

B. 4. ACUTE TOXICITY: DERMAL IRRITATION/CORROSION

1. METHOD

This method is equivalent to the OECD TG 404 (2002).

1.1 INTRODUCTION

In the preparation of this updated method 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 method 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 an Annex to this method. 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 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)(5a). 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.

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 an Annex to this Method. The strategy was developed at, and unanimously recommended by the participants of, an OECD workshop (6), and has been adopted as the recommended testing strategy in the Globally Harmonised System for the Classification of Chemical Substances (GHS) (7). 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. The use of a different testing strategy or procedure, or a decision not to use a stepwise testing approach, should be justified.

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 Annex).

1.2 DEFINITIONS

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

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 substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.

1.3 PRINCIPLE OF THE TEST METHOD

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.

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 can be found in reference (8).

1.4 DESCRIPTION OF THE TEST METHOD

1.4.1 Preparation for the *in vivo* test

1.4.1.1 Selection of animal species

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

1.4.1.2 Preparation of the animals

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.

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.

1.4.1.3 Housing and feeding conditions

Animals should be individually housed. The temperature of the experimental animal room should be 20°C (\pm 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.

1.4.2 Test procedure

1.4.2.1 Application of the test substance

The test substance should be applied to a small area (approximately 6 cm²) 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.

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.

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.

1.4.2.2 *Dose level*

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

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

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 (see Annex 1).

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 tests 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 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.

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.

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.

1.4.2.4 *Confirmatory test (In vivo dermal irritation test with additional animals)*

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.

1.4.2.5 *Observation period*

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.

1.4.2.6 *Clinical observations and grading of skin reactions*

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.

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 (9).

2. **DATA**

2.1 PRESENTATION OF RESULTS

Study results should be summarised in tabular form in the final test report and should cover all items listed in section 3.1.

2.2 EVALUATION OF RESULTS

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.

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.

3. REPORTING

3.1 TEST REPORT

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

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TABLE I: 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.

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ANNEX

A Sequential Testing Strategy for Dermal Irritation and Corrosion

GENERAL CONSIDERATIONS

In the interest of sound science and animal welfare, it is important to avoid the unnecessary use of animals and to minimise any testing that is likely to produce severe responses in animals. All information on a substance relevant to its potential skin corrosivity/irritancy should be evaluated prior to considering *in vivo* testing. Sufficient evidence may already exist to classify a test substance as to its dermal corrosion or irritation potential without the need to conduct testing in laboratory animals. Therefore, utilising a weight-of-the-evidence analysis and a sequential testing strategy, will minimise the need for *in vivo* testing, especially if the substance is likely to produce severe reactions.

It is recommended that a weight-of-the-evidence analysis be used to evaluate existing information regarding the skin irritation and corrosion of substances to determine whether additional studies, other than *in vivo* dermal studies, should be performed to help characterise such potential. Where further studies are needed, it is recommended that the sequential testing strategy be utilised to develop the relevant experimental data. For substances which have no testing history, the sequential testing strategy should be utilised to develop the data set needed to evaluate its dermal corrosion/irritation potential. The testing strategy described in this Annex was developed at an OECD workshop (1) and was later affirmed and expanded in the Harmonised 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, in November 1998 (2).

Although this sequential testing strategy is not an integral part of testing method B.4, it expresses the recommended approach for the determination of skin irritation/corrosion characteristics. This approach represents both best practice and an ethical benchmark for *in vivo* testing for skin irritation/corrosion. The testing method provides guidance for the conduct of the *in vivo* test and summarises the factors that should be addressed before initiating such a test. The strategy provides an approach for the evaluation of existing data on the skin irritation/corrosion properties of test substances and a tiered approach for the generation of relevant data on substances for which additional studies are needed, or for which no studies have been performed. It also recommends the performance of validated and accepted *in vitro* or *ex vivo* tests for skin corrosion/irritation under specific circumstances.

