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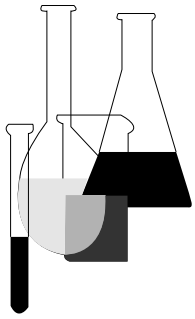
Ecological Effects Test Guidelines

OPPTS 850.1800 Tadpole/Sediment Subchronic 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 850.1800 Tadpole/sediment subchronic 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 is 40 CFR 797.1995 Tadpole/sediment subchronic toxicity test.

(b) **Purpose.** This guideline may be used to develop data on the subchronic toxicity of chemical substances and mixtures subject to environmental effects testing. This guideline prescribes tests to be used to develop data on the subchronic toxicity of chemicals sorbed to natural sediments to bullfrog tadpoles. The EPA 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 for physical, chemical, persistence, and ecological effects testing apply to this test guideline. The following definitions also apply:

Acclimation means the physiological compensation by test organisms to new environmental conditions (e.g., temperature, hardness, pH).

Carrier means a solvent or dispersant used to dissolve a test substance.

Cation exchange capacity (CEC) means the sum total of exchangeable cations that a sediment can absorb. The CEC is expressed in milliequivalents of negative charge per 100 g or milliequivalents of negative charge per gram of sediment (dry weight).

Clay mineral analysis means the estimation or determination of the kinds of clay-size minerals and the amount present in a sediment.

Conditioning means the exposure of construction materials, test chambers, and testing apparatus to dilution water or to test solutions prior to the start of a test in order to minimize the sorption of the test substance onto the test facilities or the leaching of substances from the test facilities into the dilution water or test solution.

Control means the exposure of test organisms to uncontaminated sediments.

Death means the total lack of movement by a test tadpole.

EC50 means that test substance concentration calculated from experimentally-derived growth or sublethal effects data that has affected 50 per-

cent of a test population during continuous exposure over a specified period of time.

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

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

Loading means the ratio of tadpole biomass (in grams, wet weight) to the volume (in liters) of test solution in a test chamber or passing through it in a 24–h period.

Lowest-observed-effect-concentration (LOEC) means the lowest treatment (i.e., test concentration) of a test substance that is statistically different in adverse effect on a specific population of test organisms from that observed in controls.

No-observed-effect-concentration (NOEC) means the highest treatment (i.e., test concentration) of a test substance that shows no statistical difference in adverse effect on a specific population of test organisms from that observed in controls.

Organic matter is the organic fraction of the sediment; it includes plant and animal residues at various stages of decomposition, cells and tissues of sediment-based organisms, and substances synthesized by the microbial community.

Particle size analysis is the determination of various amounts of different particle sizes in a sample (i.e., sand, silt, and clay), usually by sedimentation, sieving, micrometry, or combinations of these methods. The names and diameter ranges commonly used in the United States are provided in the following Table 1.:

Table 1.—Particle Size

Name	Diameter range
Very coarse sand	2.0 to 1.0 mm
Coarse sand	1.0 to 0.5 mm
Medium sand	0.5 to 0.25 mm
Fine sand	0.25 to 0.125 mm
Very fine sand	0.125 to 0.052 mm
Silt	0.052 to 0.002 mm
Clay	< 0.002 mm

Sediment is the unconsolidated inorganic and organic material that is suspended in and being transported by surface water, or has settled out and has deposited into beds.

Static means the test solution is not renewed during the period of the test.

Subchronic toxicity test means a method used to determine the concentration of a substance that produces adverse effects on a specified percentage of test organisms in a specified period of time (e.g., 30 days) which is a significant portion of the organism's life cycle. In this guideline, survival (i.e., death) and growth is used as the measure of toxicity.

Test slurry means the test substance and the natural sediment on which the test substance is sorbed. This sediment/test substance slurry is dosed directly into the tadpole.

