OECD/OCDE 404

Adopted:
28 July 2015

OECD GUIDELINE FOR 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 chemical in order to avoid unnecessary testing in laboratory animals. This updated version of Guideline 404 (originally adopted in 1981, revised in 1992, 2002 and 2015) includes reference to the Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Skin Irritation/Corrosion (1), proposing a modular approach for skin irritation and skin corrosion testing. The IATA describes several modules which group information sources and analysis tools, and provides guidance on (i) how to integrate and use existing testing and non-testing data for the assessment of the skin irritation and skin corrosion potentials of chemicals and (ii) proposes an approach when further testing is needed (1). In addition, where needed, 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 test chemical have been evaluated in a weight-of-the-evidence (WoE) analysis as presented in the Guidance Document on Integrated Approaches to Testing and Assessment for Skin Corrosion and Irritation, i.e. over the three Parts of this guidance and their corresponding modules (1). Briefly, under Part 1 existing data is addressed over seven modules covering human data, *in vivo* data, *in vitro* data, physico-chemical properties data (e.g. pH, in particular strong acidity or alkalinity) and non-testing methods. Under Part 2, WoE analysis is performed. If this WoE is still inconclusive, Part 3 should be conducted with additional testing, starting with *in vitro* methods, and *in vivo* testing is used as last resort. This analysis should therefore decrease the need for *in vivo* testing for dermal corrosivity/irritation of test chemicals for which sufficient evidence already exists from other studies as to those two endpoints.

PRINCIPLE OF THE *IN VIVO* TEST

4. The test chemical 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.

5. Animals showing continuing signs of severe distress and/or pain at any stage of the test should be humanely killed, and the test chemical assessed accordingly. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document (2).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of animal species

6. 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

7. 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.

8. 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

9. 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 chemical

10. The test chemical 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 chemical 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 chemical 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 test chemical on the skin. Access by the animal to the patch and ingestion or inhalation of the test chemical should be prevented.

11. Liquid test chemicals are generally used undiluted. When testing solids (which may be pulverised, if considered necessary), the test chemical 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 chemical should be minimal, if any.

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

Dose level

13. 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)

14. When a test chemical has been judged to be corrosive, irritant or non-classified on the basis of a weight of evidence analyses or of previous *in vitro* testing, further *in vivo* testing is normally not necessary. However, in the cases where additional data are felt warranted, the *in vivo* test is performed initially using one animal and applying 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.

15. 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.

16. In those cases in which the test chemical 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)

17. 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

18. 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

19. 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.

20. 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 (3).

DATA AND REPORTING

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

Evaluation of results

22. 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.

23. 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 chemical should be considered an irritant.

Test report

24. 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 chemical:

- Mono-constituent substance: chemical identification, such as IUPAC or CAS name,

CAS number, SMILES or InChI code, structural formula, purity, chemical identity of impurities as appropriate and practically feasible, etc;
- Multi-constituent substance, mixture and substances of unknown or variable composition, complex reaction products or biological materials: characterised as far as possible by chemical identity (see above), quantitative occurrence and relevant physico-chemical properties of the constituents;
- Physical appearance, water solubility, and additional relevant physico-chemical properties;
- Source, lot number if available;
- Treatment of the test chemical/control substance prior to testing, if applicable (*e.g.* warming, grinding);
- Stability of the test chemical, limit date for use, or date for re-analysis if known;
- Storage conditions.

Vehicle:
- Identification, concentration (where appropriate), volume used;
- Justification for choice of vehicle.

Test animal(s):
- Species/strain used, rationale for using animal(s) other than albino rabbit;
- Number of animal(s) of each sex;
- Individual animal weight(s) at start and conclusion of test;
- Age at start of study;
- Source of animal(s), housing conditions, diet, etc.

Test conditions:
- Technique of patch site preparation;
- Details of patch materials used and patching technique;
- Details of test chemical 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

Conclusions

LITERATURE

1) OECD (2014). Guidance document on Integrated Approached to Testing and Assessment for Skin Irritation/Corrosion. Environmental Health and Safety Publications, Series on Testing and Assessment, (No. 203.), Organisation for Economic Cooperation and Development, Paris.

2) OECD (1998) Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, November 1998 (http://www.oecd.org/ehs/Class/HCL6.htm).

3) OECD (2000). Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Environmental Health and Safety Publications, Series on Testing and Assessment (No. 19.), Organistion for Economic Cooperation and Development, Paris.

TABLE: GRADING OF SKIN REACTIONS

Erythema and Eschar Formation

No erythema 0
Very slight erythema (barely perceptible) 1
Well defined erythema 2
Moderate to severe erythema 3
Severe erythema (beef redness) to eschar formation preventing grading of erythema 4

Maximum possible: 4

Oedema Formation

No oedema 0
Very slight oedema (barely perceptible) 1
Slight oedema (edges of area well defined by definite raising) 2
Moderate oedema (raised approximately 1 mm) 3
Severe oedema (raised more than 1 mm and extending beyond area of exposure) 4

Maximum possible: 4

Histopathological examination may be carried out to clarify equivocal responses.

ANNEX

DEFINITIONS

1. Dermal irritation is the production of reversible damage of the skin following the application of a test chemical for up to 4 hours.

2. Dermal corrosion is the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test chemical for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.