Arsine

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Arsine*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-5131/JR/pg/246-D12
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Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van arsine. 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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Arsine

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. 2008/05OSH, 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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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 arsine onder de loep. De stof wordt voor diverse industriële doeleinden gebruikt.

Op basis van de beschikbare gegevens leidt de commissie af dat arsine 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 waakzaamheid geboden. De commissie adviseert daarom arsine te classificeren als *verdacht kankerverwekkend voor de mens*. Dit is vergelijkbaar met een classificatie in categorie 3 volgens de richtlijnen van de Europese Unie. Binnen deze categorie komt de situatie het meest overeen met subcategorie b.

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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 arsine. The agent is used for various industrial purposes.

Based on the available information, the committee is of the opinion that arsine 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. Therefore, the committee recommends classifying arsine as *a suspected human carcinogen*. This recommendation is comparable to the EU classification in category 3. The situation is, furthermore, comparable with subcategory b of this category.

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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 D). The criteria used for classification are partly based on an EU-directive (see Annex E). 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 arsine.

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 H.E. Buist of TNO Quality of Life, location Zeist, 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 arsine no such an IARC-monograph is available. However, IARC evaluated arsenic and arsenic compounds as a group.

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

Arsine is produced when metalic arsenides are decomposed by water or reducing acids. It is used as a pure gas or in mixtures with an inert background gas: in the semiconductor industry for epitaxial growth of gallium arsenide; as a doping agent for silicon-based solid-state electronic devices; and, in the manufacture of light-emitting diodes.[1,2] Furthermore, it is regularly formed by accident, in particular in the chemical and non-ferrous metallurgical industries. Finally, it has also been reported to be used as a chemical warfare gas.

Below is given the identity and some of its physical and chemical properties.[1,2]

Chemical name	:	arsine
CAS registry number	:	7784-42-1
EINECS number	:	232-066-3
Synonyms	:	arsenic hydride, arsenic trihydride, arsenous hydride, hydrogen arsenide, arseniuretted hydrogen
Description	:	colourless, extremely flammable gas with a garlic-like odour
Occurrence	:	arsine is a vapour heavier than air and accumulates close to the surface; decomposes on exposure to light, or when it comes into contact with moisture in the air, depositing shiny black arsenic; in water, it rapidly hydrolyses to other arsenic compounds
Molecular formula	:	AsH_3
Molecular weight	:	77.95

Melting point	:	-116.9 °C
Boiling point	:	-62 °C
Relative vapour density	:	2.7 (air = 1)
Vapour pressure	:	1043 kPa at 20 °C
Solubility	:	soluble in benzene and chloroform; slightly soluble in alcohol, and alkalis; and, slightly soluble in water (saturation, 8.93 mM)
Conversion factors (20 °C)	:	1 mg/m^3 = 0.309 ppm 1 ppm = 3.24 mg/m^3
EU risk phrases	:	R12: extremely flammable R26: very toxic by inhalation R48/20: harmful: danger of serious damage to health by prolonged exposure through inhalation R50/53: very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

2.2 IARC classification

In 1980, the Working Group of IARC evaluated arsenic and arsenic compounds.[2] Apart from chemical, physical, production, and acute toxicity data no information was presented on the carcinogenicity of arsine, due to a lack of information.

However, regarding the group of arsenic compounds, IARC concluded that "there is inadequate evidence for the carcinogenicity in animals, but there is sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens in humans. The data suggesting an increased risk for cancer at other sites are inadequate for evaluation." As a result, in 1987 IARC classified the group of arsenic and arsenic compounds in group 1, indicating that these are carcinogenic to humans.[3] In addition, the same conclusion was made by the Working Group when considering arsenic in drinking water.[4] IARC emphasized that the classification applies to the group of chemicals as a whole, and not necessarily to all individual chemicals within the group.

Chapter 3

Carcinogenicity

3.1 Observations in humans

No data were available on the carcinogenicity of arsine in humans.

3.2 Carcinogenicity studies in animals

Holland and Acevedo (1964) reported in an abstract of a carcinogenicity study in rabbits.[5] The animals (species and number of animals not specified) inhaled arsine gas for 20 to 26 months. One exposed rabbit developed a malignant mesothelioma of the pleura in the lungs. No further details were given. The committee considers this study inadequate for evaluation, due to insufficient reporting.

