CARCINOGENS, GERM CELL MUTAGENS, REPRODUCTIVE TOXICANTS

EUROPEAN REGULATORY CLASSIFICATION CRITERIA, HAZARD COMMUNICATION ELEMENTS

Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing directives 67/548/EEC and 1999/45/EC, and amending regulation (EC) No 1907/2006 – Annex I: classification and labelling requirements for hazardous substances and mixtures.

1 – Carcinogens: three categories

Carcinogenicity means the induction of cancer or an increase in the incidence of cancer occurring after exposure to a substance or mixture. Substances and mixtures 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.

Classification of a substance or mixture as posing a carcinogenic hazard is based on its intrinsic properties and does not provide information on the level of the human cancer risk which the use of the substance or mixture may represent.

1.1 – Definitions of the categories

First category

Known or presumed human carcinogens.

A substance is classified in category 1 for carcinogenicity on the basis of epidemiological and/or animal data.

Category 1A

Substances known to have carcinogenic potential for humans.

The classification in this category is largely based on human evidence, human studies that establish a causal relationship between human exposure to a substance and the development of cancer.

Category 1B

Substances presumed to have carcinogenic potential for humans.

The classification in this category is largely based on animal evidence, animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity.

Second category: 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.

1.2 – Hazard communication

SGH08 pictogram "*Health hazard*" applies regardless of the category.

The signal word "*Danger*" applies for categories 1A and 1B. The signal word "*Warning*" applies for category 2.

The hazard statement H3540: "*May cause cancer"* applies for categories 1A and 1B.

The hazard statement H351: "*Suspected of causing cancer"* applies for category 2.

For all categories, the route of exposure must be stated if it is conclusively proven that no other routes of exposure cause the hazard.

1.3 – Specific considerations for classification of substances as carcinogens

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

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.

1.3.1 – Evaluations of strength of evidence

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.

1.3.2 – Additional considerations

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.

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.

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.

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.

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.

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.

2 – Germ cell mutagens: three categories

This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests *in vitro* and in mammalian somatic and germ cells *in vivo* are also considered in classifying substances and mixtures within this hazard class.

Germ cell mutagenicity means heritable gene mutations, including heritable structural and numerical chromosome aberrations in germ cells occurring after exposure to a substance or mixture.

A mutation means a permanent change in the amount or structure of the genetic material in a cell. The term 'mutation' applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term 'mutagenic' and 'mutagen' will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

The more general terms 'genotoxic' and 'genotoxicity' apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects.

2.1 – Definitions of the categories

First category

Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans.

Category 1A

Substances known to induce heritable mutations in the germ cells of humans.

The classification in category 1A is based on positive evidence from human epidemiological studies.

Category 1B

Substances to be regarded as if they induce heritable mutations in the germ cells of humans.

The classification in category 1B is based on:

- positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or
- positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

Second category: category2

Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans.

The classification in category 2 is based on positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

- somatic cell mutagenicity tests *in vivo*, in mammals; or
- other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as category 2 mutagens.

2.2 – Hazard communication

SGH08 pictogram "*Health hazard*" applies regardless of the category.

The signal word "*Danger*" applies for categories 1A and 1B. The signal word "*Warning*" applies for category 2.

The hazard statement H340: "*May cause genetic defects"* applies for categories 1A and 1B.

The hazard statement H341: "*Suspected of causing genetic defects"* applies for category 2.

For all categories, the route of exposure must be stated if it is conclusively proven that no other routes of exposure cause the hazard.

2.3 – Specific considerations for classification of substances as germ cell mutagens

To arrive at a classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in *in vitro* tests shall also be considered.

The system is hazard based, classifying substances on the basis of their intrinsic ability to induce mutations in germ cells. The scheme is, therefore, not meant for the (quantitative) risk assessment of substances.

Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in Regulation (EC) No 440/2008 adopted in accordance with Article 13 (3) of Regulation (EC) No 1907/2006 ("Test Method Regulation") such as those listed in the following paragraphs. Evaluation of the test results shall be done using expert judgement and all the available evidence shall be weighed in arriving at a classification.

Possible tests:

- *In vivo* heritable germ cell mutagenicity tests, such as:
  - rodent dominant lethal mutation test;
  - mouse heritable translocation assay.
- *In vivo* somatic cell mutagenicity tests, such as:
  - mammalian bone marrow chromosome aberration test;
  - mammalian erythrocyte micronucleus test.
- Mutagenicity/genotoxicity tests in germ cells, such as:
  - mutagenicity tests:
    - mammalian spermatogonial chromosome aberration test;
    - spermatid micronucleus assay;
  - Genotoxicity tests:
    - sister chromatid exchange analysis in spermatogonia;
    - unscheduled DNA synthesis test (UDS) in testicular cells.
- Genotoxicity tests in somatic cells such as:
  - liver Unscheduled synthesis test (UDS) *in vivo*;
  - mammalian bone marrow Sister Chromatid Exchanges (SCE);
- *In vitro* mutagenicity tests such as:
  - *in vitro* mammalian chromosome aberration test;
  - *in vitro* mammalian cell gene mutation test;
  - bacterial reverse mutation tests.

