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# Iodoform

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Evaluation of the carcinogenicity and genotoxicity

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Aan de minister van Sociale Zaken en Werkgelegenheid

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Onderwerp : Aanbieding advies *Iodoform*  
Uw kenmerk : DGV/MBO/U-932542  
Ons kenmerk : U-1480/JR/pg/246-U11  
Bijlagen : 1  
Datum : 12 december 2007

Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van iodoform. 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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Bezoekadres  
Parnassusplein 5  
2511 VX Den Haag  
Telefoon (070) 340 66 31  
E-mail: [jolanda.rijnkels@gr.nl](mailto:jolanda.rijnkels@gr.nl)

Postadres  
Postbus 16052  
2500 BB Den Haag  
Telefax (070) 340 75 23  
[www.gr.nl](http://www.gr.nl)



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# Iodoform

Evaluation of the carcinogenicity and genotoxicity

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

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to:

the Minister of Social Affairs and Employment

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No. 2007/08OSH, The Hague, December 12, 2007

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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.



**INAHTA**

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*.

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This report can be downloaded from [www.healthcouncil.nl](http://www.healthcouncil.nl).

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## Samenvatting

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Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie iodoform onder de loep. Iodoform is een antiseptisch en desinfecterend middel.

De commissie meent dat iodoform onvoldoende is onderzocht. Hoewel de gegevens het niet toelaten de stof te classificeren als *kankerverwekkend voor de mens* of als *moet beschouwd worden als kankerverwekkend voor de mens*, is de commissie van mening dat waakzaamheid geboden is. De commissie adviseert daarom iodoform te classificeren als *verdacht kankerverwekkend voor de mens*. Volgens de richtlijnen van de Europese Unie komt dit overeen met een classificatie in categorie 3. Binnen deze categorie komt de situatie het meest overeen met subcategorie b.

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## Executive summary

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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 iodoform. Iodoform is an antisepticum and desinfectance.

The committee concludes that iodoform has been insufficiently investigated. While the available data do not warrant a classification as *carcinogenic to humans* or as *should be regarded as carcinogenic to humans*, they indicate that there is *cause for concern for man*. This recommendation corresponds to EU classification in category 3. This situation is, furthermore, comparable with subcategory b of this category.

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# Scope

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## 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 with reference to an EU-directive (see annex A and E). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question. The assessment and the proposal for a classification are expressed in the form of standard sentences (see annex D). This report contains the evaluation of the carcinogenicity of iodoform.

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## 1.2 Committee and procedures

The evaluation is performed by the committee 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 J.Th.J. Stouten 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.

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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.

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### **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). In the case of iodoform, no such an IARC-monograph is available.

Data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in March 2007. Data, which were considered relevant according to the committee, were included in this report.

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## General information

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### 2.1 Identity and physico-chemical properties

Iodoform is used as a germicide in human medicine.<sup>1</sup> Furthermore, in veterinary medicine, it is used as an antiseptic, and a disinfectant on superficial lesions and for lesions in the female reproductive tract. Occupational exposure may occur during manufacturing or packaging, or during the final preparation and administration to patients or animals.

Below is given the identity and some of its physico-chemical properties.<sup>1</sup>

Name	: iodoform
CAS no	: 75-47-8
EINECS no	: 200-874-5
EEC no	: -
CAS name; IUPAC name	: methane, triiodo; triiodomethane
Description	: yellow or greenish-yellow powder or crystalline solid
Chemical formula	: $\text{CHI}_3$
Molecular weight	: 393.78
Boiling point (101.3 kPa)	: decomposes at high temperature with evolution of iodine (210 °C)
Melting point (101.3 kPa)	: 119-123 °C
Vapour pressure	: not available
Relative density	: 4.01 g/mL (20°/4°C)
Solubility in water	: slightly soluble in water (0.1 g/L at 25°C)

Solubility in organic sol- : soluble in ether (134 g/L at 25°C), ethyl alcohol (78 g/L at 78°C), chloro-  
vents form, benzene, acetone, ethanol, diethyl ether, olive oil, carbon disul-  
phide, and glycerine

Conversion factors : 1 ppm = 16.4 mg/m<sup>3</sup>  
(101.3 kPa; 20°C) 1 mg/m<sup>3</sup> = 0.06 ppm

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## 2.2 IARC classification

IARC did not evaluate the carcinogenic and genotoxic properties of iodoform.



