Health Council of the Netherlands

2,6-Xylidine

Re-evaluation of the carcinogenicity and genotoxicity

2015/27

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *2,6-Xylidine*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-863062/DC/fs/246-S20
Bijlagen : 1
Datum : 13 november 2015

Geachte minister,

Graag bied ik u hierbij het advies *2,6-Xylidine* 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 de voorliggende adviezen 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 Gezondheid en omgeving 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

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2,6-Xylidine

Re-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. 2015/27, The Hague, November 13, 2015

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 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. 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 2,6-xylidine. 2,6-Xylidine wordt vooral gebruikt als chemisch intermediair in the productie van bestrijdingsmiddelen, kleurstoffen, antioxidantia, medicijnen, synthetische harsen en andere producten.

De commissie concludeert dat 2,6-xylidine beschouwd moet worden als verdacht kankerverwekkend voor de mens en beveelt aan de stof in categorie 2 te classificeren.* Op basis van de beschikbare gegevens beveelt de commissie verder aan om 2,6-xylidine te classificeren als mutageen voor geslachtscellen in categorie 2 (stof die reden geeft tot bezorgdheid voor de mens omdat zij mogelijk erfelijke mutaties in de geslachtscellen van mensen veroorzaakt). De stof kan kanker veroorzaken via een stochastisch genotoxisch werkingmechanisme.

* 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, such a re-evaluation was made for 2,6-xylidine. 2,6-Xylidine is mainly used as chemical intermediate in the manufacture of pesticides, dyestuffs, antioxidants, pharmaceuticals, synthetic resins and other products.

The committee concludes that 2,6-xylidine is suspected to be carcinogenic to man, and recommends classifying the compound in category 2.* Based on the available data, the committee furthermore recommends classifying the substance as a germ cell mutagen in 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 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 2,6-xylidine. An earlier evaluation of this substance was published in 2002.[1] The re-evaluation now includes a proposal for classification on germ cell mutagenicity.

1.2 Committee and procedures

The re-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. The submission letter (in English) to the State Secretary 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 2,6-xylidine, such an IARC-monograph is available, of which the summary and conclusion of IARC (1993) is inserted in Annex E.

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

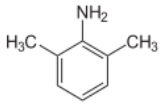
Additional data were obtained from the online databases Toxline, Medline and Chemical Abstracts, covering the period up to October 2015, using 2,6-xylidine, 2,6-dimethylaniline and CAS no 87-62-7 as key words in combination with key words representative for carcinogenesis and mutagenesis.

Chapter 2

Identity of the substance

2.1 Name and other identifiers of the substance

Table 1 Substance identity.

EC number	:	201-758-7
EC name	:	2,6-xylidine
CAS number (EC inventory)	:	87-62-7
CAS number	:	87-62-7
CAS name	:	2,6-xylidine
IUPAC name	:	2,6-dimethylaniline
CLP Annex I Index number	:	612-161-00-X
Molecular formula	:	$C_8H_{11}N$
Molecular weight range	:	121.2 g/mol
Structural formula	:	

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 the substance	: yellow liquid	IPCS INCHEM[2]	
Melting/freezing point	: 11.2°C	IPCS INCHEM[2]	
Boiling point	: 215°C	IPCS INCHEM[2]	
Relative density	: 0.98	IPCS INCHEM[2]	
Vapour pressure	: 0.02 kPa at 20°C	IPCS INCHEM[2]	
Surface tension	: -		
Water solubility	: 0.7 g/100 ml (20°C)	IPCS INCHEM[2]	
Partition coefficient n-octanol/water	: 1.84 log Pow	IPCS INCHEM[2]	
Flash point	: 91°C	IPCS INCHEM[2]	
Flammability	: -		
Explosive properties	: 1.3 - 6.9 vol. % in air	IPCS INCHEM[2]	
Self-ignition temperature	: 520°C	IPCS INCHEM[2]	
Oxidising properties	: No	ECHA[3]	
Granulometry	: -		
Stability in organic solvents	: Yes	ECHA[3]	
Dissociation constant (pK_a)	: 3.95 at 25°C	ECHA[3]	
Viscosity	: 1.7 mPa/s at 50°C; 1.16 mPa/s at 70°C	ECHA[3]	

2.4 International classifications

2.4.1 European Commission

2,6-Xylidine 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 substance is not classified for mutagenic activity. The classification by the European Commission dates from March 1999.

