Trichlormethine hydrochloride

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Trichlormethine hydrochloride*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-5137/JR/pg/246-J12
Bijlagen : 1
Datum : 1 april 2008

Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van trichlormethine hydrochloride. 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

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 66 31
E-mail: jolanda.rijnkels@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23
www.gr.nl

Trichlormethine hydrochloride

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. 2008/10OSH, The Hague, April 1, 2008

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

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

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

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

The Health Council of the Netherlands is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international collaboration of organisations engaged with *health technology assessment*.

INAHTA

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

Preferred citation:
Health Council of the Netherlands. Trichlormethine hydrochloride; Evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2008; publication no. 2008/10OSH.

ISBN: 978-90-5549-697-6

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 trichlormethine hydrochloride onder de loep. De stof wordt onder andere gebruikt als cytostaticum bij de behandeling van kanker en artritis, maar ook bij de productie van kleurstoffen voor textiel.

Op basis van de beschikbare gegevens leidt de commissie af dat trichlormethine hydrochloride *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.

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 trichlormethine hydrochloride. The agent is used as a cytostatic agent in the treatment of cancer and arthritis, and is furthermore used in the production of textile dyes.

Based on the available information, the committee is of the opinion that trichlormethine hydrochloride *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 trichlormethine hydrochloride acts by a stochastic genotoxic mechanism.

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 trichlormethine hydrochloride.

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 trichlormethine hydrochloride, 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

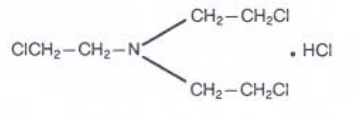
2.1 Identity and physico-chemical properties

Trichlormethine hydrochloride belongs to the class of nitrogen mustards, which are related to mustard gas.

The agent is used as a cytostatic agent in the treatment of certain cancers, such as Hodgkin's disease and leukemia. It is furthermore tested for use in the treatment of arthritis. Moreover, it is used as a fixing agent in textile dyes.[1] Occupational exposure may occur during manufacturing or packaging, or during the final preparation and administration to patients.

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

Chemical name	:	Trichlormethine hydrochloride
CAS registry number	:	817-09-4
EEC number	:	212-442-3
IUPAC name	:	Ethanamine-2-chloro-N,N-bis(2-chloroethyl) hydrochloride
Synonyms	:	HN3; HN3 hydrochloride; NSC-30211; R-47; SK-100; tri(β-chloroethyl)amine hydrochloride; trichlorotriethylamine hydrochloride; 2,2', 2"-trichlorotriethylamine hydrochloride; trimustine; trimustine hydrochloride; tris(2-chloroethyl)-amine hydrochloride; tri (β-chloroethyl)amine hydrochloride; tri(2-chloroethyl)amine monohydrochloride; tris(β-chloroethyl)amine monohydrochloride; tris-*N*-lost; TS-160; Lekamin; Sinalost; Trichlormethine; Trillekamin; Trimitan

Description	:	Crystals prepared by treating triethanolamine with thionyl chloride. Trichlormethine is not known to occur naturally
Molecular formula	:	$C_6H_{12}Cl_3N.HCl$
Structure	:	
Molecular weight	:	241.0
Melting point	:	130-131 °C
Solubility	:	Very soluble in water; soluble in ethanol
Stability	:	Aqueous solutions deteriorate rapidly

2.2 IARC classification

In 1990, IARC concluded that there is *sufficient evidence* for the carcinogenicity of trichlormethine hydrochloride in animals, but that no data were available from studies in humans.[1] Therefore, it classified the agent in group 2B, indicating that it *possibly carcinogenic to humans*.

Chapter 3

Carcinogenicity

3.1 Observations in humans

No data were available to evaluate the carcinogenicity of trichlormethine hydrochloride in humans.

3.2 Carcinogenicity studies in animals

Animal carcinogenicity data is mainly limited to dermal exposure.

The Working Group of IARC evaluated the study of Boyland and Horning (1949).[1,2] In that study, twenty mice (strain not specified) received subcutaneous injections of the agent at a dose of 1 mg/kg bw, once per week for ten weeks. At the end of the exposure period only four mice were still alive. At about 18 months of survival, three of these mice had tumours in the lungs (one animal, adenoma; two animals, carcinomas), and one of them had a spindle-cell sarcoma at the site of the injection. A control group of forty mice was killed between 14 and 18 months. In this group six animals had lung adenomas, two had hepatomas, and three had enlarged lymph nodes. The working group noted the very small number of treated animals that survived.

