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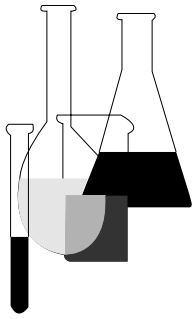
Health Effects Test Guidelines OPPTS 870.1200 Acute Dermal Toxicity



“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 870.1200 Acute dermal toxicity.

(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 material used in developing this harmonized OPPTS test guideline are 40 CFR 798.1100 Acute Dermal Toxicity; OPP 81–2 Acute Dermal Toxicity (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD 402 Acute Dermal Toxicity.

(b) **Purpose.** In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. It provides information on health hazards likely to arise from short-term exposure by the dermal route. Data from an acute study may serve as a basis for classification and labeling. It is an initial step in establishing a dosage regimen in subchronic and other studies and may provide information on dermal absorption and the mode of toxic action of a substance by this route. An evaluation of acute toxicity data should include the relationship, if any, between the exposure of animals to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects.

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

Acute dermal toxicity is the adverse effects occurring within a short time of dermal application of a single dose of a substance or multiple doses given within a 24–h period.

Dosage is a general term comprising the dose, its frequency and the duration of dosing.

Dose is the amount of test substance applied. Dose is expressed as weight of test substance (grams, milligrams) per unit weight of test animal (e.g. milligrams per kilogram).

Dose-effect is the relationship between the dose and the magnitude of a defined biological effect either in an individual or in a population sample.

Dose-response is the relationship between the dose and the proportion of a population sample showing a defined effect.

LD50 (median lethal dose), dermal, is a statistically derived estimate of a single dose of a substance that can be expected to cause death in 50 percent of treated animals when applied to the skin. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (milligrams per kilogram).

(d) **Approaches to the determination of acute toxicity.** (1) EPA recommends the following means to reduce the number of animals used to evaluate acute effects of chemical exposure while preserving its ability to make reasonable judgments about safety:

(i) Using data from substantially similar mixtures. In order to minimize the need for animal testing, the Agency encourages the review of existing acute toxicity information on mixtures that are substantially similar to the mixture under investigation. In certain cases it may be possible to glean enough information to make preliminary hazard evaluations that may reduce the need for further animal testing.

(ii) Limit test. When data on structurally related chemicals are inadequate, a limit test may be considered. If rodents are used, a limit dose of at least 2,000 mg/kg bodyweight may be administered to a single group of five males and five females using the procedures described under paragraph (e) of this guideline. If no lethality is demonstrated, no further testing for acute dermal toxicity is needed (Under current policy for pesticide products, precautionary statements may still be required unless there are data to indicate the LD50 is greater than 5,000 mg/kg.) If compound-related mortality is produced, further study may need to be considered.

(2) [Reserved]

(e) **Conventional acute toxicity test**—(1) **Principle of the test method.** The test substance is applied dermally in graduated doses to several groups of experimental animals, one dose being used per group. The doses chosen may be based on the results of a range finding test. Subsequently, observations of effects and deaths are made. Animals that die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied. This guideline is directed primarily to studies in either rats, rabbits, or guinea pigs but may be adapted for studies in other species. Animals showing severe and enduring signs of distress and pain may need to be humanely killed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out.

(2) **Substance to be tested.** The composition of each lot of the test substance must be determined, including the name and quantities of known contaminants and impurities, as far as is technically feasible. The determinations should include quantities of known materials, if any, so that 100 percent of the test sample is accounted for. Test, control, and reference substances are discussed at 40 CFR 792.

(3) **Test procedures**—(i) **Preparations.** Healthy young adult animals are acclimatized to the laboratory conditions for at least 5 days prior to the test before the test animals are randomized and assigned to the treatment groups.

(ii) **Animal selection**—(A) **Species and strain.** The rat, rabbit, or guinea pig may be used. The albino rabbit is preferred because of its size, ease of handling, skin permeability, and extensive data base. Commonly used laboratory strains should be employed. If a species other than rats, rabbits, or guinea pigs is used, the tester should provide justification and reasoning for its selection.

