OECD GUIDELINE FOR TESTING OF CHEMICALS

Adopted: 12 May 81

"Acute Dermal Toxicity"

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1. INTRODUCTORY INFORMATION

° Prerequisites

- Solid or liquid test substance
- Chemical identification of test substance
- Purity (impurities) of test substance
- Solubility characteristics
- Melting point/boiling point
- pH (where appropriate)

° Standard documents

There are no relevant international standards.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. It provides information on health hazards likely to arise from a short term exposure by the dermal route. Data from an acute dermal toxicity study may serve as a basis for classification and labelling. It is an initial step in establishing a dosage regimen in subchronic and other studies and may provide information on dermal absorption and the mode of toxic action of a substance by this route.

° Definitions

Acute dermal toxicity is the adverse effects occurring within a short time of dermal application of a single dose of a test substance.

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

The LD50 (median lethal dose), dermal, is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of treated animals when applied to the skin. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Users of this Test Guideline should consult the Preface, in particular paragraphs 3, 4, 7 and 8.

Dosage is a general term comprising the dose, its frequency and the duration of dosing.

Dose-response is the relationship between the dose and the proportion of a population sample showing a defined effect.

Dose-effect is the relationship between the dose and the magnitude of a defined biological effect either in an individual or in a population sample.

° 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 the surviving animals are sacrificed and necropsied as necessary.

B. DESCRIPTION OF THE TEST PROCEDURE

° Preparations

Healthy young adult animals are acclimatised to the laboratory conditions for at least 5 days prior to the test. Before the test, animals are randomised and assigned to the treatment groups. Approximately 24 hours before the test, 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.

Not less than 10 per cent 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.

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.

° Experimental animals

Selection of species

The adult rat, rabbit or guinea pig may be used. Other species may be used but their use would require justification. The following weight ranges are suggested to provide animals of a size which facilitates the conduct of the test: rats, 200 to 300 g; rabbits 2.0 to 3.0 kg; guinea pigs 350 to 450 g.

Number and sex

Equal numbers of animals of each sex with healthy intact skin are required for each dose level. At least 10 animals (5 female and 5 male) should be used at each dose level. The females should be nulliparous and non-pregnant. A smaller number of animals may sometimes be used, especially in the case of the rabbit, but this may prevent the determination of an acceptable LD50.

Housing and feeding conditions

Animals should be caged individually. The temperature of the experimental animal room should be 22°C (± 3°) for rodents, 20°C (± 3°) for rabbits, and the relative humidity 30-70 per cent. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

° Test conditions

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. The data should be sufficient to produce a dose-response curve and, where possible, permit an acceptable determination of the LD50.

Limit test

If a test at one dose level of at least 2000 mg/kg body weight, using the procedures described for this study, produces no compound-related mortality, then a full study using three dose levels may not be necessary.

Observation period

The observation period should be at least 14 days. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset and length of recovery period, and 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.

° Procedure

The test substance should be applied uniformly over an area which is approximately 10 per cent 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 a 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. 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 an appropriate solvent.

° Clinical examinations

Observations should be recorded systematically as they are made. Individual records should be maintained for each animal. Following application of the test substance, the animals should be observed frequently during the first day and then a careful clinical examination should be made at least once each day. Additional observations should be made daily with appropriate actions taken to minimise loss of animals to the study, e.g. necropsy or refrigeration of those animals found dead and isolation of sacrifice of weak or moribund animals. Cageside observations should include changes in fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, 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.

Individual weights of animals should be determined 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. At the end of the test surviving animals are weighed and then sacrificed.

° Pathology

Consideration should be given to gross necropsy of all animals where indicated by the nature of the toxic effects observed. All gross pathological changes should be recorded. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours should also be considered because it may yield useful information.

3. DATA AND REPORTING

° Treatment of results

Data may be summarised in tabular form, showing for each test group the number of animals at the start of the test, time of death of individual animals at different dose levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings.

The LD50 may be determined by any accepted method, e.g. Bliss(4), Litchfield and Wilcoxon (3), Finney (5), Weil (6), Thompson (7), Miller and Tainter (8).

° Evaluation of results

The dermal LD50 value should always be considered in conjunction with the observed toxic effects and the necropsy findings. The LD50 value is a relatively coarse measurement, useful only as a reference value for classification and labelling purposes, and an expression of the lethal potential of the test substance following dermal exposure.

Reference should always be made to the experimental animal species in which the LD50 value was obtained. An evaluation should include an evaluation of relationships, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities, including behavioural and clinical abnormalities, gross lesions, body weight changes, effect on mortality, and any other toxic effects.

° Test report

The test report should include the following information:

- species/strain used;
- tabulation of response data by sex and dose level (i.e. number of animals dying, number of animals showing signs of toxicity, number of animals exposed);
- time of death after dosing;
- LD50 value for each sex (intact skin) determined at 14 days with the method of determination specified;
- 95 per cent confidence interval for the LD50 (where this can be provided);
- dose-mortality curve and slope (where permitted by the method of determination); and
- pathology findings.

° Interpretation of the results

A study of acute toxicity by the dermal (percutaneous) route and determination of a dermal LD50 provides an estimate of the relative toxicity of a substance by the dermal route of exposure.

Extrapolation of the results of acute dermal toxicity studies and dermal LD50 values in animals to man is valid only to a limited degree. The results of an acute dermal toxicity study should be considered in conjunction with data from acute toxicity studies by other routes.

4. LITERATURE

(1) WHO Publication : Environmental Health Criteria 6, Principles and Methods for Evaluating the Toxicity of Chemicals. Part I, Geneva, 1978.

(2) National Academy of Sciences, Committee for the Revision of NAS Publication 1138, Principles and Procedures for Evaluating the Toxicity of Household Substances, Washington, 1977.

(3) Litchfield, J.T. and Wilcoxon, F., J. Pharmacol., Exp. Ther., 96, 99-113, 1949.

(4) Bliss, C.I., Quart.J. Pharm. Pharmacol., 11, 192-216, 1938.

(5) Finney, D.G., Probit Analysis. (3rd Ed.) London, Cambridge University Press, 1971.

(6) Weil, C.S., Biometrics, 8, 249-263, 1952.

(7) Thompson, W., Bact. Rev., 11, 115-141, 1947.

(8) Miller, L.C. and Tainter, M.L., Proc. Soc. Exp. Biol. Med. NY, 57, 261-264, 1944.