OECD GUIDELINE FOR TESTING OF CHEMICALS

"Acute Eye Irritation/Corrosion"

1. INTRODUCTORY INFORMATION

- Prerequisites
  - Solid or liquid test substance
  - Chemical identification of test substance
  - Purity (impurities) of test substance
  - Solubility characteristics
  - pH (where appropriate)
  - Melting point/boiling point

- Standard documents

There are no relevant international standards.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant and/or corrosive effects on eyes of mammals is an important initial step. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the eyes and associated mucous membranes to the test substance.

- Definitions

Eye irritation is the production of reversible changes in the eye following the application of a test substance to the anterior surface of the eye.

Eye corrosion is the production of irreversible tissue damage in the eye following application of a test substance to the anterior surface of the eye.

- Principle of the test method

The substance to be tested is applied in a single dose to one of the eyes in each of several experimental animals; the untreated eye is used to provide control information. The degree of irritation/corrosion is evaluated and scored at specific intervals and is further described to provide a complete evaluation of

Users of this Test Guideline should consult the Preface, in particular paragraphs 3, 4, 7 and 8.
the effects. The duration of the study should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed.

B. DESCRIPTION OF THE TEST PROCEDURE

° Preparations

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.

Strongly acidic or alkaline substances, for example with a demonstrated pH of 2 or less or 11.5 or greater, need not be tested owing to their probable corrosive properties.

Materials which have demonstrated definite corrosion or severe irritation in a dermal study need not be further tested for eye irritation. It may be presumed such substances will produce similarly severe effects in the eyes.

° Experimental animals

Selection of species

A variety of experimental animals have been used, but it is recommended that testing should be performed using healthy adult albino rabbits.

Number of animals

At least 3 animals should be used. Additional animals may be required to clarify equivocal responses.

Housing and feeding conditions

Animals should be individually housed. The temperature of the experimental animal room should be 22°C (± 3°C) for rodents, 20°C (± 3°C) for rabbits, and the relative humidity 30 to 70 per cent. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. Conventional laboratory diets are suitable for feeding and an unrestricted supply of drinking water should be available.
Test conditions

Dose level

For testing liquids, a dose of 0.1 ml is used. In 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 weight must always be recorded). If the test material is solid or granular it should be ground to a fine dust. The volume of particulates should be measured after gently compacting them, e.g. by tapping the measuring container. To test a substance contained in a pressurised aerosol container the eye should be held open and the test substance administered in a single burst of about one second from a distance of 10 cm directly in front of the eye. The dose may be estimated by weighing the container before and after use. Care should be taken not to damage the eye. Pump sprays should not be used but instead the liquid should be expelled and 0.1 ml collected and instilled into the eye as described for liquids.

Observation period

The duration of the observation period should not be fixed rigidly but should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed. It normally need not exceed 21 days after instillation.

Procedure

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. If it is thought that the substance could cause extreme pain, a local anaesthetic may be used prior to instillation of the test substance. The type and concentration of the local anaesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use. The control eye should be similarly anaesthetised.

The eyes of the test animals should not be washed out for 24 hours following instillation of the test substance. At 24 hours a washout may be used if considered appropriate.
For some substances shown to be irritating by this test, additional tests using rabbits with eyes washed soon after instillation of the substance may be indicated. In these cases it is recommended that 6 rabbits be used. Four seconds after instillation of the test substance, the eyes of 3 rabbits are washed, and 30 seconds after instillation the eyes of the other 3 rabbits are washed. For both groups, the eyes are washed for 5 minutes using a volume and velocity of flow which will not cause injury.

**Clinical observations and scoring**

The eyes should be examined at 1, 24, 48 and 72 hours. If there is no evidence of irritation at 72 hours the study may be ended. Extended observation may be necessary if there is persistent corneal involvement or other ocular irritation in order to determine the progress of the lesions and their reversibility or irreversibility. In addition to the observations of the cornea, iris and conjunctivae, any other lesions which are noted should be recorded and reported. The grades of ocular reaction (Table 1) should be recorded at each examination.

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

The grading of ocular responses is subject to various interpretations. To promote harmonization and to assist testing laboratories and those involved in making and interpreting the observations an illustrated guide in grading eye irritation should be used. (Such an illustrated guide is in use in the United States and can be obtained from the Consumer Product Safety Commission, Washington D.C.)

3. **Data and reporting**

**Treatment of results**

Data may be summarised in tabular form, showing for each individual animal the irritation scores at the designated observation time; a description of the degree and nature of irritation; the presence of serious lesions and any effects other than ocular which were observed.
TABLE 1: GRADES FOR OCULAR LESIONS

CORNEA

Opacity: degree of density (area most dense taken for reading).

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*

Nacular area, no details of iris visible, size of pupil barely discernible .............. 3*

Opaque cornea, iris not discernible through the opacity ................................ 4*

IRIS

Normal ................................................................. 0

Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive) ...................... 1*

No reaction to light, haemorrhage, gross destruction (any or all of these) .......... 2*

CONJUNCTIVAE

Redness (refers to palpebral and bulbar conjunctivae, cornea and iris).

Blood vessels normal .............................................. 0

Some blood vessels definitely hyperaemic (injected) ..................................... 1

Diffuse, crimson colour, individual vessels not easily discernible ................... 2*

Diffuse beefy red .................................................. 3*

Chemosis: lids and/or nictating membranes

No swelling ......................................................... 0

Any swelling above normal (includes nictating membranes) ............................. 1

Obvious swelling with partial eversion of lids .............................................. 2*

Swelling with lids about half closed .................................................. 3*

Swelling with lids more than, half closed .................................. 4*

* Starred figures indicate positive effect
Evaluation of the results

The ocular irritation scores should be evaluated in conjunction with the nature and reversibility or otherwise of the responses observed. The individual scores do not represent an absolute standard for the irritant properties of a material. They should be viewed as reference values and are only meaningful when supported by a full description and evaluation of the observations.

Test report

The test report should include the following information:

- species/strain used;
- physical nature and, where applicable, concentration and pH value for the test substance;
- tabulation of irritant/corrosive response data for each individual animal at each observation time (e.g. 1, 24, 48 and 72 hours);
- description of any serious lesions observed;
- narrative description of the degree and nature of irritation or corrosion observed;
- description of the method used to score the irritation at 1, 24, 48 and 72 hours (e.g. hand slit-lamp, biomicroscope, fluorescein); and
- description of any non-ocular topical effects noted.

Interpretation of the results

Extrapolation of the results of eye irritation studies in animals to man is valid only to a limited degree. The albino rabbit is more sensitive than man to ocular irritants or corrosives in most cases. Similar results in tests on other animal species can give more weight to extrapolation from animal studies to man.

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