DESCRIPTION OF THE EVALUATION AND TESTING STRATEGY

Prior to undertaking tests as part of the sequential testing strategy (Figure), all available information should be evaluated to determine the need for *in vivo* skin testing. Although significant information might be gained from the evaluation of single parameters (e.g., extreme pH), the totality of existing information should be considered. All relevant data on the effects of the substance in question, or its analogues, should be evaluated in making a weight-of-the-evidence decision, and a rationale for the decision should be presented. Primary emphasis should be placed upon existing human and animal data on the substance, followed by the outcome of *in vitro* or *ex vivo* testing. *In vivo* studies of corrosive substances should be avoided whenever possible. The factors considered in the testing strategy include:

Evaluation of existing human and animal data (Step 1). Existing human data, e.g. clinical or occupational studies and case reports, and/or animal test data, e.g. from single or repeated dermal exposure toxicity studies, should be considered first, because they provide information directly related to effects on the skin. Substances with known irritancy or corrosivity, and those with clear evidence of non-corrosivity or non-irritancy, need not be tested in *in vivo* studies.

Analysis of structure activity relationships (SAR) (Step 2). The results of testing of structurally related substances should be considered, if available. When sufficient human and/or animal data are available on structurally related substances or mixtures of such substances to indicate their skin corrosion/irritancy potential, it can be presumed that the test substance being evaluated will produce the same responses. In those cases, the test substance may not need to be tested. Negative data from studies of structurally related substances or mixtures of such substances do not constitute sufficient evidence of non-corrosivity/non-irritancy of a substance under the sequential testing strategy. Validated and accepted SAR approaches should be used to identify both dermal corrosion and irritation potential.

Physicochemical properties and chemical reactivity (Step 3). Substances exhibiting pH extremes such as ≤ 2.0 and ≥ 11.5 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive to skin, then its acid/alkali reserve (or buffering capacity) may also be taken into consideration (3)(4). If the buffering capacity suggests that a substance may not be corrosive to the skin, then further testing should be undertaken to confirm this, preferably by the use of a validated and accepted *in vitro* or *ex vivo* test (see steps 5 and 6).

Dermal toxicity (Step 4). If a chemical has proven to be very toxic by the dermal route, an *in vivo* dermal irritation/corrosion study may not be practicable because the amount of test substance normally applied could exceed the very toxic dose and, consequently result in the death or severe suffering of the animals. In addition, when dermal toxicity studies utilising albino rabbits have already been performed up to the limit dose level of 2000 mg/kg body weight or higher, and no dermal irritation or corrosion has been seen, additional testing for skin irritation/corrosion may not be needed. A number of considerations should be borne in mind when evaluating acute dermal toxicity in previously performed studies. For example, reported information on dermal lesions may be incomplete. Testing and observations may have been made on a species other than the rabbit, and species may differ widely in sensitivity of their responses. Also the form of test substance applied to animals may not have been suitable for assessment of skin irritation/corrosion (e.g., dilution of substances for testing dermal toxicity (5)). However, in those cases in which well-designed and conducted dermal toxicity studies have been performed in rabbits, negative findings may be considered sufficient evidence that the substance is not corrosive or irritating.

Results from in vitro or ex vivo tests (Steps 5 and 6). Substances that have demonstrated corrosive or severe irritant properties in a validated and accepted *in vitro* or *ex vivo* test (6)(7) designed for the assessment of these specific effects, need not be tested in animals. It can be presumed that such substances will produce similar severe effects *in vivo*.

In vivo test in rabbits (Steps 7 and 8). Should a weight-of-the-evidence decision be made to conduct *in vivo* testing, it should begin with an initial test using one animal. If the results of this test indicate the substance to be corrosive to the skin, further testing should not be performed. 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 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 the two additional animals simultaneously.

