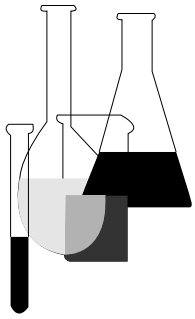
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–127
April 1996

Ecological Effects Test Guidelines

OPPTS 850.1710
Oyster BCF



“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 850.1710 Oyster BCF.

(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 797.1830 Oyster Bioconcentration Test and OPP 72–6 Aquatic Organism Accumulation Tests (Pesticide Assessment Guidelines, Subdivision E—Hazard Evaluation; Wildlife and Aquatic Organisms) EPA report 540/09-82-024, 1982.

(b) **Purpose.** This guideline is to be used for assessing the propensity of chemical substances to bioconcentrate in tissues of estuarine and marine molluscs. This guideline describes a bioconcentration test procedure for the continuous exposure of Eastern oysters (*Crassostrea virginica*) to a test substance in a flow-through system. EPA will use data from this test in assessing the hazard a chemical or pesticide may present to the 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 are applicable to this test guideline. The following definitions also apply:

(1) *Acclimation* is the physiological compensation by test organisms to new environmental conditions (e.g. temperature, salinity, pH).

(2) *Bioconcentration* is the net accumulation of a chemical directly from water into and onto aquatic organisms.

(3) *Bioconcentration factor (BCF)* is the quotient of the concentration of a test chemical in tissues of aquatic organisms at or over a discrete time period of exposure divided by the concentration of test chemical in the test water at or during the same time period.

(4) *Depuration* is the elimination of a test chemical from a test organism.

(5) *Depuration phase* is the portion of a bioconcentration test after the uptake phase during which the organisms are in flowing water to which no test chemical is added.

(6) *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 period of exposure (which should be stated).

(7) *Loading* is the ratio of the number of oysters to the volume (liters) of test solution passing through the test chamber per hour.

(8) *Organic chlorine* is the chlorine associated with all chlorine-containing compounds that elute just before lindane to just after mirex during gas chromatographic analysis using a halogen detector.

(9) *Organochlorine pesticides* are those pesticides which contain carbon and chlorine, such as aldrin, DDD, DDE, DDT, dieldrin, endrin, and heptachlor.

(10) *Steady-state* is the time period during which the amounts of test chemical being taken up and depurated by the test oysters are equal, i.e. equilibrium.

(11) *Steady-state bioconcentration factor* is the mean concentration of the test chemical in test organisms during steady-state divided by the mean concentration of the test chemical in the test solution during the same period.

(12) *Stock solution* is the concentrated solution of the test substance which is dissolved and introduced into the dilution water.

(13) *Test chamber* is the container in which the test oysters are maintained during the test period.

(14) *Test solution* is dilution water containing the dissolved test substance to which test organisms are exposed.

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

(16) *Uptake* is the sorption of a test chemical into and onto aquatic organisms during exposure.

(17) *Uptake phase* is the initial portion of a bioconcentration test during which the organisms are exposed to the test solution.

(18) *Valve height* is 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.** Oysters are continuously exposed to a minimum of one constant, sublethal concentration of a test chemical under flow-through conditions for a maximum of 28 days. During this time, test solution and oysters are periodically sampled and analyzed using appropriate methods to quantify the test chemical concentration. If, prior to day 28, the tissue concentrations of the chemical sampled over three consecutive sampling periods have been shown to be statistically similar (i.e. steady-state has been reached), the uptake phase of the test is terminated, and the remaining oysters are transferred to untreated flowing water until 95 percent of the accumulated chemical residues have been eliminated, or for a maximum depuration period of 14 days. The mean test chemical concentration in the oysters at steady-state

is divided by the mean test solution concentration at the same time to determine the bioconcentration factor (BCF). If steady-state is not reached during 28 days of uptake, the steady-state BCF should be calculated using non-linear parameter estimation methods.

(2) [Reserved]

(3) **Range-finding test**. The oyster acute toxicity test is used to determine the concentration levels to be used in the oyster bioconcentration test.

(4) **Definitive test**. (i) The following data on the test chemical should be known prior to testing:

(A) Solubility in water.

(B) Stability in water.

(C) Octanol-water partition coefficient.

(D) Acute toxicity (e.g. propensity to inhibit shell deposition) to oysters.

(E) The validity, accuracy, minimum detection, and minimum quantification limits of selected analytical methods.

(ii) At least two concentrations should be tested to assess the propensity of the compound to bioconcentrate. The concentrations selected should not stress or adversely affect the oysters and should be less than one-tenth the EC50 or <EC10 determined in either the rangefinding or 96-h definitive test under OPPTS 850.1025 of these guidelines. The test concentration should be less than the solubility limit of the test substance in water and should be close to the potential or expected environmental concentration. The limiting factor of how low one can test is based on the detection and quantification limits of the analytical methods. The concentration of the test material in the test solution should be at least 10 times greater than the detection limit in water. The mean measured concentration of the test material should be 80 percent of the nominal concentration. However, this may be difficult to achieve for chemicals with high octanol-water partition coefficients.