(B) **Age**. Young adult animals, rats 6– to 10–weeks–old on receipt and between 8 and 12 weeks old at the beginning of dosing; rabbits and guinea pigs at least 12 weeks old at the beginning of dosing should be used. The weight variation of animals used in a test should not be greater or less than 20 percent of the mean weight for each sex.

(C) **Number and sex of animals.** (*1*) At least five experimentally naive animals with healthy intact skin are used at each dose level. They should all be of the same sex. After completion of the study in one sex, at least one group of five animals of the other sex is dosed to establish that animals of this sex are not markedly more sensitive to the test substance. The use of fewer animals may be justified in individual circumstances. Where adequate information is available to demonstrate that animals of the sex tested are markedly more sensitive, testing in animals of the other sex may be dispensed with.

(*2*) The females should be nulliparous and nonpregnant.

(*3*) In acute toxicity tests with animals of a higher order than those mentioned above, the use of smaller numbers should be considered.

(D) **Assignment of animals.** Each animal must be assigned a unique identification number. A system to randomly assign animals to test groups and control groups is required.

(E) **Housing.** Animals should be housed in individual cages.

(*1*) The temperature of the experimental animal rooms should be at 22 ± 3 °C for rodents, 20 ± 3 °C for rabbits.

(*2*) The relative humidity of the experimental animal rooms should be 30 to 70 percent.

(*3*) Where lighting is artificial, the sequence should be 12–h light/ 12–h dark.

(*4*) For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

(iii) **Dose levels and dose selection.** (A) Three dose levels should be used and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data should be sufficient to produce a dose-response curve and permit an acceptable estimation of the median lethal dose. Range finding studies using single animals may help to estimate the positioning of the dose groups so that no more than three dose levels will be necessary.

(B) Limit test. This test is described under paragraph (d)(2)(ii) of this guideline.

(C) Vehicle. Solids should be pulverized when possible, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with skin. If a vehicle or diluent is needed, it should not elicit toxic effects itself nor substantially alter the chemical or toxicological properties of the test substance. In addition, the influence of the vehicle on penetration of skin by the test substance should be taken into account. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of a solution in oil (e.g. corn oil), and then by consideration of possible solution in other vehicles. For nonaqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test.

(iv) **Exposure and exposure duration.** The test substance should be administered over a period of 24 h.

(v) **Preparation of animal skin.** Fur should be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out at least 24 h before dosing. Care must be taken to avoid abrading the skin, which would alter its permeability.

(vi) **Application of test substance.** (A) The test substance should be applied uniformly over a shaved area which is approximately 10 percent of the body surface area. The area starting at the scapulae (shoulders) to the wing of the ileum (hip bone) and half way down the flank on each side of the animal should be shaved. The volume of application should be kept constant, e.g. less than 300 μL for the rat; different concentrations of the test solution should be prepared for different dose levels. With highly toxic substances, the surface area covered may be less, but as much of the area as possible should be covered with as thin and uniform a film as practical. The test material is not removed after application. In the case where less than 10 percent of the surface area is covered an approximation of the exposed areas should be determined.

(B) The test substance should be held in contact with the skin with a porous gauze dressing (<8 ply) and nonirritating tape throughout a 24–h exposure period. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that

the animals cannot ingest the test substance. Restrainers may be used to prevent the ingestion of the test substance, but complete immobilization is not a recommended method. Although a semiocclusive dressing is preferred, an occlusive dressing will also be acceptable.

(C) At the end of the exposure period, residual test substance should be removed where practicable using water or an appropriate solvent.

(vii) **Observation period.** Although 14 days is recommended as a minimum observation period, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset, and length of recovery period, and may thus be extended when considered necessary. The time at which signs of toxicity appear, their duration, and the time to death are important, especially if there is a tendency for deaths to be delayed.

(viii) **Observation of animals.** (A) A careful clinical examination should be made at least once each day.

