Xylidine (isomers)

Evaluation of the carcinogenicity and genotoxicity

Dutch Expert Committee on Occupational Standards,

a committee of the Health Council of the Netherlands

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies over xylidine (isomeren)
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-451/JR/tvdk/459-N36
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Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In dat kader bied ik u hierbij een advies aan over de kankerverwekkende eigenschappen van xylidine (isomeren). Het is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

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

Hoogachtend,

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Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

Nr 2002/10OSH, The Hague, 16 April 2002

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. In het voorliggende rapport neemt de Commissie WGD van de Raad, die deze beoordelingen verricht, enkele xylidine-isomeren onder de loep. De commissie heeft haar oordeel gegoten in door de Europese Unie aangegeven termen.

2,3-Xylidine

De commissie is van mening dat 2,3-xylidine onvoldoende is onderzocht. Zij adviseert daarom 2,3-xylidine niet te classificeren.

2,4-Xylidine

De commissie is van mening dat 2,4-xylidine onvoldoende is onderzocht. Zij adviseert daarom 2,4-xylidine niet te classificeren.

2,5-Xylidine

De commissie is van mening dat 2,5-xylidine onvoldoende is onderzocht. Zij adviseert daarom 2,5-xylidine niet te classificeren.

2,6-Xylidine

De commissie concludeert dat 2,6-xylidine beschouwd moet worden als kankerverwekkend voor de mens (vergelijkbaar met EU-categorie 2). De genotoxische eigenschappen zijn onvoldoende onderzocht. Het is daarom niet bekend of de stof een genotoxisch carcinogeen is. De commissie raadt voorzichtigheidshalve aan om 2,6-xylidine voorlopig als een genotoxische stof te beschouwen.

3,4-Xylidine

De commissie is van mening dat 3,4-xylidine onvoldoende is onderzocht. Zij adviseert daarom 3,4-xylidine niet te classificeren.

3,5-Xylidine

De commissie is van mening dat 3,5-xylidine onvoldoende is onderzocht. Zij adviseert daarom 3,5-xylidine niet te classificeren.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the carcinogenic properties of substances at the workplace and proposes a classification with reference to the EU-directive. This evaluation is performed by the Dutch Expert Committee on Occupational Standards. The present report contains an evaluation by the committee on the carcinogenicity of xylidine (isomers).

2,3-Xylidine

The committee concludes that 2,3-xylidine was insufficiently investigated. Therefore the committee recommends not to classify 2,3-xylidine.

2,4-Xylidine

The committee concludes that 2,4-xylidine was insufficiently investigated. Therefore the committee recommends not to classify 2,4-xylidine.

2,5-Xylidine

The committee concludes that 2,5-xylidine was insufficiently investigated. Therefore the committee recommends not to classify 2,5-xylidine.

2,6-Xylidine

The committee concludes that 2,6-xylidine should be regarded as carcinogenic to humans (comparable with EU category 2). Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is a genotoxic carcinogen. As a way of precaution, the committee recommends, for the time being, considering 2,6-xylidine as a genotoxic carcinogen.

3,4-Xylidine

The committee concludes that 3,4-xylidine was insufficiently investigated. Therefore the committee recommends not to classify 3,4-xylidine.

3,5-Xylidine

The committee concludes that 2,5-xylidine was insufficiently investigated. Therefore the committee recommends not to classify 3,5-xylidine.

Chapter 1

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. The Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to study the carcinogenic properties of substances and to propose a classification with reference to an EU-directive (annex A and I). This task is carried out by the Council's Dutch Expert Committee on Occupational Standards, hereafter called the committee.

The evaluation of the carcinogenicity of a substance is based, if possible, on IARC* evaluations. The original publications are not reviewed and evaluated in the text of the report, but the overall conclusion of the IARC on the carcinogenic properties is included (annex D).

In addition to classifying substances with respect to their possible carcinogenicity according to the EU Guidelines, the committee also assesses the genotoxic properties of the substances in question. The committee expresses its conclusions in the form of standard sentences (annex H).

