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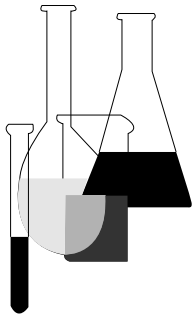
Ecological Effects Test Guidelines

OPPTS 850.1035 Mysid Acute Toxicity Test



“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 850.1035 Mysid acute toxicity test.

(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 797.1930 Mysid Shrimp Acute Toxicity Test and OPP 72–3 Acute Toxicity Test for Estuarine and Marine Organisms (Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation; Wildlife and Aquatic Organisms) EPA report 540/09-82-024, 1982.

(b) **Purpose.** This guideline prescribes a test using mysids as test organisms to develop data on the acute toxicity of chemicals. The Environmental Protection Agency will use data from these tests in assessing the hazard of a chemical to the aquatic environment.

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

Concentration-response curve is the curve produced from toxicity tests when percent response (e.g. mortality) values are plotted against concentration of test substance for a given length of exposure.

Death means the lack of reaction of a test organism to gentle prodding.

Flow-through means a continuous or an intermittent passage of test solution or dilution water through a test chamber or a holding or acclimation tank, with no recycling.

LC50 means the experimentally derived concentration of test substance that is calculated to kill 50 percent of a test population during continuous exposure over a specified period of time.

Loading means the ratio of test organisms biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber.

No observed effect concentration (NOEC) is the highest tested concentration in an acceptable toxicity test which did not cause the occurrence of any specified adverse effect (statistically different from the control at 95 percent level), and below which no tested concentration caused such an occurrence.

Retention chamber means a structure within a flow-through test chamber which confines the test organisms, facilitating observation of test organisms, and eliminating loss of organisms in outflow water.

Static system means a test chamber in which the test solution is not renewed during the period of the test.

(d) **Test procedures**—(1) **Summary of the test**. In preparation for the test, test chambers are filled with appropriate volumes of dilution water. If a flow-through test is performed, the flow of dilution water through each chamber is adjusted to the rate desired. The test substance is introduced into each test chamber. ln a flow-through test, the rate at which the test substance is added is adjusted to establish and maintain the desired concentration of test substance in each test chamber. The test is started by randomly introducing mysids acclimated in accordance with the test design into the test chambers. Mysids in the test chambers are observed periodically during the test, dead mysids are removed, and the findings recorded. Dissolved oxygen concentration, pH, temperature, salinity, the concentration of test substance, and other water quality characteristics are measured at specified intervals in test chambers. Data collected during the test are used to develop concentration-response curves and LC50 values for the test substance.

(2) **Range-finding test.** (i) A range-finding test should be conducted to determine:

(A) Which life stage (juvenile or young adult) is to be utilized in the definitive test.

(B) The test solution concentrations for the definitive test.

(ii) The mysids should be exposed to a series of widely spaced concentrations of test substance (e.g. l, l0, l00 mg/L, etc.), usually under static conditions.

(iii) This test should be conducted with both newly hatched juvenile (<24 h old) and young adult (5 to 6 days old) mysids. For each age class (juvenile or young adult), a minimum of l0 mysids should be exposed to each concentration of test substance for up to 96 h. The exposure period may be shortened if data suitable for the purpose of the range-finding test can be obtained in less time. The age class which is most sensitive to the test substance in the range-finding test should be utilized in the definitive test. When no apparent difference in sensitivity of the two life stages is found, juveniles should be utilized in the definitive test. No replicates are required, and nominal concentrations of the test chemical are acceptable.

(3) **Definitive test.** (i) The purpose of the definitive test is to determine the concentration-response curves and the 48– and 96–h LC50 values with the minimum amount of testing beyond the range-finding test.

(ii) The definitive test should be conducted on the mysid life stage (juveniles or young adults) which is most sensitive to the test substance being evaluated.