(iii) It should be documented that the potential to bioconcentrate is independent of the test chemical concentration, and at least two concentrations should be tested which are at least a factor of 10 apart.

(iv) To determine the duration of this test, an estimation of the uptake phase should be made prior to testing based upon either previous experience with the same chemical in a different species, a test with a similar material, the results of a preliminary range-finding test, or, from the water solubility or octanol-water partition coefficient of the test chemical. This

estimate should also be used to designate a sampling schedule. The uptake phase should continue until steady-state has been reached. The uptake phase should continue for at least 4 days, but need not be longer than 28 days.

(A) The time to steady-state (S in hours) can be estimated from the water solubility of the octanol-water partition coefficient for chemicals whose uptake and depuration follow a two-compartment, two-parameter model (ASTM, 1986, under paragraph (g)(1) of this guideline). The following equations were developed from data on fish but are considered useful in this test as well:

$$S = 3.0/\text{antilog}(0.431 \log W - 2.11)$$

or

$$S = 3.0/\text{antilog}(-0.414 \log P + 0.122)$$

where

W = water solubility (mg/L)

P = octanol-water partition coefffient

For example, S for a chemical of log P 4.0 would be estimated as 3.0/antilog(-0.414(4.0) + 0.122) = 3.0/0.029 = 103.4 h.

Bioconcentration kinetic studies have also been performed specifically for molluscs, e.g. as investigated by Hawker and Connell (under paragraph (g)(2) of this guideline) and these may also be consulted.

(B) The depuration phase should continue until at least 95 percent of the accumulated test substance and metabolites have been eliminated, but no longer than 14 days.

(C) Based on the estimate of the time to steady-state, one of the following sampling schemes may be used to generate the appropriate data.

Table—Time to Steady-State in Days

Test Period	S<4	S>4<14	S>15<21	S>21
	Sampling days	Sampling days	Sampling days	Sampling days
Exposure[2]	1[1]	4[1]	1	1
.....	6[1]	1	3	3
.....	1	3	7	7
.....	2	7	10	10
.....	3	10	14	14
.....	4	12	18	21
.....		14	22	28

Table—Time to Steady-State in Days—Continued

Test Period	S<4	S>4<14	S>15<21	S>21
	Sampling days	Sampling days	Sampling days	Sampling days
Depuration[2]	1[1]	1	1	1
.....	6[1]	2	3	3
.....	12[1]	4	7	7
.....	1	6	10	10
.....				14

[1] Hours
[2] Additional sampling times may be needed to confirm that steady-state has been attained

(v) The following criteria should be met in order for the test to be valid:

(A) If it is observed that the stability or homogeneity of the test chemical cannot be maintained in the test solution, care should be taken in the interpretation of the results and a note should be made that these results may not be reproducible.

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

(C) The dissolved oxygen concentration should be >60 percent of saturation throughout the test.

(D) There should be evidence (using measured test chemical concentrations) that the concentration of the chemical being tested has been satisfactorily maintained over the test period.

(E) If evidence of spawning is observed, the test should be discontinued and later repeated.

(F) Temperature variations from 20 °C should be held to a minimum, preferably ±2 °C.

(vi) The following methodology should be followed:

(A) The test should not be started until the test chemical delivery system has been observed to be functioning properly and the test chemical concentrations have equilibrated (i.e. the concentration does not vary more than 20 percent). Analyses of two sets of test solution samples taken prior to test initiation should document this equilibrium. At initiation (time 0), test solution samples should be collected immediately prior to the addition of oysters to the test chambers.

(B) The appropriate number of oysters (see paragraph (d)(4)(vii)(A) of this guideline) should be brushed clean and should be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. The number of oysters used in ths test will depend on the length of the test, number of replicate test chambers used, and if, in addition to a nonsolvent control, a solvent-control is used. Also important are the size of each oyster and the size of the test chamber. For example, in a 28–day test, a minimum of 28 oysters in the uptake (exposure) phase and an additional 20 oysters in the depuration phase per test chemical concentration would be needed. These oysters could be distributed among two or more replicates at each concentration. A minimum of 48 oysters would be required for each control. The oysters should be spread out equidistant from one another and placed with the left (cupped) valve down and the unhinged ends (opposite from umbo) all oriented in the same direction facing the incoming flow.

