

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Eye 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 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 updated version of Guideline 405 (adopted in 1981 and first revised in 1987) 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 a Supplement to the Guideline. 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 this Guideline.

2. Definitions of acute eye 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 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)(7). The studies may have been conducted prior to, or as a result of, a weight-of-the-evidence analysis.

4. 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 Guideline 404 (8). 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.

5. A preferred sequential testing strategy, which includes the performance of validated *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 (9), and has been adopted as the recommended testing strategy in the Globally Harmonised System for the Classification of Chemical Substances (GHS) (10). 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.

PRINCIPLE OF THE *IN VIVO* TEST

6. 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.

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 (11).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of species

8. 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.

Preparation of animals

9. 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.

Housing and feeding conditions

10. 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.

TEST PROCEDURE

Application of the test substance

11. 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.

Irrigation

12. 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 paragraph 16), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate.

13. 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.

Dose level

(1) Testing of liquids

14. 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.

(2) Testing of solids

15. 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.

(3) Testing of aerosols

16. It is recommended that all pump sprays and aerosols be collected prior to installation 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.

17. 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.

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

18. As articulated in the sequential testing strategy (Supplement to Guideline), it is strongly recommended that the *in vivo* test be performed initially using one animal.

19. 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.

Local anaesthetics

20. 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.

Confirmatory test (in vivo eye irritation test with additional animals)

21. 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.

Observation period

22. 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.

Clinical observations and grading of eye reactions

23. 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.

24. 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.

25. 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.

26. 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.

27. 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.

DATA AND REPORTING

Evaluation of results

28. 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.

Test report

29. 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.**Interpretation of the results**

30. 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.

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

LITERATURE

- (1) Barratt, M.D., Castell, J.V., Chamberlain, M., Combes, R.D., Dearden, J.C., Fentem, J.H., Gerner, I., Giuliani, A., Gray, T.J.B., Livingston, D.J., Provan, W.M., Rutten, F.A.J.J.L., Verhaar, H.J.M., Zbinden, P. (1995). The Integrated Use of Alternative Approaches for Predicting Toxic Hazard. ECVAM Workshop Report 8. ATLA 23, 410 - 429.
- (2) de Silva, O., Cottin, M., Dami, N., Roguet, R., Catroux, P., Toufic, A., Sicard, C., Dossou, K.G., Gerner, I., Schledè, E., Spielmann, H., Gupta, K.C., Hill, R.N. (1997). Evaluation of Eye Irritation Potential: Statistical Analysis and Tier Testing Strategies. Food Chem. Toxicol 35, 159 - 164.
- (3) Worth A.P. and Fentem J.H. (1999). A general approach for evaluating stepwise testing strategies ATLA 27, 161-177.
- (4) Young, J.R., How, M.J., Walker, A.P., Worth W.M.H. (1988). Classification as Corrosive or Irritant to Skin of Preparations Containing Acidic or Alkaline Substance Without Testing on Animals. Toxicol. *In Vitro*, 2, 19 - 26.
- (5) Neun, D.J. (1993). Effects of Alkalinity on the Eye Irritation Potential of Solutions Prepared at a Single pH. J. Toxicol. Cut. Ocular Toxicol. 12, 227 - 231.

- (6) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Edsail, D.J., Holzhutter, H.G. and Liebsch, M. (1998). The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the Management Team. *Toxicology in Vitro* 12, pp.483 – 524.
- (7) EU (2000). Official Journal of The European Communities L136/91 of 8 June 2000, Method B.40 Skin Corrosion.
- (8) OECD (2000). Test Guideline 404. Acute Dermal Irritation/Corrosion.
- (9) OECD (1996). OECD Test Guidelines Programme: Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods. Held in Solna, Sweden, 22 - 24 January 1996 (<http://www.oecd.org/ehs/test/background.htm>).
- (10) 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>).
- (11) OECD (2000). Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. OECD Environmental Health and Safety Publications. Series on Testing and Assessment No. 19 (<http://www.oecd.org/ehs/test/monos.htm>).