(d) **Test procedures**—(1) **Summary of the test.** (i) Test chambers are filled with appropriate volumes of dilution water, or appropriate amounts of contaminated natural sediments and dilution water. If a flow-through test is performed, the flow of dilution water through each chamber is adjusted to the rate desired.

(ii) This toxicity test may be performed by either of two methods:

(A) Dosing the tadpole directly with a sediment/test substance slurry and maintaining tadpoles in test chambers with only clean dilution water.

(B) Maintaining tadpoles in test chambers containing contaminated sediments and allowing tadpoles to ingest contaminated sediments ad libitum.

(iii) Tadpoles which have been acclimated in accordance with the test design are introduced into the test and control chambers by stratified random assignment.

(iv) Tadpoles in the test and control chambers should be observed daily during the test. Dead tadpoles should be removed at least twice each day and the findings recorded.

(v) Live tadpoles in the test and control chambers should be weighed at least every 3 days.

(vi) The dissolved oxygen (DO) concentration, pH, temperature, and the concentration of test substance in contaminated sediments and/or water should be measured at intervals in selected test chambers.

(vii) Concentration-response curves, LC50, EC50, LOEC, and NOEC values for the test substance are developed from the survival and growth data collected during the test.

(2) **Range finding test.** If the toxicity of the test substance is not already known, a range-finding test should be performed to determine the range of concentrations to be used in the definitive test.

(3) **Definitive test.** (i) This toxicity test may be conducted by either of two methods:

(A) Dosing the tadpole directly with a sediment/test substance slurry and maintaining tadpoles in test chambers with only clean dilution water.

(B) Maintaining tadpoles in test chambers containing sediments and allowing tadpoles to ingest contaminated sediments ad libitum.

(ii) If this test is to be performed by dosing the tadpoles directly, the sediment/test chemical slurry should be placed directly into the buccal cavity of the tadpole with a pipet. The slurry should be shaken or mixed and 50 μL of the slurry should be placed directly into the posterior portion of the buccal cavity. The dosed tadpole should be held out of the water for about 1 minute after dosing to ensure ingestion and then returned to the test chamber. The test slurry should be prepared by adding 5 mL of distilled water to 1 g of dry sediment; the test chemical is added and the final volume is brought to 10 mL. This test slurry should be mixed on a mechanical shaker for at least 8 h before dosing.

(iii) If this test is to be conducted by maintaining tadpoles in test chambers containing contaminated sediments and allowing tadpoles to ingest contaminated sediments ad libitum, appropriate amounts of contaminated sediments sufficient to cover the bottom of each test chamber with about 3 to 5 cm of the contaminated sediment should be prepared. An appropriate amount of clean dilution water (i.e., about 10 to 20 cm above the sediment) should be added carefully to each chamber followed by tadpoles.

(iv) It is recommended that this test be performed three times, each time with a different natural sediment depending on the organic carbon content: Low (0.1 to 0.2 percent), medium (0.5 to 1.0 percent), and high (2.0 to 3.0 percent) organic carbon content (refer to OPPTS guideline 835.1220). However, natural sediments with a medium organic carbon content should be used if this test is to be done only once. Sediments selected for testing should be characterized by sampling location, general clay fraction mineralogy, percent sand, silt, and clay (particle size analysis), percent organic matter, percent organic carbon, pH (1:1 solids:water), and CEC.

(v) A minimum of 20 tadpoles should be exposed to each of five or more test substance concentrations (i.e., treatments) and a control. Test concentrations should be chosen in a geometric series in which the ratio is between 1.5 and 2.0 mg/kg (e.g., 2, 4, 8, 16, 32, and 64 mg/kg). All test concentrations should be based on milligrams of test chemical (100 percent active ingredient (AI)) per kilogram of sediment (dry weight). The concentration range should be selected to determine the concentration-response relationship, EC50 values, LOEC, and NOEC values for survival, sublethal effects, and growth.