* International Agency for Research on Cancer

1.2 Committee and procedures

The present report contains evaluations by the committee of the carcinogenicity of different xylidine isomers. The members of the committee are listed in annex B. The first draft of this report was prepared by MI Willems, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

In 2000 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

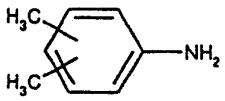
1.3 Data

The evaluation of the carcinogenicity of the xylidine isomers has been based on IARC evaluations (IARC89, IARC87). The conclusion of the IARC on mutagenic or carcinogenic properties of xylidine isomers, if available, is included in this report. Where relevant, the original publications were reviewed and evaluated in the text.

In addition, literature has been retrieved from the on-line data bases Cancerlit, Toxline, and Medline, covering the period 1966 to May 2001.

Chapter **2**

Xylidine (different isomers)*

A chemical name	:	xylidine
CAS registry number	:	1300-73-8 (mixed isomers)
EINECS number	:	215-091-4
EEC number	:	612-027-00-0
IUPAC name	:	xylidine
Synonyms	:	dimethylaniline; aminodimethylbenzene; aminoxylene
Description	:	pale yellow to brown liquid Commercial xylidine is a mixture in which 2,4-, 2,5-, and 2,6-isomers dominate
Application	:	Commercial xylidine is used as a raw material in the manufacture of dyes, pharmaceuticals and other organic compounds
Molecular formula	:	$C_8H_{11}N$
Structure		
Molecular weight	:	121.2
Boiling point (101.3 kPa)	:	213-226 °C (commercial)

* data from ACG91

Relative density (101.3 kPa)	:	0.97-0.99 (commercial)
Vapour pressure (20°C)	:	< 0.13 kPa (commercial)
Solubility in water	:	sparingly soluble
Solubility in organic solvents	:	miscible with ethanol and diethyl ether
Flash point	:	94.5 °C (commercial)
EU classification	:	T : toxic R23/24/25 : toxic by inhalation, in contact with skin, and if swallowed R33 : danger of cumulative effects

An overview of the physico-chemical properties of the different xylidine isomers is given in annex E. The main mutagenicity studies with the xylidine isomers are summarised in annex F and a list on the conclusions about carcinogenicity is given in annex G.

Chapter 3

2,3-Xylidine

3.1 IARC conclusion

No IARC evaluation on the carcinogenicity of 2,3-xylidine is available.

3.2 Human data

3.2.1 IARC data

No data were available.

3.2.2 Additional data

No additional human carcinogenicity data were retrieved.

3.3 Animal data

3.3.1 IARC data

No data were available.

3.3.2 Additional data

No additional animal carcinogenicity data were retrieved from literature databases.

3.4 Mutagenicity and genotoxicity

3.4.1 IARC data

No data were available

3.4.2 Additional information

2,3-Xylidine was mutagenic in a plate incorporation assay with *Salmonella typhimurium* TA100 with, but not without metabolic activation (Noh84, Zim80). The compound did not increase the number of revertants in a spot test using strains TA98, TA100, TA1535, and TA1537 with or without S-9 mix (Flo80). This spot test is considered of restricted value, because it will detect only strong mutagens.

In a briefly reported *in vivo* test in male mice (n= 3 or 4), no inhibition of testicular DNA synthesis, as measured by incorporation of ^{3}H-thymidine, was observed after a single oral dose of 200 mg of 2,3-xylidine per kg bw (Sei77).

3.5 Evaluation

No data on carcinogenicity in humans and animals are available.

Data on the mutagenicity and genotoxicity are insufficient. The committee considers 2,3-xylidine as a bacterial mutagen. No conclusion on the genotoxicity of 2,3-xylidine according to EC standards is possible.

3.6 Recommendation for classification

The committee concludes that 2,3-xylidine was insufficiently investigated. The committee, therefore, recommends not to classify 2,3-xylidine.

Chapter 4

2,4-Xylidine

4.1 IARC conclusion

In 1978, IARC concluded that there was no adequate evidence for carcinogenicity of 2,4-xylidine in humans and inadequate evidence in experimental animals. 2,4-Xylidine could not be classified as to its carcinogenicity to humans (Group 3) (IARC78, IARC87).

4.2 Human data

4.2.1 IARC data

No data were presented.

4.2.2 Additional data

No additional human carcinogenicity data were retrieved.

4.3 Animal data

4.3.1 IARC data

Data reported in an abstract showed that 2,4-xylidine increased the incidence of subcutaneous fibromas or fibrosarcomas and hepatomas in rats in a 2-year feeding study (IARC78).