Sykora *et al*. (1981) treated groups of ten male and ten female random-bred SPF Wistar rats with trichlormethine hydrochloride, by giving them daily subcutaneous injections at doses of 0.1 or 0.25 mg/kg bw, or by given them weekly subcutaneous injections at a dose of 1 mg/kg bw, for six months.[3] After the end of

exposure the animals were observed for one year before the experiment was terminated. In males receiving daily injections survival was decreased. The main types of tumours observed were spindle-cell sarcomas at the injection site: 70% in 0.1 mg/kg bw group, 79% in 0.25 mg/kg bw group, and 45% in 1.0 mg/kg bw group. Furthermore, 21% of the animals receiving daily injections of 0.25 mg/kg bw had mucus-secreting intestinal adenocarcinomas. No tumours were observed in a nontreated group.

The same group also administered trichlormethine hydrochloride to newborn rats by intraperitoneal (0.27 mg/kg bw) or subcutaneous (0.005 mg/kg bw) injections, from the first day after birth up to 20 days.[4] After the last injection the animals were observed for one year. No increased tumour incidences were observed. The committee does not consider this study for evaluation on carcinogenicity, because of the short exposure time and the insufficient reporting on study design and results.

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

The Working Group of IARC reported on induction of forward point mutations in *Schizosaccharomyces pombe* strains.[5] Furthermore, trichlormethine clearly induced forward mutations in *Escherichia coli* K12/343/113 strain, when a 24-hour period of liquid holding was interpolated between treatment and growth phase.[6] Trichlormethine hydrochloride did not induce mutations in the conventional Ames test using *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535, in the presence nor in the absence of an exogenous metabolic system.[7]

Using a liquid micromethod modification of the DNA-repair test, Marhan (1995) found positive outcomes when *E. coli* strains WP100, CM571 and CM611 were used, in the presence and absence of an exogenous metabolic system.[8] Negative scores were found in *E. coli* strains WP*uvrA*, WP67, and CM561.

Trichlormethine hydrochloride inhibited DNA synthesis, and induced mutations in the *hprt* locus of Chinese hamster V79 cells.[1,9] The Working Group also reported on increased frequencies of chromosomal aberrations in transplanted Walker rat carcinoma cells, and in transplanted Ehrlich and Krebs tumour cells following intraperitoneal injection into animals carrying these cells.[1] However, detailed evaluation was not possible due to insufficient data presentation.

4.2 *In vivo* assays

Trichlormethine hydrochloride showed to be highly mutagenic in the sex-linked lethal test using wild type *Drosophila melanogaster* flies.[10,11] It also decreased fertility, which was probably related to chromosome rearrangements. However, regarding the latter, the authors remarked that the agent was tested on a too small scale to make the results convincing.

A single intraperitoneal injection of 5 mg trichlormethine hydrochloride/kg bw led to dominant lethal mutations in male mice.[1,12] Also, intraperitoneal injections at a dose of 0.5 mg/kg bw, for seven consecutive days, resulted in an increased frequency of dominant lethals.[13] The dose tested was equivalent to the human therapeutic dosage, and is below the LD_{50} for this compound. Furthermore, the results in the latter study suggest that the agent not only showed genotoxic, but also cytotoxic effects. The dominant lethal test is used to demonstrate genetic risk through chromosome segregation or integrity, and is mainly associated to chromosomal breaks.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity of trichlormethine hydrochloride in humans were available, nor were there any data available on inhalation exposure in animals.

A few animal studies concerned subcutaneous injections of the agent. In these studies increased incidences of tumours at the site of injection were observed, as well as tumour development at other sites of the body. Although the study designs and reporting were limited, the overall picture gives sufficient evidence for the carcinogenicity of trichlormethine hydrochloride.

