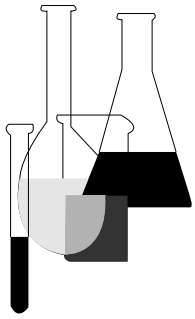
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–160
April 1996

Ecological Effects Test Guidelines

OPPTS 850.1055 Bivalve Acute Toxicity Test (Embryo-Larval)



"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 850.1055 Bivalve acute toxicity test (embryo-larval).

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is 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 to be used to develop data on the acute toxicity of chemical substances and mixtures (''chemicals'') to Eastern oysters (*Crassostrea virginica*), Pacific oysters (*Crassostrea gigas*), quahogs (*Mercenaria mercenaria*), or bay mussels (*Mytilus edulis*). The Environmental Protection Agency will use data from these tests in assessing the hazard of a chemical to the environment.

(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 guideline. The following definitions also apply to this test guideline.

Acute toxicity is the discernible adverse effects induced in an organism within a short period of time (days) of exposure to a chemical. The effects (lethal or sublethal) occurring may usually be observed within the period of exposure with aquatic organisms. In this test guideline, abnormal development or death is used as the measure of toxicity.

48–h EC50 (Effective Median Concentration) is that experimentally derived concentration of a chemical in water in which 50 percent of the larvae exposed to test material are dead or abnormally developed compared to larvae in the controls (not exposed to test material) after a 48–h exposure.

Embryo is the stage between the fertilization of the egg and the trochophore (2 to 8 cell stage).

Larva includes the trochophore and the straight hinge stage.

LOEC is the lowest observed effect concentration.

NOEC is the no observed effect concentration.

Veliger is the larval stage in which the ciliated velum (swimming organ) is present.

(d) **Test procedures**—(1) **Summary of the test**. (i) The water solubility and the vapor pressure of the test chemical should be known. Prior to testing, the structural formula of the test chemical, its purity, stability

in water and light, *n*-octanol/water partition coefficient, and pK values should be known. The results of a biodegradability test and the method of analysis for the quantification of the chemical in water is also desirable.

(ii) It may be possible to determine an EC50 for a chemical with limited solubility under the test conditions. If the stability or homogeneity of the test chemical cannot be maintained, care should be taken in the interpretation of the results and a note made that these results may not be reproducible.

(iii) This study consists of a static 48–h exposure that is used to evaluate the proportion of living and normal D-shaped veligers exposed to the test material compared to the proportion of the same in controls not exposed to test material. The concentration-response curve and EC50 value for the test chemical are developed from these data.

(2) **Range-finding test**. A range-finding test should be conducted to establish test chemical concentrations for the definitive test. The test is conducted in the same way as the definitive test except a widely spaced chemical concentration series (i.e. log-interval) is used.

(3) **Definitive test.** (i) The test is started about 4 h after fertilization while the embryos are in the 2– to 4–cell stage (determined microscopically). At this stage embryos (15–30 embryos/mL/replicate) are added to the test solution. The endpoint for this test is the determination of a 48–h EC50. This will be based on the proportion of normal larvae (those that are alive with completely developed shells containing meat) exposed to test solution as compared to normal larvae in controls. An LOEC and an NOEC are also to be calculated. Constant conditions should be maintained in the test facilities as much as possible throughout the test. The preparation and storage of the test material, the holding of the oysters, and all operations and tests should be carried out in an environmental free from harmful concentrations of dust, vapors, and gases and in such a way to avoid cross-contamination. Any disturbances that may change the behavior of the test organisms should be avoided.

(ii) The test chemical concentrations are to be documented in all tests. At least five test concentrations are to be used with a dose separation factor not to exceed 1.8 between concentrations.

(iii) Test organisms are to be impartially distributed among test chambers in such a manner that the test results show no significant bias from the distributions.

(iv) Test organisms are inspected at regular intervals. Dead bivalves are removed when observed.

(v) The criteria for a valid definitive test are:

(A) Mortality or aberrant development in the controls are not exceed 30 percent percent for oysters or 40 percent for clams at the end of each test.

(B) The dissolved oxygen concentration should be at least 60 percent of air saturation throughout all tests.

(C) Embryos were not more than 4–h old from fertilization at the beginning of the test.

(D) The difference between the time-weighted-average (TWA) measured temperatures for any two test chambers from the beginning to the end of the test should not be greater than 1 °C. No single measured temperature in any test chamber should be more than 3 °C different from the mean of the TWA measured temperatures for the individual test chambers. The difference between the measured temperatures in any two test chambers should not be more than 2 °C at any one time.

(e) **Test conditions**—(1) **Test species**—(i) **Selection**. (A) Eastern oysters (*C. virginica*) are the preferred test species, but Pacific oysters (*C. gigas*), quahogs (*M. mercenaria*), or bay mussels (*M. edulis*) may also be used.

