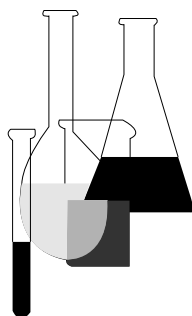




Ecological Effects Test Guidelines

OPPTS 850.1045

Penaeid 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.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202-512-0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines.”

OPPTS 850.1045 Penaeid 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 797.1970 Penaid 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 tests using penaeid shrimp 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 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this guideline:

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

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

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

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

Loading is the ratio of test organism 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 the 95 percent level), and below which no tested concentration caused such an occurrence.

ppt is parts per thousand (salinity units).

(d) **Test procedures**—(1) **Summary of the test.** Prior to testing, the bottoms of the test chambers are covered with 2 to 3 cm of sand and

then filled with appropriate volumes of dilution water. The flow is adjusted to the rate desired to achieve loading requirements. Penaeids are introduced into the test chambers according to the experimental design. The shrimp are acclimated by maintaining them in the test chambers for a period of 3 to 7 days prior to the beginning of the test. The test begins when the test substance is introduced into the test chambers. The rate of flow is adjusted to maintain the desired test substance concentration in each chamber. The shrimp are observed during the test; dead shrimp are counted, removed, and the findings recorded. Dissolved oxygen concentration (DOC), pH, temperature, salinity, test substance concentration, and other water quality characteristics are measured at specified intervals in selected test chambers. The concentration of test substances with low water solubility may have to be determined with more frequency. 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 the test substance concentrations to be used for the definitive test. Substances which have low solubility and/or unusual adsorbance characteristics may require special handling procedures (physical procedures or the use of carrier substances) and attention to the type of materials used in the testing chambers to enhance solubility or decrease adsorption.

(ii) The shrimp should be exposed to a series of widely spaced concentrations of test substance (e.g. 1, 10, 100 mg/L, etc.).

(iii) A minimum of five penaeid shrimp should be exposed to each concentration of test substance for up to 96 h. No replicates are required and nominal concentrations of the 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) A minimum of 20 shrimp per concentration should be exposed to five or more concentrations of the 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 shrimp 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 requested concentration-response curves and LC50 values. The concentration of test substance in test solutions should be determined

prior to use and at designated times. Abnormal or unexpected observations should trigger chemical analysis of the test water. If a specific test chamber seems to be affected, its water should be analyzed.

(iii) Every test should include controls consisting of the same dilution water, conditions, procedures, and shrimp from the same population or culture container, except that none of the chemical is added. If carriers are used, a separate carrier control should also be included.

(iv) The DOC, temperature, salinity, and pH should be measured at the beginning of the test and at 24, 48, 72, and 96 h in each test chamber.

(v) The test duration is 96 h. The test is unacceptable if more than 10 percent of the control organisms die or appear to be stressed or diseased during the 96-h test period. Each test chamber should be checked for dead shrimp at 3, 6, 12, 24, 48, 72, and 96 h after the beginning of the test. Concentration-response curves and 48- and 96-h LC50 values should be determined along with their 95 percent confidence limits.

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

(vii) Distribution of shrimp among test chambers should be randomized. In 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.

(viii) The concentration of dissolved test substance (that which passes through a 0.45 μm filter) in the test chambers should be measured as often as is feasible during the test. The concentration of test substance should be measured:

(A) In each chamber at the beginning of the test and at 48 and 96 h after the start of the test.

(B) In at least one chamber containing the next to the lowest test substance concentration at least once every 24 h during the test.

(C) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

(ix) Observations on compound solubility should be recorded. The investigator should report the appearance of surface slicks, precipitates, or material adhering to the sides of the test chambers.

(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 before beginning the test by appropriate laboratory practices. 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 corrected mathematically.

(ii) **Numerical.** The number of dead shrimp 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 intervals 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.** This test should be conducted using one of three species of penaeid: *Penaeus aztecus* (brown shrimp), *Penaeus duorarum* (pink shrimp), or *Penaeus setiferus* (white shrimp). Post-larval juvenile shrimp should be utilized. Shrimp may be reared from eggs in the laboratory or obtained directly as juveniles or adults. Shrimp used in a particular test should have been obtained from the same source, be of similar age, and be of normal size and appearance. Shrimp 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 control group.

(ii) **Acclimation.** During acclimation, shrimp should be maintained in facilities with background colors and light intensities similar to those of the testing areas. In addition, any change in the temperature and chemistry of the dilution water used for holding and acclimating 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 2 percent.

(iii) **Care and handling.** Upon arrival at the test facility, the shrimp should be transferred to water closely matching the temperature and salinity of the transporting medium. Shrimp should be held in glass tanks of 30 L capacity or larger. No more than 22 to 24 shrimp should be placed in a 30 L tank unless the flow-through apparatus can maintain dissolved oxygen levels above 60 percent of saturation. With species of the genus *Penaeus*, a minimum flow rate of 7.5 L/g body weight day should be provided. Larger flows, up to 22 L/g body weight day, may be desirable to ensure dissolve oxygen concentrations above 60 percent of saturation and the removal of metabolic products. The period of acclimation to ambient laboratory conditions should be at least 4 to 7 days.

(iv) **Feeding.** Penaeid shrimp should not be fed during testing. Every 2 or 3 days during the acclimation period, shrimp should be fed fish pieces approximately 1 cm². Uneaten food should be removed daily.

(2) **Facilities**—(i) **Apparatus.** (A) Facilities which may be needed to perform this test include: Flow-through tanks for holding and acclimating penaeid shrimp; a mechanism for controlling and maintaining the water temperature and salinity during the holding period; apparatus for straining particulate matter, removing air bubbles, or aerating water when necessitated by water quality requirements; and an apparatus providing a 12-h light and 12-h dark photoperiod with a 15- to 30-min transition period. Facilities should be well ventilated, free of fumes, and free of all other disturbances that may affect test organisms.

(B) Acid-washed sand, free of excess organic matter, should be placed in the bottom of test chambers to a depth of 2–3 cm.

(C) Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation, minimize entry of dust and other particles, and prevent escape of the shrimp.

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

(iii) **Construction materials.** 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.

(iv) **Dilution water.** (A) Natural or artificial seawater is acceptable as dilution water if shrimp will survive in it without signs of stress, such as unusual behavior or discoloration. Shrimp should be acclimated and tested in dilution water from the same origin.

(B) Natural seawater should be filtered through a 5 μm filter with a pore size $< 20 \mu\text{m}$ 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 0.1 mS/m 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.** Proportional diluters, metering pumps, or other suitable systems should be used to deliver test substance to the test chambers. The system 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 94-h flow through a test chamber should be equal

to a 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 as specified below:

(i) Temperature of 23 ± 1 °C.

(ii) DOC 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 shrimp placed in a test solution should not be so great as to affect results of the test. Loading requirements will vary depending on the flow rate of dilution water. The loading should not cause the DOC to fall below the recommended levels.

(iv) Photoperiod of 12-h light and 12-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 by the test that are suggestive or predictive of acute toxicity and all other toxicological manifestations. In addition to the general reporting requirements prescribed under Good Laboratory Practice Standards, 40 CFR part 792, subpart J, the reporting of test data should include the following:

(1) The nature of the test, laboratory, name of the investigator, test substance, and dates of the 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, 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 DOC 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, FL 1978).

(2) [Reserved]