2.4.2 The Health Council of the Netherlands

In 2002, the Dutch Expert Committee on Occupational Standards, a Committee of the Health Council of the Netherlands concluded that 2,6-xylidine should be regarded as carcinogenic to humans (comparable with EU category 1B). Its potential genotoxicity was insufficiently investigated. Therefore, it was unclear

whether it was a genotoxic carcinogen. As a way of precaution, the Committee recommended to consider 2,6-xylidine as a genotoxic carcinogen at that time.[1]

2.4.3 IARC

In 1993, IARC concluded that there was inadequate evidence in humans for the carcinogenicity of 2,6-xylidine, and that there was sufficient evidence in experimental animals (see Annex E). Therefore, IARC classified the compound in Group 2B ('possibly carcinogenic to humans').[4]

Chapter 3

Manufacture and uses

3.1 Manufacture

Not relevant for classification.

3.2 Identified uses

2,6-Xylidine is used as a chemical intermediate in the manufacture of pesticides, dyestuffs, antioxidants, pharmaceuticals, synthetic resins, fragrances and other products (Ethyl corp. 1990, Kuney 1991).[5,6]

Chapter 4

Summary of toxicokinetics

The data presented below is a summary from evaluations and reviews by others, such as DECOS, IARC, ACGIH, IPCS and DFG.[1,2,4,7,8]

In humans haemoglobin adducts of 2,6-xylidine were found to be present at high levels in non-smokers with no known exposure to this compound. The adduct levels were somewhat lower in cigarette smokers (Gan et al., 2004 in Tao 2013).[9] The fact that these adducts were found in non-smokers may indicate environmental and iatrogenic exposure to 2,6-xylidine and its metabolite N-hydroxy-2,6-dimethylaniline, which, upon entry in eryhrocytes, may be oxidized to 2,6-dimethylnitrosobenzene and form a sulfinamide adduct with haemoglobin (Biyant et al., 1988 in IARC 1993).[4]

It has indeed been shown that 2,6-xylidine-haemoglobin adduct levels were elevated substantially in patients receiving lidocaine treatment: the drug lidocaine is known to be metabolised mainly to 2,6-xylidine. Methaemoglobinaemia has also been reported following lidocaine treatment in humans; like haemoglobin adduct formation, it can be attributed to a circulating N-hydroxy metabolite. A recent publication of Tao et al. (2013) concluded that hemoglobin adducts of 4-aminobiphenyl and 2,6-xylidine were significantly and independently associated with increased bladder cancer risk among lifelong nonsmokers in Shanghai, China.[9] This confirmed the results of the earlier study in Los Angeles (Gan et al., 2004 in Tao 2013).[9]

Metabolism studies in rat and dog showed that 2,6-xylidine is readily absorbed from the gastrointestinal tract and excreted mainly in the form of metabolites with the urine. Neither rats nor dogs demonstrated any differences in total urine excretion for 2,6-xylidine exposure over a 10-days trial period (Short et al., 1989).[10]

After oral administration of 200 mg/kg bw 2,6-xylidine to Osborne Mendel rats and beagle dogs, 4-hydroxy-2,6-xylidine and 3-methyl-2-aminobenzoic acid (dogs only) were detected as major and minor urinary metabolites, respectively (Short et al., 1989).[10]

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 + positive	Klimisch Score**	References
Micro-organisms					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E coli* WP2 *uvrA* YG1024 and YG1029 (O-acetyltransferase overexpressing strains)	0, 3, 10, 33, 100, 333,1,000, 2,500, 5,000 µg/plate Various trials: +/- 10% and 30% human liver S9; 10% and 30% rat liver S9	- (for both 2,6-xylidine and its metabolites)	1	Kirkland 2012[11]

Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, E coli WP2 uvrA	-S9 mix, TA100: 0, 39.1, 78.1, 156, 313, 625, 1,250, 2,500 µg/plate; –S9 mix TA1535, WP2 uvrA, TA98, TA1537: 0, 156, 313, 625, 1,250, 2,500, 5,000 µg/plate; +S9 mix TA100, TA1535, TA1537: 0, 39.1, 78.1, 156, 313, 625, 1250, 2,500 µg/plate +S9 mix WP2 uvrA, TA98: 0, 156, 313, 625, 1,250, 2,500, 5,000 µg/plate	+ (for TA100, TA1535 with S9-metabolic activation)	1	MHLW, Japan, 2005 (SIDS***report)[12]
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 100, 333, 1,000, 3,333, 9,900 µg/plate with rat and hamster S9 metabolic activation	-	1	NTP 1990, TR278[13]
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	TA98 and TA100: 0, 3 µmol/plate (calculated 0, 363 µg/plate); TA1535 and TA1537: 0, 0.03, 0.3, 3 and 30 µmol/plate (calculated as 0, 3.6, 36.4, 363, 3,636 µg/plate)	-	2	Florin et al., 1980[14]
Reverse mutation	*S. typhimurium* TA98, TA100	0, 4.1, 8.3, 12.4, 16.5, 24.6, 33.1 µmol/plate (calculated as 0, 497, 1,006, 1,503, 2,000, 2,981, 7,024 µg/plate)	± (TA100 with S9 only)	2	Kugler-Steigmeier et al., 1989[15]
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0, 33,100, 333, 666, 1,000, 3,333, 9,990 µg/plate	± (TA100 with S9 only)	2	Zeiger et al., 1988[16]
Reverse mutation	*S. typhimurium* TA100	0.83 µmoles	-	3 (only one strain, one dose and no positive control)	Hartman et al., 1979 [17]
Reverse mutation	*S. typhimurium* TA98, TA100,	+S9: 0, 4, 8 µmole/plate (calculated as 0, 485, 969 µg/plate -S9: 0, 5, 8 nmol/plate (calculated as 0, 0.6, 0.97 µg/plate)	+ (TA100 only)	3 (positive control missing)	Nohmi et al.,1984[18]
Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	0-15 µmole/plate (calculated as 0, 1818 µg/plate)	-	4 (controls missing; test details lacking)	Zimmer et al., 1980[19]
Transforming DNA or Rec assay B subtilis	*B subtilus*	0, 5, 10 mM	-	3 (test validity unknown)	Nohmi et al., 1984[18]
Mammalian cells					
Chromosome aberration	Chinese hamster ovary cells (CHO-W-B1)	-S9: 0, 900, 1,200 µg/mL; +S9: 0, 1,200-1,400 µg/mL	+ (at toxic doses)	2	Galloway et al., 1987[20]
Chromosome aberration	Chinese hamster lung cells (CHL/IU)	-S9: 303, 606, 1212 µg/mL; +S9: 0, 633, 744, 876 µg/mL	+	2	MHLW, Japan, 2005 (SIDS***report)[12]

Chromosome aberration	Chinese hamster lung cells	- S9 mix (short-term treatment); 0, 303, 606, 1,212 µg/mL +S9 mix (short-term treatment): 0, 633, 744, 876 µg/mL	+	2	Echa registration data, vitro 004 study report 2009 (echa.europe.eu)
Gene mutation	Mouse lymphoma L5178Y cells, *tk locus*	Concentrations not given; with and without S9 mix	+	4 (abstract only)	Rudd et al., 1983[21]

* + or -S9, with or without metabolic activation system;
** See Annex H;
*** SIDS (Screening Information DataSet for High Production Volume Chemicals) studies are internationally accepted studies which receive a Klimisch score 2 according to the Committee.