(C) Oysters should be exposed to the test chemical during the uptake phase until steady state has been reached or for a maximum of 28 days. The uptake phase should be a minimum of at least 4 days. Then the remaining oysters should be transferred to untreated flowing water and sampled periodically to determine if depuration of the test chemical occurs. Every test should include a control consisting of the same dilution water, conditions, procedures, and oysters from the same group used in the test, except that none of the test chemical is added. If a carrier is present in the test chamber, a separate carrier control is required.

(D) Oysters should be observed (and data recorded) at least daily for feeding activity (deposition of feces) or any unusual conditions such as excessive mucus production (stringy material floating suspended from oysters), spawning, or appearance of shell (closure or gaping). If gaping is noted, the oyster(s) should be prodded. Oysters which fail to make any shell movements when prodded are to be considered dead, and should be removed promptly with as little disturbance as possible to the test chamber(s) and remaining live oysters.

(E) For oysters sampled, careful examination of all the tissues should be made at the time of shucking for any unusual conditions, such as a watery appearance or differences in color from the controls.

(F) Observations on compound solubility should also be recorded. These include the appearance of surface slicks, precipitates, or material adsorbing to the test chamber.

(vii) **Sampling.** (A) At each of the designated sampling times, triplicate water samples and enough oysters should be collected from the test chamber(s) to allow for tissue analyses of at least four oysters. The concentration of test chemical should be determined in a minimum of four oysters analyzed individually at each sampling period. If individual analy-

sis is not possible, due to limitations of the sensitivity of the analytical methods, then pairs, triplicates or more oysters may be pooled to constitute a sample for measurement. A similar number of control oysters should also be collected at each sample point, but only those collected at the first sampling period and weekly thereafter, should be analyzed. Triplicate control water samples should be collected at the time of test initiation and weekly thereafter. Test solution samples should be removed from the approximate center of the water column.

(B) At each sampling period the appropriate numbers of oysters are removed and treated as follows:

(*1*) The valve height of each oyster should be measured.

(*2*) Oysters should be shucked as soon as practical after removal and should never be refrigerated or frozen in the shell. The shell should be opened at the hinge, the adductor muscle severed and the top valve removed. The remaining adductor muscle should be severed where it attaches to the lower valve and the entire oyster removed.

(*3*) The shucked oysters should then be drained 3 min, blotted dry, weighed and analyzed immediately for the test chemical. If analyses are delayed, the shucked oysters should be wrapped individually in aluminum foil (for organic analysis) or placed in plastic or glass containers (for metal analysis) and frozen.

(C) If a radiolabeled test compound is used, a sufficient number of oysters should also be sampled at termination to permit identification and quantitation of any major (greater than 10 percent of parent) metabolites present. It is crucial to determine how much of the activity present in the oyster is directly attributable to the parent compound, and to correct the bioconcentration factor appropriately.

(5) **Test results** (i) Steady-state has been reached when the mean concentrations of test chemical in whole oyster tissue for three consecutive sampling periods are statistically similar (F test, P = 0.05). A BCF is then calculated by dividing the mean tissue residue concentration during steady-state by the mean test solution concentration during the same period. A 95 percent confidence interval should also be derived from the BCF. This should be done by calculating the mean oyster tissue concentration at steady-state (X_0) and its 97.5 percent confidence interval $X_0 \pm t$ (S.E.) where t is the t statistic at P = 0.025 and S.E. is the one standard error of the mean. This calculation would yield lower and upper confidence limits (L_0 and U_0). The same procedure should be used to calculate the mean and 97.5 percent confidence interval for the test solution concentrations at steady-state, XS ± t (S.E.), and the resulting upper and lower confidence limits (L_s and U_s). The 95 percent confidence interval of the BCF would then be between L_0/U_s and U_0/L_s. If steady-state was not reached during the maximum 28-day uptake period, the maximum BCF should be cal-

culated using the mean tissue concentration from that and all the previous sampling days. An uptake rate constant should then be calculated using appropriate techniques. This rate constant is used to estimate the steady-state BCF and the time to steady-state.

(ii) If 95 percent elimination has not been observed after 14 days depuration then a depuration rate constant should also be calculated. This rate constant should be based on the elimination of the parent compound.

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

(6) **Analytical measurements.** (i) All samples should be analyzed using EPA methods and guidelines whenever feasible. The specific methodology used should be validated before the test is initiated. The accuracy of the method should be measured by the method of known additions. This involves adding a known amount of the test chemical to three water samples taken from an aquarium containing dilution water and a number of oysters equal to that to be used in the test. The nominal concentration of these samples should be the same as the concentration to be used in the test. Samples taken on two separate days should be analyzed. The accuracy and precision of the analytical method should be checked using reference or split samples or suitable corroborative methods of analysis. The accuracy of standard solutions should be checked against other standard solutions whenever possible.