(iii) A minimum of 20 mysids per concentration should be exposed to five or more concentrations of the test chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, and 64 mg/L). An equal number of mysids are introduced into the test and control chambers by stratified random assignment and should be placed in two or more replicates. If solvents, solubilizing agents, or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test substance. Preferred carriers are dimethyl formamide, triethylene glycol, acetone, or ethanol. Use of carriers should be avoided, if possible, as they may serve as a carbon source for bacteria. The concentration of solvent should not exceed 0.1 mL/L. The concentration ranges should be selected to determine the concentration-response curves and LC50 values at 48 and 96 h.

(iv) Every test should include controls consisting of the same dilution water, conditions, and procedures, and mysids from the same population or culture container, except that none of the test chemical is added.

(v) The dissolved oxygen concentration, temperature, salinity, and pH should be measured at the beginning and end of the test in each chamber.

(vi) The test duration is 96 h. The test is unacceptable if more than 10 percent of the control organisms die or exhibit abnormal behavior during the 96–h test period. Each test chamber should be checked for dead mysids at 24, 48, 72, and 96 h after the beginning of the test. Concentration-response curves and 24–, 48–, 72– and 96–h LC50 values should be determined along with their 95 percent confidence limits.

(vii) In addition to death, any abnormal behavior or appearance should also be reported.

(viii) Test organisms should be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. ln addition, test chambers within the testing area should be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

(ix) The concentration of the test substance in the chambers should be measured as often as is feasible during the test. During static tests, the concentration of test substance should be measured at a minimum at the beginning and at the end of the tests. During the flow-through test, the concentration of test substance should be measured at the beginning and end of the test and in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

Equal aliquots of test solution may be removed from each replicate chamber and pooled for analysis. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

(4) **Analytical measurements**—(i) **Test chemical.** Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample should be validated by appropriate laboratory practices before beginning the test. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and mathematically corrected.

(ii) **Numerical**. The number of dead mysids should be counted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration-response curves. A 48– and 96–h LC50 and corresponding 95 percent interval should be calculated. An NOEC and the slope of the dose-response curve should also be determined.

(e) **Test conditions**—(1) **Test species**—(i) **Selection.** (A) The mysid, *Mysidopsis bahia*, is the organism specified for these tests. Either juvenile (< 24 h old) or young adult (5 to 6 days old) mysids are to be used to start the test. It has recently been proposed, under paragraph (g)(2) of this guideline, to place this species in a new genus, *Americamysis*.

(B) Mysids to be used in acute toxicity tests should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. Because of similarities with other mysid species, taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.

(C) Mysids used in a particular test should be of similar age and be of normal size and appearance for their age. Mysids should not be used for a test if they exhibit abnormal behavior or if they have been used in a previous test, either in a treatment or in a control group.

(ii) **Acclimation.** (A) Any change in the temperature and chemistry of the dilution water used for holding or culturing the test organisms to those of the test should be gradual. Within a 24–h period, changes in water temperature should not exceed 1 °C, while salinity changes should not exceed 5 percent.

(B) During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.

(iii) **Care and handling.** Methods for the care and handling of mysids such as those described under paragraph (g)(l) of this guideline can be used during holding, culturing, and testing periods.

(iv) **Feeding.** Mysids should be fed daily during testing. Any food utilized should support survival, growth, and reproduction of the mysids. A recommended food is live *Artemia* spp. (48–h–old nauplii).

(2) **Facilities**—(i) **Apparatus.** (A) Facilities which may be needed to perform this test include:

(*1*) Flow-through or recirculating tanks for holding and acclimating mysids.

(*2*) A mechanism for controlling and maintaining the water temperature during the holding, acclimation, and test periods.

(*3*) Apparatus for straining particulate matter, removing gas bubbles, or aerating the water, as necessary.

(*4*) An apparatus for providing a 14–h light and 10–h dark photoperiod with a 15 to 30 min transition period. In addition, for flow-through tests, flow-through chambers and a test substance delivery system are required. Furthermore, it is recommended that mysids be held in retention chambers within test chambers to facilitate observations and eliminate loss of test organisms through outflow water. For static tests, suitable chambers for exposing test mysids to the test substance are required. Facilities should be well ventilated and free of fumes and disturbances that may affect the test organisms.

(B) Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(ii) **Cleaning.** Test substance delivery systems and test chambers should be cleaned before each test following standard laboratory practices.

