Dinitrobenzene isomers

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Dinitrobenzene isomers*
Uw kenmerk : DGV/MBO/U-932342
Ons kenmerk : U 6374/JR/fs/246-P14
Bijlagen : 1
Datum : 25 februari 2011

Geachte staatssecretaris,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan isomeren van dinitrobenzeen.

Dit 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 getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. L.J. Gunning-Schepers,
voorzitter

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Dinitrobenzene isomers

Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the Classification of Carcinogenic Substances of
the Dutch Expert Committee on Occupational Safety,
a Committee of the Health Council of the Netherlands

to:

the State Secretary of Social Affairs and Employment

No. 2011/04OSH, The Hague, February 25, 2011

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 and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, Agriculture, & Innovation, and Education, Culture & Science. 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

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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 isomeren van dinitrobenzeen onder de loep. Dinitrobenzenen worden ondermeer gebruikt bij de productie van kleurstoffen, explosieven, vezels en als vervanger van kamfer in cellulosenitraat.

De commissie concludeert dat de gegevens over isomeren van dinitrobenzeen niet voldoende zijn om de kankerverwekkende eigenschappen te kunnen evalueren en beveelt aan de stoffen in categorie 3 te classificeren*.

* Volgens het nieuwe classificatiesysteem van de Gezondheidsraad (zie bijlage D). Dit system is gebaseerd op richtlijn 1272/2008 van de Europese Unie, die op 20 Januari 2009 van kracht werd.

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 Safety of the Health Council, hereafter called the Committee. In this report, the Committee evaluated dinitrobenzene isomers. Dinitrobenzenes are used in the production of dyes, explosives, fibres, and as camphor substitute in cellulose nitrate.

The Committee concludes that the data on dinitrobenzene isomers are insufficient to evaluate the carcinogenic properties, and recommends classifying the compounds in category 3*.

* According to the new classification system of the Health Council (see Annex D), which is based on regulation 1272/2008 of the European Union. This regulation entered into force on 20 January 2009.

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 dinitrobenzene isomers.

1.2 Committee and procedures

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. The members of the Committee are listed in Annex B.

In 2010 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 point of the Committees' report is, 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 dinitrobenzene isomers, such an IARC-monograph is not available.

More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in May 2010. The new relevant data were included in this report.

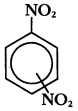
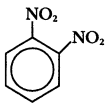
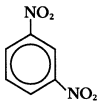
Chapter 2

General information

2.1 Identity and physico-chemical properties

Dinitrobenzenes are isomeric derivatives, which are substituted with two nitro-groups in the ortho (1,2-dinitrobenzene), meta (1,3-dinitrobenzene), and para (1,4-dinitrobenzene) positions. The compounds are used: as intermediates in the production of dyes, and explosives; as camphor substitute in cellulose nitrate; in the production of aramid and spandex fibres (1,3-dinitrobenzene); and in the production of celluloid (1,2-dinitrobenzene).

Below are given the identity and some of its physical and chemical properties.[6,12,25]

	dinitrobenzene	1,2-dinitrobenzene	1,3-dinitrobenzene	1,4-dinitrobenzene
CAS reg. No.	25154-54-5	528-29-0	99-65-0	100-25-4
EINECS no.	246-673-6	208-431-8	202-776-8	202-833-7
Synonyms	dinitrobenzol (mixed isomers)	o-dinitrobenzene; 1,2-dinitrobenzol	m-dinitrobenzene; 1,3-dinitrobenzol; binitrobenzene	p-nitrobenzene; 1,4-dinitrobenzol; dithane-A-4
Description	white to pale yellow crystals, with characteristic odour	white to yellow crystals	yellow crystals	white to pale yellow crystals
Molecular formula $C_6H_4N_2O_4$				

Molecular weight	168.12	168.12	168.12	168.12
Boiling point	≈ 300 °C	319 °C	≈ 300 °C	299 °C
Melting point	90 - 174 °C	118 °C	90 °C	≈ 174 °C
Vapour pressure at 20 °C	< 0.1 kPa	< 0.1 kPa	< 0.1 kPa	< 0.1 kPa
Vapour density at 20 °C (air = 1)	1.01	5.8	5.8	5.8
Log P_{ow}	1.46 - 1.58	1.69	1.49	1.46 - 1.49
Solubility in water	poor	very poor	very poor	not soluble
Conversion factor at 20 °C	1 ppm = 6.98 mg/m^3 1 mg/m^3 = 0.14 ppm			
EU classification	H330: Fatal if inhaled. H310: Fatal in contact with skin. H300: Fatal if swallowed. H373: May cause damage to organs. (Based on Regulation (EC) No. 1272/2008 of the European Parliament of the Council on Classification, labelling, and packaging of substances and mixtures; 16 December 2008).			

2.2 IARC classification

IARC did not evaluate dinitrobenzenes.