4.3.2 Additional data

In a carcinogenicity study, male rats (n= 25/group) received 2,4-xylidine in their diet at dose levels of 2,000 or 4,000 mg/kg diet for 13 months. A group of 25 animals served as matched controls and a group of 111 rats as pooled controls. The animals were observed for two years. No carcinogenic effect of the compound was reported. The committee noted that necropsy was done only on animals that survived for more than six months. No further information was provided (Wei78).

Two groups of mice (n= 25/group/sex) received 2,4-xylidine in the diet for 18 months (125 or 250 mg/kg diet). Groups of 25 animals of each sex served as matched controls and a group of 102 females and 99 males as pooled controls. The incidence of animals having lung tumours (no histopathological identification reported) was significantly increased in female mice only at the high dose group (11/19 compared with 5/22 in matched controls and 32/102 in pooled controls). In male mice, no carcinogenic effect of the compound was reported. Again, the committee noted that necropsy was done only on animals that survived for more then six months (Wei78).

No additional animal carcinogenicity data were retrieved.

4.4 Mutagenicity and genotoxicity

4.4.1 IARC data

No data on the mutagenicity of 2,4-xylidine were included in the IARC monographs (IARC78, IARC87).

4.4.2 Additional information

2,4-Xylidine is positive in a plate incorporation assay with *Salmonella typhimurium* TA100 with a metabolic activation system (Noh84, Zim80).

Primary DNA damage in Chinese hamster V79 lung fibroblasts was studied in the presence of a metabolic activation system (S9) by means of alkaline elution. In this assay, 2,4-xylidine was found negative at all concentrations tested (1.0 and 3.0 mM) for 2- and 4-hour exposure periods (Zim80).

In a briefly reported DNA-repair test in cultured primary rat hepatocytes, 2,4-xylidine was found positive at concentrations of more than 10 µM (Wil89).

In a briefly reported *in vivo* test in male mice (n=3 or 4), a significant inhibition of testicular DNA synthesis as measured by incorporation of ^{3}H-thymidine, was observed after a single oral dose of 200 mg 2,4-xylidine per kg bw (Sei77).

In a recently published *in vivo* study, B6C3F1 male mice (n=6) were once treated with 100 or 200 mg 2,4-xylidine/kg bw by intraperitoneal injection. In the liver cells of the treated mice, significantly more DNA damage was measured than in negative control animals (Prz99). DNA damage was measured with the 'Comet' assay, which is a rapid and sensitive method for the detection of DNA single- and double-strand breaks as well as alkali-labile sites.

4.5 Evaluation

No data on carcinogenicity in humans are available.

There is inadequate evidence on the carcinogenicity of 2,4-xylidine from animal experiments. The incidence of lung tumours was increased in female mice, fed 250 mg 2,4-xylidine per kg diet for 18 months, compared with controls. However, the committee finds this study inadequate, because necroscopy was done only on animals that survived for more than six months and the type of tumours were not identified.

There is limited evidence on the mutagenicity en genotoxicity of 2,4-xylidine. The compound is mutagenic in bacteria in the presence of a metabolic activation system. *In vitro*, 2,4-xylidine increased DNA-repair in rat hepatocytes. No information is available on *in vivo* mutagenicity or on DNA interaction in somatic cells, but inhibition of testicular DNA synthesis is reported following oral treatment of mice. Based on the available genotoxicity tests, 2,4-xylidine cannot be classified as a mutagen according to EC standards.

4.6 Recommendation for classification.

Concerning the carcinogenic effects, the committee concludes that 2,4-xylidine was insufficiently investigated. The committee, therefore, recommends not to classify 2,4-xylidine.

Chapter 5

2,5-Xylidine

5.1 IARC conclusion

In 1978, IARC concluded that there was no adequate evidence for carcinogenicity of 2,5-xylidine in humans and inadequate evidence in experimental animals. 2,5-Xylidine could not be classified as to its carcinogenicity to humans (Group 3) (IARC78, IARC87).

5.2 Human data

5.2.1 IARC data

No data were presented

5.2.2 Additional data

No additional human carcinogenicity data were retrieved.

