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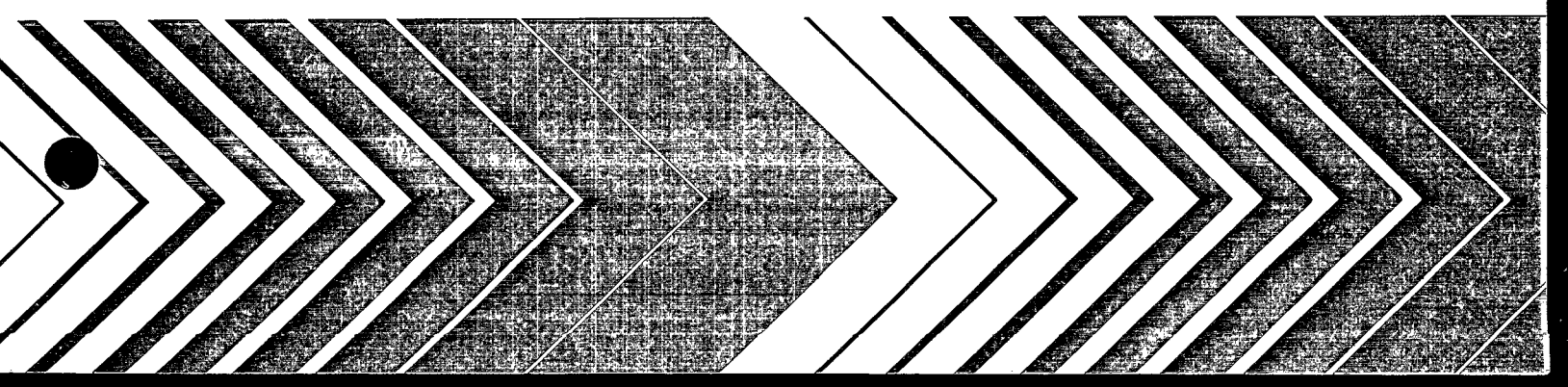
# Manual for the Evaluation of Laboratories Performing Aquatic Toxicity Tests



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# MANUAL FOR THE EVALUATION OF LABORATORIES PERFORMING AQUATIC TOXICITY TESTS

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This document has been reviewed in accordance with U. S. Environmental Protection Agency Policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

# FOREWORD

Environmental measurements are required to determine the chemical and biological quality of drinking water, surface waters, groundwaters, wastewaters, sediments, sludges, and solid waste. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- Develop and evaluate methods to identify and measure the concentration of chemical pollutants.
- Identify and quantitate the occurrence of viruses, bacteria, and other human pathogens and indicator organisms.
- Measure the toxicity of pollutants to representative species of aquatic organisms and determine the effects of pollution on communities of indigenous freshwater, estuarine, and marine organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish.
- Develop and operate a quality assurance program to support achievement of data quality objectives for environmental measurements.

The Federal Water Pollution Control Act Amendments of 1972 (PL 92-500), the Clean Water Act (CWA) of 1977 (PL 95-217), and the Water Quality Act (WQA) of 1987 (PL 100-4) explicitly state that it is national policy that the discharge of toxic substances in toxic amounts be prohibited. Determination of the toxicity of effluents, therefore, plays an important role in identifying and controlling toxic discharges into surface waters. The guidelines in this manual were developed for use by the U.S. Environmental Protection Agency (USEPA) regional and state programs, and the National Pollutant Discharge Elimination System (NPDES) compliance monitoring program to provide standardized procedures for conducting on-site audits and evaluations of laboratories performing toxicity tests of effluents and surface waters.

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# PREFACE

This document provides guidelines for evaluation of biological laboratories involved in toxicity testing and in the culturing of freshwater and marine fish, invertebrates, and plants for use in effluent and surface water toxicity tests. The subjects covered include: evaluation criteria, preparation for the audit and evaluation, organizational history, laboratory personnel, facilities, equipment and supplies, methodology, sample collection, handling, and preservation, quality assurance, records and data reporting, safety, and report preparation. The evaluator performing on-site audits and evaluations of aquatic biology laboratories must have working knowledge of the NPDES program and sufficient knowledge and experience with biomonitoring and toxicity testing methodology. The manual was developed to aid the National Pollutant Discharge Elimination System (NPDES) evaluator/inspector in performing the Compliance Evaluation Inspections (CEI) and the Performance Audit Inspection (PAI) specified in the USEPA (1988a), NPDES Compliance Inspection Manual.