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

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

3.2 Carcinogenicity studies in animals

No data were available to evaluate the carcinogenicity of the agents in animals.

Chapter 4

Genotoxicity

4.1 *In vitro* assays

4.1.1 Dinitrobenzene (isomer mixture)

The mutagenic activity of dinitrobenzenes as a mixture was tested in the common *Salmonella typhimurium* mutagenicity assay, in the presence of an exogenous metabolic activation system.[2] In strains TA98, TA100, TA1535 and TA1538 no increased frequencies of reverse mutations were detected at dose levels up to 2,500 µg/plate. No other data on mutagenicity were available.

No data were available on clastogenic activity.

4.1.2 1,2-dinitrobenzene

Overall, 1,2-dinitrobenzene scored negative in the common *S. typhimurium* mutagenicity assay at dose levels up to 5,000 µg/plate, in the presence or absence of an exogenous metabolic activation system, except for one study (TA100).[3,7,13,15,23,26]

It, furthermore, did not bind to calf thymus DNA nor did it increase the unscheduled DNA synthesis in liver cells isolated from rats.[7]

Regarding clastogenic activity, in two studies 1,2-dinitrobenzene increased the frequency of chromosomal aberrations in human peripheral lymphocytes at a dose of as low as 1 mmol/L.[11,17]

4.1.3 1,3-dinitrobenzene

Overall, positive findings were reported for 1,3-dinitrobenzene regarding induction of reverse mutations in various strains of *S. typhimurium* (TA98, TA100, TA1535, TA1538), except in strain TA1535, in the presence and in the absence of an exogenous metabolic activation system.[3,4,7,8,13,14,18-21,23,26,27]

Furthermore, increased DNA repair activity in various bacterial strains (*S. typhimurium* strains, TA1535/pSK1002, NM1000, NM1011, and NM3009; *E. coli* strains, W3110/polA$^+$, and p3478/polA$^-$) have been reported, all in the absence of metabolic activation.[19-22]

It is suggested that nitroreductase and acetyltransferase are essential metabolic enzymes for generating mutagenic activity of the compound, as demonstrated by nitroreductase (TA100NR, TA100NR3, NM1000, NM1011) and acetyltransferase (NM2000, NM2009, NM3009) deficient or overexpressing bacterial strains.[14,20-22,27]

No increased induction of unscheduled DNA synthesis was observed in isolated rat liver cells, which were exposed to up to 1,000 mmol/mL.[23,24]

Lee *et al.* (2009) showed that 1,3-dinitrobenzene induces apoptotic cell death, and G2/M phase cell cycle arrest in TM4 mouse Sertoli cells.[16]

Regarding clastogenic activity, in human peripheral lymphocytes, 1,3-dinitrobenzene did induce chromosomal aberrations at doses as lower as 1.0 mmol/L.[10,11]

4.1.4 1,4-dinitrobenzene

Overall, 1,4-dinitrobenzene was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1538, with and without an exogenous metabolic activation system.[3,5,7,13,24,26,27] However, negative scores were reported in strains TA1535 and TA1537, and also in one study using strain TA1538.[26,27] Also for 1,4-dinitrobenzene, it is suggested that nitroreductase is an essential enzyme for generating mutagenic activity, as demonstrated by nitroreductase deficient strains TA100NR3 and TA1538NR.[5,27]

1,4-Dinitrobenzene did not bind to isolated calf thymus DNA.[7] Nor did it induce an increase in unscheduled DNA synthesis in isolated primary liver cells of rats.[23,24]

Regarding clastogenic activity, in human peripheral lymphocytes, the compound did induce chromosomal aberrations at doses as lower as 1.0 mmol/L.[11,17]

4.2 *In vivo* assays

No data available.

4.3 Additional information

Regarding non-carcinogenic toxicity, 1,3-dinitrobenzene is known to induce methemoglobin formation in red blood cells and disruption of spermatogenesis.[1,9] It is proposed that the mechanism of action is reduction of the nitro groups, resulting in reactive nitroaromatic radical anions, which redox cycle to produce other reactive species, such as superoxide anions. Probably, this toxic mechanism is also responsible for the genotoxic activity observed *in vitro*. However, further research is needed to confirm this idea.

The European Union has evaluated the mutagenicity of structural related compounds to propose a mutagenicity classification for dinitrobenzene isomers. Apart from 1-chloro-4-nitrobenzene, none of the other substances listed in Annex I to Council Directive 67/548/EEC were classified. These include nitrobenzene, 1,3,5-trinitrobenzene, 4-nitrobiphenyl, 2-chloro-1,3,5-trinitrobenzene and chlorodinitrobenzene. Combined with the limited data available on mutagenicity *in vitro*, and lack of data on mutagenicity *in vivo*, and according to the EU Classification and Labelling Guidelines, classification of dinitrobenzene isomers was considered not possible.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the carcinogenicity of dinitrobenzene isomers in humans were available, nor were there any carcinogenicity data available in animals.