As summarized in Table 3, studies on the mutagenicity in *Salmonella typhimurium* are conflicting; some positive responses are observed for TA100 and TA1535 with S9 metabolic activation system. 2,6-Xylidine induces chromosomal aberrations in Chinese Hamster Ovary and Lung cells in vitro.

5.1.2 *In vivo data*

Data on the in vivo mutagenicity testing are presented in Table 4.

Table 4 Summary of in vivo mutagenicity studies (animal studies).

Method	Animal	Exposure conditions	Results	Klimisch score*	References
Somatic cell mutagenicity					
Transgenic Rodent Gene Mutation Assay	Mouse, Muta, 4-5 males/group	100 mg/kg bw, (oral gavage, 4x at weekly intervals); DNA extraction from nasal tissue bone marrow and liver; analysis of total and mutant plaques	+ (more than 2x increase in mutation frequency of lacZ and cII genes in nasal tissue; Transitions AT to GC and transversions GC to TA)	2	Hayashi et al., 2000 (SIDS** report)[12]
Micronucleus	ICR mouse, bone marrow 6 mice/dose	87.5, 175, 350 mg/kg bw (oral) Cytotoxicity tested by PCE/NCE ratio	- (no cytotoxicity observed; systemic availability)	2	Parton et al., 1988[22]
Micronucleus	ICR mouse, bone marrow 6 mice/dose	75, 375 mg/kg bw (oral; 1,2 or 3 applications of each dose); Cytotoxicity tested by PCE/NCE ratio	- (no cytotoxicity observed; limit value for evaluation; systemic availability)	2	Parton et al., 1990[23]
Micronucleus	ddY mouse, male, Peripheral blood, 3 male/group	200 mg/kg bw (once oral gavage) Cytotoxicity tested by PCE/NCE ratio	- (no positive control, no cytotoxicity observed)	2	Hayashi et al., 2000 (SIDS** report)[12]

Micronucleus	Mouse, Muta, Bone marrow 5 male/group	100 mg/kg bw (once oral gavage)	- (no data on cytotoxicity/ only one dose tested)	2	Hayashi et al., 2000 (SIDS** report)[12]
Germ cell mutagenicity					
Sex-linked recessive lethal mutations	*Drosophila melanogaster*, male	0, 100 ppm feeding; 0, 4,000 ppm injection	-	3 (classification based on studies in mammalians; no OECD guideline anymore)	Foureman et al.,1994[24]
Sex-linked recessive lethal mutations	*Drosophila melanogaster*, male	0, 330 ppm feeding	-	3 (classification based on studies in mammalians; no OECD guideline anymore) 3 (classification based on studies in mammalians; no OECD guideline anymore)	Zimmering 1989[25]

* See Annex H.
** SIDS (Screening Information DataSet for High Production Volume Chemicals) studies are internationally accepted studies which receive a Klimisch score 2 according to the Committee.

Germ cells

2,6-Xylidine was negative in the sex-linked recessive lethal mutation test in *Drosophila melanogaster* .[24,25] However, the Committee considers this test species not relevant for humans.

Somatic cells

In a transgenic muta-mice assay 2,6-xylidine showed an increased mutant frequency of lacZ and cII genes in the nasal tissue.[12] No increase in the frequency of micronuclei in the bone marrow or peripheral blood of mice was observed.

5.2 Human information

No mutagenicity studies in humans were found.

5.3 Other relevant information

Tabel 5 shows studies on DNA damage.

Table 5 Summary of other information on DNA damage.

