
**"Subchronic Oral Toxicity - Non-rodent:
90-day Study"**

oral study is conducted as a preliminary to a long-term study, the same species/breed should be used in both studies.

Number and sex

At least 8 animals (4 female and 4 male) should be used each dose level. The number of animals at the termination of the study must be adequate for a meaningful evaluation of toxic effects.

Housing and feeding conditions

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. Caging should be appropriate to the species.

• 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 fatalities. The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure the lowest dose level should exceed this. Ideally, the intermediate dose levels should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects. In the low and intermediate groups and in the controls there should also not be fatalities.

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. In the case of a substance administered by capsule, the dose should be given

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at similar times each day and adjusted as necessary at weekly intervals to maintain a constant dose level in terms of animal body weight. Where a subchronic study is used as a preliminary to a long-term study, a similar diet should usually 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 procedure 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 experimental animals should be observed for at least 90 days. A careful clinical examination 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.

• Procedure

The animals are dosed with the test substance ideally on 7 days per week, over a period of 90 days. However, based primarily on practical considerations, dosing in gavage or capsule studies on a 5-day per week basis is considered to be acceptable. Signs of toxicity should be recorded as they are observed, including the time of onset, degree and duration. 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. At the end of the exposure period, all surviving animals are sacrificed. Any moribund animals should be removed and sacrificed when noticed.

• Clinical examinations

The following examinations should be made:

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- (a) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study, preferably in all animals but at least in the high dose and control groups. If changes in the eyes are detected all animals should be examined.
- (b) 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 beginning, then either at monthly intervals or midway through the test period and finally at the end of the test period.
- (c) Clinical biochemistry determinations on blood should be carried out at the beginning, then either at monthly intervals or midway through the test and finally at the end of the test period. Test areas which are considered appropriate to all studies are electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the test substance. Suggested determinations are calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species/breed), 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 inhibition. Additional clinical biochemistry may be employed where necessary to extend the investigation of observed effects. Non-rodents should be fasted for a period (not more than 24 hours) before taking blood samples.
- (d) Urinalysis is not required on a routine basis but only when there is an indication based on expected or observed toxicity.

* Now known as serum alanine aminotransferase.

** Now known as serum aspartate aminotransferase.

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- Pathology

Gross necropsy

All animals 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, thyroid (with parathyroids), 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: all gross lesions, brain - including sections of medulla/pons, cerebellar cortex and cerebral cortex, pituitary, thyroid (parathyroid), thymus, (trachea), lungs, heart, aorta, salivary glands, liver, spleen, kidneys, adrenals, pancreas, gonads, uterus, (accessory genital organs), (skin), gall bladder, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, representative lymph node, (female mammary gland), (thigh musculature), peripheral nerve, (eyes), sternum with bone marrow, (femur - including articular surface), and (spinal cord at three levels - cervical, midthoracic and lumbar). (The tissues mentioned between brackets need only be examined if indicated by signs of toxicity or target organ involvement.)

Histopathology

Full histopathological examination should be carried out on organs and tissues of all animals in the control and high dose groups. Further histopathology in other dose groups should be carried out on organs which show lesions in the high dose group or for which clinical observations indicate such a need.

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 types of lesions and the percentage of animals displaying each type of lesion.

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

• Evaluation of results

The findings of a subchronic oral toxicity study should be evaluated in conjunction with the findings of preceding studies and 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 subchronic test should provide a satisfactory estimation of a no-effect level.

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/breed 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;
- results of ophthalmological examination;
- haematological tests employed and all results;
- clinical biochemistry tests employed and all results;

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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 subchronic oral toxicity study will provide information on the effects of repeated oral exposure to a substance. Extrapolation of the results of the study to man is valid to a limited degree, but it can provide useful information on no-effect levels and permissible human exposure.

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.
3. Food Safety Council, "Proposed System for Food Safety Assessment", *Food and Cosmetic Toxicology*, 16, 2, 1978.
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.G., *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.