While no formal national USEPA certification program is in place for aquatic biology laboratories performing toxicity tests as part of the NPDES program, guidelines are needed that describe an overall laboratory evaluation program capable of producing valid toxicity data for use in a NPDES permit. Guidelines have been used for several years to assess the capability of the Regional EPA laboratories to provide biological data of acceptable quality.

An overall laboratory evaluation program consisting of four phases is described. Phase I includes preliminary contact between the laboratory and the regulating authority to determine mutually agreeable dates for the on-site inspection, submission of completed pre-survey information forms, and submission of available data on the use of reference toxicants or performance evaluation samples by the laboratory prior to the on-site visit. Phase II is the on-site visit by a qualified evaluator, consisting of a meeting with the senior laboratory staff to explain the audit or evaluation process, a tour of the laboratory facility, one-on-one discussions with the technical staff, examination of documents and records, completion of the on-site evaluation forms and checklists, and an exit debriefing by the evaluator providing a verbal laboratory performance rating of acceptable, minimally acceptable, or unacceptable. Phase III is submission of a final report by the evaluator to the laboratory with a rating indicating reconciliation of any differences and corrective actions required by the laboratory. The final report must clearly state the capabilities of the laboratory to provide acceptable biological data. Phase IV consists of follow-up activities such as technical assistance, resolving major deficiencies, and revisiting the laboratory, if required, to inspect corrected deficiencies and major changes in the laboratory.

## ABSTRACT

This manual describes quidelines and standardized procedures for conducting on-site evaluations of laboratories performing toxicity tests. Included are pre-survey information activities, on-site evaluation activities, evaluation criteria, organizational history and laboratory staff, facilities, equipment, instruments, supplies, culturing and testing methodology, sample collection, handling, preservation, preparation, quality assurance and data handling, safety and general practices, evaluation report and performance rating. Supplementary information on chain-of-custody guidelines, quality control checklist for self-biomonitoring toxicity tests, standard operating procedures (SOPs) format, culturing criteria SOP format, pre-survey information forms, on-site laboratory evaluation forms and checklists, and on-site toxicity test conditions and test acceptability criteria checklists is provided in the Appendices.

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The manual was prepared in part using the following sources: toxicity test manuals, "Methods for measuring the acute toxicity of effluents to freshwater and marine organisms," "Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms," "Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms," by EMSL-Cincinnati; the draft outlines "Performance audit inspection guidance document for the NPDES permit program," by EMSL-Cincinnati; the draft outline "Aquatic toxicity test laboratory evaluation criteria" by EMSL-Cincinnati; the draft document "Evaluation of aquatic biology programs" by EMSL-Cincinnati; "Manual for the certification of laboratories analyzing drinking water. Criteria and procedures quality assurance," 1990a, by the Office of Drinking Water, Washington, D.C.; previous inspections of toxicity testing programs, by USEPA, Regions I, III, and IV; and manuals and checklists for performance audit inspection, evaluation, or certification for aquatic toxicity testing used by USEPA Regions I, III, IV, and state regulatory agencies, i.e., North Carolina, South Carolina, Massachusetts, New Jersey, and New York.

This manual was reviewed by the following members of the EMSL-Cincinnati Bioassay Subcommittee and its parent committee, the Biological Advisory Committee. The reviewers represented individuals from EMSL-Cincinnati, USEPA regional and headquarters programs, and other research and development (ORD) laboratories.

