

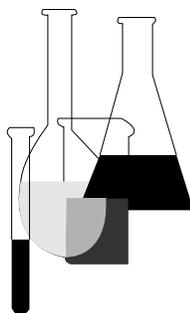


---

# Ecological Effects Test Guidelines

## OPPTS 850.1025

### Oyster Acute Toxicity Test (Shell Deposition)



**“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](http://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](mailto: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](mailto: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](http://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](http://gopher.epa.gov)) under the heading “Environmental Test Methods and Guidelines.”

**OPPTS 850.1025 Oyster acute toxicity test (shell deposition).**

(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 797.1800 Oyster 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 to be used to develop data on the acute toxicity of chemical substances and mixtures (“chemicals”) to Eastern oysters, *Crassostrea virginica* (Gmelin). 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 test 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. For aquatic animals this usually refers to continuous exposure to the chemical in water for a period of up to 4 days. The effects (lethal or sublethal) occurring may usually be observed within the period of exposure with aquatic organisms. In this test guideline, shell deposition is used as the measure of toxicity.

*EC50* is that experimentally derived concentration of a chemical in water that is calculated to induce shell deposition 50 percent less than that of the controls in a test batch of organisms during continuous exposure within a particular exposure period which should be stated.

*Shell deposition* is the measured length of shell growth that occurs between the time the shell is ground at test initiation and test termination 96 h later.

*Umbo* means the narrow end (apex) of the oyster shell.

*Valve height* means the greatest linear dimension of the oyster as measured from the umbo to the ventral edge of the valves (the farthest distance from the umbo).

(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<sub>a</sub> values should be known prior to testing. The results of a biodegradability test and the method of analysis for the quantification of the chemical in water should also be known.

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

(iii) Test chambers are filled with appropriate volumes of dilution water. The flow of dilution water through each chamber is adjusted to the rate desired. The test chemical is introduced into each test chamber and the flow-rate adjusted to establish and maintain the desired concentration in each test chamber. Test oysters, which have been acclimated and prepared by grinding away a portion of the shell periphery, are randomly introduced into the test and control chambers. Oysters in the test and control chambers are observed daily during the test for evidence of feeding or unusual conditions, such as shell gaping, excessive mucus production or formation of fungal growths in the test chambers. The observations are recorded and dead oysters removed. At the end of 96 h the increments of new shell growth are measured in all oysters. 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) Oysters which meet condition criteria (age, size, reproductive status, health) and which have been acclimated to test conditions should have approximately 3 to 5 mm of the shell periphery, at the rounded (ventral) end, ground away with a small electric disc grinder or other appropriate device, taking care to remove the shell rim uniformly to produce a smooth, rounded, blunt profile. The oyster's valves should be held together tightly during grinding to avoid vibrating the shell and injuring the adductor muscle. Oysters from which so much of the shell rim has been removed that an opening into the shell cavity is visible should not be used.

(ii) It is desirable to have shell growth values for the low and high concentrations relatively close to, but different from, 0 and 100 percent. Therefore, the range of concentrations to which the oysters are exposed should be such that in 96 h relative to the controls, very little shell growth occurs in oysters exposed to the highest concentration and shell growth is slightly less than controls at the lowest concentration. Oysters in the

remaining concentrations should have increments of shell growth such that the concentration producing 50 percent shell growth relative to the growth is bracketed with at least one concentration above and one below it.

(iii) The test should be carried out without adjustment of pH unless there is evidence of marked change in the pH of the solution. In this case, it is advised that the test be repeated with pH adjustment to that of the dilution water and the results reported.

(iv) The test begins when at least 20 prepared oysters are placed in each of the test chambers containing the appropriate concentrations of test substance and controls. The steady-state flows and test chemical concentrations should be documented. At least five test chemical concentrations should be used. The dilution factor between concentrations should not exceed 1.8.

(v) Test oysters should be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. The oysters should be spread out equidistantly from one another so that the entire test chamber is used. The oysters should also be placed with the left (cupped) valve down and the open, unhinged ends all oriented in the same direction facing the incoming flow of test solution.

(vi) The oysters are inspected at least after 24, 48, 72, and 96 h. Oysters are considered dead if touching of the gaping shell produces no reaction. Dead oysters are removed when observed and mortalities are recorded. Observations at 3 h and 6 h are also desirable.

(vii) Shell growth is the primary criterion used in this test guideline to evaluate the toxicity of the test chemical. Shell growth increments in all oysters should be measured after 96-h exposure. Record the length of the longest “finger” of new shell growth to the nearest 0.1 mm. Oysters should be handled very gently at this stage to prevent damage to the new shell growth.

(viii) Records should be kept of visible abnormalities such as loss of feeding activity (failure to deposit feces), excessive mucus production (stringy material floating suspended from oysters), spawning, or appearance of shell (closure or gaping).

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

(A) The mortality in the controls should not exceed 10 percent at the end of the test.

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

(C) If evidence of spawning is observed, the test should be repeated.

