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OECD GUIDELINE FOR TESTING OF CHEMICALS

"Repeated Dose Dermal Toxicity: 21/28-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 exposures by the dermal route over a limited period of time.

There is sufficient similarity between the considerations involved in the conduct of a 21-day or 28-day repeated dose dermal study to allow one Guideline to cover both test durations. The main difference lies in the time over which dosing takes place (indicated in the text).

• Definitions

Dose in a dermal test is the amount of test substance applied to the skin. Dose is expressed as weight (g, mg) or as weight of 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 tissues.

- 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 21/28 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, unless a requirement for abraded skin is part of the test design. 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.

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- 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 repeated dose 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 10 animals (5 female and 5 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. In addition, a satellite group of 10 animals (5 animals per sex) may be treated with the high dose level for 21/28 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for 14 days post-treatment.

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 the 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 may 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 higher dose level), using the procedures described forthis 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 21/28 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 a further 14 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 and also 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 on all animals:

(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 deteminations 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.

(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 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: normal and treated skin, liver, kidney and target organs, that is, those organs showing gross lesions or changes in size.

Histopathology

Histological examination should be performed on the preserved organs and 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 investigate the changes observed in the high dose

* Now known as serum alanine aminotransferase.

** Now known as serum aspartate aminotransferase.

dose group. Animals in the 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.

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 the results

The findings of a repeated dose dermal 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 21/28-day study will provide information on the effects of repeated dermal application of a substance 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.

• 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 repeated dose 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.

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. 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.