

B. 5. ACUTE TOXICITY: EYE IRRITATION/CORROSION

1. METHOD

This method is equivalent to the OECD TG 405 (2002)

1.1 INTRODUCTION

In the preparation of this updated method special attention was given to possible improvements through the evaluation of all existing information on the test substance in order to avoid unnecessary testing in laboratory animals and thereby address animal welfare concerns. This method includes the recommendation that prior to undertaking the described *in vivo* test for acute eye irritation/corrosion, a weight-of-the-evidence analysis be performed (1) on the existing relevant data. Where insufficient data are available, it is recommended that they be developed through application of sequential testing (2)(3). The testing strategy includes the performance of validated and accepted *in vitro* tests and is provided as an Annex to the testing method. In addition, the use of an *in vivo* dermal irritation/corrosion test to predict eye corrosion prior to consideration of an *in vivo* eye test is recommended.

In the interest of both sound science and animal welfare, *in vivo* testing should not be considered until all available data relevant to the potential eye corrosivity/irritation of the substance has 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 high acidity or alkalinity of the substance (4)(5), and results from validated and accepted *in vitro* or *ex vivo* tests for skin corrosion and irritation (6)(6a). The studies may have been conducted prior to, or as a result of, a weight-of-the-evidence analysis.

For certain substances, such an analysis may indicate the need for *in vivo* studies of the ocular corrosion/irritation potential of the substance. In all such cases, before considering the use of the *in vivo* eye test, preferably a study of the *in vivo* dermal effects of the substance should be conducted first and evaluated in accordance with testing method B.4 (7). The application of a weight-of-the-evidence analysis and the sequential testing strategy should decrease the need for *in vivo* testing for eye corrosivity/irritation of substances for which sufficient evidence already exists from other studies. If a determination of eye corrosion or irritation potential cannot be made using the sequential testing strategy, even after the performance of an *in vivo* study of dermal corrosion and irritation, an *in vivo* eye corrosion/irritation test may be performed.

A preferred sequential testing strategy, which includes the performance of validated *in vitro* or *ex vivo* tests for corrosion/irritation, is included in the Annex to this testing method. The strategy was developed at, and unanimously recommended by the participants of, an OECD workshop (8), and has been adopted as the recommended testing strategy in the Globally Harmonised System for the Classification of Chemical Substances (GHS) (9). 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 skin and eye corrosion/irritation, the strategy should be used to fill missing data gaps. The use of a different testing strategy or procedure, or the decision not to use a stepwise testing approach, should be justified.

1.2 DEFINITIONS

Eye irritation: is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

Eye corrosion: is the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application

1.3 PRINCIPLE OF THE TEST METHOD

The substance to be tested is applied in a single dose to one of the eyes of the experimental animal; the untreated eye serves as the control. The degree of eye irritation/corrosion is evaluated by scoring lesions of conjunctiva, cornea, and iris, at specific intervals. Other effects in the eye and adverse systemic effects are also described 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.

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 (10).

1.4 DESCRIPTION OF THE TEST METHOD

1.4.1 **Preparation for the *in vivo* test**

1.4.1.1 *Selection of species*

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

1.4.1.2 *Preparation of animals*

Both eyes of each experimental animal provisionally selected for testing should be examined within 24 hours before testing starts. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

1.4.1.3 *Housing and feeding conditions*

Animals should be individually housed. The temperature of the experimental animal room should be 20°C ($\pm 3^\circ\text{C}$) 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 placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control.

1.4.2.2 *Irrigation*

The eyes of the test animals should not be washed for at least 24 hours following instillation of the test substance, except for solids (see section 1.4.2.3.2), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate.

Use of a satellite group of animals to investigate the influence of washing is not recommended unless it is scientifically justified. If a satellite group is needed, two rabbits should be used. Conditions of washing should be carefully documented, e.g., time of washing; composition and temperature of wash solution; duration, volume, and velocity of application.

1.4.2.3 *Dose level*

1.4.2.3.1 *Testing of liquids*

For testing liquids, a dose of 0.1 ml is used. Pump sprays should not be used for instilling the substance directly into the eye. The liquid spray should be expelled and collected in a container prior to instilling 0.1 ml into the eye.

1.4.2.3.2 *Testing of solids*

When testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 ml or a weight of not more than 100 mg. The test material should be ground to a fine dust. The volume of solid material should be measured after gently compacting it, e.g., by tapping the measuring container. If the solid test substance has not been removed from the eye of the test animal by physiological mechanisms at the first observation time point of 1 hour after treatment, the eye may be rinsed with saline or distilled water.

1.4.2.3.3 *Testing of aerosols*

It is recommended that all pump sprays and aerosols be collected prior to instillation into the eye. The one exception is for substances in pressurised aerosol containers, which cannot be collected due to vapourisation. In such cases, the eye should be held open, and the test substance administered to the eye in a simple burst of about one second, from a distance of 10 cm directly in front of the eye. This distance may vary depending on the pressure of the spray and its contents. Care should be taken not to damage the eye from the pressure of the spray. In appropriate cases, there may be a need to evaluate the potential for “mechanical” damage to the eye from the force of the spray.

