the carcinogenic potential of 4-vinylcyclohexene diepoxide. In the NTP studies 4-vinylcyclohexene diepoxide was carcinogenic for F344/N rats and B6C3F1 mice of both sexes, causing skin (application site) squamous cell neoplasms (predominantly carcinomas) and basal cell neoplasms (adenomas and carcinomas) in rats and skin squamous cell carcinomas in mice. In addition, 4-vinylcyclohexene diepoxide induced ovarian neoplasms (benign or malignant granulosa cell tumours, benign mixed tumours)

and possibly lung neoplasms (alveolar/bronchiolar adenomas or carcinomas) in female mice. Two dermal studies in transgenic mice provided supportive evidence for the carcinogenicity of 4-vinylcyclohexene diepoxide in mice. P53-deficient C57BL/6 mice developed the same type of skin squamous cell tumours at the application site as did normal mice in the two-year mouse study by the NTP. In *ras*H2 ($CB6F_1$) mice 4-vinylcyclohexene diepoxide induced skin papillomas around the site of application, forestomach papilloma, thymic lymphoma, lung adenoma, squamous cell carcinoma and spleen hemangiosarcoma. Most of these tumours was also induced in the treated non-transgenic $CB6F_1$ included in this study.

6.2 Human information

There is no literature available regarding human exposure to 4-vinylcyclohexene diepoxide leading to carcinogenicity.

6.3 Other relevant information

No transformation studies on the potential carcinogenicity of 4-vinylcyclohexene diepoxide were available to the committee.

6.4 Summary and discussion on carcinogenicity

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

The 2-year bioassays conducted by NTP showed that skin application of 4-vinylcyclohexene diepoxide produced squamous cell neoplasms (predominantly carcinomas) and basal cell neoplasms (adenomas and carcinomas) in male and female rats and skin squamous cell carcinomas in male and female mice. In female mice 4-vinylcyclohexene diepoxide also induced ovarian neoplasms (benign or malignant granulosa cell tumours, benign mixed tumours) and possibly lung neoplasms (alveolar/bronchiolar adenomas or carcinomas). The tumours in the skin and ovaries are considered to be relevant for humans. An increase in the incidence of lung tumours in a mouse carcinogenicity study is generally considered to have little relevance to man. Moreover, in the mouse study with 4-vinylcyclohexene diepoxide the incidence of lung tumours was increased in only one sex and this finding was not unequivocally related to treatment.

The carcinogenic mechanism through which 4-vinylcyclohexene diepoxide exerts its effect on ovarian follicles is not completely understood. The results of the genotoxicity studies in the previous section provide evidence for a stochastic mechanism. Further it has been proposed that elevated levels of gonadotropins in response to oocyte depletion (due to the loss of negative feed-back on the hypothalamic-pituitary axis) act as promotors of ovarian tumour development. However, this hypothesis is not uniformly supported by experimental results.[10]

Based on these findings, the committee concludes that there is sufficient evidence for carcinogenicity in animals. The committee did not find indications that the observations in animals and the proposed carcinogenic mechanism would not occur in humans. The committee further expects that 4-vinylcyclohexene diepoxide when applied by other application routes than dermal would result in other local tumours.

6.5 Comparison with criteria

No data on the genotoxicity and carcinogenicity of 4-vinylcyclohexene diepoxide in humans were available. Adequate studies on carcinogenicity in experimental animals were available for the dermal route. In these studies 4-vinylcyclohexene diepoxide was carcinogenic in rats and mice of both sexes, causing skin (application site) squamous cell neoplasms (predominantly carcinomas) and basal cell neoplasms (adenomas and carcinomas) in rats and skin squamous cell carcinomas in mice. In addition, 4-vinylcyclohexene diepoxide induced ovarian neoplasms (benign and malignant granulosa cell tumours, benign mixed tumours) and lung neoplasms (alveolar/bronchiolar adenomas or carcinomas) in female mice.

According to the CLP criteria, 4-vinylcyclohexene diepoxide should, therefore, be classified as “presumed to be as carcinogenic to humans”, which corresponds to classification in catergory 1B. Supporting evidence is that the substances shows genotoxic properties in bacterial and mammalian cells in vitro and DNA adducts in vitro and in vivo.

The committee noticed that from 1991, the European Commission classified the substance as a carcinogen in category 2 (according to the current CLP-system). The reason for this could not be retrieved. The current classification is the same as the previous classification by the Health Council in 2008.[1]

6.6 Conclusions on classification and labelling

The committee concludes that 4-vinylcyclohexene diepoxide is “presumed to be carcinogenic to man”, and recommends classifying the substance in category 1B.