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## **Carcinogenicity studies**

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### **3.1 Observations in humans**

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

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### **3.2 Carcinogenicity studies in animals**

The American National Cancer Institute (1978) reported on an animal carcinogenicity study, in which Osborne-Mendel rats and B6C3F1 mice (n=50/group/sex/strain) received iodoform by oral administration.<sup>2</sup> During the study, dose levels were adjusted (decreased in male rats after 28 weeks, increased in female rats after 18 weeks, increased in male and female mice after 8 and 10 weeks) resulting in the administration of time-weighted average doses of 142 and 71 mg/kg bw per day for male rats, 55 and 27 mg/kg bw per day for female rats, and 93 and 47 mg/kg bw per day for male and female mice. They received the test compound for 5 days per week during 78 weeks, and were thereafter left untreated during 34 weeks for rats and 13 or 14 weeks for mice. For both species, there were vehicle- and untreated-control groups (n=20/sex/control group).

In male rats, a significant dose-related high mortality was observed (mortality high dose: 50% by week 46; mortality low dose: 52% by week 76), indicating that the maximum tolerated dose was exceeded. Unfortunately, the increased mortality was not explained further in the report, possibly due to a lack of parameters tested, *i.e.*, haematology and clinical chemistry. In these animals, there was

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an increase in the incidence of follicular cell tumours (carcinomas + adenomas) of the thyroid, despite the high mortality (incidence in vehicle control, low-, and high-dose males: 0/16, 8/35\*, 4/18, respectively; \*  $p < 0.05$  Fischer exact test). The incidences of thyroid tumours in both test groups were much higher than historical control incidences from the test facility (historical control incidence: 11 thyroid tumours/200 male rats). Incidences of non-neoplastic changes in the thyroid (*viz.*, follicular cysts, follicular-cell hyperplasia) were not or only weakly indicative of a relationship between exposure and thyroid pathology. During the dosing period, body weights of the exposed male animals were slightly lower than those of controls (graphic presentation only). Data on organ weights were not presented. Finally, there were no other differences between exposed and control male rats with respect to inflammatory, degenerative, or proliferative lesions.

In exposed female rats and exposed male and female mice, no toxicity, as presented by body weight changes or clinical signs, was observed, indicating that these animals may not have been treated with the maximum tolerated dose. No effect on thyroid was observed in female rats and in male and female mice. The absence of a thyroid effect in female rats may have been caused by the lower doses used. No other treatment-related toxic or neoplastic effects were identified.

No other animal carcinogenicity studies were available to the committee.

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## Mutagenicity and genotoxicity

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### 4.1 *In vitro* assays

Iodoform was tested in the *Salmonella typhimurium* mutagenicity assay. A summary of the outcomes is presented in table 4.1. Overall, iodoform showed to be mutagenic in various strains.

In Syrian hamster embryo cells, iodoform induced a treatment-related increase in unscheduled DNA synthesis (dose range tested: 1-30  $\mu\text{g/mL}$ ), and morphological transformations (dose range: 3-30  $\mu\text{g/mL}$ ; exposure duration: 48 hours).<sup>3</sup> It furthermore increased, in a dose-related manner, the frequency of sister chromatid exchanges (dose range: 1-30  $\mu\text{g/mL}$ ; exposure duration: 18-20 h,  $p < 0.05$ ).<sup>3</sup> However, this increase never reached a double level of the vehicle-control.

Hikiba *et al.* (2005) did not find increased levels of chromosomal aberrations in Syrian hamster embryo cells after iodoform exposure (0-240  $\mu\text{M}$ ).<sup>4</sup> The committee noted the low relative colony forming efficiency at the higher exposure doses.

No other data on the genotoxicity *in vitro* was available.

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### 4.2 *In vivo* assays

No genotoxicity studies using *in vivo* test assays were available.

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Table 4.1 Iodoform mutagenicity in *Salmonella typhimurium* test.

