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Prevention, Pesticides
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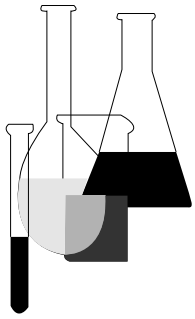
Health Effects Test Guidelines

OPPTS 870.2500
Acute Dermal Irritation



“Public Draft”

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.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 870.2500 Acute dermal 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 40 CFR 798.4470 Primary Dermal Irritation; OPP 81–5 Primary Dermal Irritation (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD 404 Acute Dermal Irritation/ Corrosion .

(b) **Purpose.** Determination of the irritant and/or corrosive effects on skin of mammals is useful in the assessment and evaluation of the toxic characteristics of a substance where exposure by the dermal route is likely. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the skin to the test substance. Data on primary dermal irritation are required by 40 CFR part 158 to support the registration of each manufacturing-use product and end-use product. See specifically §§158.50 and 158.340 to determine whether these data must be submitted.

(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.

Dermal corrosion is the production of irreversible tissue damage in the skin following the application of the test substance.

Dermal irritation is the production of reversible inflammatory changes in the skin following the application of a test substance.

Pharmacological effect means any chemically induced physiological changes in the test animal.

Target organ means any organ of a test animal showing evidence of an effect of chemical treatment.

(d) **Principle of the test methods.** (1) The substance to be tested is applied in a single dose to the skin of several experimental animals, each animal serving as its own control (except when severe irritation/corrosion is suspected and the stepwise procedure is used (see paragraph (f)(1)(iii) of this guideline)). The degree of irritation is read and scored at specified intervals and is further 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 but need not exceed 14 days.

(2) When testing solids (which may be pulverized if considered necessary), the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle, to ensure good contact with the skin. When vehicles are used, the influence of the vehicle on irritation of skin by the test substance should be taken into account. Liquid test substances are generally used undiluted.

(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 for primary dermal irritation, owing to their predictable corrosive properties.

(2) It is unnecessary to test materials which have been shown to be highly toxic (LD50 less than 200 mg/kg) by the dermal route or have been shown not to produce irritation of the skin at the limit test dose level of 2000 mg/kg body weight.

(3) It may not be necessary to test in vivo materials for which corrosive properties are predicted on the basis of results from well validated and accepted in vitro tests. If an in vitro test is performed before the in vivo test, a description or reference to the test, including details of the procedure, must be given together with results obtained with the test and reference substances.

(4) It may not be necessary to test materials for which corrosive potential is predicted from structure-activity relationships.

(f) **Test procedures—**(1) **Animal selection—**(i) **Species and strain.** The albino rabbit is recommended as the preferred species. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) **Number of animals.** At least six healthy adult animals should be used unless justification/reasoning for using fewer animals is provided. It is recommended that a stepwise procedure be used to expose one animal, followed by additional animals to clarify equivocal responses.

(iii) **Stepwise exposure of animals**. A single rabbit may be used if it is suspected that the test material might produce severe corrosion. Three test patches are applied concurrently or sequentially to the animal. The first patch is removed after 3 min. If no serious skin reaction is observed, the second patch is removed after 1 h. If observations indicate that exposure can be continued humanely, the third patch is removed after 4 h and the responses graded. If a corrosive effect is observed after either 3 min or 1 h of exposure, the test is immediately terminated by removal of the remaining patches. If a corrosive effect is observed after an exposure of up to 4 hours, then further animal testing is not required. If no corrosive effect is observed in one animal after a 4–h exposure, the test is completed using two additional animals, each with one patch only, for an exposure

period of 4 h. If it is expected that the test substance will not produce severe irritancy or corrosion, the test may be started using three animals, each receiving one patch for an exposure period of 4 h.

(2) **Control animals.** Separate animals are not recommended for an untreated control group. Adjacent areas of untreated skin of each animal may serve as a control for the test.

(3) **Dose level.** A dose of 0.5 mL of liquid or 500 mg of solid or semisolid is applied to the test site.

(4) **Preparation of test area.** Approximately 24 h before the test, fur should be removed from the test area by clipping or shaving from the dorsal area of the trunk of the animals. Care should be taken to avoid abrading the skin. Only animals with healthy intact skin should be used.

(5) **Application of the test substance.** (i) The recommended exposure duration is normally 4 h. unless corrosion is observed (see paragraph (f)(1)(iii) of this guideline). Longer exposure may be indicated under certain conditions (e.g. expected pattern of human use and exposure). At the end of the exposure period, residual test substance should generally be removed, where practicable, using water or an appropriate solvent, without altering the existing response or the integrity of the epidermis.