(vi) An equal number of tadpoles should be placed in two or more replicates. The distribution of individual tadpoles among the test chambers should be randomized. Test concentrations in sediment and/or dilution water should be analyzed for test chemical concentrations prior to the start of the test and at designated times during the test.

(vii) Every test should include a control consisting of uncontaminated sediments, the same dilution water, conditions, procedures, and tadpoles from the same group used in the test, except that none of the test substance is added.

(viii) The test duration is 30 days.

(ix) It is recommended that this test be performed under flow-through conditions.

(x) The number of dead tadpoles should be recorded daily. In addition, the number of tadpoles showing sublethal effects and the type of effect (e.g., any abnormal behavior or appearance) should also be recorded daily. Each tadpole should be weighted every 3 days. Data on survival, sublethal effects, and growth which are collected during the test are used to calculate the LC50 value for survival, the EC50 value for sublethal effects, the EC50 value for growth, and to determine the LOEC and NOEC values on days 10, 20, and 30.

(xi) Tadpoles should be fed a suitable food every day. Food which sinks to the bottom should be used; food which floats on the water surface should not be used. In tests in which the tadpoles are dosed with a sediment/test chemical slurry and held in dilution water without sediments, any excess food or fecal material should be removed when observed. In tests in which tadpoles are allowed to feed ad libitum on contaminated sediments, excess food should not be given.

(4) **Test results.** (i) Survival and growth should be the primary criteria used in this test guideline to evaluate the toxicity of the test substance.

(ii) In addition to death, any abnormal behavior such as, but not limited to, erratic swimming, loss of reflex, increased excitability, lethargy, or any changes in appearance or physiology, such as discoloration (e.g., reddened leg), excessive mucous production, opaque eyes, curved spine, or hemorrhaging should be recorded.

(iii) Each test and control chamber should be checked for dead or effected tadpoles and observations recorded every 24 h after the beginning of the test or within 1 h of the designated times. Dead tadpoles should be removed at least twice a day.

(iv) Live tadpoles in the test and control chambers should be weighted at least every 3 days and fresh weights recorded.

(v) The mortality data should be used to calculate LC50 values and their 95 percent confidence limits, and to plot concentration-response curves at 10, 20, and 30 days. The statistical methods recommended for use in calculating LC50 values include probit, logit, moving average angle, and binomial.

(vi) The sublethal effects and growth (i.e., fresh weight) data should be used to plot concentration-response curves, calculate EC50 values, and determine LOEC and NOEC values. The statistical methods recommended for use in calculating the EC50 values include probit, logit, moving average angle, and binomial. Appropriate statistical methods (e.g., analysis of variance and multiple comparison test) should be used to test for significant differences between treatment means and determine LOEC and NOEC values.

(vii) A test is unacceptable if:

(A) More than 20 percent of the control tadpoles die or appear to be stressed, or are seen to be diseased during the test.

(B) The tadpoles in the control lose a significant amount of weight during the test, i.e. 30 percent.

(5) **Analytical measurements**—(i) **Water quality analysis**. (A) The hardness, acidity, alkalinity, pH, conductivity, total organic carbon (TOC) or chemical oxygen demand (COD), and particulate matter of the dilution water should be measured in the control test chambers at the beginning of each static test and at the beginning and end of each flow-through test. The month-to-month variation of the above values should be less than 10 percent and the pH should vary less than 0.4 units.

(B) During static tests, the DO concentration, temperature, and pH should be measured in each test chamber at the beginning of the test, and as often as needed thereafter, to document changes from the initial levels. The dilution water volume should not be reduced by more than 10 percent as a result of these measurements.

(C) During flow-through tests, the DO, temperature, and pH measurements should be made in each chamber at the beginning of the test and every 48 hours thereafter until the end of the test. It is recommended that this test be done under flow-through conditions.