5.3 Animal data

5.3.1 IARC data

Data reported in an abstract show that 2,5-xylidine produced an increased incidence of subcutaneous fibromas or fibrosarcomas and hepatomas in rats in a 2-year feeding study (IARC78).

5.3.2 Additional information

In a carcinogenicity study, male rats (n=25/group) received 6 or 12 g 2,5-xylidine per kg of diet for 13 months. A group of 25 animals served as matched controls and a group of 111 rats as pooled controls. The animals were observed for two years. A significantly increased incidence of subcutaneous fibromas and fibrosarcomas was reported in both dose-groups compared with pooled controls ($p<0.025$), but not with matched controls (low 7/17, high 9/17, pooled controls 18/111, matched controls 8/17). The committee noted that necropsy was performed only on animals that survived for more than six months. No further information was provided (Wei78).

Two groups of mice (n=25/group/sex) were administered 6 or 12 g 2,5-xylidine per kg of diet for 18 months. Groups of 25 animals of each sex served as matched controls and a group of 102 females and 99 males as pooled controls. In low-dosed female mice, the incidence of liver tumours (no microscopic identification reported) was non-significantly enhanced compared with both pooled and matched control groups (low 5/16, high 2/20, pooled 1/102, matched 0/11). In male mice, vascular tumours were elevated in both dose groups compared with that of pooled controls ($p<0.025$) (low 5/18, high 7/19, matched controls 2/16, pooled 5/99). The incidence of liver tumours in males was not elevated compared with any of the control groups. The committee noted that the number of animals in the study is limited and necropsy was done only on animals that survived for more than six months (Wei78).

No additional animal carcinogenicity data were retrieved.

5.4 Mutagenicity and genotoxicity

5.4.1 IARC data

No data on the mutagenicity of 2,5-xylidine were included in the IARC monographs (IARC78, IARC87).

5.4.2 *Additional information*

2,5-Xylidine was mutagenic in a plate incorporation assay with *Salmonella typhimurium* TA100 with metabolic activation (Noh84, Zim80). In a spot test with TA98, TA100 TA1535, and TA1537 with or without an metabolic activation system (S-9 mix), the compound did not increase the number of revertants (Flo80). The committee considers this test of restricted value, because it detects only strong mutagens.

Primary DNA damage in lung fibroblasts of Chinese hamsters (V79 cells) was studied in presence of a metabolic activation system by means of alkaline elution. In this assay, 2,5-xylidine was found negative at all concentrations tested (1.0 and 3.0 mM) for 2- and 4-hour exposure periods (Zim80).

2,5-Xylidine was positive in a DNA-repair test using cultured primary rat hepatocytes at a concentration of more than 10 µM (Wil89).

In a briefly reported *in vivo* test in male mice (n= 3 or 4), significant inhibition of testicular DNA synthesis, as measured by incorporation of ^{3}H-thymidine, was seen after a single oral dose of 200 mg of 2,5-xylidine per kg bw (Sei77).

5.5 Evaluation

No data on carcinogenicity in humans are available.

There is inadequate evidence on the carcinogenicity of 2,5-xylidine of animal experiments.

Evidence for mutagenicity or genotoxicity is very limited. 2,5-Xylidine is mutagenic in a bacterial test system. *In vitro*, the compound increased DNA repair activity in mammalian cells. No *in vivo* information on mutagenicity or genotoxicity of 2,5-xylidine is available. In a limited *in vivo* test, inhibition of testicular DNA synthesis is reported following oral treatment of mice. Based on the available genotoxicity tests, 2,5-xylidine cannot be classified as a mutagen according to EC standards.

5.6 Recommendation for classification

The committee concludes that 2,5-xylidine was insufficiently investigated. The committee, therefore, recommends not to classify the 2,5-xylidine.

Chapter 6

2,6-Xylidine

6.1 IARC conclusion

In 1993, IARC concluded that concerning the carcinogenicity of 2,6-xylidine there was inadequate evidence in humans and sufficient evidence in experimental animals. 2,6-Xylidine is classified as possibly carcinogenic to humans (Group 2B). See annex D (IARC93).

6.2 Human data

6.2.1 IARC data

No data were presented

6.2.2 Additional data

No additional human carcinogenicity data were retrieved.