Method	Cell type	Concentration	Results and remarks	Klimisch Score*	References
In vivo tests					
DNA-binding study (Covalent Binding Index)	Rats, 6 /group; Two treatment regimes for each target tissue, liver and nasal epithelial cells (ethmoid turbinate)	One group pretreated orally with unlabelled 262.5 mg/kg bw/day for 9 days followed by a single dose of labelled compound (intraperitoneal 87.2 µCi ^{14}C) The other group treated once with labelled compound (intraperitoneal 87.2 µCi ^{14}C)	+ (only in pretreatment group in the ethmoid turbínate tissue of the nose)	2	Short et al., 1989[26]
Comet assay**	Mouse male ddY 3 males/group; Bone marrow, liver, kidney and lung tissue isolation 3 hr after last treatment; Examination 3 hr and 24 hr after last treatment	200 mg/kg bw, 4 times at weekly intervals, oral gavage	+ (in lung, kidney and liver at 3hr after treatment)	2 (no positive control)	Hayashi et al., 2000 (SIDS*** report)[12]
Comet assay**	Mouse male ddY 4/group; Examination of stomach, colon, liver, kidney, bladder, lung, brain and bone marrow sampled 3, 8 and 24 h after treatment	350 mg/kg bw; Single oral gavage	+ (in stomach, urinary bladder, lung and brain at 8 h after treatment)	2	Sasaki et al., 1999[27]
Unscheduled DNA synthesis	Rats F344 hepatocytes 3 male/group	0, 40, 200, 850 mg/kg bw, single oral gavage	-	2	Mirsalis 1989[28]
Testicular DNA synthesis test	Mouse, male testis	200 mg/kg bw, single oral gavage	-	3 (method not validated)	Seiler, 1977 [29]
In vitro tests					
DNA repair, host mediated assay, in vitro	Repair-deficient *E coli* K12 343/636 uvrB+/recA+/Lac-; *E coli* 343/591 uvrB-/recA-/lac+	Up to 812 mmol/L; + and -S9; positive and negative controls included.	-	3 (method not validated)	Hellmer and Bolcsfoldi 1992[30]

* See Annex H.

** Comet assay and alkaline elution assay: DNA single and double strand breaks, DNA cross-links.

*** SIDS (Screening Information DataSet for High Production Volume Chemicals) studies are internationally accepted studies which receive a Klimisch score 2 according to the Committee.

Table 6 Summary of genotoxicity studies.

Method	Cell type	Concentration	Results and remarks	Klimisch Score*	References
In vitro tests using rodent cells					
Sister chromatide exchange	CHO cells	-S9: 0, 301, 348 and 400 µg/mL +S9: 0, 33, 327, 1,510 µg/mL	+ (+/-S9; -S9 a dose related increase)	2	Galloway et al., 1987[20]

*See Annex H.

Germ cells

No DNA damage tests in germ cells were found.

Somatic cells

The studies listed in Table 5 show that unlabelled 2,6-xylidine bound covalently to the DNA of the ethmoid turbinate tissue of the nose of rats after oral pretreatment. 2,6-Xylidine does not induce unscheduled DNA synthesis in hepatocytes of male Fisher-344 rats. Two comet assays in mice at 200 and 350 mg/kg bw showed that 2,6- xylidine induces DNA strand breaks in various organs such as the lung, the liver, the kidney, the stomach, the urinary bladder and the brain. A dose-related increase in the incidence of sister chromatid exchanges in CHO cells was observed in a study of Galloway et al., (1987).[20]

5.4 Summary and discussion of mutagenicity

Below, only data are summarized of reliable (with or without restrictions) experimental design according to the Klimisch criteria (See Annex H).[31]

Germ cell genotoxicity

As no relevant genotoxicity studies of 2,6-xylidine in germ cells were found, the Committee can not conclude that 2,6-xylidine is genotoxic in germ cells.

Mutagenicity in bacteria and mammalian cells

Studies on the mutagenicity of 2,6-xylidine in *Salmonella typhimurium* are conflicting, but show some positive results for TA100 and TA1535. 2,6-Xylidine was reported to induce an increase in cells with chromosomal aberrations in

hamster ovary and lung cells in vitro. In vivo a negative micronucleus tests in mice bone marrow and peripheral blood was reported. In a transgenic mutation assay wit Muta^{MT}mice, however, an increased mutant frequency in the nasal tissue was observed.

DNA damage and cytogenicity

In vitro 2,6-xylidine showed a dose-related increase in the incidence of sister chromatid exchanges in CHO cells. Two comet assays with 2,6-xylidine in mice showed DNA strand breaks in various organs. Also covalent binding to the DNA of the nasal tissue of rats was found.