(ii) **Collection of samples for measurement of test substance.** Samples of sediment to be analyzed for the test substance should be taken with a coring device. Samples of dilution water to be analyzed for desorbed test substance should be taken midway between the top, bottom, and sides of the test chamber. These samples should not include any surface scum or material dislodged from the bottom or sides. Samples should be analyzed immediately or handled and stored in a manner which mini-

mizes loss of test substance through microbial degradation, photodegradation, chemical reaction, volatilization, or sorption.

(iii) **Measurement of test substance.** (A) The concentration of test substance in sediment and/or dilution water should be measured at a minimum in each test chamber at the beginning (zero-hour, before tadpoles are added) and every 10 days thereafter.

(B) The analytical methods used to measure the amount of test substance in a sample should be validated before beginning the test. The accuracy of a method should be verified by a method such as using known additions. This involves adding a known amount of the test substance to three samples of dilution water or sediment taken from a chamber containing dilution water and the same number of tadpoles as are used in the test. The nominal concentration of the test substance in those samples should span the concentration range to be used in the test. Validation of the analytical method should be performed on at least 2 separate days prior to starting the test.

(C) An analytical method is not acceptable if likely degradation products of the test substance give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test.

(D) In addition to analyzing samples of dilution water and sediment, at least one reagent blank, containing all reagents used, should also be analyzed.

(E) Among replicate test chambers, the measured concentrations in sediment should not vary more than 20 percent. The measured concentration of the test substance in sediment in any chamber during the test should not vary more than 30 percent from the measured concentration prior to initiation of the test.

(F) The mean measured concentration of test substance in sediment (dry weight) should be used to plot all concentration-response curves and to calculate all LC50, EC50, LOEC, and NOEC values.

(e) **Test conditions**—(1) **Test species**—(i) **Selection.** The test species for this test is the bullfrog tadpole (*Rana catesbeiana*).

(ii) **Age and condition of tadpoles.** (A) Tadpoles having the morphological characteristics of premetamorphic stages VI through IX as described by Taylor and Kollros (1946) under paragraph (g)(3) of this guideline, characterized by the emergence of hind paddles and respiration by gills, should be used. Tadpoles used in a test should be the same age, weight (i.e., 2 to 5 g), and be of normal size and appearance for their age. The longest tadpole should not be more than twice the length of the shortest tadpole.

(B) All newly acquired tadpoles should be quarantined and observed for at least 14 days prior to use in a test.

(C) Tadpoles should not be used for a test if they appear stressed or if more than 5 percent die during the 48 h immediately prior to the test.

(iii) **Acclimation of test tadpoles.** (A) If the holding water is not from the same source as the test dilution water, acclimation to the dilution water should be done gradually over a 48–h period and tadpoles should be held an additional 14 days in the dilution water prior to testing. Any changes in water temperature should not exceed about 1 °C per hour or 3 °C per day. Tadpoles should be held for a minimum of 7 days at the test temperature prior to testing.

(B) During the final 48 h of acclimation, tadpoles should be maintained in facilities with background colors and light intensities similar to those of the testing area.

(2) **Facilities**—(i) **General.** Facilities needed to perform this test include:

(A) Flow-through tanks for holding and acclimating tadpoles.

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

(C) Apparatus for straining particulate matter, removing gas bubbles, or insufficiently dissolved oxygen, respectively.

(D) Apparatus for providing a 16–h light/8–h dark photoperiod with a 15– to 30–min transition period.

(E) Chambers for exposing test tadpoles to the test substance.

(F) A dilution water delivery system for flow-through tests.

(ii) **Construction materials.** Construction materials and commercially purchased equipment that may contact the stock solution or dilution water should not contain substances that can be leached or dissolved into aqueous solutions in quantities that can alter the test results. Materials and equipment that contact stock or test solutions should be chosen to minimize sorption of test chemicals. Glass, no. 316 stainless steel, and perfluorocarbon plastic should be used whenever possible. Concrete, fiberglass, or plastic (e.g., PVC) may be used for holding tanks, acclimation tanks, and water supply systems, but they should be thoroughly conditioned before use. If cast iron pipe is used in freshwater supply systems, colloidal iron may leach into the dilution water and strainers should be used to remove rust particles. Rubber, copper, brass, galvanized metal,

epoxy glues, and lead should not come in contact with the dilution water or stock solution.

