
**"Repeated Dose Oral Toxicity - Rodent:
28-day or 14-day Study"**

not more than 8, weeks old. At the commencement of the study the weight variation of animals used should not exceed ± 20 per cent of the mean weight. Where a repeated dose oral study is conducted as a preliminary to a long-term study, the same species and strain should be used in both studies.

Number and sex

At least 10 animals (5 female and 5 male) should be used at each dose level. The females should be nulliparous and non-pregnant. If interim sacrifices are planned the number should be increased by the number of animals scheduled to be sacrificed before the completion of the study. In addition, a satellite group of 10 animals (5 animals per sex) may be treated with the high dose level for 28 days or 14 days and observed for reversibility, persistence, or delayed occurrence, of toxic effects for 14 days post-treatment.

Housing and feeding conditions

The temperature in the experimental animal room should be 22°C ($\pm 3^{\circ}$) and the relative humidity 30-70 per cent. When 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. The choice of diet may be influenced by the need to ensure a suitable admixture of a test substance when administered by this method. Animals may be caged in groups by sex or individually; for group caging not more than five animals should be housed per cage.

• **Test conditions**

Dose levels

At least three dose levels and a control should be used. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects. The highest dose level should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation. The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure the lowest level should exceed this. Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used the dose

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levels should be spaced to produce a gradation of toxic effects. In the low and intermediate groups and in the controls the incidence of any fatalities should be low in order to permit a meaningful evaluation of results.

For substances of low toxicity it is important to ensure that when administered in the diet the quantities of the test substance involved do not interfere with normal nutrition. When the test substance is administered in the diet either a constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight may be used; the alternative used must be specified. For a substance administered by gavage, the dose should be given at similar times each day, and adjusted at intervals (weekly or bi-weekly) to maintain a constant dose level in terms of animal body weight. Where a repeated dose study is used as a preliminary to a long term study, a similar diet should be used in both studies.

Limit test

If a test at one dose level of at least 1000 mg/kg body weight (but expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary.

Observations

The observation period should be for 14 or 28 days. The selection of duration is not fixed rigidly, but should be determined based on the expected toxic reactions and characteristics of the chemical. Animals in a satellite group scheduled for follow-up observations should be kept for a further 14 days without treatment to detect recovery from, or persistence of, toxic effects. 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.

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- Procedure

The animals are dosed with the test substance ideally on 7 days per week for a period of 28 days or 14 days. Signs of toxicity should be recorded as they are observed, including the time of onset, degree and duration. Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern. Measurements should be made of food consumption (or water consumption when the test substance is administered in the drinking water) weekly and the animals weighed weekly. Regular observation of the animals is necessary to ensure that animals are not lost from the study due to causes such as cannibalism, autolysis of tissues or misplacement. At the end of the study period all survivors in the non-satellite treatment groups are sacrificed. Moribund animals should be removed and sacrificed when noticed.

- Clinical examinations

The following examinations should be made:

- (a) Haematology, including haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count should be investigated at the end of the test period.
- (b) Clinical biochemistry determination on blood should be carried out at the end of the test period. Blood parameters of liver and kidney function are appropriate. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations are: calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum glutamic-pyruvic transaminase*, serum glutamic-oxaloacetic transaminase**, ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen, blood creatinine, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methaemoglobin, cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

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- (c) Urinalysis is not required on a routine basis, but only when there is an indication based on expected or observed toxicity.

If historical baseline data are inadequate, consideration should be given to determination of haematological and clinical biochemistry parameters before dosing commences.

- Pathology

Gross necropsy

All animals in the study should be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals and testes should be weighed wet as soon as possible after dissection to avoid drying. The following organs and tissues should be preserved in a suitable medium for possible future histopathological examination: liver, kidney, spleen, adrenals, heart and target organs, that is, those showing gross lesions or changes in size.

Histopathology

Histological examination should be performed on the preserved organs or tissues of the high dose group and the control group. These examinations may be extended to animals of other dosage groups, if considered necessary to further investigate changes observed in the high dose group. Animals in a satellite group should be examined histologically with particular emphasis on those organs and tissues identified as showing effects in the other treated groups.

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, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.

* Now known as serum alanine aminotransferase.

** Now known as serum aspartate aminotransferase.

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All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical method may be used; the statistical methods should be selected during the design of the study.

- Evaluation of results

The findings of a repeated dose oral toxicity study should be considered in terms of the observed toxic effects and the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence or absence, the incidence and severity of abnormalities, including behavioural and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted 28-day or 14-day study will provide information on the effects of repeated doses and can indicate the need for further longer term studies. It can also provide information on the selection of dose levels for longer term studies.

In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

- Test report

The test report must include the following information:

- species/train used;
- toxic response data by sex and dose;
- time of death during the study or whether animals survived to termination;
- toxic or other effects;
- the time of observation of each abnormal sign and its subsequent course;
- food and body weight data;
- haematological tests employed and results with relevant baseline data;
- clinical biochemistry tests employed and results with relevant baseline data;

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- necropsy findings;
- a detailed description of all histopathological findings; and
- statistical treatment of results where appropriate.
- Interpretation of the results

A repeated dose oral study will provide information on the effects of repeated oral exposure to a substance. Extrapolation from the results of the study to man is valid to a limited degree, but it can provide useful information on the mode of action of the substance.

4. LITERATURE

1. WHO Publication: Environmental Health Criteria No. 6, *Principles and Methods for Evaluating the Toxicity of Chemicals*. Part I, Geneva, 1978.
2. United States National Academy of Sciences, Committee for the Revision of NAS Publication 1138, *Principles and Procedures for Evaluating the Toxicity of Household Substances*, Washington, 1977.
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4. Boyd, E.M., *Predictive Toxicometrics*, Chapters 14, 15 and 16. Sciencetchnia Publications, 1972.
5. Draize, J.H., *The Appraisal of Chemicals in Food, Drugs and Cosmetics*, 26-30. Association of Food and Drug Officials of the United States, Austin, Texas, 1959.
6. Hagan, E.C., *Appraisal of the Safety of Chemicals. Appraisal of Chemicals in Foods, Drugs and Cosmetics*, 17-25. Association of Food and Drug Officials of the United States, Topeka, Kansas, 1965.