CHAPTER 4 TOXICITY STUDIES

TOXICITY STUDIES
AS PER OECD GUIDELINE FOR TESTING OF CHEMICALS

Acute Oral Toxicity – Acute Toxic Class Method
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The acute toxic class method set out is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods. The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated in vivo against LD50 data obtained from the literature, both nationally and internationally.

4.1 PRINCIPLE OF THE TEST

It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;
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- No further testing is needed,
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose level.

4.2 DESCRIPTION OF THE METHOD

Selection of animal species
The preferred rodent species is the rat, although other rodent species may be used. Normally females are used. This is because literature surveys of conventional LD50 tests show that, although there is little difference in sensitivity between the sexes, in those cases where differences are observed females are generally slightly more sensitive (11). However if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive, then this sex should be used. When the test is conducted in males adequate justification should be provided.

Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within + 20 % of the mean weight of any previously dosed animals.

Housing and feeding conditions
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, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.
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Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions.

4.3 PROCEDURE
   Administration of doses.
   The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

   Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. 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.

   Number of animals and dose level.
   Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals.
   The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals.
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4.4 OBSERVATIONS
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, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation 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. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should 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. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document should be taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

Body weight
Individual weights of animals should be determined shortly before the test substance is administered, and at least weekly thereafter. Weight changes should be calculated and recorded. At the end of the test surviving animals are weighed and humanely killed.

Pathology
All test animals (including those that die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing
evidence of gross pathology in animals surviving 24 or more hours may also be considered because it may yield useful information.

4.5 DATA AND REPORTING DATA
Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group 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.
Accordingly Parkinsonia aculeata was evaluated and the Test report is as follows.
The test report include the following information.

Test substance: Parkinsonia aculeata
Vehicle (distilled water)
Test animals: Rats
Species/strain used: Albino Wistar
Number: 9
Age: 8-12 weeks
Sex of animals: Female
Source: Wockhart Aurangabad.
Housing conditions: Standard (Room Temp and Humidity)
Diet: Standard pellet diet.

Test conditions:
Test substance formulation: Parkinsonia aculeata emulsified with Tween 40 in diatilled water. Administration of the test substance dosing volumes and time of dosing: Given orally in dose of 50,300 and 2000 mg/kg once.
The rationale for the selection of the starting dose: as no reported toxicity, so 50 mg instead of 5 mg was taken.
4.6 Results:

Animals showing signs of toxicity including mortality; nature, severity, and duration of effects

No adverse effects observed tabulation of body weight and body weight changes: No change in body wt observed individual weights of animals at the day of dosing, in weekly intervals thereafter, and at the time of death or sacrifice: No change in body wt observed date and time of death if prior to scheduled sacrifice: No mortality observed.

Time course of onset of signs of toxicity, and whether these were reversible for each animal

No signs of mortality till 2000 mg/kg

Necropsy findings and Histopathological findings for each animal: Not required

Discussion and interpretation of results. Since no signs of toxicity were observed with 50,300 and 2000mg/kg body wt dose, it was found to be a very safe drug

4.7 Conclusions: Based on this observation 500 mg/kg was decided as a very safe dose without any toxicity observed in animals.