(ii) An analytical method should not be used if likely degradation products of the test chemical, such as hydrolysis and oxidation products, give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test. Atomic absorption spectrophotometric methods for metal and gas chromatographic methods for organic compounds are preferable to colorimetric methods. Spectrophotometry is also acceptable provided Beer's law is followed and an acceptable extinction coefficient can be determined.

(iii) In addition to analyzing samples of test solution at least one reagent blank should also be analyzed when a reagent is used in the analysis.

(iv) When radiolabelled test compounds are used, total radioactivity should be measured in all samples. At the end of the uptake phase, water and tissue samples should be analyzed using appropriate methodology to identify and estimate the amount of any major (at least 10 percent of the parent compound) degradation products or metabolites that may be present.

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

(ii) 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.

(iii) Oysters used in the same test should be from the same source and from the same holding and acclimation tank(s).

(iv) Oysters should be in a prespawn 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.

(v) 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.

(vi) The holding and acclimation of the oysters should be as follows:

(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 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 waters 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, such that test results are not affected, as confirmed by previous testing. Oysters should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible.

(C) 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, bared, or gaping shells should not be used. Oysters infested with mudworms (*Polydora* sp.) or boring sponges (*Cilona cellata*) should not be used.

(2) **Facilities**—(i) **Apparatus.** (A) An oxygen meter, dosing equipment for delivering the test chemical, adequate apparatus for temperature control, test tanks made of chemically inert material and other normal laboratory equipment 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.

(C) The test chambers should be made from materials that will not absorb the test substance. Delivery systems and test chambers should be cleaned before and after each use. If absorption of the test substance occurs, those applicable parts of the delivery system should be discarded.

(D) The test substance delivery system used should accommodate the physical and chemical properties of the test substance and the selected exposure concentrations. The apparatus used should accurately and precisely deliver the appropriate amount of stock solution and dilution (sea) water to the test chambers. The introduction of the test substance should be done in such a way as to maximize the homogeneous distribution of the test substance throughout the test chamber.

(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 (algae) 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 flow rate of at least one, and preferably 5 L/h per oyster. The flowrate should be ± 10 percent of the nominal flow. A dilution water is acceptable if oysters will survive and grow normally over the period in which the test is conducted 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 similiar 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 units. Artificial seawater should not vary more than 2 ppt nor more than 0.5 pH units. Oysters should be tested in dilution water from the same origin. If natural sea water is used, it should meet the following specifications, measured at least twice a year.

Substance	Concentration
Suspended solids	<20 mg/L
Un-ionized ammonia	<20 mg/L

Substance	Concentration
Residual chlorine	<3 μg/L
Total organophosphorus pesticides	<50 μg/L
Total organophosphorus pesticides plus PCB's	<50 μg/L

(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 (100 mg/L).

(ii) **Dissolved oxygen.** This dissolved oxygen concentration should be at least 60 percent of the air saturation value and should be measured daily in each chamber.

(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 excursions (less than 8 h) within ± 5 °C are permissible. Temperature should be recorded continually.

(v) **pH.** The pH should be measured daily in each test chamber.

(vi) The amount of total organic carbon (TOC) in the dilution water can affect the bioavailability of some chemicals. Thus, TOC should be measured daily.

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

(1) The source of the dilution water, the mean, standard deviation and range of the salinity, pH, TOC, 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 age, size (i.e. height), weight (blotted dry), source, history, method of confirmation of prespawn condition, acclimation procedures, and food used.

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

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

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

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

(8) Description of sampling, sample storage (if required) and analytical methods of water and tissue analyses for the test chemical.

(9) The mean, standard deviation and range of the concentration of test chemical in the test solution and oyster tissue at each sampling period.

(10) The time to steady-state.

(11) The steady-state or maximum BCF and the 95 percent confidence limits.

(12) The time to 95 percent elimination of accumulated residues of the test chemical from test oysters.

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

(14) If the test was not done in accordance with the prescribed conditions and procedures, all deviations should be described in full.

(g) **References.**

(1) American Society for Testing and Materials. ASTM E 1022–84. Standard practice for conducting bioconcentration tests with fishes and saltwater bivalve molluscs. In 1986 Annual Book of ASTM Standards, vol. 11.04: Pesticides; resource recovery; hazardous substances and oil spill response; waste disposal; biological effects, pp. 702–725 (1986).

(2) Hawker, D.W. and D.W. Connell, Bioconcentration of lipophilic compounds by some aquatic organisms, *Ecotoxicology and Environmental Safety* 11:184–197 (1986).

(3) Schimmel, S.C. and R.L. Garnas, Interlaboratory comparison of the ASTM bioconcentration test method using the eastern oyster, pp. 277–287. In R.C. Bahner and R.T. Hansen (eds.), Aquatic Toxicology and Hazard Assessment: Eighth Symposium, ASTM STP 891, American Society for Testing and Materials, Philadelphia, PA (1985).