OECD GUIDELINE FOR TESTING OF CHEMICALS

401

Adopted:
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"Acute Oral Toxicity"

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 oral toxicity is usually an initial step. It provides information on health hazards likely to arise from a short-term exposure by the oral route. Data from an acute 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 initial information on the mode of toxic action of a substance.

• Definitions

Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours.

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

LD50 (median lethal dose), oral, 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).

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 administered orally by gavage in graduated doses to several groups of experimental animals, one dose being used per group. The doses chosen may be based on the results of a range finding test. 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. This guideline is directed primarily to studies in rodent species but may be adapted for studies in non-rodents. 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.

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.

Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of a solution in oil (e.g. corn oil) and then by consideration of possible solution in other vehicles. For non-aqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not exceed 1 ml/100 g body weight, except in the cases of aqueous solutions where 2 ml/100 g may be used. Variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels.

- Experimental animals

Selection of species

Although several mammalian test species may be used, the rat is the preferred rodent species. Commonly used laboratory strains should be employed. The weight variation in animals used in a test should not exceed ± 20 per cent of the mean weight.

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.

Number and sex

At least 5 rodents 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.

Housing and feeding conditions

The temperature of the experimental animal room should be 22°C (± 3°) and the relative humidity 30-70 per cent. Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g. morbidity, excitability) may indicate a need for individual caging. 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

When rodents are used, a limit test at one dose level of a least 2000 mg/kg bodyweight may be carried out in a group of 5 males and 5 females using the procedures described above.

If compound-related mortality is produced, a full study may need to be considered.

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 the 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.

- Procedure

Animals should be fasted prior to substance administration. For the rat, food should be withheld over-night; for other rodents with higher metabolic rates a shorter period of fasting is appropriate. Following the period of fasting, the animals should be weighed and then the test substance administered in a single dose to animals by groups by gavage using a stomach tube or a suitable intubation cannula. If a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. After the substance has been administered, food may be withheld for a further 3-4 hours. Where a dose is administered in fractions over a period, it may be necessary to provide the animals with food and water depending on the length of the period. Following administration, observations are made and recorded systematically with individual records being maintained for each animal.

- Clinical examinations

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 or sacrifice of weak or moribund animals. Cageside observations should include changes in the skin and 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 observation of 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 shortly before the test substance is administered, 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

Necropsy of all animals should be carried out, and 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.

- 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.

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.

Animals which are humanely killed due to compound-related distress and pain are recorded as compound-related deaths.

The LD50 may be determined by any accepted method, e.g. Bliss (7), Litchfield and Wilcoxon (4), Finney (8), Weil (9), Thompson (10), Miller and Tainter (11).

- Evaluation of results

The LD50 value should always be considered in conjunction with the observed toxic effects and any necropsy findings. The LD50 value is a relatively coarse measurement, useful only as a reference value for classification and labelling purposes, and for an expression of the lethal potential of the test substance by the ingestion route. Reference should always be made to the experimental animal species in which the LD50 value was obtained.

An evaluation should include the relationship, 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, effects on mortality, and any other toxic effects.

• Test report

The test report should include the following information:

– species/strain/source used; diet; environmental conditions;

– sex of animals dosed;

– tabulation of response data by 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 dosing and time of death after dosing;

– LD50 values for the sex dosed, determined at 14 days (with the method of determination specified);

– 95 per cent confidence interval for the LD50;

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

– pathology findings; and

– results of any test on the other sex.

• Interpretation of the results

A study of acute toxicity by the oral route and determination of an LD50 provides an estimate of the relative toxicity of a substance. Extrapolation of the results of acute oral toxicity studies and oral LD50 values in animals to man is valid only to a very limited degree.

4. LITERATURE

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