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**A GUIDE TO THE CLASSIFICATION OF
CARCINOGENS, MUTAGENS AND TERATOGENS
UNDER THE SIXTH AMENDMENT**

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A. SUMMARY

The Sixth Amendment to the European Communities' Council Directive 67/548/EEC relating to the classification, labelling and packaging of dangerous substances requires a manufacturer or importer to propose a classification of such substances according to their toxic potential. The classification, which is based on physico-chemical and toxicological properties, leads to labelling and packaging requirements.

In Annex VI of the 6th Amendment, clear guidance is given for classifying substances with regard to their LD₅₀ or LC₅₀, and it is noted that the classification may, alternatively, have to be based on other effects. The 5th Adaptation of the Directive gives further guidance on classification with respect to acute effects and also provides definitions of 3 Categories of carcinogens, 3 of mutagens and 2 of teratogens. However, it gives no detailed guidance on the criteria which would lead to classification in these Categories. Unlike substances whose classification is solely defined by a numerically-expressed acute effect, the classification of carcinogenic, mutagenic or teratogenic substances has to be based on studies the results of which can rarely be expressed in numerical terms, plus other relevant information. This means that such classifications have to be derived by an expert interpretation of all relevant information on each individual substance.

In this report ECETOC has set out general guidance for the classification of new and existing carcinogenic, mutagenic and teratogenic substances as an aid to those who have responsibilities in this area. The evidence which leads to the inclusion in, or exclusion from, each Category, or to non-classification, is discussed.

B. INTRODUCTION

In 1979 the European Communities published Council Directive 79/831/EEC amending for the sixth time the Directive 67/548/EEC relating to the classification, packaging and labelling of dangerous substances, henceforth referred to as the "6th Amendment". This amendment has been incorporated into legislation by the member states and where there are differences in the text between the 6th Amendment and the national versions the English text as issued by the European Commission was used in producing this report.

In Article 2.1(a), substances are defined as "chemical elements and their compounds as they occur in the natural state or as produced by industry, including any additives required for the purpose of placing them on the market."

In the 6th Amendment there is a well-defined procedure for notifying the intention to place a new substance on the market. According to the tonnage to be marketed, information relevant to potential adverse effects on humans or environmental organisms has to be developed from a series of experimental measurements or studies as recommended in Annexes VII and VIII of the Amendment (see ECETOC, 1984). The manufacturer or importer has also to propose a classification of the substance, based on the results of the experimental measurements and studies, and according to the Categories referred to in Annex VI, section II.D. of the 6th Amendment. The detailed requirements for categorisation are set out in the 5th Adaptation of the Directive (83/467/EEC of 29.7.83), henceforth referred to as the "5th Adaptation". It is also required to classify substances already on the market according to the same Categories (see Annex I of the 6th Amendment).

In Annex VI, guidance is given for classifying substances with regard to their LD_{50} or LC_{50} . In part 1, A,(b) of this Annex it is stated that "if facts show that for the purposes of classification it is inadvisable to use the LD_{50} or LC_{50} values as a principal basis because the substances or preparations produce other effects the substances or preparations shall be classified according to the magnitude of these effects". This means that for some substances the classification has to reflect their other acute effects, or chronic effects, rather than the lethal dose. In the 5th Adaptation, 3 Categories of carcinogens, 3 of mutagens and 2 of teratogens are defined, and the procedure for notifying the classification is given (see Article 3.1.2). Unlike substances whose classification can be defined by a numerically-expressed acute effect (eg. by an LD_{50}), classification of carcinogenic, mutagenic or teratogenic substances has to be based on the results of toxicological or epidemiological studies which can rarely be expressed in numerical terms. It is emphasised that such classifications have to be derived by a critical evaluation of the results of the studies undertaken and an expert interpretation of all relevant information on each individual substance.

The tests or studies used for classification purposes should be compatible with the relevant OECD test guidelines and Good Laboratory Practice (GLP), or their adequacy should be judged on a case by case basis.

In this report, produced by a Task Force whose members are given in Section G, ECETOC has set out guidance for the classification of substances as carcinogens, mutagens and teratogens, as an aid to those who have responsibilities in this area.

C. CLASSIFICATION OF CARCINOGENS

In the 5th Adaptation, carcinogenic substances are required to be classified in 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".

In the following sections the factors to be considered before substances are placed in one or other of the above Categories, or are not classified, are given.