The classification of individual substances shall be based on the total weight of evidence available, using expert judgement. In those instances where a single well-conducted test is used for classification, it shall provide clear and unambiguously positive results.

If new, well validated, tests arise these may also be used in the total weight of evidence to be considered. The relevance of the route of exposure used in the study of the substance compared to the route of human exposure shall also be taken into account.

2.4 – Additional classification considerations

It is increasingly accepted that the process of chemical-induced tumorigenesis in humans and animals involves genetic changes for example in proto-oncogenes and/or tumour suppresser genes of somatic cells. Therefore, the demonstration of mutagenic properties of substances in somatic and/or germ cells of mammals *in vivo* may have implications for the potential classification of these substances as carcinogens.

3 – Reproductive toxicants: two differentiations

Reproductive toxicity means adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring, occurring after exposure to a substance or mixture.

Differentiation "adverse effects on sexual function and fertility, or on development"

- *Adverse effects on sexual function and fertility*

  Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

- *Adverse effects on development of the offspring*

  Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

Differentiation "effects on or via lactation"

Adverse effects on or via lactation are also included in reproductive toxicity, but for classification purposes, such effects are treated separately. This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

Relation with the germ cell mutagenicity classification

For classification purposes, the known induction of genetically based inheritable effects in the offspring is addressed in *Germ Cell Mutagenicity*, since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

3.1 – Definitions of the categories

3.1.1 – Differentiation "adverse effects on sexual function and fertility, or on development"

First category

Known or presumed human reproductive toxicant.

Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (category 1B).

Category 1A

Known human reproductive toxicant.

The classification of a substance in category1A is largely based on evidence from humans.

Category 1B

Presumed human reproductive toxicant.

The classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.
However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate.

Second category: category 2

Suspected human reproductive toxicant.

Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

3.1.2 – Differentiation "effects on or via lactation"

Hazard category for lactation effects.

Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the:

- human evidence indicating a hazard to babies during the lactation period; and/or
- results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
- absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

3.2 – Hazard communication

3.2.1 – Differentiation "adverse effects on sexual function and fertility, or on development"

SGH08 pictogram "*Health hazard*" applies regardless of the category.

The signal word "*Danger*" applies for categories 1A and 1B. The signal word "*Warning*" applies for category 2.

The hazard statement H360: "*May damage fertility or the unborn child"* applies for categories 1A and 1B.

The hazard statement H361: "*Suspected of damaging fertility or the unborn child"* applies for category 2.

For all categories, the specific effect must be stated if it is known and the route of exposure must be stated if it is conclusively proven that no other routes of exposure cause the hazard.

3.2.2 – Differentiation "effects on or via lactation"

There is no pictogram applying for this category.

There is no signal word applying for this category.

Hazard Statement H362*: "May cause harm to breast-fed children"* applies for this category.

3.3 – Specific considerations for classification of substances as reproductive toxicants

Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence. Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

The classification of a substance is derived from the hazard categories in the following order of precedence: category 1A, category 1B, category 2 and the additional category for effects on or via lactation.

If a substance meets the criteria for classification into both of the main categories (for example category 1B for effects on sexual function and fertility and also category 2 for development) then both hazard differentiations shall be communicated by the respective hazard statements. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into category 1A, category 1B or category 2.

In the evaluation of toxic effects on the developing offspring, it is important to consider the possible influence of maternal toxicity.

For human evidence to provide the primary basis for a category 1A classification there must be reliable evidence of an adverse effect on reproduction in humans. Evidence used for classification shall ideally be from well-conducted epidemiological studies which include the use of appropriate controls, balanced assessment, and due consideration of bias or confounding factors. Less rigorous data from studies in humans shall be supplemented with adequate data from studies in experimental animals and classification in category 1B shall be considered.

3.3.1 – Weight of evidence

Classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence. This means that all available information that bears on the determination of reproductive toxicity is considered together, such as epidemiological studies and case reports in humans and specific reproduction studies along with sub-chronic, chronic and special study results in animals that provide relevant information regarding toxicity to reproductive and related endocrine organs.

