

## **OECD GUIDELINE FOR TESTING OF CHEMICALS**

### **Proposal for a New DRAFT GUIDELINE 434:**

#### **Acute Dermal Toxicity – Fixed Dose Procedure**

#### **INTRODUCTION**

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress and animal welfare considerations. The original acute Dermal Toxicity Guideline TG 402 (1) was adopted in 1987. Development of a dermal Fixed Dose Procedure (FDP) was considered appropriate, following adoption of the revised Oral FDP, OECD Guideline 420 (2) and deletion of OECD Guideline 401 in December 2001. This FDP guideline will allow the use of a series of fixed doses for the determination of acute dermal toxicity in only one sex (usually females).

2. Traditional methods for assessing acute toxicity use death of animals as the sole endpoint. In 1984, a new approach to acute toxicity testing was suggested by the British Toxicology Society based on the administration of test material at a series of fixed dose levels (3). This approach avoided using death of animals as either an exclusive or an intended endpoint by incorporating evident clinical signs of toxicity at one of a series of fixed dose levels, as an endpoint on which to base classification of the test material. This approach is also taken for this Guideline. In agreement with the OECD Guidance Document on Humane Endpoints (4) refinements are introduced in order to minimise any suffering and distress by the animals and, to the extent feasible, reduce the number of animals used. The statistical properties of the FDP have been evaluated using mathematical modelling (5).

3. Definitions used in the context of this Guideline are set out in Annex 1.

4. The method provides information on hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity (6).

#### **INITIAL CONSIDERATIONS**

5. All available information on the test substance should be considered by the testing laboratory prior to conducting the study. Such information will include the identity and chemical structure of the substance; its physico-chemical properties; the results of any other *in vitro* or *in vivo* toxicity tests on the substance; available (Q)SAR data and toxicological data on structurally related substances; the anticipated use(s) of the substance and the potential for human exposure. This information will assist in the selection of an appropriate starting dose.

#### **PRINCIPLE OF THE TEST**

6. It is a principle of the method that only moderately toxic doses are used, and that administration of doses that are expected to be lethal should be avoided. Also, dose levels that are expected to cause marked pain and distress, due to corrosive<sup>1</sup> or severely irritant actions, should not be administered. Moribund animals, or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for

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<sup>1</sup> Determined using a validated test method or an acceptable prediction

making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document (4).

7. Groups of animals, of a single sex, are exposed to the test substance in a stepwise procedure using the appropriate fixed doses as set out in Annexes 2 and 3. The initial dose level is selected on the basis of a sighting study at the concentration expected to produce clear signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death, are described in detail in a separate OECD Guidance Document on humane end-points (4). Further groups of animals may be tested at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose.

## **DESCRIPTION OF THE TEST METHOD**

### **Selection of animal species**

8. The adult rat, rabbit or guinea pig may be used. Justification should be provided for the use of other rodent or non-rodent species. In considering the most appropriate sex, surveys of conventional acute oral (6) and acute inhalation (7) toxicity tests show that usually there is little difference in sensitivity between the sexes, but in those cases where differences are observed, females are generally slightly more sensitive. Such information is not available for the dermal route, but it can be assumed that sex sensitivity differences are likely to be similar for this route. Therefore, it is recommended that females should normally be used. However, if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive then this sex should be used. When the test is conducted in males, adequate justification should be provided.

9. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of testing, should be between 8 and 12 weeks old and its weight should fall within an interval of  $\pm 20\%$  of the average body weight recorded at the laboratory for the particular strain used.

### **Housing and feeding conditions**

10. The temperature of the experimental animal room should be  $22\pm 3^{\circ}\text{C}$ . Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

### **Preparation of animals**

11. The animals are acclimatised to the laboratory conditions for at least five days prior to the start of the study. Animals are randomly selected for use in the study and marked to provide individual identification.

12. Approximately 24 hours before the study, fur should be removed from the dorsal area of the trunk of the test animals by clipping or shaving. Care must be taken to avoid abrading the skin, which could alter its permeability. At least 10% of the body surface area should be

clear for the application of the test substance. The weight of the animal should be taken into account when deciding on the area to be cleared and on the dimensions of the covering.

