

APPENDIX C4
HEALTH EFFECTS TEST GUIDELINES OPPTS 870.2400:
ACUTE EYE IRRITATION

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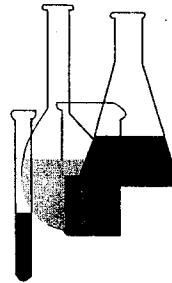
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Health Effects Test Guidelines OPPTS 870.2400 Acute Eye Irritation



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512-0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 870.2400 Acute eye irritation.

(a) **Scope—(1) Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPTS 798.4500 Primary Eye Irritation; OPP 81-4 Acute Eye Irritation—Rabbit (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09-82-025, 1982; and OECD 405 Acute Eye Irritation/Corrosion.

(b) **Purpose.** (1) 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.

(2) Data on primary eye irritation are required by 40 CFR 158.340 to support the registration of each manufacturing-use product and end-use product. (See § 158.50 to determine whether these data must be submitted and which purity/grade of the test substance should be tested.)

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

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.

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.

(d) **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 specified intervals and is fully described to provide a complete evaluation of the effects. The duration of the study should be sufficient to permit a full evaluation of the reversibility or irreversibility of the effects observed. The period of observation should be at least 72 h, but need not exceed 21 days. Animals showing severe and enduring signs of distress and pain may need to be killed in a humane fashion.

(e) **Initial considerations.** (1) 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 predictable corrosive properties. Buffer capacity should also be taken into account.

(2) 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 that such substances will produce similarly severe effects in the eyes.

(3) Results from well validated and accepted *in vitro* test systems may serve to identify corrosives or irritants such that the test material need not be tested *in vivo*.

(f) **Test procedures—(1) Animal selection—(i) Species and strain.** A variety of experimental animals has been used, but it is recommended that testing should be performed using healthy adult albino rabbits. Commonly used laboratory strains should be used. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) **Number of animals.** A single animal should be considered if marked effects are anticipated. If the results of this test in one animal suggest the test substance to be a severe irritant (reversible effect) or corrosive (irreversible effect) to the eye using the procedure described, further tests may not need to be performed. In cases other than a single animal test, at least three animals should be used. Occasionally, further testing in additional animals may be appropriate to clarify equivocal responses.

(2) **Dose level.** For testing liquids, a dose of 0.1 mL is recommended. 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 pressurized aerosol container, the eye should be held open and the test substance administered in a single burst of about 1 sec 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. For volatile substances, the dose may be estimated by weighing the container before and after use.

(3) **Examination of eyes prior to test.** Both eyes of each experimental animal provisionally selected for testing should be examined within 24 h before testing starts by the same procedure to be used during the

test examination. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

(4) Application of the test substance. (i) 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 1 sec in order to limit loss of the material. The other eye, which remains untreated, serves as a control. If it is thought that the substance may cause extreme pain, local anesthetic may be used prior to instillation of the test substance. The type and concentration of the local anesthetic 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 anesthetized.

(ii) The eyes of the test animals should not be washed out for 24 h following instillation of the test substance. At 24 h, a washout may be used if considered appropriate. This is to show whether washing with water palliates or exacerbates irritation.

(iii) For some substances shown to be irritating by this test, additional testing using animals with eyes washed soon after instillation of the substance may be indicated. Half a minute after instillation, the eyes of the animals are washed with water for 30 sec, using a volume and velocity of flow which will not cause injury.

(5) Observation period. The duration of the observation period is at least 72 h, and should not be fixed rigidly, but should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed. The observation period normally need not exceed 21 days after instillation.

(6) Clinical examination and scoring. (i) The eyes should be examined at 1, 24, 48, and 72 h. If there is no evidence of irritation at 72 h, the study may be ended. Extended observation (e.g. at 7 and 21 days) 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 using the following table should be recorded at each examination.

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 luster), details of iris clearly visible	*1
Easily discernible translucent area, details of iris slightly obscured	*2
Nacrous area, no details or 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 hyperemia, or injection, any 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
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae, excluding cornea and iris).	
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	*2
Diffuse beefy red	*3
Chemosis (refers to lids and/or nictitating membranes)	
No swelling	0
Any swelling above normal (includes nictitating 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 grades.

(ii) 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 h, the eyes of any or all rabbits may be further examined with the aid of fluorescein.

(iii) 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.

(g) **Data and reporting—(1) Data summary.** Data should be summarized in tabular form, showing for each individual animal the irritation scores at observation time up until reversal (nonpositive grades) or 21 days when the test is concluded; a description of the degree and nature of irritation; the presence of serious lesions and any effects other than ocular which were observed.

(2) **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.

(3) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Species, strain, sex, age, and source of test animal.

(ii) Rationale for selection of species (if species is other than the species preferred).

(iii) Tabulation of irritant/corrosive response data for each individual animal at each observation time point (e.g. 1, 24, 48, and 72 h until reversibility of lesions or termination of the test).

(iv) Description of any lesions observed.

(v) Narrative description of the degree and nature of irritation or corrosion observed.

(vi) Description of the method used to score the irritation at 1, 24, 48, and 72 h (e.g. hand slit-lamp, biomicroscope, fluorescein stain).

(vii) Description of any nonocular effects noted.

(viii) Description of any pre-test conditioning, including diet, quarantine, and treatment of disease.

(ix) Description of caging conditions including number (and any change in number) of animals per cage, bedding material, ambient temperature and humidity, photoperiod, and identification of diet of test animal.

(x) Manufacturer, source, purity, and lot number of test substance.

(xi) Physical nature, and, where appropriate, concentration and pH value for the test substance.

(xii) Identification, composition, and characteristics of any vehicles (e.g., diluents, suspending agents, emulsifiers, and anesthetics) or other materials used in administering the test substance.

(xiii) A list of references cited in the body of the report, i.e., references to any published literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

(h) **References.** The following references should be consulted for additional background information on this test guideline

(1) Buehler, E.V. and Newmann, E.A. A Comparison of Eye Irritation in Monkeys and Rabbits. *Toxicology and Applied Pharmacology* 6:701-710 (1964).

(2) Draize, J.H. *Dermal Toxicity. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*. The Association of Food and Drug Officials of the United States (1959) 3rd printing 1975, pp. 49–52.

(3) Draize, J.H. et al. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics*. 83:377–390 (1944).

(4) Loomis, T.A. *Essentials of Toxicology*. Lea and Febiger, Philadelphia 3rd ed. 1978 pp. 226–232.

(5) Kay, J.H. and Calandra, J.C., Interpretation of eye irritation tests. *Journal of the Society of Cosmetic Chemists* 13:281–289 (1962).

(6) National Academy of Sciences. *Principles and Procedures for Evaluating the Toxicity of Household Substances*. A report prepared by the Committee for the revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(7) World Health Organization. *Part I. Environmental Health Criteria 6. Principles and Methods for Evaluating the Toxicity of Chemicals*. World Health Organization, Geneva (1978).