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# SECTION 1

# INTRODUCTION

1.1 The Federal Water Pollution Control Act (FWPCA) Amendments of 1972 (PL 92-500), the Clean Water Act (CWA) of 1977 (PL 95-217), and the Water Quality Act of 1987 (PL 100-4) were enacted to restore and maintain the chemical, physical, and biological integrity of the Nation's waters (Section 101[a]). The legislation contains other specific or implied requirements for the collection of biomonitoring data in at least 15 sections. The Declaration of Goals and Policy, Section 101(a)(3) of these laws, states that "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited." The principal mechanism for reducing and eliminating the discharge of toxic substances is through implementation of the National Pollutant Discharge Elimination System (NPDES) program established by Section 402(a)(1) of the FWPCA.

1.2 During the 1970's and early 1980's, acute toxicity tests were used by the U.S. Environmental Protection Agency (USEPA) regional programs and states to estimate the safe concentration of toxic effluents in receiving waters (USEPA, 1973; 1975; 1978c; 1985a). These methods were supplemented later with short-term (sub-chronic or chronic) toxicity tests (nine days or less) to estimate the chronic toxicity of effluents (USEPA, 1988c; 1989c).

1.3 Based on the growing use of effluent toxicity tests for the control of toxic discharges, USEPA issued a national statement: "Policy for the Development of Water Quality Based Permit Limitations for Toxic Pollutants," in the Federal Register Vol. 49, No. 48, Friday, March 9, 1984. The policy proposed the use of toxicity data to assess and control the discharge of toxic substances through the NPDES permits program. The policy also states that "biological testing of effluents is an important aspect of the water quality-based approach for controlling toxic pollutants. Effluent toxicity data, in conjunction with other data, can be used to establish control priorities, assess compliance with state water quality standards and set permit limitations to achieve those standards." A technical support document and permit writer's guide on the use of effluent and receiving water toxicity data were prepared by the Office of Water Enforcement and Permits (OWEP) and the Office of Water Regulations and Standards (OWRS) to provide additional guidance on the implementation of the biomonitoring policy (USEPA, 1985b; 1988a; 1990a).

1.4 Dischargers of pollutants are issued permits under Section 402 of the Act which set specific limits and operating conditions to be met by the permittee. Section 308 of the Act authorizes inspections and monitoring to determine whether NPDES permit conditions are being met. The Section provides for self-monitoring and USEPA monitoring. USEPA monitoring consists of either evaluating self-monitoring data or performing on-site monitoring. Also, Section 308 and 402 of the Act provide for the delegation of Federal NPDES program authority to States

to issue permits and conduct permit compliance monitoring. Section 122.44 (d)(1)(IV)(V), in addition, specifies that there are some conditions that require the permit to contain effluent limits for whole effluent toxicity.

1.5 USEPA Regional Laboratories are currently audited by the EMSL-Cincinnati. USEPA Regional Laboratories are in turn responsible for auditing and evaluating state laboratories, and USEPA Regional Laboratories and/or some state laboratories are responsible for auditing or evaluating permittee laboratories, such as county, municipal, industrial, utility, and contract service laboratories.

1.6 Presently there is no formal USEPA certification program for laboratories performing aquatic toxicity tests as part of the NPDES permits program. This manual is to provide uniform guidelines for the evaluation of biological laboratories to ensure high quality data. USEPA Regions and several states have requested common guidelines that can be used to evaluate the capability of federal, state, university, private, and local laboratories to produce data on the toxicity of effluents and receiving waters.

1.7 Proposed operation of the certification program is as follows: EMSL-Cincinnati is to certify USEPA Regional laboratories for biological analyses. Regional certification officers are responsible for certification of state laboratories and in turn the state laboratories are responsible for certifying local laboratories. Local laboratories include any state, county, industrial, municipal, utility, federal or private consulting laboratory excluding USEPA Regional and principal state laboratories. EMSL-Cincinnati would also offer an annual training program for all evaluators.

1.8 Regional laboratories and principal state laboratories should annually provide toxicity data using reference toxicants supplied by EMSL-Cincinnati and pass an on-site evaluation every three years.

## SECTION 2

## EVALUATION CRITERIA FOR TOXICITY TESTING LABORATORIES

2.1 To comply with the criteria set forth in this manual, laboratories performing toxicity tests must:

2.1.1 Maintain a qualified staff (see Section 4).

2.1.2 Develop, implement, and maintain a document describing the quality assurance and quality control program, including a laboratory quality assurance plan to ensure that precision, accuracy, completeness, comparability, and representativeness of data are known and documented.