1. Category 1 : Substances Known to be Carcinogenic to Man

Evidence for the inclusion of a substance in this Category is provided by sound epidemiological studies. It must be recognised that such studies can

only suggest or demonstrate an association between an increased incidence of cancer in a human population and exposure to a particular substance. To demonstrate beyond reasonable doubt that exposure has actually caused cancer is difficult. The following observations support the existence of a causal relationship :

- a) A marked increase in the incidence of one or more types of cancer in the exposed, compared to the non-exposed, population.
- b) The occurrence of an unusually high incidence of a rare cancer in a population exposed to a substance, particularly where such tumours do not occur normally in geographical clusters.
- c) The observation of an increase in cancer incidence in more than one study, carried out on populations from different locations or workplaces. This reinforces the evidence of a causal relationship suggested by the findings under a) and b), above.
- d) An increase in cancer incidence associated with exposure to increasing amounts of a substance or with increasing periods of exposure prior to the development of the disease, i.e. evidence of dose-time-response relationships. Supporting evidence is provided by the observation of a decrease in the incidence of cancer following a reduction in the exposure of populations to a substance.

Limitations in the epidemiological data can make it difficult to determine whether a causal relationship exists. For example :

- i) the sample size may be too small to provide the statistical power necessary for detecting an excess incidence of cancer;
- ii) information on the level and duration of exposure may be inadequate;
- iii) the study period may be too short for a latency period to elapse;
- iv) an excess incidence of tumours or an elevated death-rate among the exposed persons may be due to confounding factors (such as exposure to other substances, smoking habits or alcohol consumption) which were not, or could not be taken into account.

2. Category 2 : Substances Which Should be Regarded as if They Are Carcinogenic to Man

Evidence leading to a classification in this Category will come from appropriate long-term animal studies together with other relevant information. Substances in this Category fall into the class of "Putative

Human Chemical Carcinogens", defined by ECETOC (1982) as "a clearly-defined chemical substance which causes cancers in adequate animal experimentation, under exposure conditions which correspond to those in man or where the relevance of exposure conditions can be deduced". The phrase in the 5th Adaptation definition "...a strong presumption that human exposure to a substance may result in the development of cancer...", and the ECETOC definition, can be interpreted to exclude from Category 2 those substances that produce cancers only under conditions which are irrelevant to the likely human exposure, or where the relevance cannot be deduced. (see C.2.2.2).

When the adequacy of animal data for a Category 2 classification is considered, two aspects have to be taken into account : the reliability of the animal data, and the criteria for a Category 2 classification based on them. These are discussed below.

2.1 Assessment of the reliability of animal data

Generally speaking, current studies for investigating carcinogenic potential follow recognised guidelines such as those of the OECD, EPA or FDA. However, none of these guidelines can be regarded as comprising rigid requirements and the reliability of data from any study (including those carried out before these guidelines were published) should be determined on a case by case basis. The following criteria should be borne in mind when reliability is assessed :

- a) The test substance should have been clearly specified and the amount administered should have been monitored. For example, during inhalation studies the concentration of the test substance and the characteristics of the atmosphere (e.g. air flow, concentration, temperature, humidity and, where relevant, particle size) should have been carefully monitored, and the substance should have been administered in a form similar to that involved in human exposure. If the test substance was administered in the feed, its concentration, homogeneity and stability in the feed should have been determined.
- b) The number of animals (normally rodents) used should have been high enough to identify a small increase in neoplasms above the background incidence (50 per sex, per dose in the exposed and control groups are usually required).

- c) Ideally both sexes should have been examined. This may not be necessary in some types of study, e.g. in skin painting in mice to assess topical carcinogenicity, for which the use of a single sex is generally regarded as acceptable because there are substantial background data on such studies.
- d) When there has been no increase in the incidence of tumours, the highest dose level should have produced some toxic effects without significantly reducing the survival. Where there is an increase in tumour incidence, the survival of test animals should not have been significantly less than that of the controls, unless the animals died early because of treatment-related tumours.
- e) The control groups should have been of the same size as, or larger than, the dosed groups, and treated in the same way as the dosed groups except for the absence of test material. In specific cases it may be necessary to include a positive-control group to demonstrate the susceptibility of the animal strain used.
- f) The substance should have been administered, either continuously or with adequate frequency, over a high proportion of the total life span of the animal.
- g) Because of the variability of tumour incidence in control animals, adequate background data should have been available to assist in interpreting the results.

Some substances are sufficiently potent to provide adequate evidence of carcinogenicity even if many of the above criteria are not fulfilled, eg. the use of fewer animals is adequate when there is a clear increase in the incidence of tumours in the exposed as compared to control animals.

2.2 Criteria for a Category 2 classification based on findings from animal studies.

- 2.2.1 Inclusion criteria. For a classification in Category 2 it is recommended that at least one of the following conditions described by the International Agency for Research on Cancer (IARC, 1984) should be met, ie. there should be "an increased incidence of malignant tumours : (a)

in multiple species or strains, or (b) in multiple experiments (preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumour, or age at onset. Additional evidence may be provided by data on dose-response effects".