### **Administration of doses**

13. The test substance should be applied uniformly over an area which is approximately 10 % of the total body surface area. With highly toxic substances the surface area covered may be less, but as much of the area should be covered with as thin and uniform film as possible. Test substances should be held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance. When testing solids, which may be pulverised if appropriate, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on penetration of skin by the test substance should be taken into account. Liquid test substances are generally used undiluted.

14. Restrainers may be used to prevent the ingestion of the test substance, but complete immobilisation is not recommended. At the end of the exposure period, residual test substance should be removed, where practicable using water or an appropriate solvent.

## **PROCEDURE**

### **Sighting study**

15. The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study. The test substance is administered to single animals in a sequential manner following the flow charts in Annex 2. The sighting study is completed when a decision on the starting dose for the main study can be made (or if a death is seen at the lowest fixed dose).

16. The starting dose for the sighting study is selected from the fixed dose levels of 50, 200, 1000 and 2000 mg/kg as a dose expected to produce evident toxicity based, when possible, on evidence from existing data on the same chemical and for structurally related chemicals. In the absence of such information, the starting dose will be 1000 mg/kg.

17. A period of at least 24 hours will be allowed between the testing of each animal. All animals should normally be observed for at least 14 days.

18. In cases where an animal tested at the lowest fixed dose level (50 mg/kg) in the sighting study dies or exhibits clear clinical signs of toxicity, the normal procedure is to terminate the study and assign the substance to GHS Category 1 (as shown in Annex 2). However, if further confirmation of the classification is required, an optional supplementary procedure may be conducted, as follows. A second animal is dosed at 50 mg/kg. If this second animal dies, then GHS Category 1 will be confirmed and the study will be immediately terminated. If the second animal survives, then a maximum of three additional animals will be dosed at 50 mg/kg. Because there will be a high risk of death, these animals should be dosed in a sequential manner to protect animal welfare. The time interval between dosing each animal should be sufficient to establish that the previous animal is likely to survive. If a second death occurs, the dosing sequence will be immediately terminated and no further animals will be dosed. The classification will be as shown in Annex 3 at the 50 mg/kg fixed dose: Category 1 if there are 2 or more deaths (outcome A) at 50 mg/kg, or Category 2 if there is 1 death (outcome B) at 50 mg/kg.

19. Exceptionally, and only when justified by specific regulatory needs, the use of an additional upper fixed dose level of 5000 mg/kg may be considered (see Annex 4). For reasons of animal welfare concern, testing of animals in GHS Category 5 ranges (2000-5000 mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.

## **MAIN STUDY**

### **Numbers of animals and dose levels**

20. The action to be taken following testing at the starting dose is indicated by the flow charts in Annex 2. One of three actions will be required; either stop testing and assign the appropriate hazard classification category, test at a higher fixed dose or test at a lower fixed dose. However, a dose which caused death in the sighting study will not be revisited in the main study, to protect animal from unnecessary suffering (see Annex 3). Experience has shown that the most likely outcome at the starting dose level will be that the substance can be classified and no further testing will be necessary. When testing a descending series and 2-3 deaths are observed (within the scope of outcome A), then in the interests of animal welfare the test should be halted and the substance classified according to outcome C of the next dose in the series.

21. A total of five animals of one sex will normally be used for each dose level investigated. The five animals will be made up of one animal from the sighting study dosed at the selected dose level together with an additional four animals (except, unusually, if a dose level used on the main study was not included in the sighting study).

22. The time interval between dosing at each level is determined by the onset, duration, and severity of toxic signs. Exposure of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. A period of 3 or 4 days between dosing at each dose level is recommended to allow for the observation of delayed toxicity. The time interval may be adjusted as appropriate, *e.g.*, in case of an inconclusive response.

23. When the use of an upper fixed dose of 5000 mg/kg is considered, the procedure outlined in Annex 4 should be followed

### **Limit Test**

24. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be non-toxic, *i.e.*, having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed.

25. Using the normal procedure, a sighting study starting dose of 2000 mg/kg (or exceptionally 5000 mg/kg) followed by dosing of a further four animals at this level serves as a limit test for this guideline. In some cases, as required by some regulatory authorities, testing up to the limit of GHS Category 5 may be conducted (see Annex 4).

