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AMERICAN SOCIETY FOR TESTING AND MATERIALS
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Standard Test Method for Evaluation of Eye Irritation in Albino Rabbits[1]

This standard is issued under the fixed designation E 1055; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method determines the duration and the numerical degree of eye irritation in young, adult albino rabbits. The test is an alternative method to the standard Draize eye irritation method. Experimentation indicates that this procedure provides more realistic data, and therefore a better basis for making predictions of eye responses from accidental exposure in man. This test method is applicable to both liquids and solids; it is not applicable to aerosols or vapors.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility the user of this standard to establish appropriate safety and health practices and determine the applicability and regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 609 Definition of Terms Relating to Pesticides[2]

E 943 Terminology Relating to Biological Effects and Environmental Fate[2]

2.2 *Federal Standards:*[3]

Title 40 Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticides Programs; Part 160, Good Laboratory Practice Standards

Title 21 CFR, Food and Drug Administration, Part 58, Laboratory Practice for Nonclinical Studies

Title 40 CFR, Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards

3. Summary of Test Method

3.1 The test material is placed directly on the cornea of one eye in each of six rabbits while the untreated eye serves as a control. The eyelid is released immediately after instillation without forced blinking or manipulation.

3.2 The eyes are scored in accordance with the method of Draize at 1, 2, 3, 7, 14, and 21 days following treatment. Scoring at other time intervals may be added as deemed necessary for certain materials. Scores are discontinued at any time during the 21-day observation period if there is no perceivable evidence of irritation or at the end of the 21-day observation period. The daily total score for each eye is calculated, and the daily arithmetic mean score for all eyes in test group is calculated. When the test is completed, the highest average score is determined.

4. Significance and Use

4.1 This test method evaluates the eye irritation elicited by a test substance when introduced into the eye of a rabbit. For terminology see Terminology Standards E 609 and E 943.

4.2 Results of testing by this test method can be used to estimate the potential for eye irritation or injury in humans when this material is accidentally introduced into the eye.

4.3 The dose volume of 0.01 mL is used because this amount provides a very good discrimination between substances of moderate irritancy plus the ability to detect potentially severe irritants or corrosives.[4,5]

4.4 The dose volume of 0.01 is only one-tenth the volume specified in the Draize rabbit eye irritation test and thereby produces less ocular injury than the latter test.[3] This results in a more humane test with better discrimination between moderate irritants than with a 0.1 mL volume.[6,7]

5. Interpretation of Results

5.1 When the exposure of the eyes to a test material under the specified conditions causes no significant ocular changes at 24 h, the material is considered to produce *no irritation.*

5.2 An eye response is characterized as *moderate irritation* when any of the following responses are present at 24-h and clear within 7 days: opacity of the cornea (other than a slight dulling of the normal luster), hyperemia of the iris, or swelling of the conjunctiva.

5.3 An eye response is characterized as *substantial irritation* when any changes/injuries in the ocular tissues persist for

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[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.26 on Safety to Man.

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[2] *Annual Book of ASTM Standards,* Vol 11.05.

[3] Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.

[4] Committee for the Revision of NAS Publication #1138, "Principles and Procedures for Evaluating the Toxicity of Household Substances," *National Academy of Sciences*, 1977, pp. 41–54.

[5] Griffith, J. F., et al., "Dose-Response Studies with Chemical Irritants in the Albino Rabbit Eye as a Basis for Selecting Optimum Testing Conditions for Predicting Hazard to the Human Eye," *Toxicity and Applied Pharmacology*, Vol 55, 1980, pp. 501–513.

[6] Freeberg, F. E., et al., "Human and Rabbit Eye Responses to Chemical Insult," *Fundamental and Applied Toxicology*, Vol 7, 1986, pp. 626–634.

[7] Freeberg, F. E., et al., "Correlation of Animal Eye Test Data with Human Experience for Household Products: an Update," *Journal of Toxicology-Cutaneous and Ocular Toxicology*, Vol 5, 1986, pp. 115–123.

more than 7 days but clear within 21 days.

5.4 An eye reponse is characterized as *severe irritation* when the exposure of the eye results in injury persisting beyond 21 days.

5.5 An eye response is characterized as *corrosive* when the exposure of the eye results in significant necrosis or other injuries that adversely affect the visual process.