Evaluation of substances chemically related to the substance under study may also be included, particularly when information on the substance is scarce. The weight given to the available

evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, the presence of maternal toxicity in experimental animal studies, level of statistical significance for inter-group differences, number of endpoints affected, relevance of route of administration to humans and freedom from bias. Both positive and negative results are assembled together into a weight of evidence determination. A single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification.

Toxicokinetic studies in animals and humans, site of action and mechanism or mode of action study results may provide relevant information which reduces or increases concerns about the hazard to human health. If it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified.

If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments.

Data from animal studies ideally shall provide clear evidence of specific reproductive toxicity in the absence of other systemic toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects shall be assessed to the extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternally toxic doses shall not be automatically discounted. Discounting developmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis when a causal relationship is established or refuted.

If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects.

This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. In some situations it can be assumed that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, if the substance is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups; or they are prostrate or dying.

3.3.2 – Maternal toxicity

Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms. In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification.

Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the

relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.

Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated.
In such a case, classification in category 2 may be considered more appropriate than category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

Some of the end points used to assess maternal effects are provided below. Data on these end points, if available, need to be evaluated in light of their statistical or biological significance and dose response relationship.

- Maternal mortality: an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10% is considered excessive and the data for that dose level shall not normally be considered for further evaluation.
- Mating index (No animals with seminal plugs or sperm/No mated × 100) (it is recognised that the Mating index and the Fertility index can also be affected by the male).
- Fertility index (No animals with implants/No of matings × 100).
- Gestation length (if allowed to deliver).
- Body weight and body weight change: consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available. The calculation of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.
- Food and water consumption (if relevant): the observation of a significant decrease in the average food or water consumption in treated dams compared to the control group is useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption need to be evaluated in conjunction with maternal body weights when determining if the effects noted are reflective of maternal toxicity or more simply, unpalatability of the test material in feed or water.
- Clinical evaluations (including clinical signs, markers, haematology and clinical chemistry studies): the observation of increased incidence of significant clinical signs of toxicity in treated dams relative to the control group is useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree and duration of clinical signs shall be reported in the study. Clinical signs of maternal intoxication include: coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.
- Post-mortem data: increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data, including absolute organ weight, organ-to-body weight ratio, or

organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared to those in the control group, may be considered evidence of maternal toxicity.

3.3.3 – Animal and experimental data

A number of internationally accepted test methods are available; these include methods for developmental toxicity testing (e.g. OECD Test Guideline 414), and methods for one or two-generation toxicity testing (e.g. OECD Test Guidelines 415, 416, 443).

Results obtained from Screening Tests (e.g. OECD Guidelines 421 – *Reproduction/Developmental Toxicity Screening Test*, and 422 – *Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test*) can also be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads.

Evidence from *in vitro* assays, or non-mammalian tests, and from analogous substances using structure-activity relationship (SAR), can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. Inadequate data shall not be used as a primary support for classification.

It is preferable that animal studies are conducted using appropriate routes of administration which relate to the potential route of human exposure. However, in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity. However, if it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals shall not be classified.

Studies involving routes of administration such as intravenous or intraperitoneal injection, which result in exposure of the reproductive organs to unrealistically high levels of the test substance, or elicit local damage to the reproductive organs, including irritation, must be interpreted with extreme caution and on their own are not normally the basis for classification.

There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model.

In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Further guidance appears in the section related to maternal toxicity.

However, specification of the actual “limit dose” will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

4 – CMR Classification criteria for mixtures

4.1 – Classification when data are available for all ingredients or only for some ingredients of the mixture

The mixture shall be classified as a carcinogen and/or mutagen and/or toxic for reproduction when at least one ingredient has been classified and is present:

- at or above the appropriate specific concentration limit as shown in annex VI of the regulation (EC) No 1272/2008 for the considered substances;
- at or above the appropriate generic concentration limit as shown in the *ad hoc* table as shown in annex I of the regulation (EC) No 1272/2008 if the substance does not appear in annex VI of the same regulation or if it appears in annex VI without specific concentration limit.

These tables are:

- table 3.6.2: generic concentration limits of ingredients of a mixture classified as carcinogen that trigger classification of the mixture;
- table 3.5.2: generic concentration limits of ingredients of a mixture classified as germ cell mutagens that trigger classification of the mixture;
- table 3.7.2: generic concentration limits of ingredients of a mixture classified as reproduction toxicants or for effects on or via lactation that trigger classification of the mixture.

4.2 – Classification when data are available for the complete mixture

Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the classified ingredients. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of the test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

4.3 – Classification of mixtures when data are not available for the complete mixture: bridging principles

Where the mixture itself has not been tested, but there are sufficient data on the individual ingredients and similar tested mixtures (subject to the previous paragraph), to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3, annexe I of regulation (EC) No 1272/2008.

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