2.1.3 Develop data quality objectives (DQOs) so that determination of data quality is accomplished and met (USEPA, 1984b; 1989a; 1989b). DQOs are qualitative and quantitative statements developed by data users to specify the quality and precision of data needed to support specific decisions or regulatory actions. Establishment of DQOs involves interaction of decision-makers and the technical staff.

2.1.4 Develop and maintain detailed written standard operating procedures (SOPs) for all toxicity tests, culture methods, equipment and instruments, glassware cleaning procedures, sample collection, sample preservation, preparation, chain-of-custody procedures, chemical analyses, quality control, and data analyses. Quality control procedures and techniques must also be included in the SOPs.

2.1.5 Use approved or recommended methods (see Section 7). The culturing, toxicity testing conditions, physical and chemical analyses, test acceptability criteria, and statistical methods for data analyses are found in the following toxicity test manuals (unless otherwise noted):

*EPA/600/4-85/013 - Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, Third Edition or latest edition. NTIS (#PB 85-205383) $31.00

*EPA/600/4-87/028 - Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, First Edition or latest edition. NTIS (#PB 89-220503) $45.00

*EPA/600/4-89/001 - Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Second Edition or latest edition. NTIS (#PB 89-207013) $31.00

*Available from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22165.

2.1.6 Calculate the LC50 (or EC50) for acute toxicity tests using the statistical methods described in the appropriate Agency toxicity test manual.

2.1.7 Calculate the No-Observed-Effect Concentration (NOEC) and Lowest-Observed-Effect Concentration (LOEC) and the linear interpolation method for chronic toxicity tests as described in the appropriate Agency toxicity test manual.

2.1.8 Conform to the specified toxicity test methods or the approved NPDES methodology for analytical methods, sample collection, preservation, and preparation, sample containers, sample holding times, and chain-of-custody of samples.

2.1.9 Provide at least 150-200 square feet of laboratory space and 15-20 linear feet of laboratory bench space (see Section 5). Laboratory space must be appropriate to the types and numbers of tests performed. The building must provide adequate lighting, cooling, and heating to maintain appropriate environmental conditions for culturing organisms and toxicity tests. Hot and cold running water must be available for equipment cleaning.

2.1.10 Provide and maintain separate, compartmentized areas in the laboratory for culturing, toxicity testing, and other chemical analyses (e.g. extractions, ammonia analyses).

2.1.11 Maintain the facilities, instruments, equipment, and supplies so that environmental controls of test conditions meets the criteria for the tests (see Sections 5 and 6).

2.1.12 Report to the regulatory authority, equipment changes or other changes that would affect the ability of the testing laboratory to meet culturing or toxicity test criteria.

2.1.13 Provide appropriate glassware, chemicals, apparatus, disposable supplies, and equipment necessary for culturing and toxicity testing conditions.

2.1.14 Have available the instrumentation for measurements of dissolved oxygen, temperature, conductivity, salinity, and pH. The capability to determine alkalinity and total hardness and to detect total residual chlorine may also be required.

2.1.15 Maintain daily records for physical, chemical, and biological data including all culturing and toxicity testing. Records and data reporting must be kept by the laboratory for not less than three years (USEPA, 1982) or as required by the regulating authority.

2.1.16 Conduct a standard reference toxicant testing program on organisms cultured by the laboratory in order to verify the health and sensitivity of the offspring. The health and sensitivity of the offspring must be determined at least once each month or as recommended in the acute or chronic toxicity test methods or as specified in the NPDES permit. If the laboratory is performing acute and chronic toxicity tests, a reference toxicant(s) must be used for both tests. Each testing program must be described in a separate section of the SOP.

2.1.17 Develop and maintain control charts for each reference-toxicant-organism combination, and successive toxicity values should be plotted and examined to determine if results are within prescribed limits.

2 1.18 Maintain reproducing cultures of test organisms in the laboratory. Use of test organisms for regulatory purposes that are not maintained as a viable laboratory culture may be accepted on a case-by-case basis upon receipt of written permission from regulatory authority. Organisms purchased must be acclimated. A reference toxicant must be used to determine and document health and sensitivity of the purchased organisms.

