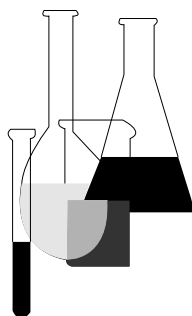




Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity



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 870.1100 Acute oral 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 materials used in developing this harmonized OPPTS test guideline are 40 CFR 798.1175 Acute Oral Toxicity; OPP 81–1 (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD 401 Acute Oral Toxicity.

(b) **Purpose.** In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from short-term exposure by the oral route. Data from an acute study may serve as a basis for classification and labeling. It is traditionally a step in establishing a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a substance. 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 oral toxicity is the adverse effects occurring within a short period of time after oral administration of either a single dose of a substance or multiple doses given within a 24-hour period.

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

Dose is the amount of test substance administered. Dose is expressed as weight of test substance (milligrams, grams) 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) is a statistically derived estimate of single dose of a substance that can be expected to cause death in 50 percent

of animals when administered by the oral route. 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) Use of appropriate alternative test protocols when available. Thus, for example, acute oral toxicity testing may be performed using the Fixed Dose Method (OECD Guideline 420, see paragraph (f)(1) of this guideline), or the Acute Toxic Class Method (OECD Guideline 423, see paragraph (f)(2) of this guideline), or the Up-and-Down Method (OECD Guideline 425, see paragraph (f)(3) of this guideline). Abbreviated methods are not yet available through OECD for acute toxicity by other routes of exposure. However, OECD is actively revising its approaches to testing for dermal and ocular irritation and when this is done, the OPPTS guidelines will be updated to harmonize with the OECD revisions.

(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 per kilogram of body weight 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 oral toxicity is needed. (Under current policy and regulations 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 in the limit test, further study may need to be considered.

(iii) Estimation of acute oral toxicity. When further study is warranted, EPA generally supports limiting such tests to those using the lowest number of animals feasible. Given the approval internationally through OECD of three alternative test methods to the “traditional” acute oral toxicity test, it is time to reassess the status of acute toxicity testing. The three OECD alternatives include the following. The fixed dose procedure is a refinement of the traditional acute oral test that employs nonlethal endpoints. In contrast, the acute toxic class and up-and-down procedures estimate lethality within a dose range and as a point estimate, respectively, and reduce animal usage in comparison to the “traditional” test.

(A) The up and down procedure as described in OECD Guideline 425 referenced in paragraph (f)(4) of this guideline. This method is highly recommended.

(B) The acute toxic class method as described in OECD Guideline 423 and referenced in paragraph (f)(6) of this guideline.

(C) A three-dose method described as the conventional acute toxicity test under paragraph (e) in this guideline, and in OECD Guideline 401 referenced in paragraph (f)(7) of this guideline.

(D) The fixed dose method as described in OECD Guideline 420 and referenced in paragraph (f)(5) of this guideline.

(e) **Conventional acute toxicity test**—(1) **Principle of the test method.** The test substance is administered orally by gavage 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 rodent species but may be adapted for studies in nonrodents. 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.** Test, control and reference substances are discussed in 40 CFR Part 792—Good Laboratory Practice Standards.

(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.** Although several mammalian test species may be used, the rat is the preferred species. Commonly used laboratory strains should be employed. If another species is used, the tester should provide justification and reasoning for its selection.

(B) **Age.** Young adult rats between 8- and 12-weeks-old at the beginning of dosing should be used. Rabbits should be at least 12 weeks of age at study initiation. The weight variation of animals used in a test should be within 20 percent of the mean weight for each sex.

(C) **Number and sex of animals.** (1) At least five experimentally naive rodents 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. An acceptable option would be to test at least one group of five animals per sex at one or more dose levels to definitively determine the more sensitive sex prior to conducting the main study.

(2) The females should be nulliparous and nonpregnant.

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

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

(E) **Housing.** Animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g. morbidity, excitability) may indicate a need for individual caging.

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

(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–hours light/12–hours 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, 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 LD₅₀. Range finding studies using single animals may help to estimate the positioning of dose groups so that no more than three dose levels will be necessary. An acceptable option for pesticide products would be to set the dose levels in correlation with the OPP toxicity categories (bracketing). In these cases, the determination of an LD₅₀ may not be necessary.

(B) Limit test. This test has been defined and described under paragraph (d)(2)(ii) of this guideline.

(C) Vehicle. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. 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. 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. Toxic characteristics of nonaqueous vehicles should be known, and, if not known, should be determined before the test.

(D) **Volume.** The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not exceed 1 mL/100 g body weight, except when an aqueous solution is used in which case 2 mL/100 g may be administered. Either constant volume or constant concentration administration is acceptable when dosing, provided the following guidance is employed. When possible, the liquid test material should be dosed neat. Otherwise, it may be diluted, using the highest concentration possible, although volumes less than 0.5 mL per animal would not be required. Lower dose volumes are acceptable if they can be accurately administered. Solid materials should be suspended or dissolved in the minimum amount of vehicle and dosed at the highest concentration possible.

(iv) **Exposure and exposure duration.** (A) Animals should be fasted prior to test substance administration. For the rat, feed should be withheld overnight; for other rodents with higher metabolic rates a shorter period of fasting is appropriate.

(B) The test substance should be administered in a single dose by gavage, using a stomach tube or suitable intubation cannula.

(C) If a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. Where a dose is administered in fractions, it may be necessary to provide the animals with food and water, depending on the length of the dosing period.

(D) After the substance has been administered, feed may be withheld for an additional 3–4 hours.

(v) **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.

(vi) **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 activity, gait and posture, reactivity to handling or sensory stimuli, altered 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 1 day.

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

(vii) **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 should be performed as soon as practicable, normally within a day or two.

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

(ix) **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)(1), (f)(2), and (f)(3) 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 labeling 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 pretest 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) Identification and composition of any vehicles (e.g., diluents, suspending agents, and emulsifiers) or other materials used in administering the test substance.
 - (10) 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 material on this test guideline.

- (1) Chanter, D.O. and Heywood, R. The LD₅₀ Test: Some Considerations of Precision. *Toxicology Letters* 10:303–307 (1982).
- (2) Finney, D.J. Chapter 3—Estimation of the median effective dose and Chapter 4—Maximum likelihood estimation, *Probit Analysis*, 3rd ed. Cambridge, London (1971).
- (3) Finney, D.J. The Median Lethal Dose and Its Estimation. *Archives of Toxicology* 56:215–218 (1985).
- (4) Organization for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals. OECD Guideline 425: Acute Oral Toxicity: Up-and-Down Procedure, Approved: June 1998.
- (5) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 420: Acute Oral Toxicity—Fixed Dose Method, Adopted: July 17, 1992.
- (6) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 423: Acute Oral Toxicity - Acute Toxic Class Method, Adopted: March 22, 1996.
- (7) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 401: Acute Oral Toxicity, Adopted: February 24, 1987.