OECD GUIDELINE FOR TESTING OF CHEMICALS

411

Adopted:
12 May 1981

"Subchronic Dermal Toxicity: 90-day Study"

1. INTRODUCTORY INFORMATION

• Prerequisites

– Solid or liquid test substance
– Chemical identification of test substance
– Purity (impurities) of test substance
– Solubility characteristics
– pH (where appropriate)
– Stability, including stability in vehicle when so applied
– Melting point/boiling point

• 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 chemical the determination of subchronic dermal toxicity may be carried out after initial information on toxicity has been obtained by acute testing. It provides information on possible health hazards likely to arise from repeated exposure by the dermal route over a limited period of time.

• Definitions

Subchronic dermal toxicity is the adverse effects occurring as a result of the repeated daily dermal application of a chemical to experimental animals for part (not exceeding 10 per cent) of a life span.

Dose in a dermal test is the amount of test substance applied to the skin (applied daily in subchronic tests). Dose is expressed as weight (g, mg) or as weight of the test substance per unit weight of test animal (e.g. mg/kg).

No-effect level/No-toxic-effect level/No-adverse-effect level is the maximum dose used in a test which produces no adverse effects. A no-effect level is expressed in terms of the weight of a substance given daily per unit weight of test animal (mg/kg).

Cumulative toxicity is the adverse effects of repeated doses occurring as a result of prolonged action on, or increased concentration of the administered substance or its metabolites in, susceptible tissue.

- Principle of the test method

The test substance is applied daily to the skin in graduated doses to several groups of experimental animals, one dose per group, for a period of 90 days. During the period of application the animals are observed daily to detect signs of toxicity. Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied.

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 and control groups. Shortly before testing, fur is clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out approximately 24 hours before the test. Repeat clipping or shaving is usually needed at approximately weekly intervals. When clipping or shaving the fur, care must be taken to avoid abrading the skin, which could alter its permeability. Not less than 10 per cent of the body surface area should be clear for the application of the test substance. The weight of the animal should be taken into account when deciding on the area to be cleared and on the dimensions of the covering. When testing solids, which may be pulverised if appropriate, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on penetration of skin by the test substance should be taken into account. Liquid test substances are generally used undiluted.

- Experimental animals

Selection of species

The adult rat, rabbit or guinea pig may be used. Other species may be used, but their use would require justification.

The following weight ranges at the start of the test are suggested in order to provide animals of a size which facilitates the conduct of the test:

rats, 200 to 300 g; rabbits, 2.0 to 3.0 kg; guinea pigs, 350 to 450 g.

Where a subchronic dermal 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 20 animals (10 female and 10 male) with healthy skin 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. A satellite group of 20 animals (10 animals per sex) may be treated with the high dose level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days.

Housing and feeding conditions

Animals should be caged individually. The temperature in the experimental animal room should be 22°C (± 3°) for rodents or 20°C (± 3°) for rabbits 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.

- Test conditions

Dose levels

At least three dose levels with a control and (where appropriate) a vehicle control should be used. Except for treatment with test substances, 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 levels should be spaced to produce a gradation of toxic effects. In the low and intermediate groups and in the controls the incidence of fatalities should be low, in order to permit a meaningful evaluation of the results.

If application of the test substance produces severe skin irritation the concentration should be reduced, although this may result in a reduction in, or absence of, other toxic effects at the high dose level. However, if the skin has been badly damaged early in the study, it may be necessary to terminate the study and undertake a new study at lower concentrations.

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 high 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

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.

- Procedure

The animals are treated with the test substance, ideally for at least 6 hours per day on a 7-day per week basis, for a period of 90 days. However, based primarily on practical considerations, application on a 5-day per week basis is considered to be acceptable. Animals in a satellite group scheduled for follow-up observations should be kept for at least a further 28 days without treatment to detect recovery from, or persistence of, toxic effects.

The test substance should be applied uniformly over an area which is approximately 10 per cent of the total body surface area. With highly toxic substances the surface area covered may be less, but as much of the area should be covered with as thin and uniform a film as possible.

Between applications the test substance is held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance. Restrainers may be used to prevent ingestion of the test substance, but complete immobilisation is not a recommended method.

Signs of toxicity should be recorded as they are observed, including the time of onset, the degree and duration. Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, as well as respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern. Measurements should be made of food consumption 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) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to exposure to 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 end of the test period.

(c) Clinical biochemistry determinations on blood should be carried out at the end of the test period. Test areas which are considered appropriate to all studies are electrolyte balance, carbohydrate metabolism, liver and kidney function. 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 the period of fasting appropriate to the species), serum glutamic-pyruvic transaminase*, serum glutamic oxalaocetic 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.

(d) 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 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 must 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, accessory genital organs, gall bladder (if present), oesophagus,

* Now known as serum alanine aminotransferase.

** Now known as serum aspartate aminotransferase.

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), (spinal cord at three levels - cervical, midthoracic and lumbar), and (exorbital lachrymal glands). (The tissues mentioned in brackets need only be examined if indicated by signs of toxicity or target organ involvement.)

Histopathology

(a) Full histopathology should be carried out on normal and treated skin and on organs and tissues of all animals in the control and high dose groups.

(b) All gross lesions should be examined.

(c) Target organs in other dose groups should be examined.

(d) Where rats are used, lungs of animals in the low and intermediate dose groups should be subjected to histopathological examination for evidence of infection, since this provides a convenient assessment of the state of health of the animals. Further histopathological examination may not be required routinely on the animals in these groups but must always be carried out in organs which showed evidence of lesions in the high dose group.

(e) When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in 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 types of lesions and the percentage of animals displaying each type of lesion.

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 subchronic dermal 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.

• Test report

The test report must include the following information:

– species/strain 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;

– necropsy findings;

– a detailed description of all histopathological findings; and

– statistical treatment of results where appropriate.

- Interpretation of the results

A subchronic dermal study will provide information on the effects of repeated dermal 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 degree of percutaneous absorption of a substance, no-effect levels and permissible human exposure.

4. LITERATURE

1. WHO Publications: 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. 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.

4. 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.