2.1.19 Demonstrate satisfactory performance on evaluation samples submitted by the regulatory authority requiring the toxicity test. Each laboratory must maintain a written record and report analyses of performance evaluation samples to the proper authority.

2.1.20 Comply with local, state, and federal regulations for handling and disposing of toxic and hazardous waste.

## SECTION 3

## PREPARATION FOR THE EVALUATION

3.1 The evaluator conducting on-site audits and evaluations of aquatic biology laboratories performing toxicity tests must have working knowledge of the NPDES compliance monitoring program and sufficient knowledge and experience with biomonitoring and toxicity testing methodology.

3.2 The laboratory audit or evaluation program consists of four phases.

3.2.1 Phase I includes preliminary contact between the laboratory and the regulating authority to determine mutually agreeable dates, submission of completed pre-survey information forms (Appendix E) by the laboratory prior to the on-site visit and submission of results of performance evaluation samples.

3.2.1.1 Prior to the on-site visit, the laboratory is required to submit (1) all the "Laboratory History" pre-survey information forms provided in Appendix E, (2) an organizational chart, and (3) a copy of its Quality Assurance Plan.

3.2.1.2 The pre-survey information forms (Appendix E) must be completed and returned to the evaluating authority 30 days prior to the on-site visit.

3.2.1.3. The laboratory staff is available during the on-site visit.

3.2.2 Phase II is the on-site visit by an evaluator consisting of an introduction and meeting with the senior laboratory staff involved in the toxicity program to explain the evaluation process. During the on-site visit the evaluator must:

3.2.2.1 Insure that the laboratory has a QA plan in effect by determining if the laboratory has written procedures (QA plan or equivalent) for conducting its quality assurance program.

3.2.2.2 Tour the laboratory facility.

3.2.2.3 Review and verify the items completed on the pre-survey forms during the on-site visit.

3.2.2.4 Have one-on-one discussions with the technical staff.

3.2.2.5 Review the records and written standard operating procedures for compliance with the required toxicity test methods, using the completed pre-survey forms.

3.2.2.6 Examine the quality assurance data to determine if the quality assurance program is being implemented.

3.2.2.7 Evaluate the procedures and equipment used for those specific analyses for which the laboratory has requested the evaluation, using the criteria in this manual.

3.2.2.8 Use the pre-survey forms (Appendix E) and the on-site evaluation forms and checklists (Appendix F and Appendix G) as verification checklists during the on-site visit.

3.2.2.9 Review the results of the evaluation at a debriefing conference with the laboratory director, manager, or supervisor and appropriate staff members.

3.2.2.10 Discuss deviations in the observed culturing and toxicity test precedures and records.

3.2.2.11 Recommend changes in equipment and supply needs, staffing requirements, and facility improvements.

3.2.2.12 Give a verbal laboratory performance rating of acceptable, minimally acceptable, or unacceptable.

3.2.2.13 Agree on a schedule for review of the draft report by the laboratory personnel and submission of the final evaluation report.

3.2.3 Phase III is submission of the final report to the laboratory with a rating by the evaluator indicating reconciliation of any evaluation criteria differences (see Section 2) and corrective actions required by the laboratory (see Section 11).

3.2.3.1 The report must clearly state the capabilities of the laboratory to provide acceptable biological and toxicological data.

3.2.4 Phase IV should consist of follow-up activities.

3.2.4.1 Technical assistance to help resolve deficiencies found during the audit or evaluation process.

3.2.4.2 The evaluating authority should be notified of any major changes in personnel, equipment, and laboratory facilities.

3.2.4.3 If a follow-up visit to the laboratory by the evaluator is necessary to determine whether the deficiencies have been resolved and found acceptable, a time period should be scheduled by the evaluator and proper authority of the laboratory.