6.3 Animal data

6.3.1 IARC data

IARC's conclusion is based on a well-conducted National Toxicology Program (NTP) study in CD-rats. A F_0 generation was administered 0, 0.3, 1.0 or 3.0 g 2,6-xylidine per kg of diet, beginning at five weeks of age. This is equivalent to a daily dose of 2,6-xylidine of 12, 40 or 120 mg/kg bw for male rats and of 15, 50 or 150 mg/kg bw for female rats. The animals were mated and female rats were allowed to deliver. During pregnancy and lactation treatment continued. After weaning, the F_1 generation (n= 56/group/sex) was treated for 102 weeks. After termination, all F_1 animals were necropsied and histologically examined. The tumour incidence in male animals was 22/56 in controls, 35/56 in the high-dose group, 35/56 in the mid-dose group and 49/56 in the low-dose group. The tumour incidence in female animals was 44/56 in controls, 50/56 in the low-dose group, 50/56 in the mid-dose group and 53/56 in the high-dose group. In high-dosed males and females, mainly papillary adenomas, adenocarcinomas, and carcinomas of the nasal cavity were found, with several sarcomas. Other tumours found were subcutaneous fibromas and fibrosarcomas in both sexes. An increased incidence of neoplastic nodules in the liver was seen in female rats only. Of the initial number of F_1 animals, 43 male + 33 female, 39 male + 24 female, 33 male + 30 female, and 14 male + 23 female survived until the end of the study in the control, low-, mid-, and high-dose group, respectively. No information was provided on the parent generation (IARC93).

6.3.2 Additional information

In a two-stage nasal carcinogenesis model, 2,6-xylidine exerted tumor-promoting effects in the nose. After initiation with a single subcutaneous injection of N-bis(2-hydroxypropyl)nitrosamine (DHPN), male F344 rats (n=15) were administered a diet containing 0 or 3.0 g 2,6-xylidine/kg diet for 52 weeks. None of the control animals (n=10) developed nasal lesions, whereas of the DHPN/2,6-xylidine treated animals (n=30) 27% developed adenomas and 33% carcinomas in the nose. For comparison, of the animals only treated with DHPN (n=20), 20% developed adenomas and 5% carcinomas, the last result being significantly lower than the DHPN/2,6-xylidine treated animals (Kou99).

6.4 Mutagenicity and genotoxicity

6.4.1 IARC data

Studies on the mutagenicity of 2,6-xylidine in *Salmonella typhimurium* were conflicting. 2,6-Xylidine was reported to induce mutations in mouse lymphoma cells at the *tk* locus (abstract), induced sister chromatid exchanges and chromosomal aberrations in mammalian CHO cells *in vitro*.

In vivo, unlabelled 2,6-xylidine bound covalently to the DNA of the ethmoid turbinates and liver of rats after oral pretreatment. In mouse bone marrow *in vivo*, no micronuclei were induced (IARC93).

6.4.2 Additional data

2,6-Xylidine was negative in the sex-linked recessive lethal mutation assay (feeding or injecting males with a 100 or 4000 ppm solutions) in *Drosophila melanogaster* (Fou94).

2,6-Xylidine induced morphological cell transformation in A31-1-13 Balb/c-3T3 cells. In this assay, 2,6-xylidine was tested at four different doses in two or more independent trials. The doses selected for the main test were based on cytotoxicity found in a co-culture clonal survival test (Mat93).

6.5 Evaluation

No data on carcinogenicity in humans are available.

From the information of the NTP study, the committee concludes that 2,6-xylidine has carcinogenic properties.

The results of gene mutation tests in bacteria with 2,6-xylidine were conflicting. In mammalian cells, the compound induced sister chromatid exchanges, chromosomal aberrations and morphological cell transformation. In *Drosophila melanogaster*, 2,6-xylidine did not increase the recessive lethal mutations. *In vivo*, 2,6-xylidine bound covalently to DNA in rat tissues, but it did not induce micronuclei in the bone marrow of mice.

6.6 Recommendation for classification

The committee concludes that 2,6-xylidine should be regarded as carcinogenic to humans (comparable with EU category 2). Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is a genotoxic carcinogen.

Additional consideration

The committee recommends considering 2,6-xylidine, as a way of precaution, as a genotoxic carcinogen as long as the available data do not allow an evaluation of the potential genotoxicity and the assessment of the mode of action underlying the carcinogenicity.