### **Observations**

26. Animals are observed immediately after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4

hours, and daily thereafter, for a total of 14 days, except where they are found dead. However, the duration of observation is not fixed but should be determined by the nature and time of onset of clinical signs and length of recovery period. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for signs of toxicity to be delayed (4). All observations are systematically recorded, with individual records being maintained for each animal. Animals found in a moribund condition and animals showing severe pain and/or enduring signs of severe distress should be humanely killed without delay. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

27. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document should be taken into consideration (4). Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed for animal welfare reasons. Care should be taken (*e.g.*, by using control animals exposed to air) when conducting examinations for clinical signs of toxicity that initial poor appearance and transient respiratory changes, resulting from the exposure procedure, are not mistaken for treatment-related effects.

### **Body weight**

28. Individual weights of animals should be determined on the day of, or immediately prior to the administration of the test substance and at least weekly thereafter. At the end of the test surviving animals are weighed and then humanely killed.

### **Pathology**

29. All test animals (including those that die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours after the initial dosing may also be considered because it may yield useful information.

## **DATA AND REPORTING**

### **Data**

30. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings.

### **Test Report**

31. The test report must include the following information, as appropriate:

Test substance:

- physical nature, purity, and, where relevant, physico-chemical properties

(including isomerisation);

- identification data, including CAS number (if available).

Vehicle (if appropriate):

- justification for use of vehicle and justification for choice of vehicle (if other than water).

Test animals:

- species/strain used;
- microbiological status of the animals, when known;
- number, age and sex of animals (including, where appropriate, a rationale for use of males instead of females);
- source, housing conditions, diet, historical data etc.;
- date and time of death if prior to scheduled sacrifice.

Test conditions:

- details of test substance formulation, including details of the physical form of the material administered;
- details of the administration of the test substance including dosing volumes and time of dosing;
- details of food and water quality (including diet type/source, water source);
- the rationale for the selection of the starting dose;
- method of randomisation in animal selection.

Results:

- tabulation of response data and dose level for each animal (*i.e.*, animals showing signs of toxicity including mortality, nature, severity and duration of effects);
- individual weight of animals at the day of exposure, in weekly intervals thereafter, and at time of death or euthanasia; date and time of death if prior to scheduled euthanasia; time course of onset of signs of toxicity and whether these were reversible for each animal;
- time course of onset of signs of toxicity and whether these were reversible for each animal;
- necropsy findings and histopathological findings for each animal, if available.

Discussion and interpretation of results.

Conclusions.

## **LITERATURE**

1. OECD (1987). OECD Guideline for Testing of Chemicals: Acute Dermal Toxicity 402. Available:  
[[http://www.oecd.org/document/22/0,2340,en\\_2649\\_34377\\_1916054\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/22/0,2340,en_2649_34377_1916054_1_1_1_1,00.html)]
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[[http://www.oecd.org/document/22/0,2340,en\\_2649\\_34377\\_1916054\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/22/0,2340,en_2649_34377_1916054_1_1_1_1,00.html)]
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[[http://www.oecd.org/document/22/0,2340,en\\_2649\\_34377\\_1916054\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/22/0,2340,en_2649_34377_1916054_1_1_1_1,00.html)]
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## ANNEX 1

### DEFINITIONS

**Acute dermal toxicity** is the adverse effect caused by a substance following a single uninterrupted exposure by dermal application over a short period of time (24 h or less).

**Evident toxicity** is a general term describing clear signs of toxicity following the administration of a test substance, (see Van den Heuvel *et al.* (1990) for examples\*) such that at the next highest fixed concentration either severe pain and enduring signs of severe distress, moribund condition (criteria are presented in the Humane Endpoints Guidance Document No. 19 (4)) or probable mortality in most animals can be expected.

**Dose** is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of test animal (*e.g.*, mg/kg).

**GHS**: Globally Harmonised Classification System for Chemical Substances and Mixtures. A joint activity of OECD (human health and the environment), UN Committee of Experts on Transport of Dangerous Goods (physical-chemical properties) and ILO (hazard communication) and co-ordinated by the Interorganisation Programme for the Sound Management of Chemicals (IOMC).

**Impending death** is when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, lateral position, recumbence, and tremor. (See the Humane Endpoint Guidance Document No. 19 (4) for more details).

