OECD/OCDE

404

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OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Dermal Irritation/Corrosion

INTRODUCTION

1. OECD Guidelines for Testing of Chemicals are periodically reviewed to ensure that they reflect the best available science. In the review of this Guideline, special attention was given to possible improvements in relation to animal welfare concerns and to the evaluation of all existing information on the test substance in order to avoid unnecessary testing in laboratory animals. This updated version of Guideline 404 (adopted in 1981 and first revised in 1992) includes the recommendation that prior to undertaking the described *in vivo* test for corrosion/irritation of the substance, a weight-of-the-evidence analysis be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing (1). The testing strategy includes the performance of validated and accepted *in vitro* tests and is provided as a Supplement to this Guideline. In addition, where appropriate, the successive, instead of simultaneous, application of the three test patches to the animal in the initial *in vivo* test is recommended in this Guideline.

2. Definitions of dermal irritation and corrosion are set out in the Annex to the Guideline.

INITIAL CONSIDERATIONS

3. In the interest of both sound science and animal welfare, *in vivo* testing should not be undertaken until all available data relevant to the potential dermal corrosivity/irritation of the substance have been evaluated in a weight-of-the-evidence analysis. Such data will include evidence from existing studies in humans and/or laboratory animals, evidence of corrosivity/irritation of one or more structurally related substances or mixtures of such substances, data demonstrating strong acidity or alkalinity of the substance (2)(3), and results from validated and accepted *in vitro* or *ex vivo* tests (4)(5)(6). This analysis should decrease the need for *in vivo* testing for dermal corrosivity/irritation of substances for which sufficient evidence already exists from other studies as to those two endpoints.

4. A preferred sequential testing strategy, which includes the performance of validated and accepted *in vitro* or *ex vivo* tests for corrosion/irritation, is included as a Supplement to this Guideline. The strategy was developed at, and unanimously recommended by the participants of, an OECD workshop (7), and has been adopted as the recommended testing strategy in the Globally Harmonized System for the Classification of Chemical Substances (GHS) (8). It is recommended that this testing strategy be followed prior to undertaking *in vivo* testing. For new substances it is the recommended stepwise testing approach for developing scientifically sound data on the corrosivity/irritation of the substance. For existing substances with insufficient data on dermal corrosion/irritation, the strategy should be used to fill missing data gaps. Major deviation from the testing strategy or procedure, or a decision not to use a stepwise testing approach, should be justified.

5. If a determination of corrosivity or irritation cannot be made using a weight-of-the-evidence analysis, consistent with the sequential testing strategy, an *in vivo* test should be considered (see Supplement).

PRINCIPLE OF THE *IN VIVO* TEST

6. The substance to be tested is applied in a single dose to the skin of an experimental animal; untreated skin areas of the test animal serve as the control. The degree of irritation/corrosion is read and scored at specified intervals and is further described in order to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate the reversibility or irreversibility of the effects observed.

7. Animals showing continuing signs of severe distress and/or pain at any stage of the test should be humanely killed, and the substance assessed accordingly. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document (9).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of animal species

8. The albino rabbit is the preferable laboratory animal, and healthy young adult rabbits are used. A rationale for using other species should be provided.

Preparation of the animals

9. Approximately 24 hours before the test, fur should be removed by closely clipping the dorsal area of the trunk of the animals. Care should be taken to avoid abrading the skin, and only animals with healthy, intact skin should be used.

10. Some strains of rabbit have dense patches of hair that are more prominent at certain times of the year. Such areas of dense hair growth should not be used as test sites.

Housing and feeding conditions

11. Animals should be individually housed. The temperature of the experimental animal room should be 20°C (± 3°C) for rabbits. 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 unrestricted supply of drinking water.

TEST PROCEDURE

Application of the test substance

12. The test substance should be applied to a small area (approximately 6 cm^2) of skin and covered with a gauze patch, which is held in place with non-irritating tape. In cases in which direct application is not possible (e.g., liquids or some pastes), the test substance should first be applied to the gauze patch, which is then applied to the skin. The patch should be loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of the exposure period. If the test substance is applied to the patch, it should be attached to the skin in such a manner that there is good contact and uniform distribution of the substance on the skin. Access by the animal to the patch and ingestion or inhalation of the test substance should be prevented.

13. Liquid test substances are generally used undiluted. When testing solids (which may be pulverised, if considered necessary), the test substance should be moistened with the smallest amount of water (or, where necessary, of another suitable vehicle) sufficient to ensure good skin contact. When vehicles other than water are used, the potential influence of the vehicle on irritation of the skin by the test substance should be minimal, if any.

14. At the end of the exposure period, which is normally 4 hours, residual test substance should be removed, where practicable, using water or an appropriate solvent without altering the existing response or the integrity of the epidermis.