No other data were available on the carcinogenicity of arsine in animal experiments.

3.3 Additional information

Arsine belongs to a group of inorganic arsenic compounds, which have been evaluated on carcinogenicity by various organizations.[2-4,6] In summary, in humans, long term exposure to arsenic in drinking water is causally related to increased risk of cancer in the urinary bladder, lung and skin.[4,6] Furthermore, occupational exposure to arsenic by inhalation is causally related to lung cancer. Overall, animal carcinogenicity studies are difficult to interpret, due to shortcom-

ings in study design and reporting, with some exceptions.[2-4,6] For instance, in a long-term study, (pentavalent) arsenic given in the drinking water caused increased incidences of tumours in various organs, such as in the lung, liver, gastrointestinal tract and skin of mice.[6] Furthermore, arsenic trioxide, a compound that may be formed by oxidation of arsine in lung tissue, induced lung adenomas in mice, which were given the agent by perinatal treatment; and lung adenomas, carcinomas and papillomas in the respiratory tract of hamsters after intratracheal installation.[3]

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

No specific *in vitro* studies are available on the genotoxic potential of arsine *per se*.

4.2 *In vivo* assays

No *in vivo* studies are available on the genotoxic potential of arsine *per se*.

4.3 Additional information and possible mechanism of toxicity

4.3.1 Arsine

The toxicity of arsine is thought to be dependent on its metabolism.[7] However, the metabolism and mechanism of toxic action have not been clarified yet. Below is given a brief summary of the state of the art.

Regarding metabolism, some information indicate that inhaled arsine is rapidly removed from the lung and transported to the systemic circulation.[1,7,8] Research focusing on blood revealed that the agent is rapidly distributed to the red blood cells causing hemolysis and potential global cellular hypoxia.[7] Other organs can be affected although it is not known whether the effects are a result of a direct interaction between the tissue and arsine or indirect effects from red

blood cell contents.[7] A small amount of inorganic arsenic oxides and unknown metabolites can be formed during the arsine metabolism.[7]

It is hypothesized that the major route of metabolism to the ultimate metabolite(s) is via interaction of hemoproteins in the red blood cells, and the formation of inorganic arsenic oxides.[7-9] Metabolites of arsine are mainly excreted via the urine, as shown by case reports. For instance, in the urine of workers who accidentally inhaled arsine gas, various metabolites of the agent were found, including trivalent arsenic (III), arsenobetaine, dimethylarsinate, monomethylarsonate, and to al lesser extent pentavalent arsenic (V).[1,10,11] However, it was suggested that arsenobetaine was from dietary origin. When rats inhaled arsine gas at a concentration of up to 80 mg/m^3 for one hour, in the urine the same metabolites were found, except arsenobetaine.[1,12]

Using an *in vitro* test system, Ayala-Fierro *et al.* (1999) determined whether arsine itself or its metabolite trivalent arsenic was required for toxicity.[9] For this study, they used red blood cells, primary hepatocytes, and primary renal cortical epithelial cells from rats. Based on their findings, the investigators concluded that unchanged arsine was able to produce toxicity (lactate dehydrogenase leakage, and decreases in intracellular K^+) in these cells, and that the toxic potency was tissue-dependent.

Regarding its toxic mechanism, arsine is predominantly known as a hemolytic agent. Based on studies on mainly blood cells, it was postulated that arsine or its metabolites exert their toxic effects by oxidative stress depleting glutathione. However, conflicting findings have been reported, so the subject is still a matter of debate.[9,13] Others have suggested that the toxic mechanism depends on the reaction with sulfhydryl groups of Na^+K^+-ATPase.[14] The latter causes impairment in the sodium-potassium pump mechanism, with subsequent induction of abnormalities in the structure of cell membranes, for instance resulting in hemolysis of red blood cells. It is known that trivalent arsenic has a high affinity for sulfhydryl groups.

