Chlorozotocin

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Chlorozotocin*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-1478/JR/pg/246-S11
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Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van chlorozotocine. 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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Chlorozotocin

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. 2007/07OSH, The Hague, December 12, 2007

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 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 chlorozotocine onder de loep. Chlorozotocine is een cytostaticum dat wordt gebruikt ter behandeling van kanker.

Op basis van de beschikbare gegevens leidt de commissie af dat chlorozotocine beschouwd moet worden als kankerverwekkend voor de mens. Dit komt overeen met een classificatie in categorie 2 volgens de richtlijnen van de Europese Unie. De commissie concludeert verder dat chlorozotocine stochastisch genotoxisch is.

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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 chlorozotocin. Chlorozotocin is a cytostatic agent that is used to treat cancer.

Based on the available information, the committee is of the opinion that chlorozotocin should be considered as carcinogenic to humans. This recommendation corresponds to the EU classification in category 2. The committee concludes furthermore that chlorozotocin 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 with reference to an EU-directive (see annex A and F). 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 E). This report contains the evaluation of the carcinogenicity of chlorozotocin.

1.2 Committee and procedure

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 MI Willems, from the Department of Occupational Toxicology of the TNO Nutrition and Food Research, by contract with the Ministry of Social Affairs and Employment.

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 chlorozotocin, 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 March 2007. The new relevant data were included in this report.

Chapter 2

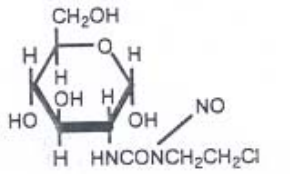
General information

2.1 Identity and physico-chemical properties

Chlorozotocin is a cytostatic agent that is used in the treatment of gastrointestinal cancers, cancer of the lungs, melanomas, and multiple myelomas.[1,2] 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.

Chemical name	:	D-glucose, 2-({[(2-chloroethyl)nitrosoamino] carbonyl}amino)-2-deoxy-
CAS registry number	:	54749-90-5
IUPAC name	:	Ethanamine-2-chloro-N,N-bis(2-chloroethyl) hydrochloride
Synonyms	:	D-Glucopyranose; 2-({[(2-chloroethyl)nitrosoamino] carbonyl}-amino)-2-deoxy-1-(2-chloroethyl)-1-nitroso-3-(D-glucos-2-yl)urea; DCNU; NSC-178248; Dome
Description	:	Ivory crystals. Chlorozotocin is synthesized by nitrosation of the urea derivative prepared from D-glucosamine and 2-chloroethylisocyanante. Chlorozotocin is not known to occur naturally. Chlorozotocin is available as a lyophilized powder in vials containing 50 mg of the compound with 48 mg citric acid and sodium hydroxide to adjust the pH.
Molecular formula	:	$C_9H_{16}ClN_3O_7$

Structure	:	
Molecular weight	:	313.69
Boiling point	:	-
Melting point	:	140-148 °C (decomposes)
Vapour pressure	:	-
Solubility	:	Soluble in water
Stability	:	Stable in solution at room temperature for 3 hours and at 2-8 °C for 24 hours; powder is stable for 24 months under refrigeration
Partition coefficient	:	3 (octanol:water)

2.2 IARC classification

In 1990, IARC concluded that there is sufficient evidence for the carcinogenicity of chlorozotocin in experimental animals, but that there were no carcinogenicity data available from studies in humans.[1] Therefore, according to the IARC guidelines, it classified chlorozotocin in Group 2A, which means that the agent is probably carcinogenic to humans. IARC also took notice of the possible genotoxic effects of chlorozotocin.

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

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

3.2 Carcinogenicity studies in animals

Habs *et al*. (1979) reported on a carcinogenicity study on Sprague-Dawley rats, which received intraperitoneal injections of chlorozotocin at doses of 0.4 and 2 mg/kg bw, once a week for more than 2 years.[3] Also vehicle-control animals were included. The median survival time was lowest for the high-dose group, and highest for the control animals. The number of animals with tumours was significantly increased in the exposed groups compared to the control group: sarcomas and mesotheliomas of the peritoneal cavity, 13/20 and 16/20 (high dose, male and female, respectively), 14/20 and 10/20 (low dose, male and females, respectively), 0/20 and 1/20 (control, male and female, respectively). No other types of tumours were reported.

In another study, thirty male Wistar rats were given ten intravenous injections of chlorozotocin at doses of 9.5, 19, and 38 mg/kg bw, one injection every six weeks. Also vehicle-control animals were included.[1,4] The median survival time was lowest for the high-dose group, and highest for the control animals. The authors reported on increased numbers of animals with malignant tumours of the

nervous system, lung, and forestomach in the exposed groups compared to the control animals: 4, 5 and 4% *versus* 1, 0, and 1% in low, mid, and high exposure group, respectively, *versus* controls. IARC noted the poor survival and limiting reporting.[1]

The carcinogenic potency, expressed as TD_{50}*, is estimated to be 0.0375.[5] No other animal carcinogenicity studies were available to the committee.

* TD_{50} is daily dose rate in mg/kg bw/day to induce tumours in half of test animals that would have remained tumour-free at zero dose.

Chapter 4

Mutagenicity and genotoxicity

The molecular mechanism by which chlorozotocin exerts its antineoplastic action is thought to be modification of DNA. However, this DNA modification may also contribute to its toxic and carcinogenic properties. The possible mutagenic and genotoxic properties are further reviewed in the next sections.