Chapter 7

3,4-Xylidine

7.1 IARC conclusion

No IARC evaluation on the carcinogenicity of 3,4-xylidine is available.

7.2 Human data

7.2.1 IARC data

No data were available.

7.2.2 Additional data

No additional human carcinogenicity data were retrieved.

7.3 Animal data

7.3.1 IARC data

No data were available.

7.3.2 Additional data

No additional animal carcinogenicity data were retrieved.

7.4 Mutagenicity and genotoxicity

7.4.1 IARC conclusion

No data were available.

7.4.2 Additional data

3,4-Xylidine was mutagenic in plate incorporation tests with *Salmonella typhimurium* TA100 with a metabolic activation system (S-9 mix) (Noh84, Zim80).

In a briefly reported *in vivo* test in male mice (n=3 or 4), a significant inhibition of testicular DNA synthesis, as measured by incorporation of ^{3}H-thymidine, was seen after a single intraperitoneal injection of 100 mg 3,4-xylidine per kg bw (Sei77).

7.5 Evaluation

No data on carcinogenicity in humans and animals are available.

Data on the mutagenicity and genotoxicity are insufficient. The committee considers 3,4-xylidine as a bacterial mutagen. No conclusion on the genotoxicity of 3,4-xylidine according to EC standards is possible.

7.6 Recommendation for classification

The committee concludes that 3,4-xylidine was insufficiently investigated. The committee, therefore, recommends not to classify 3,4-xylidine.

Chapter 8

3,5-Xylidine

8.1 IARC conclusion

No IARC evaluation on the carcinogenicity of 3,5-xylidine is available.

8.2 Human data

8.2.1 IARC data

No data were presented.

8.2.2 Additional data

No additional human carcinogenicity data were retrieved.

8.3 Animal data

8.3.1 IARC data

No data were available.

8.3.2 Additional data

No additional animal carcinogenicity data were retrieved.

8.4 Mutagenicity and genotoxicity

8.4.1 IARC conclusion

No data were available.

8.4.2 Additional data

3,5-Xylidine was not mutagenic in a plate incorporation test with *Salmonella typhimurium* strains TA100 or TA98 with or without a metabolic activation system (S9) (Zim80, Noh84).

In a briefly reported *in vivo* test in male mice (n=3 or 4), no inhibition of testicular DNA synthesis, as measured by incorporation of ^{3}H-thymidine, was seen after a single intraperitoneal injection of 100 mg 3,5-xylidine per kg bw (Sei77).

8.5 Evaluation

No data on the carcinogenicity in humans and animals are available.

Data on the mutagenicity and genotoxicity are insufficient. The committee considers 3,5-xylidine as a bacterial mutagen. No conclusion on the genotoxicity of 3,5-xylidine according to EC standards is possible.

8.6 Recommendation for classification

The committee concludes that 3,5-xylidine was insufficiently investigated. The committee, therefore, recommends not to classify 3,5-xylidine.

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Annexes

Annex **A**

Request for advice

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

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

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

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

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the

case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

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

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

Annex **B**

The committee

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- P Boogaard
  toxicologist; Shell International Petroleum Company, The Hague
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; Utrecht University, Utrecht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- TM Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of toxicology; Wageningen University, Wageningen.
- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague

- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of the present advisory report was prepared by MI Willems, from the Department of Occupational Toxicology of the TNO Nutrition and Food Research, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance was provided by mrs A van der Klugt.
Lay-out: mrs J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2000 for public review. No organisations and persons have commented on the draft document.

Annex **D**

IARC Monograph

See next page.