(D) There should be evidence that the concentration of the substance being tested has been satisfactorily maintained over the test period. The concentration of the test substance should be measured:

(1) In each chamber at time 0-h.

(2) In each chamber at 96-h; and

(3) In at least one appropriate chamber whenever a malfunction is detected in any part of the test chemical delivery system.

(E) Dissolved oxygen, temperature, salinity, and pH measurements should be made at the beginning and end of the test in each chamber.

(F) A minimum of 2 mm of new shell growth should be observed in control oysters (solvent and dilution water).

(4) **Test results.** (i) At the end of the test, appropriate statistical analysis should be conducted on the oyster shell deposition test data. The probit transformation should then be applied to the response variable and then regressed, using least squares regression, on dose or log-dose. An F Test for linearity should be conducted to determine whether the chosen regression technique adequately describes the experimental data.

(ii) Calculate the ratio of the mean shell growth for each group of test oysters (exposed to each of the test chemical concentrations) to the mean shell growth of the group of control oysters. From these data the concentration-response curve is drawn and an EC50 along with the 95 percent confidence limits on the value are determined from the curves. The mean measured concentration of test chemical should be used to calculate the EC50 and to plot the concentration-response curve.

(e) **Test conditions**—(1) **Test species**—(i) **Selection.** (A) The Eastern oyster, *Crassostrea virginica*, should be used as the test organism.

(B) Oysters used in the same test should be 30 to 50 mm in valve height and should be as similar in age and/or size as possible to reduce variability. The standard deviation of the valve height should be less than 20 percent of the mean.

(C) Oysters used in the same test should be from the same source and from the same holding and acclimation tanks.

(D) Oysters should be in a prespawm condition of gonadal development prior to and during the test as determined by direct or histological observation of the gonadal tissue for the presence of gametes.

(ii) **Acquisition.** Oysters 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.

(iii) **Acclimation.** (A) Oysters should be attended to immediately upon arrival. Oyster shells should be brushed clean of fouling organisms and the transfer of the oysters to the holding water should be gradual to reduce stress caused by differences in water quality characteristics and temperature. Oysters should be held for at least 12 to 15 days before testing. All oysters should be maintained in dilution water at the test temperature for at least 2 days before they are used.

(B) During holding, the oysters should not be crowded, and the dissolved oxygen concentration should be above 60 percent saturation. The temperature of the holding water should be the same as that used for testing. Holding tanks should be kept clean and free of debris. Cultured algae may be added to dilution water sparingly, as necessary to support life and growth and such that test results are not affected as confirmed by previous testing.

(C) Oysters should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible.

(D) A batch of oysters is acceptable for testing if the percentage mortality over the 7-day period prior to testing is less than 5 percent. If the mortality is between 5 and 10 percent, acclimation should continue for 7 additional days. If the mortality is greater than 10 percent, the entire batch of oysters should be rejected. Oysters which appear diseased or otherwise stressed or which have cracked, chipped, bored, or gaping shells should not be used. Oysters infested with mudworms (*Polydora* sp.) or boring sponges (*Cilona cellata*) should not be used.

(2) **Test facilities**—(i) **Apparatus.** (A) In addition to normal laboratory equipment, an oxygen meter, equipment for delivering the test chemical, adequate apparatus for temperature control, and test tanks made of chemically inert material are needed.

(B) Constant conditions in the test facilities should be maintained 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 environment free from harmful concentrations of dust, vapors and gases and in such a way as to avoid cross-contamination. Any disturbances that may change the behavior of the oysters should be avoided.

(ii) **Dilution water.** A constant supply of good quality unfiltered seawater should be available throughout the holding, acclimation, and testing periods. Natural seawater is recommended, although artificial seawater with food added may be used. In either case, to ensure each oyster is provided equal amounts of food, the water should come from a thoroughly mixed common source and should be delivered at a flowrate of at least 1 and preferably 5 L/h per oyster. The flowrate should be  $\pm 10$  percent of the nominal flow. A dilution water is acceptable if oysters 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. The dilution water should have a salinity in excess of 12 ppt, and should be similar to that in the environment from which the test oysters originated. 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 should 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 dispersants of low toxicity to oysters. When such carriers are used the control oysters should 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 should 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.** The test temperature should be 20 °C. Temporary fluctuations (less than 8 h) within  $\pm 5$  °C are permissible. Temperature should be recorded continuously.

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

(f) **Reporting.** In addition to the reporting requirements as specified under EPA Good Laboratory Practice Standards, 40 CFR part 792, subpart J, the following specific information should be reported:

(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 96-h shell growth measurements of each oyster; the mean, standard deviation and range of the measured shell growth at 96 h of oysters in each concentration of test substance and control.

(8) The calculated 96-h EC50 and its 95 percent confidence limits and the statistical methods used to calculate these values.

(9) When observed, the 96-h observed no-effect concentration (the highest concentration tested at which there were no mortalities, abnormal behavioral or physiological effects and at which shell growth did not differ from controls).

(10) A graph of the concentration-response curve based on the 96-h chemical concentration and shell growth measurements upon which the EC50 was calculated.

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

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

(13) 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).