Data on genotoxicity were limited to *in vitro* studies. These showed a variable outcome when the individual isomers are compared, in that only 1,3- and 1,4-dinitrobenze were shown to be able to induce mutations in several bacterial strains. Regarding these two substances, investigators suggested that mutagenic activity depends on the presence of nitroreductase and/or acetyltransferase enzymes. A limited number of studies indicated that dinitrobenzenes were clastogenic. Overall the available data on genotoxicity is too limited for a definite conclusion on genotoxicity.

5.2 Recommendation for classification

The Committee concludes that the data on dinitrobenzene isomers are insufficient to evaluate the carcinogenic properties, and recommends classifying the compounds in category 3*.

* According to the new classification system of the Health Council (see Annex D), which is based on regulation 1272/2008 of the European Union. This regulation entered into force on 20 January 2009.

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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	Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling, and packaging of substances and mixtures

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 Safety (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. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- G.J. Mulder, *chairman*
  Emeritus Professor of Toxicology, Leiden University, Leiden
- J. van Benthem
  Genetic toxicologist, National Institute for Public Health and the Environment, Bilthoven
- 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; Professor of Translational toxicology, Wageningen University and Research Centre, Wageningen
- A.A. van Zeeland
  Emeritus Professor of Molecular radiation dosimetry and radiation mutagenesis, Leiden 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 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 2010 for public review. The following organisations and persons have commented on the draft document:

- Ms V. Gálvez Pérez, Instituto Nacional de Seguridad e Higiene en el Trabajo, Madrid, Spain.
- Mr R.D. Zumwalde, National Institute of Occupational Safety and Health, Cincinnati, the USA.

Annex **D**

Carcinogenic classification of substances by the Committee

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

Category	Judgement of the Committee (GRGHS)	Comparable with EU Category	
		67/584/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to man. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	1	1A
1B	The compound is presumed to be carcinogenic to man. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
3	The available data are insufficient to evaluate the carcinogenic properties of the compound.	Not applicable	Not applicable
4	The compound is probably not carcinogenic to man.	Not applicable	Not applicable

Annex **E**

Regulation (EC) No 1272/2008

of the European Parliament and of the Council on classification, labelling, and packaging of substances and mixtures

3.6 Carcinogenicity

3.6.1 Definition

Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

3.6.2 Classification criteria for substances

See Table on the next page.

3.6.2.1 For the purpose of classification for carcinogenicity, substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence). In certain instances, route-specific classification may be warranted, if it can be conclusively proved that no other route of exposure exhibits the hazard.

3.6.2.2 Specific considerations for classification of substances as carcinogens.

3.6.2.2.1 Classification as a carcinogen is made on the basis of evidence from reliable and acceptable studies and is intended to be used for substances which have an intrinsic property to cause can-

Table 3.6.1 Hazard categories for carcinogens.

Categories	Criteria
Category 1:	Known or presumed human carcinogens. A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A:	Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B:	Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
Category 2:	Suspected human carcinogens. The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

(1) Note: See 3.6.2.2.4.

cer. The evaluations shall be based on all existing data, peer-reviewed published studies and additional acceptable data.

3.6.2.2.2 Classification of a substance as a carcinogen is a process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place substances with human cancer potential into hazard categories.

3.6.2.2.3 Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
- limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;
- limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, *e.g.* (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

3.6.2.2.4 Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here.

3.6.2.2.5 The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

3.6.2.2.6 Some important factors which may be taken into consideration, when assessing the overall level of concern are:

a tumour type and background incidence;
b multi-site responses;
c progression of lesions to malignancy;
d reduced tumour latency;
e whether responses are in single or both sexes;
f whether responses are in a single species or several species;
g structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
h routes of exposure;
i comparison of absorption, distribution, metabolism and excretion between test animals and humans;
j the possibility of a confounding effect of excessive toxicity at test doses;
k mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.

3.6.2.2.7 A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, *e.g.* for benzidine congener dyes.

3.6.2.2.8 The classification shall take into consideration whether or not the substance is absorbed by a given route(s); or whether there are only local tumours at the site of administration for the tested route(s), and adequate testing by other major route(s) show lack of carcinogenicity.

3.6.2.2.9 It is important that whatever is known of the physico-chemical, toxicokinetic and toxicodynamic properties of the substances, as well as any available relevant information on chemical analogues, i.e. structure activity relationship, is taken into consideration when undertaking classification.

3.6.4 Hazard communication

3.6.4.1 Classification for carcinogenicity:

Category 1A or Category 1B:

Hazard statement H350: May cause cancer <*state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard*>.

Category 2:

Hazard statement H351: Suspected of causing cancer <*state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard*>.