Chapter 9:
GERM CELL MUTAGENICITY

DEFINITIONS

1. The classification system is primarily concerned with chemicals which may cause mutations in the germ cells of humans and these mutations can be transmitted to the progeny. However, mutagenicity/genotoxicity tests *in vitro* and in mammalian somatic cells *in vivo* will also be considered in the sub-divisions of the classification system.

2. In the present context, commonly found definitions of the terms mutagenic, mutagen, mutations and genotoxic are used, and a mutation is defined here as a permanent change in the amount or structure of the genetic material in a cell.

3. The term "mutation" applies both for heritable genetic changes that may be manifested at the phenotypic level, and for the underlying DNA modifications when known (including, for example, 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.

4. 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.

CONSIDERATIONS

5. The purpose of the harmonised scheme for the classification of chemicals which may cause heritable mutations in germ cells in humans is to provide a common ground which could be used internationally for the classification of mutagens. All tests conducted according to validated and internationally accepted test guidelines are acceptable for the purpose of classifying substances.

6. To arrive at that classification scheme, 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 may also be considered.

7. The system is hazard based, classifying chemicals 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 chemical substances.

CLASSIFICATION CRITERIA FOR SUBSTANCES

8. The classification system comprises two different classes of germ cell mutagens to accommodate the weight of evidence available. The two-category system is described in the following.

CATEGORY 1:

CHEMICALS KNOWN TO INDUCE HERITABLE MUTATIONS OR TO BE REGARDED AS IF THEY INDUCE HERITABLE MUTATIONS IN THE GERM CELLS OF HUMANS.

CATEGORY 1A: Chemicals known to induce heritable mutations in germ cells of humans

Criteria: Positive evidence from human epidemiological studies**.**

CATEGORY 1B: Chemicals which should be regarded as if they induce heritable mutations in the germ cells of humans.

Criteria:

- 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. This supporting evidence may, for example, be derived from mutagenicity/genotoxic 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.

CATEGORY 2:

CHEMICALS WHICH CAUSE CONCERN FOR MAN OWING TO THE POSSIBILITY THAT THEY MAY INDUCE HERITABLE MUTATIONS IN THE GERM CELLS OF HUMANS.

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 to be supported by positive results from *in vitro* mutagenicity assays

Nota Bene:

- Chemicals which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, should be considered for classification as Category 2 mutagens.

Rationale

9. Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in OECD Test Guidelines. Evaluation of the test results should be done using expert judgement and all the available evidence should be weighed for classification.

10. Examples of *in vivo* heritable germ cell mutagenicity tests are:

Rodent dominant lethal mutation test (OECD 478)
Mouse heritable translocation assay (OECD 485)
Mouse specific locus test

11. Examples of *in vivo* somatic cell mutagenicity tests are:

Mammalian bone marrow micronucleus test (OECD 474)
Mammalian bone marrow chromosome aberration test (OECD 475)
Mouse spot test (OECD 484)
Mammalian erythrocyte micronucleus test (OECD 474)

12. Examples of mutagenicity/genotoxicity tests in germ cells are:

A) Mutagenicity tests:
Mammalian spermatogonial chromosome aberration test (OECD 483)
Spermatid micronucleus assay

B) Genotoxicity tests:
Sister chromatid exchange analysis in spermatogonia
Unscheduled DNA synthesis test (UDS) in testicular cells

13. Examples of genotoxicity tests in somatic cells are:

Liver Unscheduled DNA Synthesis (UDS) *in vivo* (OECD 486)
Mammalian bone marrow sister chromatid exchanges (SCE)

14. Examples of *in vitro* mutagenicity tests are:

In vitro mammalian chromosome aberration test (OECD 473)
In vitro mammalian cell gene mutation test (OECD 476)
Bacterial reverse mutation tests (OECD 471)

15. The classification of individual substances should 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 should 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 chemical compared to the route of human exposure should also be taken into account.

CLASSIFICATION CRITERIA FOR MIXTURES

Classification of Mixtures When Data are Available for the Complete Mixture.