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A Request for advice

B The Committee

C The submission letter (in English)

D Comments on the public review draft

E IARC evaluation and conclusion

F Classification on carcinogenicity

G Classification on mutagenicity

H Criteria for testing reliability of animal and in vitro studies

Annexes

Annex **A**

Request for advice

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

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

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

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

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

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

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

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  toxicologic pathologist, TNO, Zeist; professor of translational toxicology, Wageningen UR
- J. Van Benthem
  Genetic toxicologist, RIVM, Bilthoven
- P.J. Boogaard
  toxicologist, SHELL International BV, The Hague
- G.J. Mulder
  emeritus professor of toxicology, Leiden University
- M.J.M. Nivard
  molecular biologist and genetic toxicologist, LUMC, Leiden
- G.M.H. Swaen
  epidemiologist, Maastricht University, Maastricht
- E.J.J. van Zoelen
  professor of cell biology, Radboudumc, Nijmegen
- T.M.M. Coenen, *scientific secretary*
  Health Council of the Netherlands, The Hague

With respect to the data presentation and interpretation, the Committee consulted an additional expert, Mr. J.A.A. Muller, toxicologist from Bureau Reach, RIVM, Bilthoven.

The first draft of the present advisory report was prepared by Dr. I. Antolino-Lobo, Dr. M.A.C. Schults and Dr. D. Jonker from TNO by contract with SZW.

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 chairperson 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 inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter (in English)

Subject : Submission of the advisory report *4-Vinylcyclohexene diepoxide*
Your Reference: DGV/BMO/U-932542
Our reference : U-915764/DC/fs/246-D24
Enclosed : 21
Date : February 29, 2016

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to 4-vinylcyclohexene diepoxide.

This advisory report is a re-evaluation of an advisory report on the classification as a carcinogenic substance that has earlier been published by the Health Council. The Council is asked for a re-evaluation because the proposed classification differs from the classification that applies in the European Union. In addition, the Council is asked to also propose a classification for mutagenicity. The classifications are based on the European classification system.

The conclusions in the advisory report were drawn by a subcommittee of the Health Council's Dutch Expert Committee on Occupational Safety (DECOS). The subcommittee has taken comments into account from a public review, and

included the opinions by the Health Council's Standing Committee on Public Health.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,
(signed)
Professor J.L. Severens,
Vice President

Annex D

Comments on the public review draft

A draft of the present report was released in 2015 for public review. The following organization and persons have commented on the draft document:

- T.J. Lentz, S. Reynolds, National Institute for Occupational Safety and Health (NIOSH), USA.

Annex **E**

IARC evaluation and conclusion

4-Vinylcyclohexene diepoxide

Vol.: 60 (1994) (p. 361-375)[3]

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-Vinyl-cyclohexene 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-epoxyethylcyclo-hexane-1,2-diol, was not mutagenic to *Salmonella typhimurium.*

Evaluation

There is inadequate evidence in humans for the carcinogenicity of 4-vinylcylco-hexene 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).

Annex **F**

Classification on carcinogenicity

The Committee expresses its conclusions in the form of standard phrases*:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category[a]	
		(before 16 December 2008)	(as from 16 December 2008)
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be as carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	not applicable	not applicable
(4)	The compound is probably not carcinogenic to man.	not applicable	not applicable

a See Section 3.6 (Carcinogenicity) of Regulation No. 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances.

* Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.[38]

Annex **G**

Classification on mutagenicity

Source: Section 3.5 (Germ cell mutagenicity) of Regulation No. 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances.

3.5.1 Definitions and general considerations

3.5.1.1 A mutation means a permanent change in the amount or structure of the genetic material in a cell. The term ‘mutation’ applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term ‘mutagenic’ and ‘mutagen’ will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

3.5.1.2 The more general terms ‘genotoxic’ and ‘genotoxicity’ apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects.

3.5.2 Classification criteria for substances

3.5.2.1 This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from

mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class.

3.5.2.2 For the purpose of classification for germ cell mutagenicity, substances are allocated to one of two categories as shown in Table 3.5.1.

3.5.2 Specific considerations for classification of substances as germ cell mutagens

3.5.2.3.1 To arrive at a classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in in vitro tests shall also be considered.

3.5.2.3.2 The system is hazard based, classifying substances on the basis of their intrinsic ability to induce mutations in germ cells. The scheme is, therefore, not meant for the (quantitative) risk assessment of substances.

Table 3.5.1 Hazard categories for germ cell mutagens.

Categories		Criteria
CATEGORY 1:		Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.
	Category 1A:	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
	Category 1B:	The classification in Category 1B is based on: • positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or • positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or • positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

CATEGORY 2:	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: • positive evidence obtained from experiments in mammals and/ or in some cases from in vitro experiments, obtained from: • somatic cell mutagenicity tests in vivo, in mammals; or • other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. *Note*: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

3.5.2.3.3 Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in Regulation (EC) No 440/2008 adopted in accordance with Article 13(3) of Regulation (EC) No 1907/2006 ('Test Method Regulation') such as those listed in the following paragraphs. Evaluation of the test results shall be done using expert judgement and all the available evidence shall be weighed in arriving at a classification.

3.5.2.3.4 In vivo heritable germ cell mutagenicity tests, such as:

- rodent dominant lethal mutation test;
- mouse heritable translocation assay.