5.6 If a reference compound or product was also included in the test, then the test substance results are compared to those for the reference material, and a judgment is made as to the relative severity of the response.

5.7 These interpretations are for general guidance. Hazard assessment for cautionary labeling, hazard communication or other purposes should be made according to any governmental regulations or guidelines that apply.

6. Reagents and Materials

6.1 *Fluorescein Dye*, sterile ophthalmologic solution containing 0.25 to 1.0 % sodium fluorescein, or fluorescein-impregnated paper strips.

6.2 *Micropipet or microsyringe, with a teflon tip*, for administering test solution to the eye of the rabbit.

6.3 *Sieve*, 40-mesh size, for checking the particle size of powdered or ground, dry samples. The 13-cm diameter sieve is recommended.

6.4 *Mortar and Pestle*, small, for pulverizing granular test materials or dry mixes or blends.

6.5 *Reference Compound* or product of established ocular irritancy, which can be run as a reference material and used to rate the test substance.

7. Sample Preparation

7.1 The measurement of the dose will depend on the physical form of the substance to be tested. Liquids can be delivered from a micropipet or syringe. For pastes and finely divided solids, use a weighed portion of 10 mg. Other solids should be weighed to determine the amount equal to that contained in a specified volume when the material is lightly compacted. Read the volume occupied by the solids to the nearest 0.1 mL. From these data, calculate the apparent density in milligrams per millilitre and then treat as described below.

7.2 Pulverize 2 to 5 g of the granular or dry mixed material using a mortar and pestle.

7.3 Pass the ground material from step 7.2 through a 40-mesh sieve. Regrind any material not passing through the screen and resieve.

7.4 Determine the amount of solid test material to be used for dosing by multiplying the apparent density by 0.01. Weigh this quantity of pulverized substance accurately on a small weighing paper for administration to each animal.

8. Test Animal

8.1 Each test group will consist of six young, adult albino rabbits of either sex, selected on a random basis. More than six rabbits may be used for a test substance if deemed necessary by the investigator.

9. Pretest Conditioning

9.1 Examine the rabbits on arrival for overt signs of disease, and condition them to the environment for a minimum of 7 days. Select animals that have not been used for any other tests.

9.2 Maintain the animals during pretest and test periods in accordance with accepted laboratory practices for the care and handling of animals.

9.3 Identify each animal with an ear tag or other suitable means.

9.4 During acclimation, observe the animals for respiratory distress, diarrhea, emaciation, ocular and nasal discharges, skin lesions, and eye defects. Eliminate any animal demonstrating signs of spontaneous disease prior to the start of the study. Use only animals judged to be healthy. Animal species should be segregated in different rooms both during and after acclimation.

9.5 Rabbits should be housed individually from the time of receipt. Rabbit chow or the equivalent and water are to be available *ad libitum*.

10. Procedure

10.1 One day prior to testing, examine both eyes of each test animal with the aid of fluorescein dye.[2] After 1 to 2 min, flush the eye with sufficient saline solution to remove all excess dye. In a darkened room, examine the eye for fluorescence with the aid of an ophthalmoscope or penlight. Eyes that show staining should not be used for this test. Furthermore, eyes that show corneal edema, iritis, opacity, or other damage on visual evaluation should not be used. Use six rabbits for each test substance; use one eye of each animal for testing and the other eye as an untreated control.

10.2 Have an assistant immobilize the rabbit by holding the animal on a table or other work surface. Hold the eyelid gently open and place 0.01 mL of test material or the equivalent of solid test material directly on the cornea. Release the eyelid immediately. Observe and record whether the animals react abnormally upon instillation of material.

10.2.1 If it is thought that the substance will cause extreme pain, determine whether such testing is essential. If testing is necessary, use a local anaesthetic prior to installation of the test substance. Anesthetize the control eye similarly. Do not test strongly acidic (pH 2.0 or less) or strongly alkaline (pH 11.5 or greater) materials owing to their predicatble corrosive properties. Do not test substances that are corrosive in primary skin irritation tests.

10.2.2 Use a washout 24 h following application of the test substance if considered necessary.

10.3 Examine the eyes for corneal opacity, iritis, conjunctivitis, chemosis, or edema, and discharge; score the treated eyes in accordance with the method of Draize (see Table 1). Score at 1, 2, 3, 7, 14, and 21 days. Additional observations at other time intervals may be made if desired. If the eye(s) has (have) not cleared in 21 days, discontinue scoring and report as “greater than 21 days to clear.” Discontinue scoring at any time during the observation period if there is no gross evidence of any irritation to the ocular tissue.

