B.2. ACUTE TOXICITY (INHALATION)

1. METHOD

1.1. INTRODUCTION

It is useful to have preliminary information on the particle size distribution, the vapour pressure, the melting point, the boiling point, the flash point and explosivity (if applicable) of the substance.

See also General Introduction Part B (A).

1.2. DEFINITIONS

See General Introduction Part B (B).

1.3. REFERENCE SUBSTANCES

None.

1.4. PRINCIPLE OF THE TEST METHOD

Several groups of experimental animals are exposed for a defined period to the test substance in graduated concentrations, one concentration being used per group. Subsequently observations of effects and deaths are made. Animals which die during the test are necropsied and at the conclusion of the test surviving animals are necropsied.

Animals showing severe and enduring signs of distress and pain may need to be humanely killed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or severe irritating properties need not be carried out.

1.5. QUALITY CRITERIA

None.

1.6. DESCRIPTION OF THE TEST METHOD

1.6.1. Preparations

The animals are kept under the experimental housing and feeding conditions for at least five days prior to the experiment. Before the test healthy young animals are randomized and assigned to the required number of groups. They need not be subjected to simulated exposure unless this is indicated by the rype of exposure apparatus being used.

Solid test substances may need to be micronised in order to achieve particles of an appropriate size.

Where necessary a suitable vehicle may be added to the test substance to help generate an appropriate concentration of the test substance in the atmosphere and a vehicle control group should then be used. If a vehicle or other additives are used to facilitate dosing, they should be known not to produce toxic effects. Historical data can be used if appropriate.

1.6.2. Test Conditions

1.6.2.1. Experimental Animals

Unless there are contra-indications the rat is the preferred species. Commonly used laboratory strains should be employed. For each sex, at the start of the test the range of weight variation in the animals used should not exceed ± 20 % of the appropriate mean value.

1.6.2.2. Number and Sex

At least 10 rodents (five female and five male) are used at each concentration level. The females should be nulliparous and non-pregnant.

Note: In acute toxicity tests with animals of a higher order than rodents, the use of smaller numbers should be considered. Doses should be carefully selected, and every effort should be made not to exceed moderately toxic doses. In such tests administration of lethal doses of the test substance should be avoided.

1.6.2.3. Exposure Concentrations

These should be sufficient in number, at least three, and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data should be sufficient to produce a concentration mortality curve and, where possible, permit an acceptable determination of an LC_{50}.1.6.2.4. Limit test

If an exposure of five male and five female test animals to 20 mg per litre of a gas or 5 rug per litre of an aerosol or a particulate for four hours (or where this is not possible due to the physical or chemical, including explosive, properties of the test substance, the maximum attainable concentration) produces no compound related mortality within 14 days further testing may not be considered necessary. (18th ATP, dir. 93/21/EEC, Ll10/93)

1.6.2.5. Exposure time

The period of exposure should be four hours.

1.6.2.6. Equipment

The animals should be tested with inhalation equipment designed to sustain a dynamic airflow of at least 12 air changes per hour, to ensure an adequate oxygen content and an evenly distributed exposure atmosphere. Where a chamber is used its design should minimize crowding of the test animals and maximize their exposure by inhalation to the test substance. As a general rule to ensure stability of a chamber atmosphere the total 'volume' of the test animals should not exceed 5% of the volume of the test chamber. Oro-nasal, head only, or whole body individual chamber exposure may be used; the first two will help to minimize the uptake of the test substance by other routes.

1.6.2.7. Observation Period

The observation period should be at least 14 days. However, the duration of observations should not be rigidly fixed. It should be determined by the toxic reactions, their rate of onset and the length of the recovery period; it may thus be extended when considered necessary. The time at which signs of toxicity appear and disappear and the time of death are important, especially if there is a tendency for deaths to be delayed.