**LD50** (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

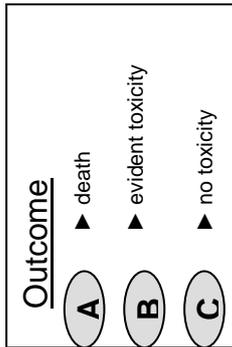
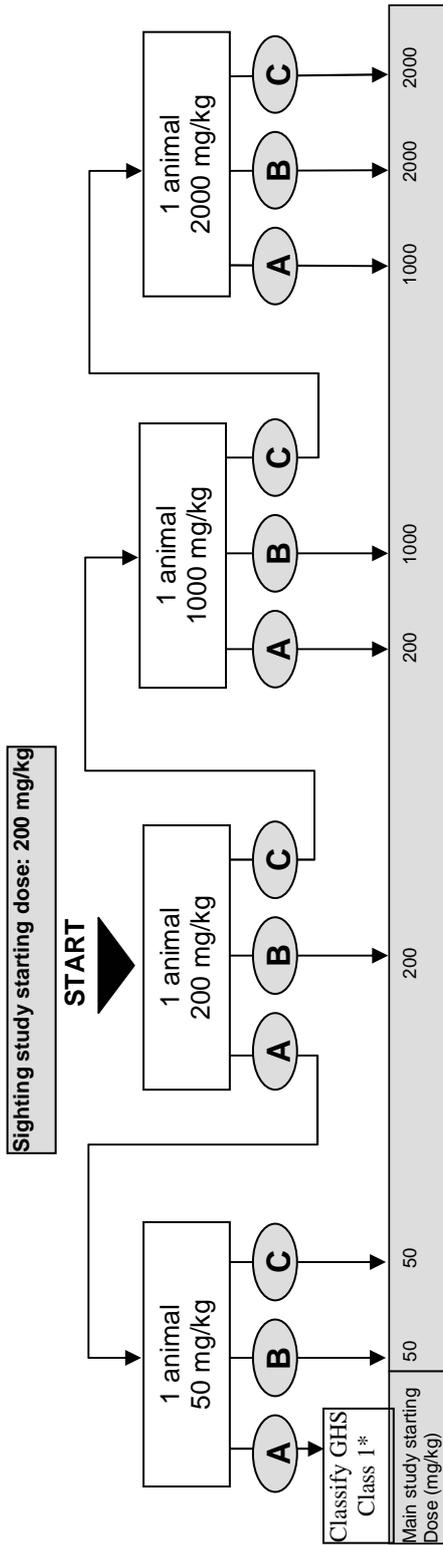
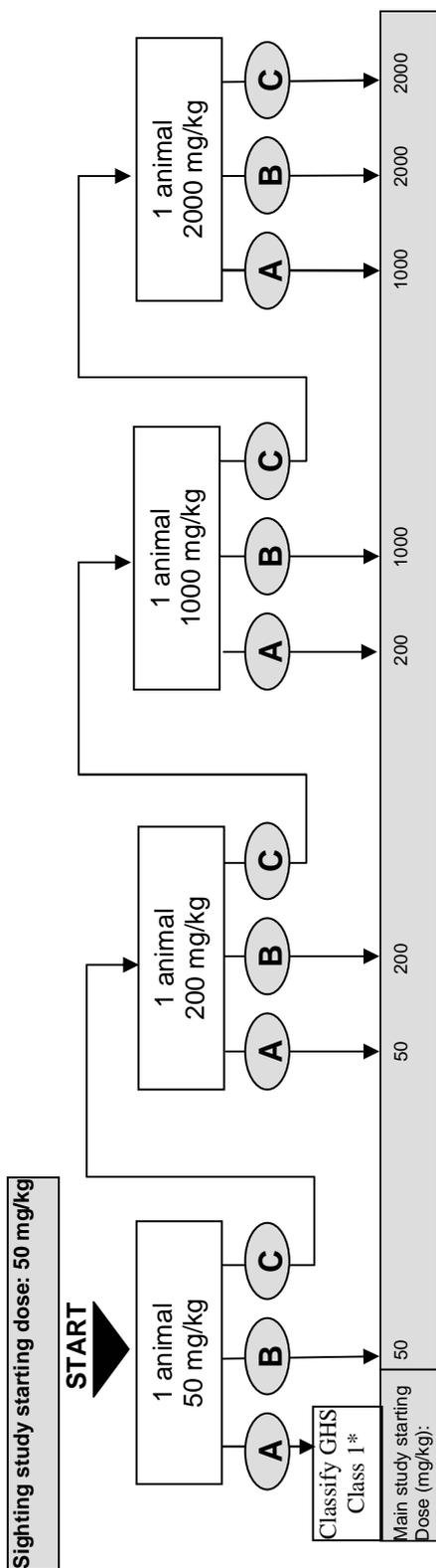
**Limit dose** refers to a dose at an upper limitation on testing (2000 or 5000 mg/kg).