Summary of data reported and evaluation on 2,6-xylidine (IARC93)

Annex **E**

Physico-chemical properties of the xylidine isomers

compound	CAS-No.	M.P (°C)	B.P. (°C)	relative density	solubility	remark	reference
2,3-xylidine	87-59-2						
2,4-xylidine	96-86-1	16	214	0.9723 (20 °C)	slightly soluble in water; soluble in ethanol, diethylether and benzene		IARC78
2,5-xylidine	95-78-3	15.5	214	0.9790 (21 °C)	slightly soluble in water and ethanol; soluble in diethylether		IARC78
2,6-xylidine	87-62-7	-1.112	216	0.98 (20 °C)	in water: 13 g/L at 20 °C	flash point: 92-97 °C autoflammability: 190-520 °C	EC96
3,4-xylidine	95-64-7						
3,5-xylidine	108-69-0						

Annex **F**

Survey of main genotoxicity tests available for the various isomers

See table next page.

testsystem indicator cells/ organisms		endpoint	2,3-xylidine	2,4-xylidine	2,5-xylidine	2,6-xylidine	3,4-xylidine	3,5-xylidine	ref.
bacteria	*S. typh.* TA100 plus rat-liver S9	reverse mutations[a]	+ (120) + (44)	+ (420) + (125)	+ (240) + (58)	+ (39) -	+ (93) + (84)	- -	Noh84 Zim80
Mammalian cells (*in vitro)*	V79	primary DNA damage (alkaline elution)		-	-				Zim80
	rat hepatocytes	primary DNA damage (UDS)		+	+				Wil89
	mouse lymphoma cells L5178Y	forward mutations on TK-locus				+			IARC93
	CHO cells	SCE				+			IARC93
	CHO cells	chromosomal aberrations		+ (with S9)		+			IARC93
	BALB/c-3T3 cells	cell transformation				+			IARC93
Drosophila		SLRL test -feeding -injection				 - -			Fou94
Mammals *(in vivo*)	male mice po or ip	testicular DNA synthesis	- po	+ po	+ po	- po	+ ip	- ip	Sei77
	mice, po	bone marrow micronucleus test				-			IARC93
	rat, ip	DNA binding (liver, ethmoid turbinates)				+			IARC93
	mice, ip	DNA damage in liver cells		+					Prz99

+ = positive in the test system; - = negative in the test system
ip = intraperitoneal; po = per os; S9 = metabolic activation system

[a] The numbers in brackets indicate the number of revertants per µmole/plate calculated from the straight part of the dose response curve after substraction of the control value

Annex **G**

Conclusions regarding the carcinogenic properties of the xylidine isomers

compound	conclusion			remark
	IARC	DECOS classification	comparable to EC class	
2,3-xylidine	-[a]	this compound cannot be classified	not classifiable	no data in animals and humans
2,4-xylidine	3	this compound cannot be classified	not classifiable	no data in humans; inadequate data in animals
2,5-xylidine	3	this compound cannot be classified	not classifiable	no data in humans; inadequate data in animals
2,6-xylidine	2B	regarded as if it were a genotoxic carcinogen	2	no data in humans; adequate data in animals
3,4-xylidine	-[a]	this compound cannot be classified	not classifiable	no data in animals and humans
3,5-xylidine	-[a]	this compound cannot be classified	not classifiable	no data in animals and humans

[a] No IARC evaluation available

Annex **H**

Classification of substances with respect to carcinogenicity

See table next page.

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

Judgement of the committee	Comparable with EU class
This compound is known to be carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	1
This compound should be regarded as carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	2
This compound is a suspected human carcinogen.	3
This compound has been extensively investigated. Although there is insufficient evidence of a carcinogenic effect to warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern.	(A)
This compound has been insufficiently investigated. While the available data do not warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is a cause for concern.	(B)
This compound cannot be classified	not classifiable

Annex I

Guideline 93/21/EEG of the European Union

4.2 Criteria for classification, indication of danger, choice of risk phrases

4.2.1 Carcinogenic substances

For the purpose of classification and labelling, and having regard to the current state of knowledge, such substances are divided into three categories:

Category 1:

Substances known to be carcinogenic to man.

There is sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer.

Category 2:

Substances which should be regarded as if they are carcinogenic to man.

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of:

- appropriate long-term animal studies
- other relevant information.

Category 3:

Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

However for substances and preparations which present a carcinogenic risk only when inhaled, for example, as dust, vapour or fumes, (other routes of exposure e.g. by swallowing or in contact with skin do not present any carcinogenic risk), the following symbol and specific risk phrase should be used:

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Limited evidence of a carcinogenic effect

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3 actually comprises 2 sub-categories:

a substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

b substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds); if the particular target is not relevant to man;
- lack of genotoxicity in short-term tests *in vivo* and *in vitro*;
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation;
- existence of a species - specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man;
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories;
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.