2,4,5-Trimethylaniline

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *2,4,5-Trimethylaniline*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-5138/JR/pg/246-K12
Bijlagen : 1
Datum : 1 april 2008

Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van 2,4,5-trimethylaniline. Het maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

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

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

Hoogachtend,

prof. dr. J.A. Knottnerus

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2,4,5-Trimethyhlaniline

Evaluation of the carcinogenicity and genotoxicity

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

to:

the Minister of Social Affairs and Employment

No. 2008/01OSH, The Hague, April 1, 2008

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues...” (Section 22, Health Act).

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

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

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INAHTA

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Contents

Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens het uitoefenen van hun beroep kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie 2,4,5-trimethylaniline onder de loep. De stof werd onder andere gebruikt bij de productie van kleurstoffen en geneesmiddelen.

Op basis van de beschikbare gegevens leidt de commissie af dat 2,4,5-trimethylaniline *beschouwd moet worden als kankerverwekkend voor de mens*. Dit is vergelijkbaar met een classificatie in categorie 2 volgens de richtlijnen van de Europese Unie. De commissie is verder van mening dat de stof een stochastisch genotoxisch werkingsmechanisme heeft.

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Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. In this report, the committee evaluated 2,4,5-trimethylaniline. The agent has been used as an intermediate for dyestuffs and pharmaceuticals.

Based on the available information, the committee is of the opinion that 2,4,5-trimethylaniline *should be considered as carcinogenic to humans*. This recommendation is comparable to the EU classification in category 2. The committee is furthermore of the opinion that 2,4,5-trimethylaniline acts by a stochastic genotoxic mechanism.

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Chapter 1

Scope

1.1 Background

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

This report contains the evaluation of the carcinogenicity of 2,4,5-trimethylaniline.

1.2 Committee and procedures

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

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

In 2007 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation and recommendation of the committee is standardly based on scientific data, which are publicly available. The starting points of the committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of 2,4,5-trimethylaniline, such an IARC-monograph is available, of which the summary and conclusion of IARC is inserted in Annex D.

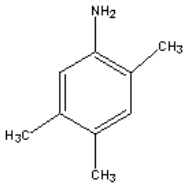
More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in June 2007. The new relevant data were included in this report.

Chapter 2

General information

2.1 Identity and physico-chemical properties

2,4,5-Trimethylaniline has been used as an intermediate for dyestuffs (red dye Ponceau 3R), and pharmaceuticals.[1] Below is given the identity and some of its physical and chemical properties.

Chemical name	:	Benzenamine, 2,4,5-trimethyl
CAS registry number	:	137-17-7
EINECS number	:	205-282-0
IUPAC name	:	2,4,5-trimethylaniline
Synonyms	:	1-amino-2,4,5-trimethylbenzene; pseudocumidine; 1,2,4-trimethyl-5-aminobenzene; 2,4,5-trimethylbenzenamine; psi-cumidine; NCI-C02299
Description	:	white to light tan crystalline solid
Molecular formula	:	$C_9H_{13}N$
Structure	:	
Molecular weight	:	135.2
Melting point	:	62-65 °C
Boiling point	:	234-235 °C

Solubility	:	Highly soluble in water; soluble in ethanol
Relative vapour density	:	3.76
Vapour pressure	:	0.13 kPa at 68.4 °C; 1.3 kPa at 109.0 °C
Stability	:	Stable under ordinary conditions
Conversion factors (20 °C)	:	1 ppm= 5.61 mg/m^3 1 mg/m^3 = 0.18 ppm
EU risk and safety phrases	:	R45: may cause cancer R23/24/25: toxic by inhalation, in contact with skin, and if swallowed R51/53: toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment S53: avoid exposure – obtain special instructions before use S45: in case of accident or when you feel unwell, seek medical advise immediately (show the label where possible) S61: avoid release to the environment. Refer to special instructions/safety data sheets
EU classification	:	Carcinogenic category 2

2.2 IARC classification

In 1982, IARC concluded that there is *limited evidence* for the carcinogenicity of 2,4,5-trimethylaniline in animals, and that no data were available from studies in humans.[1] In 1987, IARC classified the agent in group 3, indicating that it was not classifiable as to its carcinogenicity to humans. [2]

Chapter 3

Carcinogenicity

3.1 Observations in humans

No data were available on the carcinogenicity of 2,4,5-trimethylaniline in humans.