(iii) **Construction materials.** (A) Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.

(B) For use in the flow-through test, retention chambers utilized for confinement of test organisms can be constructed with netting material of appropriate mesh size.

(iv) **Dilution water.** (A) Natural or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating, and testing periods without

showing signs of stress, such as reduced growth and fecundity. Mysids should be cultured and tested in dilution water from the same origin.

(B) Natural seawater should be filtered through a filter with a pore size of < 20 μm prior to use in a test.

(C) Artificial seawater can be prepared by adding commercially available formulations or specific amounts of reagent-grade chemicals to deionized water. Deionized water with a conductivity less than 1 μohm/cm at 12 °C is acceptable for making artificial seawater. When deionized water is prepared from a ground or surface water source, conductivity and total organic carbon (or chemical oxygen demand) should be measured on each batch.

(v) **Test substance delivery system.** In flow-through tests, proportional diluters, metering pumps, or other suitable systems should be used to deliver test substance to the test chambers. The system to be used should be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24–h flow through a test chamber should be equal to at least 5× the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent among test chambers or across time.

(3) **Test parameters.** Environmental parameters of the water contained in test chambers should be maintained as specified below:

(i) The test temperature should be 25 °C. Excursions from the test temperature should be not greater than ± 2 °C.

(ii) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.

(iii) The number of mysids placed in a test solution should not be so great as to affect results of the test. Loading should not exceed 30 mysids per liter for a static test. Loading requirements for the flow-through test will vary depending on the flow rate of dilution water. The loading should not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 14 h light and 10 h darkness, with a 15 to 30 min transition period.

(v) Salinity of 20 ± 3 ppt.

(f) **Reporting.** The sponsor should submit to the EPA all data developed during the test that are suggestive or predictive of acute toxicity and

all concomitant toxicologic manifestations. ln addition to the reporting requirements as specified under Good Laboratory Practice Standards, 40 CFR part 792, subpart J, the following specific information should be reported:

(1) The nature of the test, laboratory, name of the investigator, test substance, and dates of test should be supplied.

(2) A detailed description of the test substances should be provided. This information should include the source, lot number, composition, physical and chemical properties, shelf life and storage conditions, and any carrier or additives used.

(3) Detailed information about the shrimp should be provided: Common and scientific names, source of supply, age, history, weight, acclimation procedure, and feeding history should be reported.

(4) A description of the experimental design including the number of test solution concentrations, number of replicates, and number of shrimp per replicate should be provided.

(5) The source of the dilution water, its chemical characteristics (e.g. salinity), and a description of any pretreatment.

(6) A description of the test chambers, the depth and volume of solution in the chamber, the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system and flow rate expressed as volume additions per 24 h.

(7) The concentration of the test substance in each test chamber before the start of the test and at the end.

(8) The number of dead shrimp and measurements of water temperature, salinity, and dissolved oxygen concentration in each test chamber should be recorded at the protocol-designated times.

(9) Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(10) Recorded data for the holding and acclimation period (temperature, salinity, etc.).

(11) Concentration-response curves should be fitted to mortality data collected at 24, 48, 72, and 96 h. A statistical test of goodness-of-fit should be performed.

(12) For each set of mortality data, the 48– and 96–h LC50 and 95 percent confidence limits should be calculated on the basis of the average measured concentration of the test substance. When data permits, LC50 values with 95 percent confidence limits should be computed for 24– and

72–h observations. The NOEC and slope of the dose-response curves should also be calculated.

(13) The methods used in calculating the concentration-response curves and the LC50 values should be fully described.

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

(1) Environmental Protection Agency, *Bioassay Procedures for the Ocean Disposal Permit Program*, EPA Report No. 600–9–78–010 (Gulf Breeze, Florida, 1978).

(2) Price, E.W. et al. Observations on the genus *Mysidopsis* Sars, 1864 with the designation of a new genus, *Americamysis*, and the descriptions of *Americamysis alleni* and *A. stucki* (Pericarda: Mysidacea: Mysidae), from the Gulf of Mexico. *Proceedings of the Biological Society of Washington* 107:680-698 (1994).