(iii) **Dilution water delivery system.** In flow-through tests, the system used should be calibrated before each test. Calibration includes determining the flow rate of dilution water through each chamber. The general operation of the dilution water delivery system should be checked twice daily during a test. The 24–h flow rate through a test chamber should be a minimum of six tank volumes. During a test, the flow rates should not vary more than 10 percent from one test chamber to another or from one time to any other.

(iv) **Test chambers.** Test chambers made of stainless steel should be welded, not soldered. Test chambers made of glass should be fused or bonded using clear silicone adhesive. As little adhesive as possible should be left exposed in the interior of the chamber.

(v) **Cleaning of test system.** Dilution water delivery systems and test chambers should be cleaned before each test. They should be washed with detergent and rinsed in sequence with clean water, pesticide-free acetone, clean water, and 5 percent nitric acid, followed by two or more changes of dilution water.

(vi) **Dilution water.** (A) Clean surface or ground water, reconstituted water, or dechlorinated tap water is acceptable as dilution water if the test tadpoles will survive in it for the duration of the holding, acclimating, and testing periods without showing signs of stress, such as discoloration (i.e., reddened leg), hemorrhaging, disorientation, or other unusual behavior. The quality of the dilution water should be constant and should meet the specifications in the following Table 2. when analyzed (at least twice a year).

Table 2.—Specifications for Dilution Water

Substance	Maximum Concentration
Particulate matter	20.0 mg/L
Total organic carbon (TOC)	20 mg/L
Chemical oxygen demand (COD)	50 mg/L
Un-ionized ammonia	10 µg/L
Residual chlorine	10 µg/L
Total organochlorine pesticides	50.0 ng/L
Total organochlorine pesticides. plus polychlorinated biphenyls (PCBs)	50.0 ng/L
Organic chlorine	25.0 ng/L

(B) The concentration of DO in the dilution water should be between 90 and 100 percent saturation, or >5 mg/L at sea level. If necessary, the dilution water can be aerated before the addition of the test substance.

All reconstituted water should be aerated before use. Hardness should be <180 mg/L as $CaCO_3$; pH should be 6.5 to 8.5.

(C) If disease organisms (e.g., pathogenic bacteria) are present in the dilution water in sufficient numbers to cause infection, they should be killed or removed by suitable equipment.

(D) Glass distilled or carbon filtered deionized water with a conductivity less than 1 μS/cm is acceptable for use in making reconstituted water. If the reconstituted water is prepared from a ground or surface water source, conductivity, and TOC or COD should be measured on each batch.

(vii) **Carriers.** (A) Distilled water should be used in making stock solutions of the test substance. If a carrier is absolutely necessary to dissolve the test substance, the volume used should be minimal. If the test substance is a mixture, formulation, or commercial product, none of the ingredients is considered a carrier unless an extra amount is used to prepare the stock solution. Concentrations of stock solution should be based on 100 percent AI of the test chemical.

(B) Triethylene glycol and dimethyl formamide are the preferred carriers, but acetone can also be used.

(3) **Test parameters**—(i) **Loading**. The number of tadpoles placed in a test chamber should not be so great as to affect the results of the test. The loading should not be so great that the test substance concentrations in treated sediments are decreased by more than 20 percent due to uptake by the tadpoles. Loading should not exceed one tadpole per liter of dilution water in the test chamber at any time. Loading rates should be adjusted to maintain the DO concentration above the recommended levels and the ammonia concentration below 20 μg/L.

(ii) **Dissolved oxygen concentration.** The DO in each test chamber should be greater than 5.0 mg/L.