3.5.2.3.5 In vivo somatic cell mutagenicity tests, such as:

- mammalian bone marrow chromosome aberration test;
- mouse spot test;
- mammalian erythrocyte micronucleus test.

3.5.2.3.6 Mutagenicity/genotoxicity tests in germ cells, such as:

a mutagenicity tests:
  - mammalian spermatogonial chromosome aberration test;
  - spermatid micronucleus assay;

b genotoxicity tests:
  - sister chromatid exchange analysis in spermatogonia;
  - unscheduled DNA synthesis test (UDS) in testicular cells.

3.5.2.3.7 Genotoxicity tests in somatic cells such as:

- liver Unscheduled synthesis test (UDS) in vivo;
- mammalian bone marrow Sister Chromatid Exchanges (SCE);

3.5.2.3.8 In vitro mutagenicity tests such as:

- in vitro mammalian chromosome aberration test;
- in vitro mammalian cell gene mutation test;
- bacterial reverse mutation tests.

3.5.2.3.9 The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1). In those instances where a single well-conducted test is used for classification, it shall provide clear and unambiguously positive results. If new, well validated, tests arise these may also be used in the total weight of evidence to be considered. The relevance of the route of exposure used in the study of the substance compared to the route of human exposure shall also be taken into account.

3.5.3 Classification criteria for mixtures

3.5.3.1 Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.5.3.1.1 The mixture shall be classified as a mutagen when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 mutagen and is present at or above the appropriate generic concentration limit as shown in Table 3.5.2 for Category 1A, Category 1B and Category 2 respectively.

Table 3.5.2 Generic concentration limits of ingredients of a mixture classified as germ cell mutagens that trigger classification of the mixture.

	Concentration limits triggering classification of a mixture as:		
Ingredient classified as:	Category 1A mutagen	Category 1B mutagen	Category 2 mutagen
Category 1A mutagen	≥ 0,1 %	-	-
Category 1B mutagen	-	≥ 0,1 %	-
Category 2 mutagen	-	-	≥ 1,0 %

Note. The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).

3.5.3.2 Classification of mixtures when data are available for the complete mixture.

3.5.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients classified as germ cell mutagens. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical

analysis of germ cell mutagenicity test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.5.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles.

3.5.3.3.1 Where the mixture itself has not been tested to determine its germ cell mutagenicity hazard, but there are sufficient data on the individual ingredients and similar tested mixtures (subject to paragraph 3.5.3.2.1), to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.5.4 Hazard communication

3.5.4.1 Label elements shall be used in accordance with Table 3.5.3, for substances or mixtures meeting the criteria for classification in this hazard class.

Table 3.5.3 Label elements of germ cell mutagenicity.

Classification	Category 1A or Category 1B	Category 2
GHS Pictograms		
Signal word	Danger	Warning
Hazard Statement	H340: May cause genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H341: Suspected of causing genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
Precautionary Statement Prevention	P201, P202, P281	P201, P202, P281
Precautionary Statement Response	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405
Precautionary Statement Disposal	P501	P501

3.5.5 Additional classification considerations

It is increasingly accepted that the process of chemical-induced tumourigenesis in humans and animals involves genetic changes for example in proto-oncogenes and/or tumour suppresser genes of somatic cells. Therefore, the demonstration of mutagenic properties of substances in somatic and/or germ cells of mammals in vivo may have implications for the potential classification of these substances as carcinogens (see also Carcinogenicity, section 3.6, paragraph 3.6.2.2.6).

Annex

H

Criteria for testing reliability of animal and in vitro studies

To assess the reliability of animal and in vitro studies, the committee uses the criteria set by Klimisch et al. 1997.[28] A summary of the criteria of the reliability scores is given below. Only studies with a reliability score of 1 or 2 are considered in assessing genotoxicity and carcinogenicity.

Reliability 1 (reliably without restriction)

For example, guideline study (OECD, etc.); comparable to guideline study; test procedure according to national standards (DIN, etc.).

Reliability 2 (reliable with restrictions)

For example, acceptable, well-documented publication/study report which meets basic scientific principles; basic data given: comparable to guidelines/standards; comparable to guideline study with acceptable restrictions.

Reliability 3 (not reliable)

For example, method not validated; documentation insufficient for assessment; does not meet important criteria of today standard methods; relevant methodological deficiencies; unsuitable test system.

Reliability 4 (not assignable)

For example, only short abstract available; only secondary literature (review, tables, books, etc.).

Health Council of the Netherlands

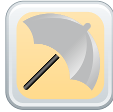
Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory opinions that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

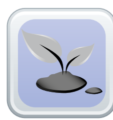
Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

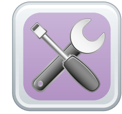


Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

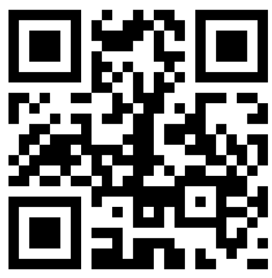


Environmental health
Which environmental influences could have a positive or negative effect on health?



Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.



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