## SECTION 4

## ORGANIZATIONAL HISTORY AND LABORATORY STAFF

### 4.1 History

4.1.1 Prior to the on-site visit, the evaluator should become familiar with the staff, facilities, and biological testing experience of the laboratory. The information, gathered during the preliminary contact phase (Phase I) and by use of pre-survey information forms and check lists, may be expanded upon and clarified during the on-site senior staff meeting (Phase II). If the evaluator is not familiar with the laboratory, a tour of the facility must be given during the initial on-site visit.

4.1.2 Laboratories should demonstrate an active biological testing program sufficient to insure that the technical staff maintains expertise in their respective fields.

4.1.3 This information includes the client served, client satisfaction, materials tested, species (organisms) used, test methods, and quality assurance program.

4.1.4 Past performance data on reference toxicants, quality control and performance evaluation samples, compliance monitoring data previously submitted, and previous evaluation reports should be reviewed to identify potential problem areas to concentrate on during the on-site visit. This review will allow the evaluator to provide assistance in areas of greatest need and more efficiently utilize the time allotted to the on-site visit.

4.1.5 The laboratory is requested to complete and return the pre-survey information forms and submit an organizational chart, a resume for the supervisor, each professional biologist/analyst, and technican involved in the culturing and toxicity testing (Appendix E).

### 4.2 Laboratory Staff

4.2.1 The importance of a competent supervisor/manager and professional staff with relevant training and experience is necessary in order to generate valid toxicity testing data.

### 4.3 Manager or Supervisor

#### 4.3.1 Qualification and Responsibilities

4.3.1.1 Is responsible for the overall performance of the laboratory in its execution and reporting of analyses.

4.3.1.2 Must have sufficient academic training and experience to properly implement testing and a quality assurance program.

4.3.1.3 Minimum of a bachelor of science degree in biological sciences or closely related science curricula and three years laboratory experience in aquatic toxicity testing or a master of science degree in biological or closely related science and at least one year laboratory experience in culturing and aquatic toxicity testing.

4.4 Professional Biologist/Analyst

4.4.1 Staff Qualifications

4.4.1.1 The biologist/analyst performs toxicological tests with no or minimal supervision.

4.4.1.2 Academic Training: Minimum of a bachelor's degree in areas of biology, zoology, fisheries, chemistry, environmental science, or related fields.

4.4.1.3 Job Training: Minimum of two weeks formal or on-the-job training each from a federal agency, state agency, or academic institution in culturing and toxicity testing of effluents and surface waters. The amount of training would depend on academic background.

4 4.1.4 Experience: At least one year of bench experience with no or minimal supervision in culturing and toxicity testing aquatic organisms used in the NPDES program.

4.5 Biological Technician

4.5.1 The technician performs toxicity tests and culturing of aquatic organisms with supervision from a professional biologist/analyst.

4.5.2 Academic training: Minimum of high school education. Two years of college in biology, zoology, chemistry, or related fields is recommended.

4.5.3 Job Training: One week of training each in toxicological testing and the culturing of aquatic organisms. Personnel should take advantage of courses available from federal and state agencies, or academic institutions.

4.5.4 Experience: At least one year of culturing aquatic organisms and bench experience in toxicity testing.

SECTION 5

LABORATORY FACILITIES

5.1 General Requirements

5.1.1 Minimum standards for the instrumentation, equipment (see Section 6), and culture units (see USEPA, 1985a; 1988c; 1989c) to perform the toxicity tests are inherent to the production of valid data from fixed or mobile laboratories.

5.1.2 The laboratory facilities should be clean, air conditioned (20°C to 25°C), well ventilated, adequately lighted at the bench top (100 + ft-c), and have adequate workspace. It is recommended that 150 to 200 square feet/person be available. The laboratory should contain at least 10 to 15 linear feet of usable bench space per biologist/analyst to accommodate periods of peak work load. The laboratory must have hot and cold running water.

5.1.3 The laboratory should be secure and be maintained in a clean, organized manner. The laboratory should provide safeguards (see Section 10) to avoid electric shock, prevent accidental chemical spills, equipment failures, and prevent fires.

5.1.4 The laboratory should have policies, procedures, and provisions for the disposal of chemical and toxic wastes. Exhaust hoods are required for the handling of toxic chemicals and samples. This includes venting for sample preparation, extractions, and toxicity testing.