The criteria for including a substance in this Category are based upon finding an increased incidence of malignant tumours (c.f. 2.2.1.). There are a few instances, however, where a precise distinction between benign and malignant tumours is not possible, eg. for certain endocrine or skin tumours. In this case, providing also that a progression from the apparently benign state to a more overtly malignant one is known for such tumours, it may be valid to combine them when assessing tumour incidence in a particular organ. Furthermore, where benign and malignant tumours occur together in a particular study and originate from one and the same cell type in an organ or tissue, it may be valid to combine them in assessing tumour incidence. Nevertheless, the finding of an increase in malignant tumours is the hallmark of carcinogenicity and for this reason it is stressed in the text.

2.2.2 Exclusion criteria. Data from an animal study would not normally be regarded as sufficient for a Category 2 classification if :

- a) the route of exposure or physical form of the substance was not relevant to the human situation, or its relevance could not be deduced;
- b) the dose level required to produce tumours in experimental animals was so high that it adversely affected their physiological state or produced recurrent injury in the tissue or organ in which tumours later appeared, but no tumours developed at exposure levels at which these effects were not produced ;
- c) tumours were produced in animals following repeated administration of a substance only at levels which would require the intake of unrealistically large quantities by man or under conditions which could not be tolerated by man;
- d) only benign tumours were induced and there was no evidence of progression to malignancy;
- e) there was an increased incidence only of tumours with a high spontaneous incidence in the species used, eg. liver, lung, lymphatic and mammary tumours in the mouse ;
- f) only hepatic nodules were induced in rats;

g) other relevant information raised doubts about the significance of the animal data to man.

In such cases the substance should be considered for classification in Category 3.

2.3 The use of other relevant information

In this context the phrase "Other relevant information" in the 5th Adaptation is interpreted to mean information other than that from the long-term animal studies. For the purpose of classification such information should not normally be used in place of data from animal studies but rather as an aid in interpreting them, especially if it helps in deciding whether humans are likely to be affected.

a) Epidemiological data.

i) The results of epidemiological studies which are inadequate to support a Category 1 classification might support a classification in Category 2, but only when the animal data are largely sufficient on their own.

ii) Adequate epidemiological studies which provide good evidence for the absence of carcinogenicity should be taken into account when the relevance of data from animal studies to man is assessed.

b) Results from short-term tests.

These tests should be such as to enable the ability of a substance to interact with DNA to be assessed. The interaction of a chemical with DNA, which may result in gene, chromosome or genome mutations in somatic cells, is thought to be a necessary step in the initial stages of chemical carcinogenesis. Many test systems have been developed for assessing the mutagenic (genotoxic) activity of a substance but the extent to which these tests have been validated as indices of carcinogenic potential varies. The results from an appropriate battery of adequately validated short-term tests may assist classification.

Substances which are clearly mutagenic and possess carcinogenic activity according to the criteria outlined above will obviously be placed in Category 2. Substances which, although positive in animal carcinogenicity studies, give negative results in an appropriate battery of short-term tests covering the above end-points may not appropriately be classified in Category 2 if the exposure levels at which tumours are

induced in the animal studies greatly exceed the likely level of human exposure.

The demonstration of DNA-adduct formation or chromosome damage in exposed human populations does not prove that the substance is a human carcinogen, but in conjunction with positive results from animal studies it could support a Category 2 classification.

- c) Data concerning the differences and similarities in metabolism, absorption and toxicokinetics between man and test species may be useful in assessing the relevance to man of the results of animal studies.
- d) Structure-activity relationships (SARs). These are not at present reliable enough to support or exclude the classification of a substance as a carcinogen, although in a few cases where particularly strong SARs have been established for series of structurally-related chemicals. eg. the alkyl nitrosamines, they may suggest that a substance possibly has carcinogenic potential.

3. Category 3 : Substances Which Cause Concern Owing to Possible Carcinogenic Effects

Substances for which the limited evidence of carcinogenicity does not meet the criteria for classification in Categories 1 or 2 as described above should be considered for this Category.