(B) The test must begin with embryos within 4-h of fertilization when embryos are in the 2– to 4–, and 8–cell stages.

(C) Embryos used to start a test should be obtained from females and males that have been maintained for at least 2 weeks in the dilution water in the laboratory before they are stimulated to spawn.

(D) The spawning of bivalve test organisms is induced by rapidly elevating the temperature 5–10 °C above the conditioning temperature. An added stimulus of heat-killed bivalve sperm may be used. To fertilize the eggs, sufficient sperm suspension should be added to the egg suspension to yield 10^5 to 10^7 sperm/mL in the final mixture. Additional guidance may be found in paragraph (g)(1) of this guideline.

(ii) **Acquisition.** Bivalves may be cultured in the laboratory, purchased from culture facilities or commercial harvesters, or collected from a natural population in an unpolluted area free from epizootic disease.

(2) **Test facilities**—(i) **Apparatus**. (A) Test vessels, equipment and facilities that contact stock solutions, test solutions, or any water into which any brood stock or test organisms will be placed should not contain substances that can be leached or dissolved by aqueous solutions in amounts that adversely affect test organisms.

(B) Test chambers are defined as the smallest physical units between which there are no water connections. Tests are usually conducted in glass chambers that are 1– to 2–L in capacity.

(ii) **Dilution water.** A constant supply of good quality unfiltered seawater should be available throughout the holding, acclimation, and testing periods. The dilution water should be acceptable to adult bivalve molluscs and their embryos and larvae. For oysters, at least 70 percent of the embryos resulting from eggs and sperm of appropriately conditioned adults result in normal larvae while being maintained in the dilution water for 48 h. For clams, this should be 60 percent of the embryos resulting in normal larvae. Also, a dilution water is acceptable if adult oysters or clams will survive and grow normally for 14 days without exhibiting signs of stress, i.e., excessive mucus production (stringy material floating suspended from oysters) lack of feeding, shell gaping, poor shell closing in response to prodding, or excessive mortality. Natural seawater is recommended, although artificial seawater with food added may be used. The dilution water is to have a salinity in excess of 12 ppt. A natural seawater should have a weekly range in salinity of less than 10 ppt and a monthly range in pH of less than 0.8 unit. Artificial seawater salinity should not vary more than 2 ppt nor more than 0.5 pH unit. Oysters are to be tested in dilution water from the same origin.

(3) **Test parameters**—(i) **Carriers**. Stock solutions of substances of low aqueous solubility may be prepared by ultrasonic dispersion or, if necessary, by use of organic solvents, emulsifiers or dispersant of low toxicity to oysters. When such carriers are used the control oysters are to be exposed to the same concentration of the carrier as that used in the highest concentration of the test substance. The concentration of such carriers should not exceed 0.1 mL/L.

(ii) **Dissolved oxygen**. The dissolved oxygen concentrations are to be at least 60 percent of the saturation value and should be recorded daily.

(iii) **Loading.** The loading rate should not crowd oysters and should permit adequate circulation of water while avoiding physical agitation of oysters by water current.

(iv) **Temperature.** Tests with *C. gigas* should be conducted at 20 °C, with *C. virginica* and *M. mercenaria* at 25 °C, and with *M. edulis* at 16 °C. The temperature for *C. gigas*, *C. virginica*, and *M. mercenaria* should never exceed 32 °C, nor 20 °C for *M. edulis* (even during spawning induction). Temperature should be recorded continuously.

(v) **pH.** The pH is to be measured at the beginning and end of the test in each test chamber.

(f) **Reporting.** In addition to the reporting requirements prescribed in 40 CFR Part 792—Good Laboratory Practice Standards, the report is to contain the following:

(1) The source of the dilution water, the mean, standard deviation and range of the salinity, pH, temperature, and dissolved oxygen during the test period.

(2) A description of the test procedures used (e.g., the flow-through system, test chambers, chemical delivery system, aeration, etc.).

(3) Detailed information about the oysters used, including the age and/or size (i.e., height), source, history, method of confirmation of prespawn condition, acclimation procedures, and food used.

(4) The number of organisms tested, the loading rate, and the flowrate.

(5) The methods of preparation of stock and test solutions, and the test chemical concentrations used.

(6) The number of dead and live test organisms, the percentage of organisms that died, and the number that showed any abnormal effects in the control and in each test chamber at each observation period.

(7) The calculated 48–h EC50 and its 95 percent confidence limits and the statistical methods used to calculate these values.

(8) The calculated LOEC and a NOEC must also be developed.

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

(10) Any incidents in the course of the test which might have influenced the results.

(11) A statement that the test was carried out in agreement with the prescriptions of the test guideline given above (otherwise a description of any deviations occurring).

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

(1) ASTM. Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Apecies of Saltwater Bivalve Molluscs. E 724-89. American Society for Testing and Materials, Philadelphia, PA. 18 pp (1989).

(2) [Reserved]