Dose level

15. A dose of 0.5 mL of liquid or 0.5 g of solid or paste is applied to the test site.

Initial test (*in vivo* dermal irritation/corrosion test using one animal)

16. It is strongly recommended that the *in vivo* test be performed initially using one animal, especially when the substance is suspected to have corrosion potential. This is in accordance with the sequential testing strategy (Supplement).

17. When a substance has been judged to be corrosive on the basis of a weight-of-the-evidence analysis, no further animal testing is needed. For most substances suspected of being corrosive, further *in vivo* testing is normally not necessary. However, in those cases where additional data are felt warranted because of insufficient evidence, limited animal testing may be carried out using the following approach: Up to three test patches are applied sequentially to the animal. The first patch is removed after three minutes. If no serious skin reaction is observed, a second patch is applied at a different site and removed after one hour. If the observations at this stage indicate that exposure can humanely be allowed to extend to four hours, a third patch is applied and removed after four hours, and the response is graded.

18. If a corrosive effect is observed after any of the three sequential exposures, the test is immediately terminated. If a corrosive effect is not observed after the last patch is removed, the animal is observed for 14 days, unless corrosion develops at an earlier time point.

19. In those cases in which the test substance is not expected to produce corrosion but may be irritating, a single patch should be applied to one animal for four hours.

Confirmatory test (*in vivo* dermal irritation test with additional animals)

20. If a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals, each with one patch, for an exposure period of four hours. If an irritant effect is observed in the initial test, the confirmatory test may be conducted in a sequential manner, or by exposing two additional animals simultaneously. In the exceptional case, in which the initial test is not conducted, two or three animals may be treated with a single patch, which is removed after four hours. When two animals are used, if both exhibit the same response, no further testing is needed. Otherwise, the third animal is also tested. Equivocal responses may need to be evaluated using additional animals.

Observation period

21. The duration of the observation period should be sufficient to evaluate fully the reversibility of the effects observed. However, the experiment should be terminated at any time that the animal shows continuing signs of severe pain or distress. To determine the reversibility of effects, the animals should be observed up to 14 days after removal of the patches. If reversibility is seen before 14 days, the experiment should be terminated at that time.

Clinical observations and grading of skin reactions

22. All animals should be examined for signs of erythema and oedema, and the responses scored at 60 minutes, and then at 24, 48 and 72 hours after patch removal. For the initial test in one animal, the test site is also examined immediately after the patch has been removed. Dermal reactions are graded and recorded according to the grades in the Table below. If there is damage to skin which cannot be identified as irritation or corrosion at 72 hours, observations may be needed until day 14 to determine the reversibility of the effects. In addition to the observation of irritation, all local toxic effects, such as defatting of the skin, and any systemic adverse effects (e.g., effects on clinical signs of toxicity and body weight), should be fully described and recorded. Histopathological examination should be considered to clarify equivocal responses.

23. The grading of skin responses is necessarily subjective. To promote harmonisation in grading of skin response and to assist testing laboratories and those involved in making and interpreting the observations, the personnel performing the observations need to be adequately trained in the scoring system used (see Table below). An illustrated guide for grading skin irritation and other lesions could be helpful (10).

DATA AND REPORTING

24. Study results should be summarised in tabular form in the final test report and should cover all items listed in paragraph 27.

Evaluation of results

25. The dermal irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated. Instead, individual scores should be viewed as reference values, which need to be evaluated in combination with all other observations from the study.

26. Reversibility of dermal lesions should be considered in evaluating irritant responses. When responses such as alopecia (limited area), hyperkeratosis, hyperplasia and scaling, persist to the end of the 14-day observation period, the test substance should be considered an irritant.

Test report

27. The test report must include the following information:

Rationale for *in vivo* testing: weight-of-evidence analysis of pre-existing test data, including results from sequential testing strategy:

- description of relevant data available from prior testing;
- data derived at each stage of testing strategy;
- description of *in vitro* tests performed, including details of procedures, results obtained with test/reference substances;
- weight-of-the-evidence analysis for performing *in vivo* study.

Test substance:

- identification data (e.g. CAS number; source; purity; known impurities; lot number);
- physical nature and physicochemical properties (e.g. pH, volatility, solubility, stability);
- if mixture, composition and relative percentages of components.

Vehicle:

- identification, concentration (where appropriate), volume used;
- justification for choice of vehicle.

Test animals:

- species/strain used, rationale for using animals other than albino rabbit;
- number of animals of each sex;
- individual animal weights at start and conclusion of test;
- age at start of study;
- source of animals, housing conditions, diet, etc.

Test conditions:

- technique of patch site preparation;
- details of patch materials used and patching technique;
- details of test substance preparation, application, and removal.

Results:

- tabulation of irritation / corrosion response scores for each animal at all time points measured;
- descriptions of all lesions observed;
- narrative description of nature and degree of irritation or corrosion observed, and any histopathological findings;
- description of other adverse local (e.g. defatting of skin) and systemic effects in addition to dermal irritation or corrosion.

Discussion of results

LITERATURE

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