Overall, the Committee concludes that 2,6-xylidine is mutagenic in mammalian cells and acts by a stochastic genotoxic mechanism.

5.5 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, and the test with drosophila was not relevant for humans. Overall, due to a lack of data the Committee concludes that there is no evidence for in vivo heritable germ cell mutagenicity of 2,6-xylidine.

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). Evidence has been found for in vivo mutagenicity testing in the transgenic mouse. Regarding the second part of the criterion, there is no evidence that 2,6-xylidine 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 2,6-xylidine 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 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).

As summarized in the previous section, according to the Committee, there is positive evidence from the transgenic assay in mice and also from in vitro chromosomal aberration experiments. There is also some evidence that 2,6-xylidine is able to induce DNA damage in vivo and sister chromatid exchanges in vitro. Therefore, the Committee recommends classifying the substance in category 2.

5.6 Conclusions on classification and labelling

Based on the available data, the Committee recommends classifying the compound as a germ cell mutagen in 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 substance acts by a stochastic genotoxic mechanism.

Chapter 6

Carcinogenicity

6.1 Non-human information

Data on animal carcinogenicity studies are summarized in Table 7.

Table 7 Summary of animal carcinogenicity studies on 2,6-xylidine exposure.

Species	Design	Exposure levels	Observations and remark (Klimisch score)*	References
Oral: feeding				
Rat CD	Multigeneration – reproduction-carcinogenicity study; F0 generation treated before and during pregnancy and lactation; F1 generation (56/sex/group) was treated for 102 weeks. Gross necropsy, haematology, clinical biochemistry, and histopathological examination	F0 and F1: 0, 300, 1,000, 3,000 ppm feeding (actual dose levels m: 0, 12, 40, 120 mg/kg, f: 0, 15, 50, 150 mg/kg bw/day)	*Klimisch-score*: 2 (no data on parent generation; instability of feed: 40% loss within one week of which 70-80% due to evaporation. So rats also exposed by inhalation *General:* Decreased body weight gain for mid dose males (5-9%) and high dose males and females (>10%). No compound related clinical signs. Reduced survival rates at 3,000 ($p < 0.001$) and 1,000 ppm males towards end of 2-yr exposure period. Acute inflammation (rhinitis), epithelial hyperplasia, and squamous metaplasia occurred at increased incidences in high dose male and female rats	NTP, 1990[13]

	Neoplastic lesions: Nasal cavity: increased incidence of adenocarcinomas and papillary adenomas in 3,000 ppm males. Undifferentiated sarcoma and rhabdomyosarcomas in 3,000 ppm animals (for tumour incidences see table 8)

* See Annex H.

Table 8 Tumour incidences in nasal cavity of rats, which were given 2,6-xylidine in the diet for 2 years.[13]

Exposure level (ppm)	0	300	1,000	3,000
Male rats				
Nasal cavity:				
• Rhapdomysarcoma	0/56	0/56	0/56	2/56
• Papillary adenoma	0/56	0/56	2/56	10/56**
• Carcinoma NOS	0/56	0/56	0/56	26/56**
• Carcinoma or adenocarcinoma	0/56	0/56	0/56	28/56**
• Adenoma, adenocarcinoma or carcinoma	0/56	0/56	2/56	33/56**
Subcutaneous tissue:				
• Fibroma	0/56	1/56	2/56	4/56
• Fibroma or fibrosarcoma	0/56	2/56	2/56	5/56*
Female rats				
Nasal cavity:				
• Sarcoma	0/56	0/56	0/56	1/56
• Rhapdomysarcoma	0/56	0/56	0/56	2/56
• Papillair adenoma	0/56	0/56	0/56	6/56*
• Adenoma NOS	0/56	0/56	1/56	0/56
• Carcinoma NOS	0/56	0/56	1/56	24/56**
• Adenoma or Carcinoma	0/56	0/56	2/56	29/56**
Subcutaneous tissue:				
• Fibroma	0/56	2/56	1/56	4/56
• Fibrosarcoma	1/56	0/56	1/56	3/56*
• Fibroma or fibrosarcoma	1/56	2/56	2/56	6/56
Liver:				
• Neoplastic nodule	0/56	1/56	2/56	4/55*
• Neoplastic nodule or hepatocellular carcinoma	1/55	1/56	3/56	5/55

Fischer exact test: * $p \leq 0.05$, ** $p \leq 0.001$.