(ii) Purity/grade for pesticides. A manufacturing-use product should be tested to support the registration of a manufacturing-use product and an end-use product should be tested to support the registration of an end-use product. The lot of the substance tested should be the same throughout the duration of the study, and the research sample should be stored under conditions that maintain its purity and stability. The composition of each lot of the test substance should be determined, including the name and quantities of known contaminants and impurities, as far as is technically feasible. The determination should include quantities of unknown materials, if any, so that 100 percent of the test sample is accounted for. The test substance should be within the limits, if any certified in accordance with OPPTS 830.1750. When vehicles are used, the influence of the vehicle on irritation of skin by the test substance should by taken into account. If a vehicle is used, it should not alter the absorption, distribution, metabolism, retention or the chemical properties of the test substance nor should it enhance, reduce, or alter its toxic characteristics. At the levels used in the study, the vehicle should not produce physiological effects, and should resemble the vehicle to be used under expected conditions of use. It should not interfere with the nutritional status of the animals, nor produce physiological effects. If the test substance is to be incorporated into a vehicle, the period during which the test substance is stable in such a mixture should be determined prior to the start of the study. Alternatively, determination of the stability of the test or control substance in statistically randomized samples of vehicle mixture should be made periodically during

the study (see 40 CFR Part 160—Good Laboratory Practice Standards (GLPS)). If the test substance is incorporated into a vehicle, its homogeneity and concentration should be determined prior to the start of the study and periodically during the study.

(iii) The test substance should be applied to a small area (approximately 6 cm^2) of skin and covered with a gauze patch, which is held in place with nonirritating tape. In the case of liquids or some pastes, it may be necessary to apply the test substance to the gauze patch and apply that to the skin. The patch should be loosely held in contact with the skin by means of a suitable semiocclusive dressing for the duration of the exposure period. Access by the animal to the patch and resultant ingestion/inhalation of the test substance should be prevented.

(6) **Observation period.** The duration of the observation period need not be rigidly fixed. It should be sufficient to fully evaluate the reversibility or irreversibility of the effects observed. It need not exceed 14 days after application.

(7) **Clinical examination and scoring.** (i) After removal of the patch, animals should be examined for signs of erythema and edema and the responses scored within 30–60 min, and at 24, 48, and 72 h after patch removal.

(ii) Dermal irritation should be scored and recorded according to the grades in the following Table. 1. Further observations may be needed, as necessary, to establish reversibility. In addition to the observation of irritation, any lesions and other toxic effects should be fully described.

Evaluation of Skin Reaction

	Value
Erythema and Eschar Formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum possible	4
Edema Formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure	4
Maximum possible	4

(g) **Data and reporting**—(1) **Data summary.** Data should be summarized in tabular form, showing for each individual animal the irritation scores for erythema and edema at 30 to 60 min, and 24, 48, and 72 h after patch removal, any lesions, a description of the degree and nature

of irritation, corrosion and reversibility, and any other toxic effects observed.

(2) **Evaluation of results.** The dermal 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 which are only meaningful when supported by a full description and evaluation of the observations.

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

(i) Species and strain used.

(ii) Rationale for selection of species (if species is other than the species preferred or required by OPP's toxicology data requirements for pesticide registration).

(iii) Rationale for selections of strain.

(iv) Source of supply of animals.

(v) Description of any pre–test conditioning including diet and quarantine.

(vi) Numbers of animals of each sex in test and control group.

(vii) Age and condition of animals at beginning of study.

(viii) 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 the test animal.

(ix) Description of treatments used to prevent or control infectious diseases if such treatment was taken during a test or shortly before a test was begun.

(x) The report should indicate all ways in which the test procedure fails to meet applicable standards for acceptable testing contained in OPP's toxicology data requirements for pesticide registration and should state the reasons for such deviations (see 40 CFR part 158).

(4) **Format.** (i) The test report should include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results. A test report should contain at least three parts: A summary and evaluation of the test results, a description of the test procedures, and the data and information required by each applicable section of OPP's toxicology data requirements for pesticide registration.

Particular information, data, or analysis may be required more than once in the test report, and it should be referenced each time that it is required.

(ii) **Test chemical.** The test report should include:

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

(B) Chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition (including names and quantities of known contaminants and impurities, so far as technically feasible; the determinations should also include quantities of unknown materials, if any, so that 100 percent of the sample tested is accounted for.

(C) Manufacturer and lot number of the test substance.

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

(iii) **Test procedures.** The test report should include:

(A) Specification of test methods, including a full description of the experimental design and procedures, the length of the study, and the dates on which the study began and ended.

(B) All dose levels administered, method and frequency of administration (including hour of dosing in relation to photoperiod), total volume of material (i.e., test substance plus vehicle), individual dosings, and duration of treatment.

(C) The method of randomization used in selecting samples to assay, the assay method used to determine the stability and homogeneity of the test substance being administered, and results of the assay if the test substance is administered by a vehicle.

(D) Include references to statistical and any other methods employed for analyzing the raw data, and references to any published literature used in developing the test protocol, performing the testing, making and interpreting the observations, and compiling and evaluating the results.

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

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