4.3.2 *Arsenic and arsenic compounds*

The metabolites of arsine, which are found in the urine, are also the major metabolites found after exposure to arsenic and other arsenic compounds.[1] For this reason it is of interest to evaluate data on mutagenicity and genotoxicity of some of these inorganic arsenic compounds. In addition, it is proposed that the trivalent species are implicated in the mechanism of arsenic-induced carcinogenicity.[4]

Andrewes *et al*. (2003) investigated the *in vitro* genotoxicity of various inorganic and organic arsenic compounds using supercoiled DNA.[15] Negative out-

comes were observed for the inorganic compounds arsine, arsenate and arsenite. The DNA damaging potency for methylated arsenic compounds varied from strong positive (dimethylarsenite, trimethylarsine oxide) to negative (monomethylarsenate).

Regarding arsenic, the Working Group of IARC evaluated several studies, and concluded that in humans arsenic has limited ability to induce point mutations, but that it has the capacity to induce large deletions and rearrangements in chromosomes.[2-4] In addition, it showed clastogenic effects (*i.e.*, micronuclei, chromosomal aberrations, aneuploidy) in humans. In *in vitro* bioassays arsenic did not act as a mutagen, but it did have clastogenic effects in mammalian cells, and it showed to be a synergistic co-mutagen.

Concerning arsenite and arsenate, these compounds did not induce mutations in various *in vitro* bioassays, except at the *tk* locus of mouse lymphoma L5178Y cells.[2-4,16] However, in various test systems they showed to be clastogenic (*i.e.*, micronuclei, sister chromatid exchanges, chromosomal aberrations) *in vitro*.

Monomethylarsonic acid and dimethylarsinic acid not only induced gene mutations at the *tk* locus of mouse lymphoma L5178Y cells, but also chromosomal aberrations and micronuclei formation in the same cells.[4,16]

Other arsenic compounds, such as trimethylarsine oxide, arsenobetaine, methylarsonous acid and dimethylarsinous acid, were also clastogenic.[4]

IARC noted that methylated trivalent arsenic had a higher genotoxic potency than trivalent inorganic arsenic, whereas methylated pentavalent arsenic had a lower potency than pentavalent inorganic arsenic.[4]

In summary, the overall weight of evidence indicate that arsenic compounds can cause clastogenic effects in exposed individuals and animals, but that results on mutagenicity are largely negative.[4,6]

Finally, several mechanisms for genotoxicity of arsenic and arsenic compounds have been proposed, including reactive oxygen radical DNA-damage, and impairment of DNA repair, but the exact genotoxic mechanism is still under debate.[4,6]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the carcinogenicity and genotoxicity of arsine in humans and animals were available, nor were there any data presented on the mutagenic and genotoxic potency *in vitro*. Due to a lack of data, there is insufficient direct evidence that arsine is carcinogenic and genotoxic.

Yet, arsine belongs to a group of arsenic and arsenic compounds, which share the same kind of metabolism and metabolites. Based on data on arsenic compounds, the committee is not able to conclude on the height of the carcinogenic potency of arsine, but the information obtained on these compounds gives insight into the possible carcinogenic and genotoxic potential of the compound. Overall, arsenic and arsenic compounds as a group are considered carcinogenic to humans. In addition, the European Union classified arsenic trioxide, arsenic oxide, and arsenic acid and its salts in carcinogenic category 1 (known to be carcinogenic to humans). Furthermore, arsenic and arsenic compounds have limited or no ability to induce mutations. However, they are clearly clastogenic, and for this reason arsenic compounds are considered genotoxic agents that mainly act by a non-stochastic mechanism. At least, the assumed metabolites of arsine have shown to possess some carcinogenic potential, although the set of data is limited.

All data considered, there is insufficient information on the carcinogenic and genotoxic potential of arsine, but the limited information on its metabolites is cause for concern to the committee.

5.2 Recommendation for classification

Based on the available information, the committee is of the opinion that arsine 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. Therefore, the committee recommends classifying arsine as *a suspected human carcinogen*. This recommendation is comparable to the EU classification in category 3. The situation is, furthermore, comparable with subcategory b of this category.

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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 93/21/EEG of the European Union

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**

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 **E**

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.