4.1 *In vitro* assays

Chlorozotocin induced base-pair substitutions but not frame shift mutations in *Salmonella typhimurium*, in the presence and absence of an exogenous metabolic activation system.[1] Also, it induced mutations at the *hprt* locus in V79 Chinese hamster cells.

Chlorozotocin alkylated the DNA in mouse leukaemia L1210 cells. It also induced DNA strand breaks in L1210 and V79 Chinese hamster cells.[1] In addition, DNA interstrand cross-links were observed in mouse leukaemia L1210 and in human embryo cells.

In mouse leukaemia L1210 cells, and in 9L rat brain tumour cells, chlorozotocin increased the frequencies of sister chromatid exchanges. It also induced mitotic gene conversion in *Saccharomyces cerevisiae*.[1]

4.2 *In vivo* assays

Chlorozotocin induced sex-linked recessive lethal mutations in *Drosophila melanogaster*.[1]

Furthermore, in the bone-marrow cells of Wistar rats, which were given a single intraperitoneal injection of 100 μmol chlorozotocin per kg bw, increased numbers of DNA strand breaks and interstrand cross links were observed.[6]

4.3 Carcinogenic mechanism

Chlorozotocin is structurally related to other chloroethyl nitrosoureas, which are listed as ‘known human carcinogens’ or ‘should be regarded as a human carcinogen’.[1,2] These agents, including chlorozotocin, exert their carcinogenic effects through the formation of mono- and bifunctional alkylating agents.[2] As such they are able to alkylate, and thus to damage, DNA.[1,7-11]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity of chlorozotocin in humans were available, nor were any data available on inhalation exposure in animals. Overall, the availability of animal carcinogenicity studies is limited, but in one of the two available, chlorozotocin induced tumours throughout the body. These findings give sufficient evidence that exposure to chlorozotocin can result in cancer development.

Chlorozotocin is an alkylating agent, and as such has been shown to cause mutations and DNA-damage in *in vitro* and *in vivo* test systems. In addition, chlorozotocin causes mutations in cultured mammalian cells, as well in *Drosophila melanogaster.* For this reason the committee considers chlorozotocin as a stochastic genotoxic carcinogen.

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

The committee is of the opinion that chlorozotocin should be considered as carcinogenic to humans. This recommendation corresponds to the EU classification in category 2. The committee concludes furthermore that chlorozotocin acts by stochastic genotoxic mechanism.

References

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2 National Toxicology Program. Chlorozotocin. Report on Carcinogens, Eleventh Editions; US Department of Health and Human Services, Public Health Services, National Toxicology Program; 2002. Internet: PM:15320326.

3 Habs M, Eisenbrand G, Schmahl D. Carcinogenic activity in Sprague-Dawley rats of 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-gluco-pyranose (chlorozotocin). Cancer Lett 1979; 8(2): 133-137.

4 Eisenbrand G, Habs M. Chronic toxicity and carcinogenicity of cytostatic N-nitroso-(2-chloroethyl) ureas after repeated intravenous application to rats. Dev Toxicol Environ Sci 1980; 8: 273-278.

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11 Vogel EW, Barbin A, Nivard MJ, Stack HF, Waters MD, Lohman PH. Heritable and cancer risks of exposures to anticancer drugs: inter-species comparisons of covalent deoxyribonucleic acid-binding agents. Mutat Res 1998; 400(1-2): 509-540.

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Annexes

Annex **A**

Request for advice

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

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

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

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

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

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

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

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

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 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.

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:

- G. Jonkers, Vereniging van Verf en Drukinktfabrikanten, the Netherlands;
- 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. 65)[1]

CAS No.: 54749-90-5
Chem. Abstr. Name: D-Glucose, 2({[(2-chloroethyl)nitrosoamino]carbonyl}-amino)-2-deoxy-

Summary of Data Reported and Evaluation

Exposure data

Chlorozotocin has been used as a cytostatic drug for the treatment of cancers at a variety of sites.

Experimental carcinogenicity data

Chlorozotocin was tested for carcinogenicity in single experiments in rats by intraperitoneal and intravenous injection. Intraperitoneal administration induced a high incidence of sarcomas and mesotheliomas in the peritoneal cavity in rats of each sex. The study by intravenous administration was inadequate for evaluation.

Human carcinogenicity data

No data were available to the Working Group.

Other relevant data

Chlorozotocin alkylates DNA and protein and causes DNA interstrand cross-links. In humans, it induces leukopenia and thrombocytopenia; in animals, it suppresses the bone marrow and affects immune response.

It is hepatotoxic in both humans and experimental animals.

Chlorozotocin induced DNA damage in bone-marrow cells of rats *in vivo*. It induced DNA damage in human, mouse and Chinese hamster cells *in vitro*, sister chromatid exchange in mouse and rat cells and gene mutation in Chinese hamster cells. It induced sex-linked recessive lethal mutations in *Drosophila* and gene conversion in *Saccharomyces cerevisiae*. Chlorozotocin induced mutations in *Salmonella typhimurium*.

Evaluation

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

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

In making the overall evaluation, the Working Group also took note of the following information. Chlorozotocin is an alkylating agent and is structurally related to other chloroethyl nitrosoureas, one of which, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU), is carcinogenic to humans (Group 1) and two of which, bischloroethyl nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), are probably carcinogenic to humans (Group 2A) (IARC, 1987). Chlorozotocin has given consistently positive results in a broad spectrum of assays for genetic and related effects, including those involving mammalian cells.

Overall evaluation

Chlorozotocin is *probably carcinogenic to humans (Group 2A)*.

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.