(B) Additional observations should be made daily, especially in the early days of the study. Appropriate actions should be taken to minimize loss of animals to the study (e.g. necropsy or refrigeration of those animals found dead and isolation of weak or moribund animals).

(C) Observations should be detailed and carefully recorded, preferably using explicitly defined scales. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength, and stereotypies or bizarre behavior (e.g. self-mutilation, walking backwards).

(D) Individual weights of animals should be determined shortly before the test substance is administered, weekly thereafter, and at death. Changes in weights should be calculated and recorded when survival exceeds one day.

(E) The time of death should be recorded as precisely as possible.

(ix) **Gross pathology.** (A) At the end of the test, surviving animals should be weighed and sacrificed.

(B) A gross necropsy should be performed on all animals under test. All gross pathology changes should be recorded.

(C) If necropsy cannot be performed immediately after a dead animal is discovered, the animal should be refrigerated (not frozen) at temperatures low enough to minimize autolysis. Necropsies shall be performed no later than 16 hours after death.

(x) **Additional evaluations.** Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 h or more should also be considered because it may yield useful information.

(xi) **Data and reporting**—(A) **Treatment of results.** Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, body weights, time of death of individual animals at different dose levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings. Any methods used for calculation of the LD50 or any other parameters should be specified and referenced. Methods for parameter estimation are described under paragraphs (f)(2), (f)(3), and (f)(4) of this guideline.

(B) **Evaluation of results.** An evaluation should include the relationship, if any, between exposure of the animals to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects. The LD50 value should always be considered in conjunction with the observed toxic effects and any necropsy findings. The LD50 value is a relatively coarse measurement, useful only as a reference value for classification and labelling purposes, and for an expression of the lethal potential of the test substance by the ingestion route. Reference should always be made to the experimental animal species in which the LD50 value was obtained.

(C) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J and 40 CFR part 160, subpart J, the following specific information should be reported. The test report should include:

(*1*) Species, strain, sex,and source of test animals.

(*2*) Method of randomization in assigning animals to test and control groups.

(*3*) Rationale for selection of species, if other than that recommended.

(*4*) Tabulation of individual and test group data by sex and dose level (e.g. number of animals exposed, number of animals showing signs of toxicity and number of animals that died or were killed during the test).

(*i*) Description of toxic effects, including their time of onset, duration, reversibility, and relationship to dose.

(*ii*) Body weights.

(*iii*) Time of dosing and time of death after dosing.

(*iv*) Dose-response curves for mortality and other toxic effects (when permitted by the method of determination).

(*v*) Gross pathology findings.

(*vi*) Histopathology findings and any additional clinical chemistry evaluations, if performed.

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

(*6*) Description of caging conditions including: Number (or change in number) of animals per cage, bedding material, ambient temperature and humidity, photoperiod, and identification of diet of test animals.

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

(*8*) Relevant properties of substance tested including physical state and pH (if applicable).

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

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

(1) American Society for Testing and Materials. *Standard Test Method for Estimating Acute Oral Toxicity in Rats*, a standard issued under the fixed designation E 1163–87, under the jurisdiction of ASTM Committee E–35 on Pesticides and is the direct responsibility of Subcommittee E3526 on Safety to Man, Philadelphia, PA (1987).

(2) Chanter, D.O. and Heywood, R., The LD50 Test: Some Considerations of Precision, *Toxicology Letters* 10:303–307 (1982).

(3) Finney, D.J. Chapter 3—Estimation of the median effective dose and Chapter 4-Maximum likelihood estimation, *Probit Analysis*, 3rd ed. Cambridge, London (1971).

(4) Finney, D.J. The Median Lethal Dose and Its Estimation. *Archives of Toxicology* 56:215–218 (1985).

(5) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 420: Acute Oral Toxicity—Fixed Dose Method. Endorsed by the Joint Meeting of the Chemicals Group and Management Committee, 20th–22nd November, 1991, ENV/EPOC(92)15.

(6) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 402: Acute Dermal Toxicity. Adopted: February 24, 1987.