3.2 Carcinogenicity studies in animals

Two series of carcinogenicity studies have been performed in rats and mice, which were fed 2,4,5-trimethylaniline-enriched diets.

In the study by Weisburger *et al*. (1978) male CD rats, and male and female CD-1 mice were given diets containing 1,000 or 2,000 ppm 2,4-5-trimethylaniline (as the hydrochloride) for 78 weeks, with a further observation period of 26 (rats) and 13 (mice) weeks on plain diet.[3] The dose of 2,000 ppm was considered the maximum tolerated dose. An additional group of 25 animals per species per sex served as matched untreated control. Also a pooled untreated control group was used to evaluate the results. Only animals that survived six months or more were examined.

In rats, subcutaneous fibromas and fibrosarcomas, and liver tumours were found. The incidences for matched controls, pooled controls, low- and high dose groups are shown in Table 3.1. Overall, no treatment-related increases in tumour incidences were observed. Although the increase in tumours in the low-dose

group was statistically significantly increased compared to pooled controls, no such significant increases were observed in the high-dose group.

In contrast, in both male and females mice, treatment-related increases of tumour incidences were observed for lung and liver tumours (see Table 3.1). In some mice also vascular tumours were observed, but these could not be related to treatment.

The committee noted the poor reporting of the study design and results of both the rat and mouse study. This plus the low survival rate in the mouse study led to the conclusion of the Working Group of IARC that the mouse study was inadequate for evaluation.[1] It is unclear to the committee why IARC discarded in particular the mouse study, since survival rates and the number of animals examined between the rat and mouse studies did not differ appreciable. Overall, despite the poor reporting, the committee considers the results of the Weisburger studies suggestive for carcinogenic activity of 2,4,5-trimethylaniline.

The second large carcinogenicity study was performed by the National Cancer Institute (NCI) of the United States.[4] Male and female F344 rats and $B6C3F_1$ mice received 200 or 800 ppm (rats), and 50 or 100 ppm (mice) 2,4,5-trimethylaniline (free amine) in the diet for 101 weeks. Plain diets were given to matched controls. Survival and tumour incidence rates are given in Table 3.2 (rats) and Table 3.3 (mice).

Table 3.1 Tumour incidences of rats and mice receiving 2,4,5-trimethylaniline-enriched diets.[3]

Dose in feed	0 ppm (matched control)	0 ppm (pooled control)	1,000 ppm	2,000 ppm
CD rats (males)				
no. of animals at start of study	25	111	25	25
liver tumours	2/22	2/111	3/17**§	2/25
skin tumours	4/22	18/111	6/17*§	1/25 (lipoma)
CD1 mice (males)				
no. of animals at start of study	25	100	25	25
liver tumours	3/18	7/99	9/14**	19/21**
lung tumours	5/18	24/99	11/14**	10/21*§
vascular tumours	0/18	5/99	3/14	3/21
CD1 mice (females)				
no. of animals at start of study	25	102	25	25
liver tumours	0/20	1/102	6/15**	14/22**
lung tumours	6/20	32/102	11/15**	12/22*§
vascular tumours	0/20	9/102	3/15	3/22

* $p<0.05$; ** $p<0.025$; § compared to pooled controls.

Table 3.2 Tumour incidences of rats receiving 2,4,5-trimethylaniline-enriched diets.[4]

Dose in feed	0 ppm (matched control)	200 ppm	800 ppm
F344 rats (males)			
no. of animals at start of study	20	50	50
survival (percentage)	80	74	86
lung: adenomas/adenocarcinomas	1/20 (1 carc)	0/49	7/50 (2 carc)
liver: hepatocellular carcinomas	0/19	3/50	11/50*
neoplastic nodules	1/19	3/50	11/50***
bile-duct carcinomas	0/19	0/50	4/50
F344 rats (females)			
no. of animals at start of study	20	50	50
survival (percentage)	70	84	84
lung: adenomas/adenocarcinomas	0/20	3/43 (2 carc	11/50* (2 carc)
liver: hepatocellular carcinomas	0/20	0/49	9/50**
neoplastic nodules	0/20	12/49	20/50***
bile-duct carcinomas	0/19	0/49	1/50