**Moribund status** is being in a state of dying or inability to survive, even if treated. (See the OECD Humane Endpoint Guidance Document No. 19 (4) for more details).

**Predictable death**: presence of clinical signs indicative of death at a known time in the future before the planned end of the experiment, for example: inability to reach water or food. (See the Humane Endpoint Guidance Document No. 19 (4) for more details).

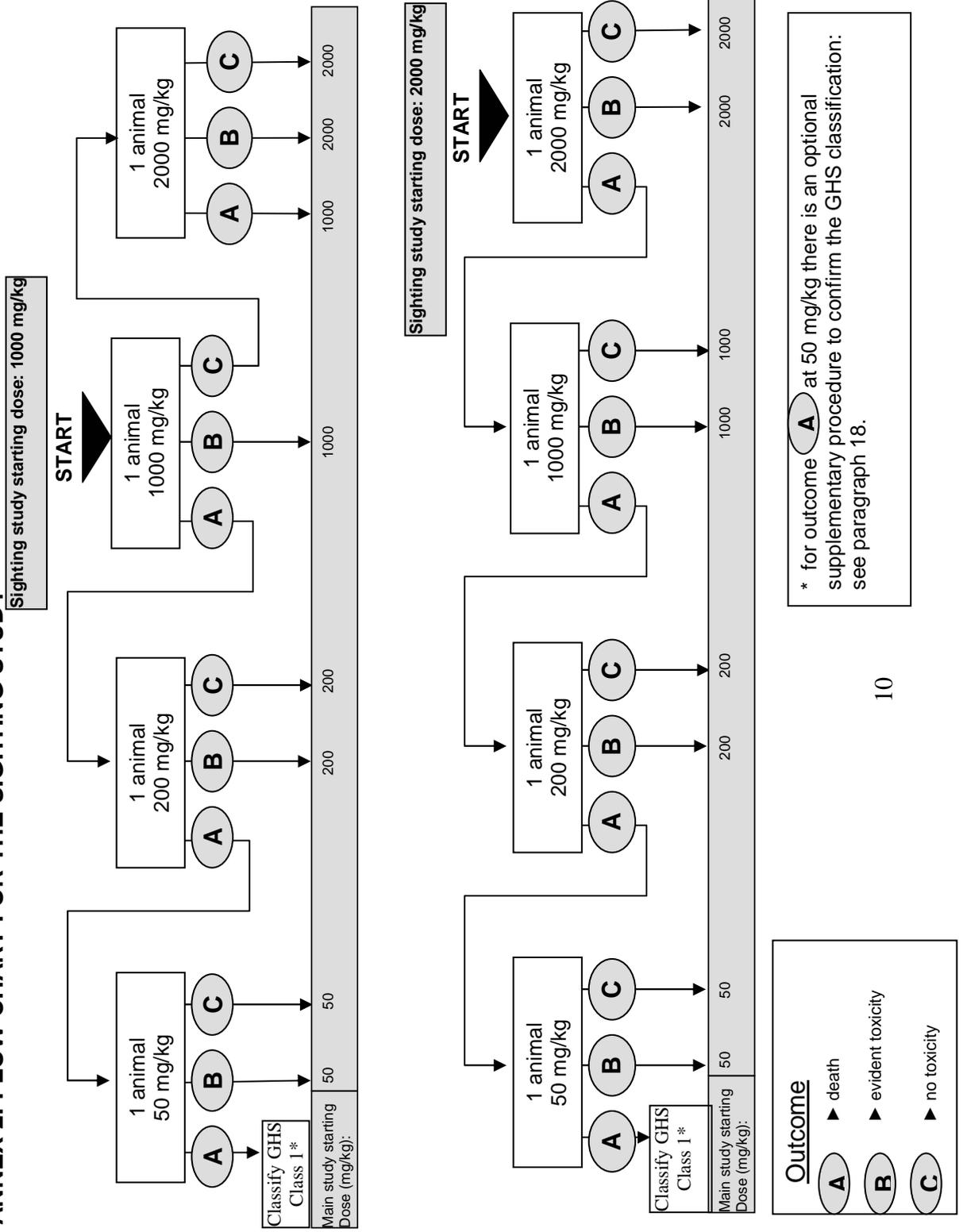
\* Van den Heuvel, M. J., Clark, D. G., Fielder, R. J., Koundakjian, P. P., Oliver, G. J. A., Pelling, D., Tomlinson, N. J. and Walker, A. P. (1990). The international validation of a fixed-dose procedure as an alternative to the classical LD<sub>50</sub> test. *Fd. Chem. Toxicol.* 28, 469-482.

## ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY

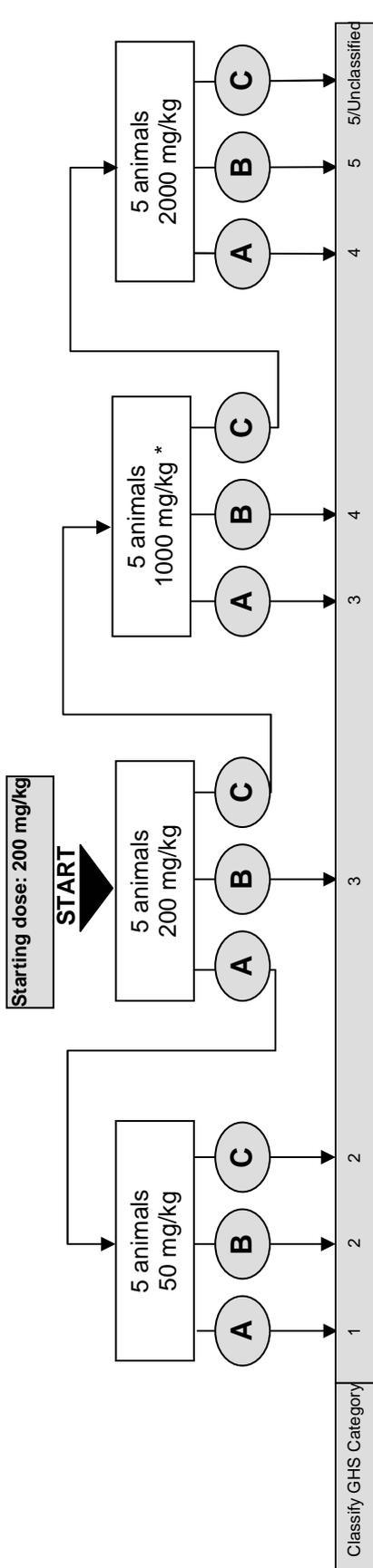
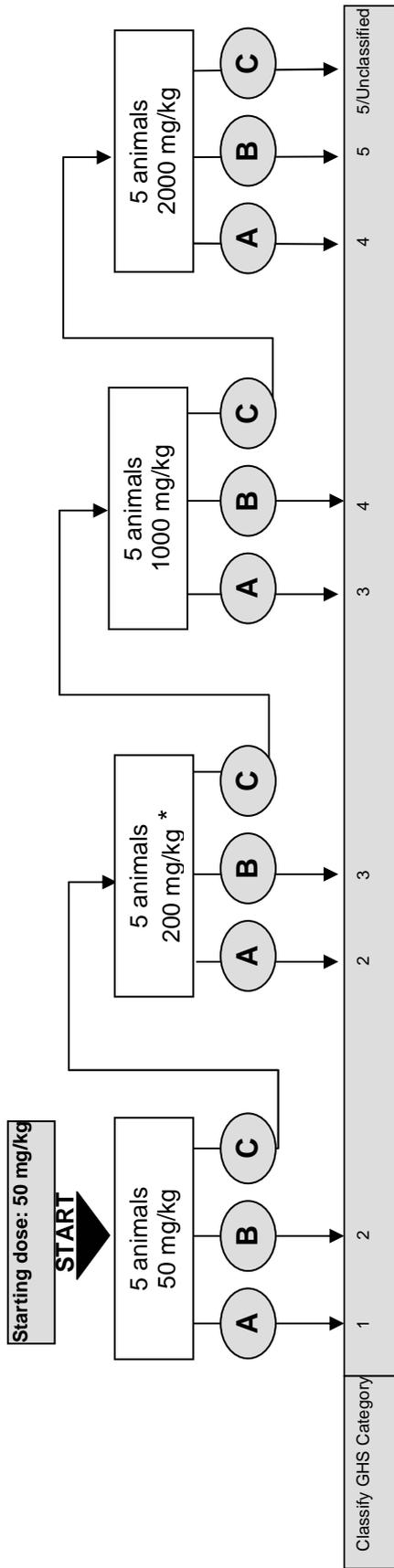


\* for outcome **A** at 50 mg/kg there is an optional supplementary procedure to confirm the GHS classification: see paragraph 18.