10.4 The use of fluorescein stain is optional, depending on the degree of certainty required by the investigator, or the presence of other grossly observable effects; instillation of the stain is unnecessary in the presence of more severe effects that can be observed without staining. Lesions or areas of involvement not detected upon visual examination may become

TABLE 1 Draize Scale for Scoring Ocular Lesions[A]

Cornea:		
A. Opacity-degree of density (area most dense taken for reading):		
No opacity		0
Scattered or diffuse area, details of iris clearly visible		1
Easily discernible translucent areas, details of iris slightly obscured		2
Opalescent areas, no details of iris visible, size of pupil barely discernible		3
Opaque, iris invisible		4
B. Area of cornea involved:		
One quarter (or less) but not zero		1
Greater than one quarter but less than half		2
Greater than half but less than three quarters		3
Greater than three quarters, up to whole area		4
opacity × area × 5	Total maximum = 80	
Iris:		
A. Values:		
Normal		0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)		1
No reaction to light, hemorrhage, gross destruction (any or all of these)		2
Value × 5	Total maximum = 10	
Conjunctivae:		
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris):		
Vessels normal		0
Vessels definitely injected above normal		1
More diffuse, deeper crimson red, individual vessels not easily discernible		2
Diffuse beefy red		3
B. Chemosis:		
No swelling		0
Any swelling above normal (includes nictitating membrane)		1
Obvious swelling with partial eversion of lids		2
Swelling with lids about half closed		3
Swelling with lids about half closed to completely closed		4
C. Discharge:		
No discharge		0
Any amount of difference from normal (does not include small amounts observed in inner anthus of normal animals)		1
Discharge with moistening of the lids and hairs just adjacent to lids		2
Discharge with moistening of the lids and hairs and considerable area around the eye		3
Score (Redness + Chemosis + Discharge) × 2	Total maximum = 20	

[A] Draize, J. H., "Appraisal of the Safety of Chemical in Foods, Drugs, and Cosmetics," Association of Food and Drug Officials of the U.S., Editorial Committee, 1959, Second Printing 1965, Topeka, Kans., pp. 46–51.

apparent following fluorescein staining. After visual examination, apply stain as specified in 10.1.

11. Totaling and Averaging of Eye Scores

11.1 The total score for each treated eye at each observation period is the sum of combined and weighed scores obtained for the cornea, iris, and conjunctivae for that eye (see Table 1). The maximum possible score is 110.

11.2 The maximum average score (MAS) is determined by selecting the highest daily mean score for each treatment group of six eyes.

12. Calculation of Median Recovery Time

12.1 To determine the median recovery time for eyes treated with the test substance, arrange the time to recovery for each rabbit in the treatment group in sequential order. Average the third and fourth numbers, rounding the time to the nearest 0.1 day.

12.2 The data, including the median recovery time for the test material should be compared with those results obtained for the reference material when the severity of the eye responses was scored.

13. Report

13.1 The report shall contain such basic information as test species, source of rabbits, and sex.

13.2 Include the name and address of the facility performing the test, the names of the Study Director and the Principal Investigator, and the names of all personnel involved in the study.

13.3 The information reported should include a detailed description of the material tested, dates of the test, the MAS, the number and the time for corneas, irides, and conjunctivae to return to normal (or if not normal, that the clearing time was greater than 21 days). Record all eye abnormalities not covered by the grading scale (Table 1) and any unusual animal responses. Any specific means of observation should also be included.

13.4 The information reported should contain the median number of days required for eyes to clear in each treatment group.

14. Quality Assurance

14.1 Use good laboratory practices to ensure the quality and reliability of data developed using this test method (see Title 21 CFR, part 58, and Title 40, CFR, Parts 160 and 792).

15. Precision and Bias

15.1 No precision data are available for this method at present. However, the committee is interested in conducting an interlaboratory test program and encourages interested parties

to contact the staff manager, Committee E-35, at ASTM Headquarters.

16. Keywords

16.1 chemicals; Draize method; eye; irritation; pesticides; rabbit