* p<0.02; ** p<0.039; *** p<0.05; carc= adenocarcinoma

Table 3.3 Tumour incidences of mice receiving 2,4,5-trimethylaniline-enriched diets.[4]

Dose in feed	0 ppm (matched control)	200 ppm	800 ppm
B6C3F$_1$ mice (males)			
no. of animals at start of study	20	50	50
survival (percentage)	80	86	76
lung: adenomas/adenocarcinomas	4/20 (4 carc)	9/50 (9 carc)	1/50 (1 carc)
liver: hepatocellular carcinomas	5/20	26/50**	27/50*
hyperplastic nodules	1/20	3/50	7/50
bile-duct carcinomas	0/20	2/50	2/51
Vasculature: hemangiosarcoma	2/20	3/50	6/50
B6C3F$_1$ mice (females)			
no. of animals at start of study	20	50	50
survival (percentage)	85	78	90
lung: adenomas/adenocarcinomas	0/19	5/49 (4 carc)	6/48*** (6 carc)
liver: hepatocellular carcinomas	0/20	18/49*	40/50*
hyperplastic nodules	0/20	4/49	13/50
bile-duct carcinomas	0/20	0/49	0/50
Vasculature: hemangiosarcoma	1/20	11/49***	7/50

* p<0.02; ** p<0.03; *** p<0.05; carc= adenocarcinoma

Statistically significant dose-related increases in tumour incidences were observed for liver and lung tumours in male and female rats, and for liver tumours in male and female mice. Overall, the committee is of the opinion that the NCI studies point to carcinogenic activity of 2,4,5-trimethylaniline in both rats and mice.

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

2,4,5-Trimethylaniline induced reverse mutations in the conventional Ames test in *Salmonella typhimurium* strains TA98 and TA100, in the presence of an exogenous metabolic system.[1,5,6] It, furthermore, induced gene mutations at the *hprt* locus of mammalian fibroblasts at a concentration of 50 μg/mL.[7]

In Chinese hamster V79 cells, exposure to 2,4,5-trimethylaniline at concentrations of up to 10 mM for 2 or 4 hours did not induce DNA strand breaks.[6]

In Chinese hamster ovary cells, the agent increased the frequency of sister chromatid exchanges (SCE) and chromosomal aberrations in the presence and absence (SCE only) of an exogenous metabolic system (maximum concentration was 5 mg/mL for 2 hours).[8]

4.2 *In vivo* assays

Kugler-Steigmeier *et al.* (1988, 1989) tested the genotoxic potential of 2,4,5-trimethylaniline in the conventional wing spot test using *Drosophila melanogaster* flies.[5,7] This test detects somatic mutations and recombinations. The agent increased the frequency of small single spots, whereas the frequency of large single spots and twin spots did not differ significantly from untreated controls. The exposure was for 24 or 48 hours to various concentrations (up to 20 mM).

4.3 Additional information on carcinogenic mechanism

In the literature attention is given to structure-activity comparisons among aromatic amines in general.[9,10] As a result the American Environmental Protection Agency indicated a 'high-moderate' concern for carcinogenicity to 2,4,5-trimethylaniline.

The possible genotoxic mechanism of 2,4,5-trimethylaniline is not clarified yet. Based on their results, Kugler-Steigmeier *et al*. suggested that mutagenic activity is dependent on metabolic activation.[5,7] At least other aromatic amines, which have been more extensively studied, are known to cause cancer by a genotoxic mechanism.[9]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity of 2,4,5-trimethylaniline in humans were available, nor were there any data available on inhalation exposure in animals.

In two independent long-term carcinogenicity studies, 2,4,5-trimethylaniline administered in the diet of rats and mice resulted in treatment-related liver and lung tumour development in part of the animals. However, in one rat group no treatment-related tumour development was observed. Although the committee noted the poor reporting of one of these studies, it is of the opinion that the data indicate sufficient evidence for the carcinogenicity of 2,4,5,-trimethylaniline in animals.

Furthermore, the agent showed to be mutagenic in bacterial mutagenicity assays and in assays using *Drosophila melanogaster* flies. It also showed clastogenic effects *in vitro* in mammalian cells. Based on these results the committee considers 2,4,5-trimethylaniline a genotoxic carcinogen that acts by a stochastic mechanism.