16. Classification of mixtures will be based on the available test data of the individual constituents of the mixture using cut-off values/concentration limits for the components of the mixture. The classification may be modified on a case-by-case basis based on the available test data for the mixture as a whole. 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 and analysis (e.g., statistical analysis, test sensitivity)

of germ cell mutagenicity test systems. Adequate documentation supporting the classification should be retained and made available for review upon request.

Classification of Mixtures When Data are not Available for the Complete Mixture.

Bridging Principles

17. Where the mixture itself has not been tested to determine its germ cell mutagenicity hazard, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, this data will be used in accordance with the following agreed bridging rules. This ensures that the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without the necessity for additional testing in animals.

Dilution

18. If a mixture is diluted with a diluent which is not expected to affect the germ cell mutagenicity of other ingredients, then the new mixture may be classified as equivalent to the original mixture.

Batching

19. The germ cell mutagenic potential of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product produced by and under the control of the same manufacture unless there is reason to believe there is significant variation in composition such that the germ cell mutagenic potential of the batch has changed. If the latter occurs, a new classification is necessary.

Substantially similar mixtures

20. Given the following:

a). Two mixtures: i.) A + B
ii.) C + B
b). The concentration of mutagen Ingredient B is the same in both mixtures.
c). The concentration of ingredient A in mixture (i) equals that of ingredient C in mixture (ii).
d). Data on toxicity for A and C are available and substantially equivalent, i.e. they are not expected to affect the germ cell mutagenicity of B.

If mixture (i) is already classified by testing, mixture (ii) can be assigned the same category.

Classification of Mixtures When Data are Available for all Components or Only for Some Components of the Mixture.

21. The mixture will be classified as a mutagen when at least one ingredient has been classified as a Category 1 or Category 2 mutagen and is present at or above the appropriate cut-off value/concentration limit as mentioned in Table 1 below for Category 1 and 2 respectively.

Table 1: Cut-off values/concentration limits of ingredients of a mixture classified as germ cell mutagens that would trigger classification of the mixture.

Ingredient classified as:	Cut-off/concentration limits triggering classification of a mixture as:	
	Category 1 mutagen	**Category 2 mutagen**
Category 1 mutagen	≥ 0.1 %	-
Category 2 mutagen	-	≥ 1.0%

Note: The cut-off values/concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).

