# Formamide

Evaluation of the carcinogenicity and genotoxicity

**Gezondheidsraad**
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Formamide*
Uw kenmerk : DGV/MBO/U-932342
Ons kenmerk : U 6367/AvdB/fs/246-G14
Bijlagen : 1
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Geachte staatssecretaris,

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

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

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 70 17
E-mail: a.vander.burght@gr.nl

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

# Formamide

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. 2011/01OSH, The Hague, February 18, 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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# 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 formamide onder de loep. Formamide is een stof dat onder andere wordt gebruikt als intermediair bij diverse industriële processen en bij de productie van geneesmiddelen en pesticiden en als oplosmiddel in de productie van plastics.

Op basis van de beschikbare gegevens leidt de commissie af dat formamide verdacht kankerverwekkend voor de mens is, en beveelt aan de stof te classificeren in categorie 2*.

* Volgens het nieuwe classificatiesysteem van de Gezondheidsraad (zie bijlage D). Dit systeem 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 (DECOS) of the Health Council, hereafter called the Committee. In this report, the Committee evaluated formamide. Formamide is an agent that is among others used as intermediate in various industrial processes, and in the production of pharmaceuticals, and pesticides, and as solvent in the production of plastics.

Based on the available information, the Committee is of the opinion that formamide is a suspected carcinogenic to man, and recommends classifying the substance in category 2*.

* 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 formamide.

## 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 relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of formamide, such an IARC-monograph is not available.

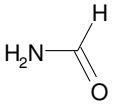
Published data were retrieved from the online databases Medline, Toxline, and Chemical Abstracts. The last updated online search was in June 2010. The relevant data were included in this report.

Chapter 2

# General information

## 2.1 Identity and physico-chemical properties

The data have been retrieved from the WHO* assessment of formamide, the European Substance Information System (ESIS, which can be accessed via the ECB-site: http://ecb.jrc.it) and the internet based Chemfinder database and search engine (http://chemfinder.cambridgesoft.com).

| | | |
|---|---|---|
| Chemical name | : | formamide |
| CAS registry number | : | 75-12-7 |
| EINECS number | : | 200-842-0 |
| Synonyms | : | Formamid, Methanamide |
| Appearance | : | Slightly viscous, colorless liquid |
| Use | : | Formamide is an important intermediate in the chemical industry; it is used for the production of heterocyclic compounds, pharmaceuticals, fungicides and pesticides; it is used as an solvent for the production of plastics. |
| Chemical formula | : | $CH_3NO$ |
| Molecular structure | : |  |
| Molecular weight | : | 45.04 |



* WHO: World health organisation.

| | | |
|---|---|---|
| Boiling point | : | 210.5°C |
| Melting point | : | 2.6°C |
| Vapour pressure | : | 0.008 kPa at 20°C |
| Vapour density (air = 1) | : | 1.56 |
| Solubility | : | Miscible in water at 20°C, soluble alcohol and acetone |
| EU Classification | : | T: Toxic |
| (100% solution) | : | H360D: May cause harm to the unborn child.<br>(Based on Regulation (EC) No 1272/2008 of the European Parliament and of the Council on Classification, labelling and packaging of substances and mixtures; 16 December 2008) |

## 2.2 IARC classification

Formamide has not been evaluated by IARC.

Chapter 3

# Carcinogenicity

## 3.1 Observations in humans

No information on human studies related to the carcinogenicity and/or mutagenicity of formamide has been retrieved from public literature.

## 3.2 Carcinogenicity studies in animals

In a review by Kennedy (2001), it is stated that formamide has been shown to produce liver tumours in rats and mice.[3] However, the Committee noticed that neither supporting literature has been referenced nor was any data found in the present literature search that could substantiate this. Therefore, this information is not considered to be reliable.

The US National Cancer Institute has nominated a class of chemicals for testing by the National Toxicology Program (NTP), which included formamide. A two-year carcinogenicity study was conducted in both rats and mice.[5]

Groups of fifty male and fifty female rats were administered 0, 20, 40, or 80 mg formamide/kg bw, five days per week for 104 to 105 weeks, in deionized water by gavage. The survival of all dosed groups of rats was similar to that of the vehicle controls. Mean body weights of the high dose males were less than those of the vehicle controls throughout most of the study. The mean body weights of females to which 40 and 80 mg/kg bw was administered, were some-

what less than those of the vehicle controls during the second year of the study. No neoplasms or non-neoplastic lesions were attributed to exposure to formamide.

Groups of fifty male and fifty female mice were administered 0, 20, 40, or 80 mg formamide/kg bw, five days per week for 104 to 105 weeks, in deionized water by gavage. The survival of all dosed groups of mice was similar to that of the vehicle controls. Mean body weights of high dose males and females were generally less than those of the vehicle controls throughout the study. NTP concluded that the incidences of hemangiosarcoma of the liver occurred with a positive trend in *males*, and the incidences were significantly increased in the groups administered 40 and 80 mg/kg bw; this was considered to be 'clear evidence' of carcinogenic potential. The significantly increased incidence of hepatocellular adenoma or carcinoma (combined) in 80 mg/kg bw (*females*) were taken as 'equivocal evidence' (see Table 3.1). In high dose males the incidence of hematopoietic cell proliferation of the spleen, and the mineralization of the testicular arteries and testicular tunic were significantly increased as well.