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

5.2 Recommendation for classification

Based on the available information, the committee is of the opinion that 2,4,5-trimethylaniline should be considered as carcinogenic to humans. This recommendation is comparable to the EU classification in category 2. The committee is furthermore of the opinion that 2,4,5-trimethylaniline acts by a stochastic genotoxic mechanism.

References

1 International Agency for Research on Cancer. 2,4,5- and 2,4,6-Trimethylaniline and their hydrochlorides. IARC Monographs on the evaluation of carcinogenic risk on humans, Lyon, France, Volume 27: pp177-188; 1982.

2 International Agency for Research on Cancer. Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42. IARC monographs on the evaluation of carcinogenic risk to humans, Lyon, France, Supplement 7; 1987.

3 Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG *et. al*. Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J Environ Pathol Toxicol 1978; 2(2): 325-356.

4 National Cancer Institute. Bioassay of 2,4,5-trimethylaniline for possible carcinogenicity. Natl.Cancer Inst, Bethesda, Maryland, USA.Carcinogenesis Technical Report Series No. 160: 1979.

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6 Zimmer D, Mazurek J, Petzold G, Bhuyan BK. Bacterial mutagenicity and mammalian cell DNA damage by several substituted anilines. Mutat Res 1980; 77(4): 317-326.

7 Kugler-Steigmeier ME, Friederich U, Graf U, Maier P, Schlatter C. Testing of 2,4,5- and 2,4,6-trimethylaniline in the Salmonella assay, in mammalian cell cultures, and in Drosophila melanogaster, and comparison of the results with carcinogenicity data. Arch Toxicol Suppl 1988; 12: 337-340.

8 Loveday KS, Anderson BE, Resnick MA, Zeiger E. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. V: Results with 46 chemicals. Environ Mol Mutagen 1990; 16(4): 272-303.

9 California Environmental Protection Agency. Evidence on the carcinogenicity of 2,4,5-trimethylaniline and its strong acid salts (draft). California EPA, Office of Environmental Health Hazard Assessment, Sacramento, USA; 1997.

10 Crabtree HC, Hart D, Thomas MC, Witham BH, McKenzie IG, Smith CP. Carcinogenic ranking of aromatic amines and nitro compounds. Mutat Res 1991; 264(4): 155-162.

A	Request for advice
B	The committee
C	Comments on the public review draft
D	IARC Monograph
E	Carcinogenic classification of substances by the committee
F	Guideline 93/21/EEG of the European Union

Annexes

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Annex **A**

Request for advice

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

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

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

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

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

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

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

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

Annex **B**

The committee

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

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

The Health Council and interests

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

Annex **C**

Comments on the public review draft

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

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

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Annex **D**

IARC Monograph

Vol.: 27 (1982) (p. 177)[1]
2,4,5-Trimethylaniline
CAS No.: 137-17-7
Chem. Abstr. Name: Benzenamine, 2,4,5-trimethyl-

Summary of Data Reported and Evaluation

Experimental data

2,4,5-Trimethylaniline and its hydrochloride were tested in two experiments in mice and in two experiments in rats by dietary administration. In one experiment in mice, 2,4,5-trimethylaniline produced an increased incidence of hepatocellular carcinomas in female mice. The other experiment in mice was considered inadequate for an evaluation. In one experiment in rats it produced an increased incidence of liver carcinomas and lung adenomas. In the other experiment in rats no significantly increased incidence of tumours was observed.

2,4,5-Trimethylaniline was mutagenic to *Salmonella typhimurium* with metabolic activation. The available data were inadequate to evaluate the mutagenicity of 2,4,6-trimethylaniline.

Human data

2,4,5-Trimethylaniline has been produced commercially in the past. No case report or epidemiological study was available to the Working Group.

Evaluation

There is *limited evidence* for the carcinogenicity of 2,4,5-trimethylaniline in experimental animals. No evaluation of the carcinogenicity of 2,4,5-trimethylaniline to humans could be made.

Subsequent evaluation: Suppl. 7 (1987) (p. 73: Group 3)

Annex **E**

Carcinogenic classification of substances by the committee

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

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

Annex **F**

Guideline 93/21/EEG of the European Union

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

4.2.1 Carcinogenic substances

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

Category 1:

Substances known to be carcinogenic to man.

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

Category 2:

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

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

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

Category 3:

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

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

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

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

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Possible risk of irreversible effects

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

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

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

Category 3 actually comprises 2 sub-categories:

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

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

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

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

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

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

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