1.6.3. Procedure

Shortly before exposure, the animals are weighed, and then exposed to the test concentration in the designated apparatus for a period of four hours, after equilibration of the chamber concentration. Time for equilibration should be short. The temperature at which the test is performed should be maintained at 22 ± 3 °C. Ideally the relative humidity should be maintained between 30% and 70 %, but in certain instances (e.g. tests of some aerosols) this may not be practicable. Maintenance of a slight negative pressure inside the chamber (≥ 5 mm of water) will prevent leakage of the test substance into the surrounding area. Food and water should be withheld during exposure. Suitable systems for the generation and monitoring of the test atmosphere should be used. The system should ensure that stable exposure conditions are achieved as rapidly as possible. The chamber should be designed and operated in such a way that a homogeneous distribution of the test atmosphere within the chamber is maintained.

Measurements or monitoring should be made:

(a) of the rate of air flow (continuously).

(b) of the actual concentration of the test substance measured in the breathing zone at least three times during exposure (some atmospheres, e.g. aerosols at high concentrations, may need more frequent monitoring). During the exposure period the concentration should not vary by more than ± 15 % of the mean value. However in the case of some aerosols, this level of control may not be achievable and a wider range would then be acceptable. For aerosols, particle size analysis should be performed as often as necessary (at least once per test group).

(c) of temperature and humidity , continuously if possible.

During and following exposure, observations are made and recorded systematically; individual records should be maintained for each animal. Observations should be made frequently during the first day. A careful clinical examination should be made at least once each working day, other observations should be made daily with appropriate actions taken to minimize loss of animals from the study, e.g. necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals.

Observations should include changes in the skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Particular attention should be directed to observation of respiratory behaviour, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The time of death should be recorded as precisely as possible. Individual weights of animals should be determined weekly after exposure, and at death.

Animals that die during the test and those surviving at the termination of the test are subjected to necropsy with particular reference to any changes in the upper and lower respiratory tract. All gross pathological changes should be recorded. Where indicated, tissues should be taken for histopathological examination.

2. **DATA**

Data should be summarized in tabular form showing for each test group the number of animals at the start of the test, time of death of individual animals, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings. Changes in weight must be calculated and recorded when survival exceeds one day. Animals which are humanely killed due to compound-related distress and pain are recorded as compound-related deaths. The LC_{50} should be determined by a recognized method. Data evaluation should
include the relationship, if any, between the animal's exposure to the test substance and the incidence and severity of all abnormalities, including behavioural and clinical abnormalities, gross lesions, body weight changes, mortality and any other toxic effects.

3. **REPORTING**

3.1. TEST REPORT

The test report shall, if possible, include the following information:

- species, strain, source, environmental conditions, diet etc.;

- test conditions: description of exposure apparatus

including design, type, dimensions, source of air, system for generating aerosols, method of conditioning air and the method of housing animals in a test chamber when this is used. The equipment for measuring temperature, humidity, and aerosol concentrations and particle size distribution should be described.

Exposure data

These should be tabulated and presented with mean values and a measure of variability (e.g. standard deviation) and shall, if possible, include:

(a) airflow rates through the inhalation equipment;

(b) temperature and humidity of the air;

(c) nominal concentrations (total amount of test substance fed into the inhalation equipment divided by volume of air);

(d) nature of vehicle, if used;

(e) actual concentrations in test breathing zone;

(f) The mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD);

(g) equilibration period;

(h) exposure period;

- tabulation of response data by sex and exposure level (i.e. number of animals that died or were killed during the test; number of animals showing signs of toxicity; number of animals exposed);

- time of death during or following exposure, reasons and criteria used for humane killing of animals;

- all observations;

- LC_{50} value for each sex determined at the end of the observation period (with method of calculation specified);

- 95 % confidence interval for the LC_{50} (where this can be provided);

- dose/mortality curve and slope (where permitted by the method of determination);

- necropsy findings;

- any histopathological findings;

- discussions of the results (particular attention should be given to the effect that humane killing of animals during the test may have on the calculated LC_{50} value);

- interpretation of the results.

3.2. EVALUATION AND INTERPRETATION

See General Introduction Part B (D).

4. **REFERENCES**

See General Introduction Part B (E).