(iii) **Temperature.** The test temperature should be about 18 °C. The temperature should be measured at least hourly in one test chamber.

(iv) **Light.** A 16–h light/8–h dark photoperiod with a 15– to 30–minute transition period should be maintained.

(e) **Reporting**. (1) The final report should include, but not necessarily be limited to, the following information.

(i) Name and address of the facility performing the study, and the dates on which the study was initiated and was completed, terminated, or discontinued.

(ii) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.

(iii) Statistical methods used for analyzing the data. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.

(iv) The test substance identified by name, Chemical Abstracts Service (CAS) registry number or code number, source, lot or batch number, strength, purity, and composition, or other appropriate characteristics.

(v) Stability of the test and, if used, control substances under the conditions of administration.

(vi) A description of the methods used, which should include the following:

(A) Description of the test chambers, the depth and volume of solution in the chamber, the specific way the test was begun (e.g., conditioning and test substance additions), and for flow-through tests, a description of the dilution water delivery system including a diagram if the design is complex.

(B) The source of the dilution water, a description of any pretreatment, and the measured hardness, acidity, alkalinity, pH, conductivity, TOC or COD, and particulate matter.

(C) The source of the natural sediment (i.e., sampling location), sediment physical-chemical properties, percent sand, silt, and clay (particle size analysis), percent organic matter, percent organic carbon, pH (1:1 solids:water), CEC, general clay fraction mineralogy, and procedures used to determine the above properties.

(D) Methods used to determine the placement of test chambers and the assignment of treatment concentrations to particular test chambers to ensure randomization of exposure.

(E) Frequency, duration, and methods of observations.

(F) Detailed information about the test tadpoles, including the scientific name and method of verification, source of test species, histories of the species, average fresh weight (grams), average size, age, observed diseases, treatments and mortalities, acclimation procedures, and food used.

(G) The number of treatments and replicates used, the number of organisms per replicate, the loading rate, and the flow rate of dilution water for flow-through tests.

(H) A description of the preparation of the sediment/test substance slurry or the treated sediments. A description of the dosing procedures if tadpoles were dosed directly.

(I) The concentration of the test substance in the test slurry or in sediments and/or dilution water in each test chamber just before the start of the test and at all subsequent sampling periods. The concentration of the test substance in the stock solution, if used, and the type and concentration of carrier solvent, if used.

(vii) The measured DO, pH, and temperature and the lighting regime.

(viii) The reported results should include:

(A) The results of the preliminary test and measurements. The number of tadpoles and concentrations of test substance used and observed effects on tadpoles should be stated.

(B) For the definitive test, in each untreated control and for each treatment concentration used:

(*1*) The number of dead and live tadpoles.

(*2*) The percentages of tadpoles that died or showed adverse sublethal effects.

(*3*) The number that showed any abnormal effects.

(*4*) The fresh weights of live tadpoles.

(*5*) The LC50, EC50, LOEC, and NOEC values at days 10, 20, and 30.

Results of the data analysis should include the concentration-response curves with 95 percent confidence limits and the results of a goodness-of-fit (e.g., X^2-square test).

(ix) A description of all circumstances that may have affected the quality or integrity of the data.

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

(xi) The name of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(xii) The signed and dated reports of each of the individual scientists or other professionals involved in the study including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(xiii) The locations where all specimens, raw data, and the final report are stored.

(xiv) The quality control statement prepared and signed by the quality assurance unit.

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

(1) National Research Council. Amphibians: Guidelines for the Breeding, Care, and Management of Laboratory Animals. National Academy of Sciences, Washington, DC (1974).

(2) Perkins, K. W. et al. Reptiles and Amphibians: Care and Culture. Carolina Biological Supply Co., Burlington, NC (1981).

(3) Taylor, A. C. and Kollros, J. J. Stages in the Normal Development of *Rana pipiens*. *Anatomy Records* 94:2 (1946).