**ANNEX 2: FLOW CHART FOR THE SIGHTING STUDY**



**ANNEX 3: FLOW CHART FOR THE MAIN STUDY**



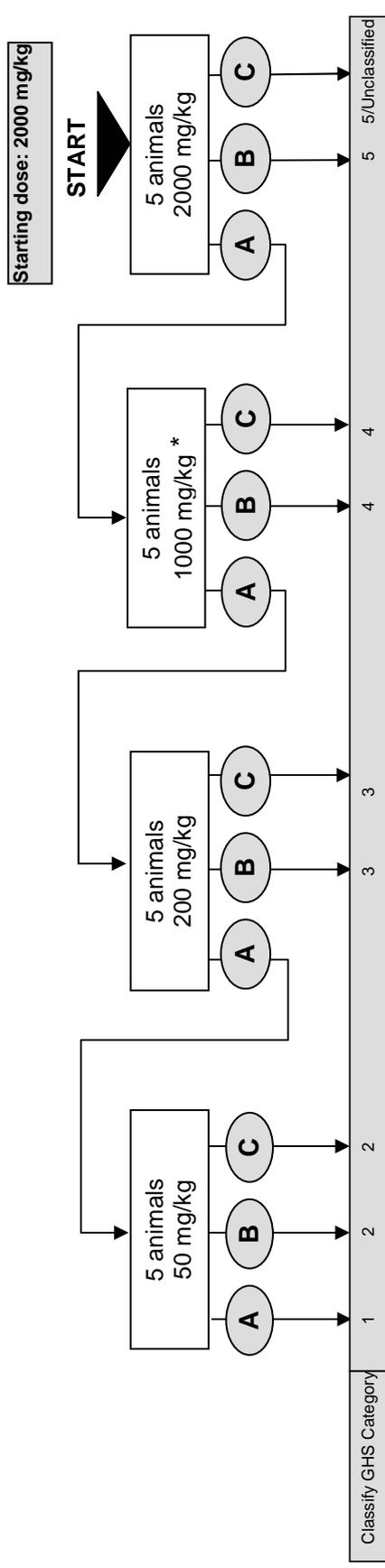
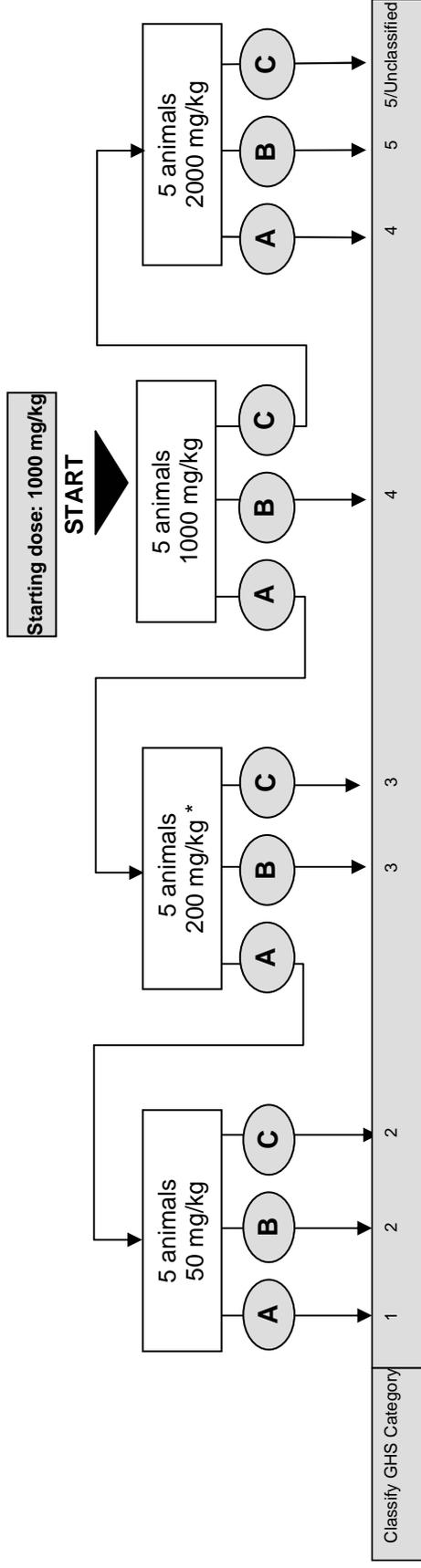
**Outcome**