A substance should be classified in Category 3 only after an expert consideration of all the available evidence. The following circumstances, taken singly or together, exemplify such evidence :

- a) there is an increase in the incidence of malignant tumours but it is only of borderline significance ;
- b) there is a treatment-related increase only of benign tumours;
- c) there is an increased incidence of malignant tumours in only 1 species or strain but the criteria for classification in Category 2 (C.2.2.1) are not met;
- d) there is an increased incidence of tumours only in organs or tissues of which the natural incidence is high, or known to be variable;
- e) there is an increased incidence of malignant tumours induced only by a route of administration which is not relevant to human exposure, or where the relevance cannot be deduced. It should be noted that some

routes of administration (e.g. subcutaneous, intravenous and intraperitoneal) may be less reliable indicators of carcinogenicity than are others. The subcutaneous route, in particular, can give false positive findings and much caution must be exercised in interpreting the data, especially when only local tumour formation is induced;

- f) there is a clear increase in the incidence of malignant tumours but only at exposure levels which are not relevant to the human situation, or where the relevance cannot be deduced;
- g) other information suggests that positive findings in animal studies are not relevant to humans, for example when extensive epidemiological studies provide a strong indication that the carcinogenic risk to man is negligible;
- h) adequately-performed animal studies at exposure levels relevant to that of humans have given results which do not meet the criteria for classification in Category 2, and genotoxic activity has not been demonstrated in an appropriate battery of short-term tests (see section C.2.3.b).

4. Circumstances Leading to Non-classification

In the absence of evidence for carcinogenicity, a substance is obviously not classified. There are also circumstances when the evidence is not sufficient for classification, as in the following examples.

- a) The experimental animals were exposed under conditions which do not occur when the substance is handled or used by humans (eg. "solid state" sarcomas from the implantation of a plastic), or which involved the use of the substance in a physical form to which humans are not exposed.
- b) There is only equivocal evidence of carcinogenicity from animal studies where, i) it is considered that the observed tumours probably did not occur as a result of exposure to the substance; or ii) where the experimental studies were either inadequately performed or could not be reproduced.
- c) Tumours were produced in animals by a substance which led to :
 - i) a marginal but statistically-significant increase in the incidence of tumours only at dose levels which greatly exceed those likely to be experienced by man, especially if the increase appeared towards the end of the animals' life-span;

- ii) an increase in the incidence of tumours only at dose levels which produced recurrent toxic damage in the target tissue.

The criteria under c), above, are especially important if the substance lacks mutagenic activity, and considerable experience is required to assess the significance of such findings to man. These findings are exemplified by substances which cause ;

- recurrent tissue damage leading to tissue regeneration (eg. skin painting with highly irritant materials);
 - an alteration in the physiological state of the animal. This may be due to the physico-chemical properties of the substance, eg. which cause its deposition as bladder stones in the rat, leading to the development of bladder cancer. Other substances administered at high dose levels may produce dietary changes or alterations in hormonal balance, both of which can influence the incidence of spontaneous tumours;
 - alterations in immunological competence. There is evidence that damage to the immune system may lead to an increase in the incidence of tumours during an animal study.
- d) The substance acted only as a promoter, ie. caused an increase in the incidence of tumours only when administered after treatment with a known carcinogen.
- e) The occurrence of tumours can be reliably attributed to changes in the metabolism of the substance due to metabolic over-loading at high doses - for example, the saturation of de-toxification processes which are normally present in man and the test species.
- f) The existence of evidence from an epidemiological study or studies which is adequate to contradict that derived from limited data in animal studies.
- g) The demonstration of carcinogenic activity in animal studies at exposure levels which greatly exceed the likely human exposure, in the absence of evidence that the substance is genotoxic.

D. CLASSIFICATION OF MUTAGENS

In the 5th Adaptation mutagenic substances are classified into three Categories as follows :

Category 1. Substances known to be mutagenic to man. There is sufficient evidence to establish a causal association between human exposure to a substance and heritable genetic damage.

Category 2. Substances which should be regarded as if they are mutagenic to man. There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in the development of heritable genetic damage, generally on the basis of appropriate animal studies.

Category 3. Substances which cause concern for man owing to possible mutagenic effects but in respect of which the available evidence does not satisfactorily demonstrate heritable genetic damage. There is evidence from appropriate mutagenicity studies, but this is insufficient to place a substance in Category 2."

Although mutagenicity tests are often conducted for carcinogenicity screening, mutagenicity is a toxicological property in its own right. In the following sections the rationale for placing mutagenic substances in one or other of the above Categories is discussed.

1. Category 1 : Substances Known to be Mutagenic to Man

Evidence for inclusion in this Category can come only from detailed observation of exposed human populations. Clinical and epidemiological studies would be required to demonstrate a causal relationship between exposure to a substance and the occurrence of heritable effects (germ-cell mutations). However, no human mutagens are known and it is extremely difficult to gain reliable information from studies on humans for the following reasons :

- a) there is a large variation within the human gene pool;
- b) the number of progeny is small;
- c) the generation time is long;
- d) not all mutations produce detectable phenotypic variations;
- e) only dominant, and some sex-linked, recessive mutations would be observed in the first generation;