B.3. ACUTE TOXICITY (DERMAL)

1. METHOD

1.1. INTRODUCTION

See General Introduction Part B (A).

1.2. DEFINITION

See General Introduction Part B (B).

1.3. REFERENCE SUBSTANCES

None.

1.4. PRINCIPLE OF THE TEST METHOD

The test substance is applied to the skin in graduated doses to several groups of experimental animals, one dose 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 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 in their experimental cages under the experimental housing and feeding conditions for at least five days prior to the experiment. Before the test, healthy young adult animals are randomized and assigned to the treatment groups. Approximately 24 hours before the test, fur should be removed by clipping or shaving from the dorsal area of the trunk of the animals. When clipping or shaving the fur, care must be taken to avoid abrading the skin which could alter its permeability. Not less than 10% of the body surface should be clear for the application of the test substance. When testing solids, which may be pulverized 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.

1.6.2. Test Conditions

1.6.2.1. Experimental Animals

The adult rat or rabbit may be used. Other species may be used but their use would require justification. 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 5 animals are used at each dose level. They should all be of the same sex. If females are used, they should be nulliparous and non-pregnant. Where information is available demonstrating that a sex is markedly more sensitive, animals of this sex should be dosed.

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. Dose Levels

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. Any irritant or corrosive effects should be taken into account when deciding on dose levels. The data should be sufficient to produce a dose/response curve and, where possible, permit an acceptable determination of the LD_{50}.

1.6.2.4. Limit Test

A limit test at one dose level of at least 2000 mg/kg bodyweight may be carried out in a group of 5 male and 5 female animals, using the procedures described above. If compound-related mortality is produced, a full study may need to be considered.

1.6.2.5. Observation Period

The observation period should be at least 14 days. However, the duration of observation 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, their duration and the time of death are important, especially if there is a tendency for deaths to be delayed.

1.6.3. Procedure

Animals should be caged individually. 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 a layer as thin and uniform 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. Restrainers may be used to prevent the ingestion of the test substance but complete immobilisation is not a recommended method.

At the end of the exposure period, residual test substance should be removed, where practicable, using water or some other appropriate method of cleansing the skin.

Observations should be recorded systematically as they are made. 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 to 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 fur, treated skin, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Particular attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy , sleep and coma. The time of death must be recorded as precisely as possible. Animals that die during the test and those surviving at the termination of the test are subjected to necropsy. All gross pathological changes should be recorded. Where indicated, tissues should be taken for histopathological examination.

Assessment of toxicity in the other sex

After completion of the study in one sex, at least one group of 5 animals of the other sex is dosed to establish that animals of this sex are not markedly more sensitive to the test substance. The use of fewer animals may be justified in individual circumstances. Where adequate information is available to demonstrate that animals of the sex tested are markedly more sensitive, testing in animals of the other sex may be dispensed with.

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. Individual weights of animals should be determined and recorded shortly before the test substance is applied, weekly thereafter, and at death; changes in weight should 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 LD_{50} should be determined by a recognized method.

Data evaluation should include an evaluation of relationships, 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 toxicological 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 (including method of skin cleansing and type of dressing: occlusive or not occlusive);

-dose levels (with vehicle, if used, and concentrations),

-sex of animals dosed;

-tabulation of response data by sex and dose 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 after dosing, reasons and criteria used for humane killing of animals;

-all observations;

-LD_{50} value for the sex subjected to a full study, determined at 14 days with the method of determination specified;

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

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

-necropsy findings;

-any histopathological findings;

-results of any test on the other sex;

-discussion of results (particular attention should be given to the effect that humane killing of animals during the test may have on the calculated LD_{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).