An estimate of the dose from an aerosol can be made by simulating the test as follows: the substance is sprayed on to weighing paper through an opening the size of a rabbit eye placed directly before the paper. The weight increase of the paper is used to approximate the amount sprayed into the eye. For volatile substances, the dose may be estimated by weighing a receiving container before and after removal of the test material.

1.4.2.4 *Initial test (In vivo eye irritation/corrosion test using one animal)*

As articulated in the sequential testing strategy (see Annex 1), it is strongly recommended that the *in vivo* test be performed initially using one animal.

If the results of this test indicate the substance to be corrosive or a severe irritant to the eye using the procedure described, further testing for ocular irritancy should not be performed.

1.4.2.5 *Local anaesthetics*

Local anaesthetics may be used on a case-by-case basis. If the weight-of-the-evidence analysis indicates that the substance has the potential to cause pain, or initial testing shows that a painful reaction will occur, a local anaesthetic may be used prior to instillation of the test substance. The type, concentration, and dose of the local anaesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use. The control eye should be similarly anaesthetised.

1.4.2.6 *Confirmatory test (In vivo eye 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. If a severe irritant effect is observed in the initial test indicating a possible strong (irreversible) effect in the confirmatory testing, it is recommended that the confirmatory test be conducted in a sequential manner in one animal at a time, rather than exposing the two additional animals simultaneously. If the second animal reveals corrosive or severe irritant effects, the test is not continued. Additional animals may be needed to confirm weak or moderate irritant responses.

1.4.2.7 *Observation period*

The duration of the observation period should be sufficient to evaluate fully the magnitude and 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 (9). To determine reversibility of effects, the animals should be observed normally for 21 days post administration of the test substance. If reversibility is seen before 21 days, the experiment should be terminated at that time.

1.4.2.7.1 *Clinical observations and grading of eye reactions*

The eyes should be examined at 1, 24, 48, and 72 hours after test substance application. Animals should be kept on test no longer than necessary once definitive information has been obtained. Animals showing continuing severe pain or distress should be humanely killed without delay, and the substance assessed accordingly. Animals with the following eye lesions post-instillation should be humanely killed: corneal perforation or significant corneal ulceration including staphyloma; blood in the anterior chamber of the eye; grade 4 corneal opacity which persists for 48 hours; absence of a light reflex (iridial response grade 2) which persists for 72 hours; ulceration of the conjunctival membrane; necrosis of the conjunctivae or nictitating membrane; or sloughing. This is because such lesions generally are not reversible.

Animals that do not develop ocular lesions may be terminated not earlier than 3 days post instillation. Animals with mild to moderate lesions should be observed until the lesions clear, or for 21 days, at which time the study is terminated. Observations should be performed at 7, 14, and 21 days in order to determine the status of the lesions, and their reversibility or irreversibility.

The grades of ocular reaction (conjunctivae, cornea and iris) should be recorded at each examination (Table I). Any other lesions in the eye (e.g. pannus, staining) or adverse systemic effects should also be reported.

Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 hours, the eyes may be further examined with the aid of fluorescein.

The grading of ocular responses is necessarily subjective. To promote harmonisation of grading of ocular 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.

2. **DATA**

2.2 EVALUATION OF RESULTS

The ocular 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 and are only meaningful when supported by a full description and evaluation of all observations.

3. REPORTING

3.1 TEST REPORT

The test report must include the following information:

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

- description of relevant data available from prior testing;
- data derived in each step of testing strategy;
- description of *in vitro* tests performed, including details of procedures, results obtained with test/reference substances;
- description of *in vivo* dermal irritation/corrosion study performed, including results obtained;
- 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, reactivity with water);
- in case of a mixture, composition and relative percentages of components;
- if local anaesthetic is used, identification, purity, type, dose, and potential interaction with test substance.

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;
- age of each animal at start of study;
- number of animals of each sex in test and control groups (if required);
- individual animal weights at start and conclusion of test;
- source, housing conditions, diet, etc.

Results:

- description of method used to score irritation at each observation time (e.g., hand slitlamp, biomicroscope, fluorescein);
- tabulation of irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test;
- narrative description of the degree and nature of irritation or corrosion observed;
- description of any other lesions observed in the eye (e.g., vascularization, pannus formation, adhesions, staining);
- description of non-ocular local and systemic adverse effects, and histopathological findings, if any.

Discussion of results.

3.2 INTERPRETATION OF THE RESULTS

Extrapolation of the results of eye irritation studies in laboratory animals to humans is valid only to a limited degree. In many cases the albino rabbit is more sensitive than humans to ocular irritants or corrosives.

Care should be taken in the interpretation of data to exclude irritation resulting from secondary infection.