6.1.1 *Carcinogenicity: oral administration*

2,6-Xylidine was tested for carcinogenicity in one study in rats by pre- and postnatal administration via the diet. Neoplastic lesions are described in detail in Table 8. The substance induced adenoma and carcinoma as well as several

sarcoma in the nasal cavity. The substance also produced subcutaneous fibroma and fibrosarcoma in both males and females and increased the incidence of neoplastic nodules in the livers of female rats.

6.2 Human information

No human carcinogenicity data were found.

6.3 Other relevant information

Table 9 Cell transformation and initiation/promotion studies with 2,6-xylidine.

Method	Cell type	Concentration	Results and remarks (Klimisch Score)*	References
Initiation/promotion studies				
Nasal carcinogenesis model (2-stage)	F344 rats, 15-30 per group Duration 52 weeks Complete necropsy; Histopathological analysis, immunohistochemical staining and electron microscopic analysis of nasal tissue	Initiation with a single subcutaneous injection of 2,400 mg/kg bw N-bis(2-hydroxypropyl)nitrosamine (DHPN). One week later exposure to 0, 3,000 ppm 2,6-xylidine by diet for 52 weeks. Positive and negative controls included. (actual intake 164.8 mg/kg bw/day for DHPN + 2,6-xylidine and 155.9 mg/kg bw/day for 2,6-xylidine-alone groups)	2 (no validated method) Significant increased incidence of carcinomas, epithelial hyperplasia and dysplastic foci in the nose; Neoplastic lesions nose at 0; DHPN alone; 3000 ppm 2,6-xylidine alone; DHPN + 3000 ppm 2,6-xylidine: Adenomas: 0/10; 4/20, 0/15, 8/30 Carcinomas: 0/10, 1/20, 0/15, 10/30 ($p< 0.001$) Immunohistochemical staining suggests that all lesions arise from epithelial cells including Bowman's glands, rather than from mesenchymal cells of olfactory neuroepithelial (sensory) cells. Electron microscopy suggests that Bowman's glands are the target of 2,6-xylidine giving rise to nasal carcinomas after DHPH-initiation	Koujitani et al., 1999[32] Koujitani et al., 2000, 2001[33,34]
Cell transformation assay				
Cell transformation assay	Balb/c-3T3cells	2.06, 4.04, 6.07, 8.09 mM (2 trials)	2 + (cytotoxic at the two highest doses)	Matthews et al., 1993[35]

* See Annex H.

2,6-Xylidine showed a positive response in a transformation assay with Balb-c/3T3 cells and exerted tumour-promoting effects in the nose of rats.

6.4 Summary and discussion of carcinogenicity

Regarding the single carcinogenicity study in rat of NTP (1990), feeding of 2,6-xylidine induced adenoma, carcinoma and sarcoma in the nasal cavity and fibroma and fibrosarcoma in subcutaneous tissue. 2,6-Xylidine further increased the incidence of neoplastic nodules in liver of female rat. The Committee considers these nasal tumours of relevance to humans. A two-stage nasal carcinogenesis study of Koujitani (1999) showed tumour-promoting activity of 2,6-xylidine.[32] Based on these findings, the Committee concludes that there is limited evidence of carcinogenicity from animal experiments.[13,32-34]

6.5 Comparison with criteria

No data on the carcinogenicity of 2,6-xylidine in humans are available. Therefore the Committee cannot take a final conclusion on the carcinogenic potential of 2,6-xylidine in humans.