<i>Salmonella typhimurium</i> strain	rat liver S9 mix	dose applied (µg/plate)	score	reference
TA98	-	10 – 333	+	Haworth <i>et al.</i> 1983 <sup>5</sup>
	+	10 – 888	+	
	+(hamster S9)	10 – 444	+	
TA100	-	10 – 333	+	Haworth <i>et al.</i> 1983 <sup>5</sup>
	+	10 – 888	+	
	+(hamster S9)	10 – 444	+	
TA1535	-	10 – 333	-	Haworth <i>et al.</i> 1983 <sup>5</sup>
	+	10 – 888	+	
	+(hamster S9)	10 – 444	-	
TA1537	-	10 – 333	-	Haworth <i>et al.</i> 1983 <sup>5</sup>
	+	10 – 888	+	
	+(hamster S9)	10 – 444	-	
BA13 (L-arabinose resistance test)	-	< 1.8 µmol/plate	+	Roldán-Arjona <i>et al.</i> 1991 and 1993 <sup>6,7</sup>
	+	< 1.8 µmol/plate	+	

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## Classification

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### 5.1 Evaluation of data on carcinogenicity and genotoxicity

In the literature, no case-reports or observational studies were available on the possible carcinogenicity of iodoform in humans. So far known, there is only one carcinogenicity study performed on animals. In that study, iodoform induced tumours of the thyroid in male rats, but not in female rats or in mice. Overall, there is insufficient evidence for carcinogenicity of iodoform, but the findings in the animal study worries the committee.

Iodoform clearly induced mutations *in vitro*, which indicates that iodoform has mutagenic and genotoxic potential. The findings concerning clastogenicity are controversial and limited. No data are available on the possible mutagenic and genotoxic properties of iodoform by *in vivo* assays.

The committee did not find information that the observations in the animal study, and the genotoxic action in the *in vitro* assays, would not occur in humans.

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### 5.2 Recommendation for classification

The committee concludes that iodoform has been insufficiently investigated. While the available data do not warrant a classification as *carcinogenic to humans* or as *should be regarded as carcinogenic to humans*, they indicate that there is *cause for concern for man*. This recommendation corresponds to EU

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classification in category 3. This situation is, furthermore, comparable with sub-category b of this category.

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## References

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- 1 Richardson M, Gangolli S. I46 Iodoform. In: Dictionary of substances and their effects. Volume 2. Cambridge, the UK: Royal Society of Chemistry; 1993.
  - 2 National Cancer Institute. Bioassay of iodoform for possible carcinogenicity (CAS No. 75-47-8). Natl Cancer Inst Carcinog Tech Rep Ser 1978; 110: 1-107.
  - 3 Suzuki H. [Assessment of the carcinogenic hazard of 6 substances used in dental practices. (II) Morphological transformation, DNA damage and sister chromatid exchanges in cultured Syrian hamster embryo cells induced by formocresol, iodoform, zinc oxide, chloroform, chloramphenicol, tetracycline hydrochloride] In Japanese. Shigaku 1987; 74: 1385-1403.
  - 4 Hikiba H, Watanabe E, Barrett JC, Tsutsui T. Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. J Pharmacol Sci 2005; 97(1): 146-152.
  - 5 Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E. Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen 1983; 5 Suppl 1: 1-142.
  - 6 Roldan-Arjona T, Garcia-Pedrajas MD, Luque-Romero FL, Hera C, Pueyo C. An association between mutagenicity of the Ara test of Salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons. Mutagenesis 1991; 6(3): 199-205.
  - 7 Roldan-Arjona T, Pueyo C. Mutagenic and lethal effects of halogenated methanes in the Ara test of Salmonella typhimurium: quantitative relationship with chemical reactivity. Mutagenesis 1993; 8(2): 127-131.
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- A Request for advice
- 
- B The committee
- 
- C Comments on the public review draft
- 
- D Carcinogenic classification of substances by the committee
- 
- E Guideline 93/31/EEG of the European Union

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## Annexes



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## Request for advice

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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.

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## 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 Nutrition and Food Research, 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.

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## 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.

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## **Comments on the public review draft**

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

- G. Jonkers, Vereniging van Verf en Drukinkfabrikanten, the Netherlands;
- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain;
- R.D. Zumwalde, National Institute for Occupational Safety and Health, the USA.





**D**


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## Carcinogenic classification of substances by the committee

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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

1

- 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

This compound should be regarded as carcinogenic to humans

2

- 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

This compound is a suspected human carcinogen.

3

- 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. (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

- There is a lack of carcinogenicity and genotoxicity data.
  - Its carcinogenicity is extensively investigated. The data indicate sufficient evidence suggesting lack of carcinogenicity.
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# Guideline 93/21/EEG of the European Union

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## 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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