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- (6a) Testing Method B.40 Skin Corrosion.
- (7) Testing method B.4. Acute toxicity: dermal irritation/corrosion.
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TABLE I: GRADING OF OCULAR LESIONS

Cornea

Opacity: degree of density (readings should be taken from most dense area)*

No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4

Maximum possible: 4

* The area of corneal opacity should be noted

Iris

Normal.....	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect).....	1
Hemorrhage, gross destruction, or no reaction to light	2

Maximum possible: 2

Conjunctivae

Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris) Normal.....	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse beefy red	3

Maximum possible: 3

Chemosis

Swelling (refers to lids and/or nictating membranes)

Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4

Maximum possible: 4

ANNEX

A Sequential Testing Strategy for Eye Irritation and Corrosion

GENERAL CONSIDERATIONS

In the interests of sound science and animal welfare, it is important to avoid the unnecessary use of animals, and to minimise testing that is likely to produce severe responses in animals. All information on a substance relevant to its potential ocular irritation/corrosivity should be evaluated prior to considering *in vivo* testing. Sufficient evidence may already exist to classify a test substance as to its eye irritation or corrosion potential without the need to conduct testing in laboratory animals. Therefore, utilizing a weight-of-the-evidence analysis and 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 pertaining to eye irritation and corrosion of substances and to determine whether additional studies, other than *in vivo* eye 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 needed to evaluate its eye corrosion/irritation. The testing strategy described in this Annex was developed at an OECD workshop (1). It was subsequently 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 testing strategy is not an integrated part of testing method B.5, it expresses the recommended approach for the determination of eye irritation/corrosion properties. This approach represents both best practice and an ethical benchmark for *in vivo* testing for eye irritation/corrosion. The Testing method provides guidance for the conduct of the *in vivo* test and summarises the factors that should be addressed before considering such a test. The sequential testing strategy provides a weight-of-the-evidence approach for the evaluation of existing data on the eye irritation/corrosion properties of 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. The strategy includes the performance first of validated and accepted *in vitro* or *ex vivo* tests and then of testing method B.4 skin irritation/corrosion studies under specific circumstances (3)(4).

DESCRIPTION OF THE STEPWISE 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* eye testing. Although significant information might be gained from the evaluation of single parameters (e.g., extreme pH), the totality of existing information should be assessed. All relevant data on the effects of the substance in question, and its structural 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 and occupational studies, and case reports, and/or animal test data from ocular studies should be considered first, because they provide information directly related to effects on the eyes. Thereafter, available data from human and/or animal studies investigating dermal corrosion/irritation should be evaluated. Substances with known corrosivity or severe irritancy to the eye should not be instilled into the eyes of animals, nor should substances showing corrosive or irritant effects to the skin; such substances should be considered to be corrosive and/or irritating to the eyes as well. Substances with sufficient evidence of non-corrosivity and non-irritancy from previously performed ocular studies should also not be tested in *in vivo* eye studies.

Analysis of structure activity relationships (SAR) (Step 2). The results of testing of structurally related chemicals 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 eye corrosion/irritancy potential, it can be presumed that the test substance will produce the same responses. In those cases, the 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 the corrosion and irritation potential for both dermal and ocular effects.

Physicochemical properties and chemical reactivity (Step 3). Substances exhibiting pH extremes such as ≤ 2.0 or ≥ 11.5 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid/alkaline reserve (buffering capacity) may also be taken into consideration (5)(6). If the buffering capacity suggests that a substance may not be corrosive to the eye, 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 section step 5 and 6).

Consideration of other existing information (Step 4). All available information on systemic toxicity via the dermal route should be evaluated at this stage. The acute dermal toxicity of the test substance should also be considered. If the test substance has been shown to be very toxic by the dermal route, it may not need to be tested in the eye. Although there is not necessarily a relationship between acute dermal toxicity and eye irritation/corrosion, it can be assumed that if an agent is very toxic via the dermal route, it will also exhibit high toxicity when instilled into the eye. Such data may also be considered between Steps 2 and 3.

Results from in vitro or ex vivo tests (Steps 5 and 6). Substances that have demonstrated corrosive or severe irritant properties in an *in vitro* or *ex vivo* test (7)(8) that has been validated and accepted for the assessment specifically of eye or skin corrosivity/irritation, need not be tested in animals. It can be presumed that such substances will produce similar severe effects *in vivo*. If validated and accepted *in vitro/ex vivo* tests are not available, one should bypass Steps 5 and 6 and proceed directly to Step 7.

Assessment of in vivo dermal irritancy or corrosivity of the substance (Step 7). When insufficient evidence exists with which to perform a conclusive weight-of-the-evidence analysis of the potential eye irritation/corrosivity of a substance based upon data from the studies listed above, the *in vivo* skin irritation/corrosion potential should be evaluated first, using testing method B.4 (4) and its accompanying Annex (9). If the substance is shown to produce corrosion or severe skin irritation, it should be considered to be a corrosive eye irritant unless other information supports an alternative conclusion. Thus, an *in vivo* eye test would not need to be performed. If the substance is not corrosive or severely irritating to the skin, an *in vivo* eye test should be performed.

In vivo test in rabbits (Steps 8 and 9): In vivo ocular testing should begin with an initial test using one animal. If the results of this test indicate the substance to be a severe irritant or corrosive to the eyes, further testing should not be performed. If that test does not reveal any corrosive or severe irritant effects, a confirmatory test is conducted with two additional animals.

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FIGURE

TESTING AND EVALUATION STRATEGY FOR EYE IRRITATION/CORROSION