*Table 3.1* Increased tumours incidences[a] in formamide treated[b] B6C3F1 mice.[5]

| Type of tumour | Control | 20 mg/kg bw | 40 mg/kg bw | 80 mg/kg bw |
|---|---|---|---|---|
| Males, liver | | | | |
| • Hemangioma | | | | 1 |
| • Hemangiosarcoma | 1 | 5 | 6 | 3 |
| • Hemangiosarcoma, multiple | - | - | 1 | 5 |
| • *Combined* | *1* | *5* | *7*[c] | *8*[c,d] |
| Females, liver | | | | |
| • Adenoma | 4 | 9 | 11 | 10 |
| • Adenoma, multiple | 2 | 3 | 2 | 2 |
| • Carcinoma | 4 | 3 | - | 5 |
| • Carcinoma, multiple | - | 1 | - | 1 |
| • *Combined* | *9* | *15* | *13* | *18*[c,e] |

[a] Number of animals with lesion per 50 treated animals.
[b] Treatment: 5 days/week per gavage for 104 weeks.
[c] Statistically significantly increased, probably $P<0.05$ (test not indicated).
[d] Clear evidence of carcinogenic activity (NTP nomenclature).
[e] Equivocal evidence of carcinogenic activity (NTP nomenclature).

Chapter 4

# Genotoxicity

## 4.1 *In vitro* assays

Formamide has been tested in a series of short-term *in vitro* assays. In three independent Ames tests, formamide was tested up to a concentration of 10% in several strains of *S. typhimurium* TA 98, 100, 1535, 1537, with and without rat or hamster liver S9 activation enzymes, or in *E. coli* strain WP uvrA pKM101, tested with and without 10% rat liver S9. In all three tests, formamide scored negative for the induction of gene mutations.[5]

Several authors report a good compatibility for formamide as a solvent in microbial mutagenicity tests.[1,2,4] Although the mutagenic potential of formamide was not the primary objective in these studies, lack of positive results in the solvent controls suggest non-genotoxic properties of formamide.

## 4.2 *In vivo* assays

Formamide was tested by the NTP in a micronucleus assay, using male and female B6C3F1 mice.[5] Animals were administered 0, 10, 20, 40, 80 and 160 mg/kg bw by gavage for 90 days (10 animals/group). In all treatment groups of both sexes, no increase of micronucleated erythrocytes was observed.

Negative results were obtained in a test for induction of sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* treated with formamide either by feeding or injection.[5]

Chapter 5

# Classification

## 5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the carcinogenicity and genotoxicity in humans were available. No signs of carcinogenicity in rats were found in one study, but in the same study using B6C3$F_1$ mice, chronic oral exposure to formamide induced hepatic hemangiosarcomas in male mice, whereas equivocal evidence for carcinogenicity was found in female mice (combined hepatocelluar adenomas and carcinomas). Although the mouse strain used in this study is known to be sensitive in developing liver tumours, the Committee considers the development of hemangiosarcomas is rather uncommon, but relevant for humans.

Regarding genotoxicity, data showed no signs of mutagenic activity in *in vitro* and *in vivo* assays.

## 5.2 Recommendation for classification

Based on the available information, the Committee is of the opinion that formamide is a suspected carcinogenic to man, and recommends classifying the substance in category 2*.

* 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.

# References


1 Abbondandolo A, Bonatti S, Corsi C, Corti G, Fiorio R, Leporini C *et al*. The use of organic solvents in mutagenicity testing. Mutat Res 1980; 79(2): 141-150.

2 Banno F, Saito S, Tsuchiya T, Hagiwara Y. Comparative examination of various solvents for the microbial mutagenicity tests. Kankyo Henígen Kenkyu 1998; 20(1): 19-27.

3 Kennedy GL, Jr. Biological effects of acetamide, formamide, and their mono and dimethyl derivatives: an update. Crit Rev Toxicol 2001; 31(2): 139-222.

4 Maron D, Katzenellenbogen J, Ames BN. Compatibility of organic solvents with the Salmonella/ microsome test. Mutat Res 1981; 88(4): 343-350.

5 National Toxicology Program. NTP Toxicology and Carcinogenesis Studies of Formamide (CAS No. 75-12-7) in F344/N Rats and B6C3F1 Mice (Gavage Studies). Natl Toxicol Program Tech Rep Ser 2008;(541): 1-192.

| | |
|---|---|
| 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

- A.S.A.M. van der Burght, *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:

- National Institute of Occupational Safety and Health (NIOSH), Cincinatti, USA
- Unidad technical de Evaluaciones Ambientales, CNNT-INSHT, Madrid, Spain

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.<br>• It acts by a stochastic genotoxic mechanism.<br>• It acts by a non-stochastic genotoxic mechanism.<br>• It acts by a non-genotoxic mechanism.<br>• 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.<br>• It acts by a stochastic genotoxic mechanism.<br>• It acts by a non-stochastic genotoxic mechanism.<br>• It acts by a non-genotoxic mechanism.<br>• 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.<br>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:<br>human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or<br>animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).<br>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*>.