Adopted:
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OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Oral Toxicity: Up-and-Down Procedure

INTRODUCTION

1.   The proposal for this guideline was submitted by the United States. The concept of the up-and-down testing approach was first described by Dixon and Mood (1)(2). In 1985, Bruce proposed to use an up-and-down procedure (UDP) for the determination of acute toxicity of chemicals (3). There exist several variations of the up-and-down experimental design for estimating an LD50. This guideline is based on the procedure of Bruce as adopted by ASTM in 1987 (4).

2.   A study comparing the results obtained with the UDP, the conventional LD50 test and the Fixed Dose Procedure (FDP, Guideline 420) was published in 1995 (5). The study showed that i) the UDP yields an estimate of the LD50 which is similar to that obtained by the conventional LD50 test and hence leads to similar classification in LD50-based classification schemes, ii) classifications in the EC scheme were similar for the UDP and the FDP, and iii) of the three protocols, the UDP required the smallest number of animals: from 6 to 10 animals of one sex. Also for the Acute Toxic Class method (Guideline 423) classifications in the EC scheme were similar to the conventional LD50 test and the ATC and UDP methods require comparably small numbers of animals (6)(7).

3.   Some terms used are defined in the Annex.

INITIAL CONSIDERATIONS

4.   This test procedure is of principal value in minimising the number of animals required to estimate the acute oral toxicity of a chemical and in estimating a median lethal dose. The median lethal dose allows for comparison with historical data. In addition to the observation of mortality, it allows the observation of signs of toxicity. The latter is useful for classification purposes and in the planning of additional toxicity tests.

5.   The procedure is easiest to apply to materials that produce death within one or two days. The method would not be practical to use when considerably delayed death (5 days or more) can be expected.

6.   During the test, animals obviously in pain or showing signs of severe distress should be humanely killed.

PRINCIPLE OF THE TEST

7.   Animals are dosed, one at a time, at 24 hour intervals. The first animal receives a dose at the level of the best estimate of the LD50. Depending on the outcome for the previous animal, the dose for the next animal is adjusted up or down. If an animal survives, the dose for the next animal is increased; if it dies, the dose for the next animal is decreased. After reaching the reversal of the initial outcome, i.e. the point where an increasing (or decreasing) dose pattern is reversed by giving a smaller (or a higher) dose, four additional animals are dosed following the same UDP. The LD50 is calculated using the method of maximum likelihood (8)(9).

DESCRIPTION OF THE METHOD

Selection of animals species

8.   The preferred rodent species is the rat although other rodent species may be used. In the normal procedure female rats are used, because literature surveys of conventional LD50 tests show that, although there is little difference of sensitivity between sexes, in those cases where differences were observed, females were in general slightly more sensitive (5). When there is adequate information to infer that males are more sensitive, they should replace females in the test.

9.   Healthy young adult animals should be employed. The females should be nulliparous and non-pregnant. At the commencement of the study, the weight variation of the animals should be minimal and not exceed ± 20 % of the mean weight for each sex. The test animals should be characterised as to species, strain, source, sex, weight and/or age.

Housing and feeding conditions

10.   The temperature in the experimental animal room should be 22° C (± 3° 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 and 12 hours dark. The animals are housed individually. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

Preparation of animals

11.   The animals are uniquely identified and kept in their cages for at lease five days prior to dosing for acclimatisation to the laboratory conditions. During acclimatisation the animals should be observed for ill health. Animals demonstrating signs of spontaneous disease or abnormality prior to the start of the study are eliminated from the study.

Preparation of doses

12.   When necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, whenever possible, the use of an aqueous solution or suspension be considered first, followed by consideration of a solution or emulsion in oil (e.g. corn oil) and then by possible solution in other vehicles. For vehicles other than water, the toxicity of the vehicle must be known.

PROCEDURE

Full test

13.   Individual animals are dosed in sequence at 24 h intervals, one at a time, and then observed for a minimum of 24 hours. However, the time intervals between dosing should not be fixed rigidly and may be adjusted as appropriate, in case of delayed mortality. The first animal is dosed at the toxicologist's best estimate of the LD50. If the animal survives, the second animal receives a higher dose, unless the limit dose was used as the starting dose. If the first animal dies or appears moribund the second animal receives a lower dose. Moribund state is characterised by symptoms such as shallow, laboured or irregular respiration, muscular weakness or tremors, absence of voluntary response to external stimuli, cyanosis and coma. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document. Animals killed for humane reasons are considered in the same way as animals that died on test.

14.   For selecting the starting dose, all available information should be used, including information on structure-activity relationships. When the information suggests that mortality is unlikely then a limit test should be conducted (see paragraph 16). When there is no information on the substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 200 or 500 mg/kg body weight.