The agent showed to be mutagenic in *in vitro* and *in vivo* bioassays, but not in the conventional Ames test. It also showed clastogenic activity *in vitro*. Overall, based on the presented results, the committee considers trichlormethine hydrochloride as a genotoxic compound 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 trichlormethine hydrochloride should be considered as carcinogenic to humans. This recommendation is comparable to the EU classification in category 2. The com-

mittee is furthermore of the opinion that trichlormethine hydrochloride acts by a stochastic genotoxic mechanism.

References

1 International Agency for Research on Cancer. Trichlormethine (trimustine hydrochloride). IARC Monographs, Evaluationa on carcinogenic risk in humans, Lyon, France, Volume 50; pp 143-149; 1990.

2 Boyland E, Horning ES. The induction of tumours with nitrogen mustards. Br J Cancer 1949; 3: 118-123.

3 Sykora I, Vortel V, Marhan O, Dynterova A. Carcinogenicity of trichloromethine hydrochloride (TS-160 Spofa) and morphological damage after its intraamniotic injection. Neoplasma 1981; 28(5): 565-574.

4 Sykora I, Vortel V. Comparability of results of postnatal and long-term tests for carcinogenicity. Neoplasma 1993; 40(5): 321-327.

5 International Agency for Research on Cancer. Some azaridines, N-, S-, and O-mustards and selenium. IARC Monographs on teh evaluation of the carcinogenic risk of chemicals to man, Lyon, France, Volume 9, pp 229-234; 1975.

6 Zijlstra JA. Liquid holding increases mutation induction by formaldehyde and some other cross-linking agents in Escherichia coli K12. Mutat Res 1989; 210(2): 255-261.

7 Marhan J. Mutagenicity of cytostatic drugs in a bacterial system. I. Ames test. Folia Microbiol (Praha) 1995; 40(5): 457-461.

8 Marhan J. Mutagenicity of cytostatic drugs in a bacterial system. II. DNA-repair test. Folia Microbiol (Praha) 1995; 40(5): 462-466.

9 Slamenova D, Dusinska M, Budayova E, Gabelova A. The genetic effects of the cytostatic drug TS160 on Chinese hamster fibroblasts in vitro. Mutat Res 1983; 116(3-4): 431-440.

10 Auerbach C, Robson JM, Carr JG. The chemical production of mutations. Science 1947; 105(2723): 243-247.

11 Auerbach C, Robson JM. Tests of chemical substances for mutagenic action. Proc R Soc Edinburgh 1947; 62B: 284-291.

12 Sykora I, Gandalovicova D. Dominant-lethal assay of selected cytostatics. Neoplasma 1978; 25(5): 523-533.

13 Sykora I, Gandalovicova D. Trichlormethine hydrochloride and correlation of its mutagenic and toxic effects on male germ cells in mice. Mutat Res 1992; 266(2): 291-297.

Annexes

Annex **A**

Request for advice

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

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

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

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

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

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

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

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

Annex **B**

The committee

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

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

The Health Council and interests

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

Annex **C**

Comments on the public review draft

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

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

Annex **D**

IARC Monograph

Vol.: 50 (1990) (p. 143)[1]
CAS No.: 817-09-4
Chem. Abstr. Name: Ethanamine-2-chloro-*N*,*N*-bis(2-chloroethyl) hydrochloride

Summary of Data Reported and Evaluation

Exposure data

Trichlormethine is a cytostatic agent that has been used since 1946 for the treatment of leukaemia and lymphoma.

Experimental carcinogenicity data

Trichlormethine was tested for carcinogenicity by subcutaneous injection in mice and rats. The study in mice was inadequate for evaluation. In rats, trichlormethine induced a high incidence of sarcomas (mostly spindle-cell type) in animals of each sex at the site of subcutaneous injection, as well as a few intestinal adenocarcinomas; neither tumour type was seen in controls.

Human carcinogenicity data

No data were available to the Working Group.

Other relevant data

In single studies, trichlormethine induced dominant lethal mutations in mice and gene mutations in Chinese hamster cells.

Evaluation

There is *sufficient evidence* for the carcinogenicity of trichlormethine in experimental animals.

No data were available from studies in humans on the carcinogenicity of trichlormethine.

Overall evaluation

Trichlormethine is *possibly carcinogenic to humans (Group 2B)*.

Previous evaluations: Vol. 9 (1975) (p. 229); Suppl. 7 (1987) (p. 73)

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.