HAZARD COMMUNICATION

Allocation of Label Elements

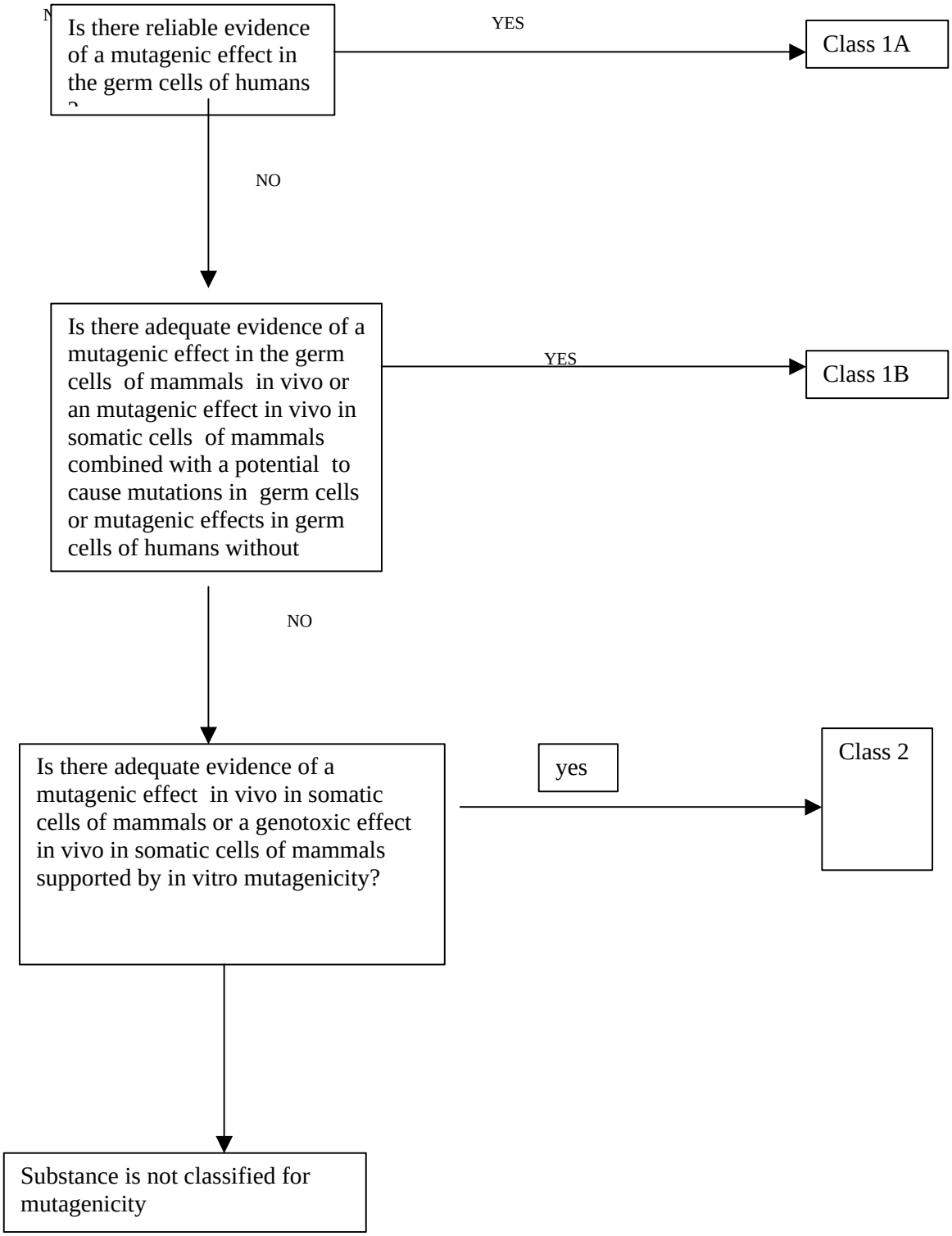
22. General and specific considerations concerning labelling requirements are provided in Chapter 4. Annex 5 contains examples of precautionary statements and pictograms which can be used where allowed by the competent authority. Additional reference sources providing advice on the use of precautionary information is also included.

Table 2: Label Elements of Germ cell mutagenicity

	Category 1A	Category 1B	Category 2
Symbol	New health hazard symbol	New health hazard symbol	New health hazard symbol
Signal Word	Danger	Danger	Warning
Hazard Statement	May cause genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	May cause genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	Suspected of causing genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)

DECISION LOGIC AND GUIDANCE

Decision Logic



Guidance

Explanatory Note

23. It becomes increasingly clear that the process of chemical-induced tumorigenesis in man and animals involves (an accumulation of) genetic changes in proto-oncogenes and/or tumour suppresser genes of somatic cells. Therefore, the demonstration of mutagenic properties of chemicals in somatic and/or germ cells of mammals *in vivo* may have implications for the potential classification of these chemicals as carcinogens (cf. chapter "Carcinogenicity").

SUMMARY TABLE

EXAMPLES

Examples mutagenicity classification

Substance 1: positive in vitro mammalian mutagenicity assays and has a SAR to known germ cell mutagens. Classification: class 2.

Substance 2: positive in vitro mammalian mutagenicity assays and positive in a UDS test in vivo. Classification: class 2.

Substance 3: positive in vitro mammalian mutagenicity assays and positive in a mammalian bone marrow chromosome aberration test in vivo. Classification: Class 2

Substance 4: positive in vivo in somatic cells and positive in a UDS test in germ cells.
Classification: Class: 1B

NB At the moment there are no known 1A substances

When present in a mixture the cut-off values / concentration limits for a Class 1 mutagen is 0.1%, for a Class 2 mutagen 1.0% (Table 1).

The classified substances and preparations are labelled according Table 2