15.   The dose for each successive animal is adjusted up or down, depending on the outcome of the previous animal. If feasible, a dose progression factor of 1.3 is used. Other factors may be used, if justified. After reaching the reversal of the initial direction (the point where a decreasing dose pattern requires an increase due to a tested animal's survival or an increasing dose pattern results in a decrease due to lethality), four additional animals are dosed using the same UDP. This is the end of the normal test.

Limit test

16.   Doses should not exceed 2000 mg/kg which is considered the upper limit dose. When the first animal is dosed with the upper limit dose and survives, the second animal receives the same dose. When a total of three animals have been dosed with the limit dose and no deaths have occurred, then three animals of the other sex should be tested at the limit dose level. If there is again no lethality, the test can be terminated.

Optional testing

17.   Information from one sex may be adequate to assess acute toxicity. However, if found desirable, comparability of response in the other sex can be evaluated by administering to generally not more than 3 animals, doses above and below the estimated LD50. The point intermediate between doses where responses change can be taken as an approximate estimate of the lethal dose.

Administration of doses

18.   The test substance is administered in a single dose by gavage, using an oral dosing needle or rubberised tubing.

19.   The animals should be fasted prior to dosing by withholding food overnight. Fasted body weight of each rat is determined and the dose is calculated according to the body weight. After dosing food may be withheld for a further 3-4 hours. The volume should not exceed 1 ml/100g body weight, except in the case of aqueous solutions where 2 ml/100g body may be used.

Observations

20.   Animals are observed individually 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. However, the duration of the observation period should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary.

21.   Observations include mortality and clinical signs. These 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. Attentions should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Body weight

22.   Individual weights of animals should be determined shortly before the test substance is administered, at least weekly thereafter, at the time of death or at day 14 in the case of survival. Weight changes should be calculated and recorded.

Pathology

23.   All animals, including those which die during the test or are killed for animal welfare reasons during the test and those that survive at day 14, are subjected to gross necropsy. The necropsy should entail a macroscopic inspection of the visceral organs. As deemed appropriate, microscopic analysis of target organs and clinical chemistry may be included to gain further information on the nature of the toxicity of the test material.

DATA AND REPORTING

Data

24.   Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test concentration 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.

Calculation of LD50

25.   The LD50 is calculated using the maximum likelihood method (8)(9). The following statistical details may be helpful in implementing the maximum likelihood calculations suggested.

All deaths, whether immediate or delayed or humane kills, are incorporated for the purpose of the maximum likelihood analysis. Following Dixon (8), the likelihood function is written as follows:

$$L = L_1 L_2 ....L_n ,$$

where

L is likelihood of the experimental outcome, given μ and σ, and n the number of animals tested.

$$L_i = 1 - F(Z_i) \quad \text{if the i}^{th}\text{ animal survived, or}$$
$$L_i = F(Z_i) \quad \text{if the i}^{th}\text{ animal died ,}$$

where

F = cumulative, standard normal density,
$Z_i = [\log(d_i) - \mu] / \sigma$
d_i = dose given to the ith animal
μ = log LD50, and
σ = standard deviation

An estimate of σ of 0.12 is used unless a better generic or case-specific value is available.

26.   The calculation can be performed using either SAS (10) or BMDP (11) computer program packages. Other computer programs may also be used. Typical instructions for these packages are given in appendices to the ASTM Standard E 1163-87 (4). The program output is an estimate of log LD50 and its standard error.

Report

27.   The test report must include the following information:

Test substance:

- physical nature, purity and physicochemical properties (including isomerisation);
- identification data.

Vehicle (if appropriate):

- 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;
- rationale for use of males instead of females;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start of the test, at day 7, and at day 14.

Test conditions:

- rationale for initial dose level selection and for follow-up dose levels;
- details of test substance formulation;
- details of the administration of the test substance;
- details of food and water quality (including diet type/source, water source).

Results:

- body weight/body weight changes;
- tabulation of response data by sex and dose level for each animal (i.e. animals showing signs of toxicity including nature, severity, duration of effects, and mortality);
- time course of onset of signs of toxicity and whether these were reversible for each animal;
- necropsy findings and any histopathological findings for each animal, if available.
- LD_{50} data;
- statistical treatment of results.

Discussion and interpretation of results.

Conclusions.

LITERATURE

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ANNEX

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.

Delayed death means that an animal does not die or appear moribund within 24 hours but dies later during the observation period.

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

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).

Moribund status of an animal is the result of the toxic properties of a test substance where death is anticipated. For making decisions as to the next step in this test, animals killed for humane reasons are considered in the same way as animals that died.

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).