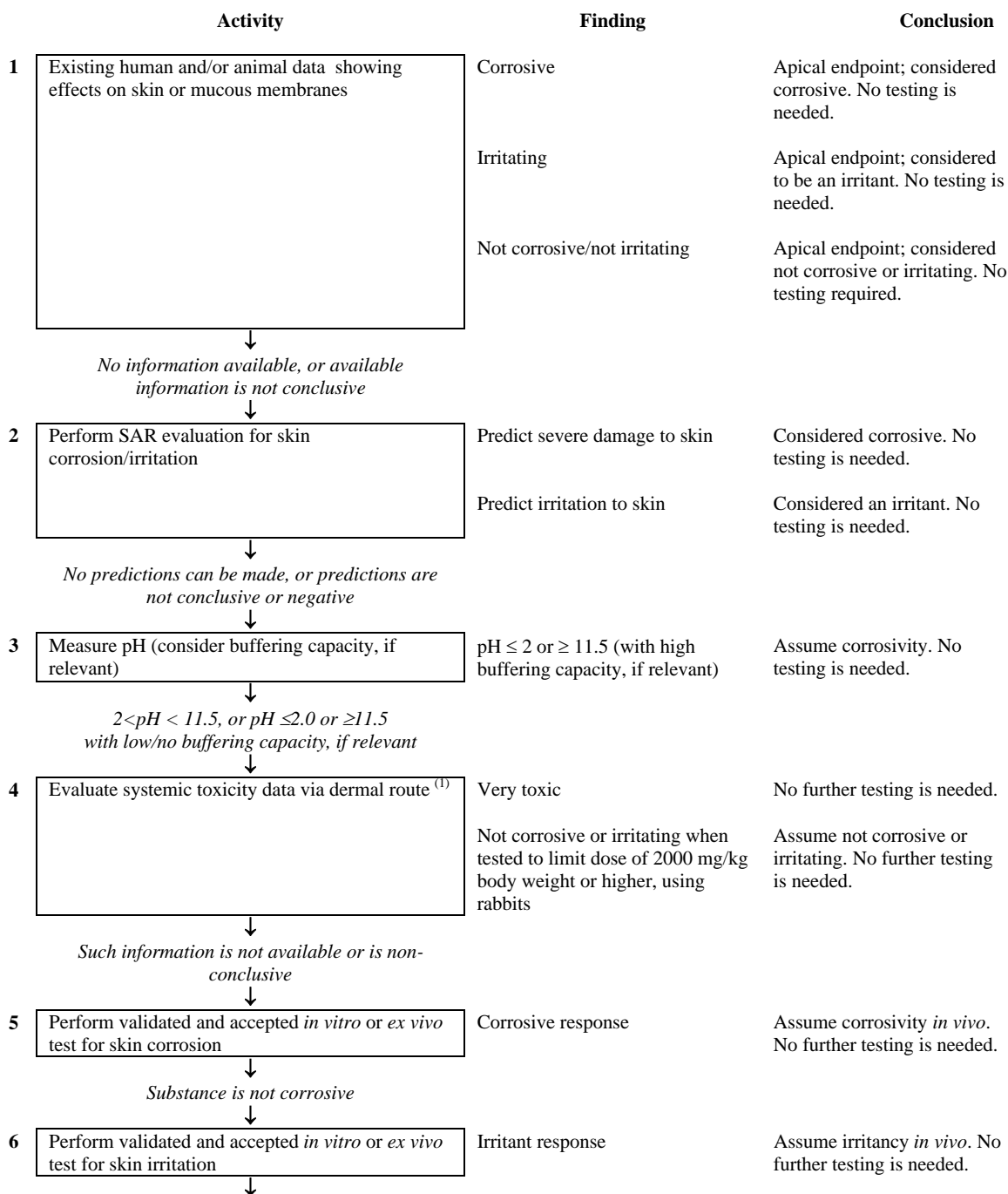
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FIGURE

TESTING AND EVALUATION STRATEGY FOR DERMAL IRRITATION /CORROSION



⁽¹⁾ can be considered before Steps 2 and 3.