- A** ▶ 2 deaths (see paragraph 19 if 2-3 deaths)
- B** ▶ 1 with evident toxicity; and/ or ≤ 1 death
- C** ▶ No evident toxicity and no deaths

**Group size**  
The 5 animals in each main study group will include any animal tested at that dose level in the sighting study

\*Animal welfare override if this concentration caused death in the sighting study, then no further animals will be tested. Go directly to outcome **A**

**ANNEX 3: FLOW CHART FOR THE MAIN STUDY**



**Outcome**

- A** ▶ 2 deaths (see paragraph 19 if 2-3 deaths)
- B** ▶ 1 with evident toxicity; and/ or ≤ 1 death
- C** ▶ No evident toxicity and no deaths

**Group size**  
The 5 animals in each main study group will include any animal tested at that dose level in the sighting study

\* Animal welfare override if this concentration caused death in the sighting study then no further animals will be tested. Go directly to outcome **A**

## ANNEX 4

### **CRITERIA FOR CLASSIFICATION OF TEST SUBSTANCES WITH EXPECTED LD50 VALUES EXCEEDING 2000 MG/KG WITHOUT THE NEED FOR TESTING.**

1. Criteria for Category 5 are intended to enable the identification of substances which are of relatively low acute toxicity hazard but which under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD50 in the range of 2000-5000 mg/kg bodyweight and equivalent doses for inhalation. The specific criteria for Category 5 are:

(i) The substance is classified in this Category if reliable evidence is already available that indicates the LD50 (or LC50) to be in the range of Category 5 values or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature.

(ii) The substance is classified in this Category, through extrapolation, estimation or measurement of data, if assignment to a more hazardous category is not warranted, and:

- reliable information is available indicating significant toxic effects in humans; or
- any mortality is observed when tested up to Category 4 values by the oral, inhalation, or dermal routes; or
- where expert judgement confirms significant clinical signs of toxicity, when tested up to Category 4 values, except for diarrhoea, piloerection or an ungroomed appearance; or
- where expert judgement confirms reliable information indicating the potential for significant acute effects from other animal studies.

### **TESTING AT DOSES ABOVE 2000 MG/KG**

2. Exceptionally, and only when justified by specific regulatory needs, the use of an additional upper fixed dose level of 5000 mg/kg may be considered. Recognising the need to protect animal welfare, testing in animals in Category 5 ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test would have a direct relevance for protecting human health.

### **Sighting Study**

3. The decision rules governing the sequential procedure presented in Annex 2 are extended to include a 5000 mg/kg dose level. Thus, when a sighting study starting dose of 5000 mg/kg is used outcome A (death) will require a second animal to be tested at 2000 mg/kg; outcomes B and C (evident toxicity or no toxicity) will allow the selection of 5000 mg/kg as the main study starting dose. Similarly, if a starting dose other than 5000 mg/kg is used then testing will progress to 5000 mg/kg in the event of outcomes B if evident toxicity is seen at 2000 mg/kg then that should be the level used for the main study or C at 2000 mg/kg; a subsequent 5000 mg/kg outcome A will dictate a main study starting dose of 2000 mg/kg and outcomes B and C will dictate a main study starting dose of 5000 mg/kg.

### **Main Study**

4. The decision rules governing the sequential procedure presented in Annex 3 are extended to include a 5000 mg/kg dose level. Thus, when a main study starting dose of 5000 mg/kg is used, outcome A ( $\geq 2$  deaths) will require the testing of a second group at 2000 mg/kg; outcome B (evident toxicity and/or  $\leq 1$  death) or C (no toxicity) will result in the substance being unclassified according to GHS. Similarly, if a starting dose other than 5000 mg/kg is used then testing will progress to 5000 mg/kg in the event of outcome C at 2000 mg/kg; a subsequent 5000 mg/kg outcome A will result in the substance being assigned to GHS Category 5 and outcomes B or C will lead to the substance being unclassified.