Testing of aerosols

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. Care should be taken not to damage the eye. In appropriate cases, aerosols may be tested in the manner already described for pump sprays.

An estimate of the dose may be made by simulating the test as follows: the substance is sprayed through a window, the size of a rabbit eye, placed directly before a weighing paper. The weight increase of the weighing paper is considered to approximate the amount sprayed into a rabbit eye. For volatile substances the dose may be estimated by weighing the container before and after use.

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

Application

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.

Local anaesthetics

If it is thought that the substance could cause unreasonable 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.

Irrigation

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 3 rabbits be used. Half a minute after instillation, the eyes of the rabbits are washed for half a minute 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 conjunctivae, cornea and iris, 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 fluoroscein.

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.

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
Nacrous 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

* The area of corneal opacity should be noted.

Table 1: GRADES FOR OCULAR LESIONS (continued)

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

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.

- 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

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