In animal data, the Committee found limited evidence of carcinogenicity, since a causal relationship was established between malignant tumours in animals, and chronic oral administration to 2,6-xylidine in a single oral carcinogenicity study in rat. According to the CLP classification criteria, 2,6-xylidine should, therefore, be classified as “suspected to be carcinogenic to man”, which corresponds to classification in category 2. Supporting evidence for its carcinogenic potential is that the substance showed tumour-promoting activity and genotoxic properties in at least mammalian cells in vivo.

6.6 Conclusions on classification and labelling

The Committee concludes that 2,6-xylidine is “suspected to be carcinogenic to man”, and recommends classifying the substance in category 2.

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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 Quality of Life, Zeist; professor of translational toxicology, Wageningen University and Research Centre, Wageningen
- J. Van Benthem
  Genetic toxicologist, National Health Institute for Public health and the Environment, Bilthoven
- P.J. Boogaard
  toxicologist, SHELL International BV, The Hague
- G.J. Mulder
  emeritus professor of toxicology, Leiden University, Leiden
- M.J.M. Nivard
  molecular biologist and genetic toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  epidemiologist, Maastricht University, Maastricht
- E.J.J. van Zoelen
  professor of cell biology, Radboud University Nijmegen, 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, J.A.A. Muller, toxicologist from Bureau Reach, National Health Institute for Public Health and the Environment, Bilthoven.

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 *2,6-xylidine*
Your Reference: DGV/MBO/U-932542
Our reference : U-863062/DC/fs/246-S20
Enclosed : 1
Date : November 13, 2015

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to 2,6-xylidine.

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 Health and the Environment.

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 organisations and persons have commented on the draft document:

- D. Coggon, University of Southampton, Southampton General Hospital, UK
- T.J. Lentz, L. Rojanasakul, National Institute for Occupational Safety and Health (NIOSH), USA .

All comments received and the response of the Committee will be publicly available (www.gezondheidsraad.nl) from the moment of presentation of the final report.

Annex **E**

IARC evaluation and conclusion

2,6-Dimethylaniline

Vol. 57 (1993) (p 323).

Summary of Data Reported and Evaluation.

Exposure data

2,6-Dimethylaniline is used as a chemical intermediate in the manufacture of pesticides, dyestuffs, antioxidants, pharmaceuticals and other products. It is a metabolite of the xylidine group of anaesthetics, including, for example, lidocaine, and is produced by the reduction of certain azo dyes by intestinal microflora. It may also enter the environment through degradation of certain pesticides.

Human carcinogenicity data

No data were available to the Working Group.

Animal carcinogenicity data

2,6-Dimethylaniline was tested for carcinogenicity in one study in rats by pre- and postnatal administration in the diet. It induced adenomas and carcinomas as well as several sarcomas in the nasal cavity. It also produced subcutaneous fibromas and fibrosarcomas in both males and females and increased the incidence of neoplastic nodules in the livers of female rats.

Other relevant data

Methaemoglobinaemia has been observed in humans and animals exposed to 2,6-dimethylaniline. The metabolism of 2,6-dimethylaniline in humans and rats appears to be similar and gives rise to a characteristic haemoglobin adduct in both species.

2,6-Dimethylaniline gave conflicting results for gene mutation in bacteria. Sister chromatid exchanges and chromosomal aberrations were included in cultured mammalian cells. The compound bound covalently to DNA in rat tissues but did not induce micronuclei in the bone marrow of mice treated in vivo.

Evaluation

There is inadequate evidence in humans for the carcinogenicity of 2,6-dimethylaniline. There is sufficient evidence in experimental animals for the carcinogenicity of 2,6-dimethylaniline.

Overall evaluation

2,6-Dimethylaniline is possibly carcinogenic to humans (Group 2B).

Synonyms: 1-Amino-2,6-dimethylbenzene, 2-Amino-1,3-dimethylbenzene, 2-Amino-1,3-xylene, 2-Amino-*meta*-xylene, 2,6-Dimethylphenylamine, *ortho*-Xylidine, 2,6-*meta*-Xylidine, 2,6-Xylylamine.

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.[36]

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.[31] 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.