5.1.5 Contamination-free work areas should be provided for handling of test materials.

5.1.6 Adequate facilities should be provided for storage of samples and test materials, including cold storage.

5.1.7 Adequate space should be available for culturing test organisms and preforming the toxicity test. Organisms should be shielded from external disturbances.

5.1.8 Culturing, toxicity testing, and chemical analyses should be done in separate areas.

5.1.9 Volatile compounds or toxic samples should not be used or stored near culture units.

5.1.10 Temperature control of the toxicity tests, culture units, and holding tanks should be achieved using circulating water baths, heat exchangers, or environmental chambers.

5.1.11 Air used for aeration must be free of oil and fumes.

5.2 Laboratory Pure Water

5.2.1 Only satisfactorily tested reagent grade water from deionization or distillation units is used to prepare media, reagents, and dilution/rinse water for culture and toxicity test methods (ASTM Standards and Standard Methods, latest editions).

5.2.2 Reconstituted or synthetic water made with laboratory pure water for culturing and toxicity testing should be tested monthly to assure it meets the following requirements and demonstrates no toxicity to the test organisms:

5.2.2.1 Conductivity ( $\leq$ 0.1 umhos conductivity or $\geq$ 17 megohm resistivity at 25°C).

5.2.2.2 pH (5.5-7.5).

5.2.2.3 Total Chlorine residual (non-detectable).

5.2.2.4 The dilution water made with laboratory pure water should demonstrate no toxicity to the test organisms.

5.2.3 If laboratory pure water does not meet the above requirements, supplementary analyses must be performed to determine the cause.

5.2.4 Toxic metals and organics must not be present in the laboratory pure water.

5.2.4.1 The "USEPA Ambient Water Quality Criteria Documents" and USEPA (1987d) Quality Criteria for Water 1986, Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, D.C., EPA 440/4/86-001 provide data and guidance on acceptability and toxicity of individual metals and organics to aquatic organisms and should be consulted.

5.3 Dilution Water

5.3.1 The choice of the dilution water (reconstituted laboratory pure water or surface water) used in the tests will depend largely on the objectives of the study, and what is required in the NPDES permit. The dilution water should demonstrate no toxicity to the test organisms (see USEPA, 1985a; 1988c; 1989c).

5.3.2 To prepare a synthetic (reconstituted) freshwater or a synthetic (artificial) seawater, use only reagent grade chemicals or recommended commercial sea salts (see USEPA, 1985a; 1988c; 1989c).

5.3.3 To prepare a synthetic seawater from manufactured synthetic sea salts, follow the directions of the manufacturer and USEPA (1988c) in making the dilution water. To prepare hypersaline brine derived from

natural seawater, see USEPA (1988c). Artificial seawater should be used only if specified in the culturing and toxicity testing methods.

5.4 Glassware Washing

5.4.1 The guidance provided below is intended to eliminate toxicity associated with glassware. If controls show toxicity additional cleaning steps may be required.

5.4.1.1 All glassware, sample containers, test vessels, pumps, tanks, and other equipment that need cleaning or come in contact with effluent must be washed and rinsed with laboratory pure water to remove surface contaminants (see USEPA, 1985a; 1988c; and 1989c).

5.4.1.2 New plastic ware, used for sample collection or organism exposure vessels, may or may not require soaking or rigorous cleaning. It may be sufficient to rinse the new containers once with deionized water and sample water before use, but control tests with the new containers may be required to see if toxicity occurs before using them in toxicity testing. If toxicity is found during the control tests, more rigorous cleaning will be required.

## SECTION 6

## EQUIPMENT, INSTRUMENTS, AND SUPPLIES

6.1 Necessary and appropriate equipment, instruments, and supplies must be available in adequate quantities for culturing test organisms and for performing the toxicity tests being conducted. The evaluator should limit the review to those tests performed routinely.

6.2 All materials in contact with a sample or the test organisms should be nontoxic. Materials used should not reduce or add to the sample toxicity. Adequate survival and growth in the cultures and test control solutions and acceptable performance on reference toxicants, would imply appropriate materials are being used. The date of receipt and lot number of disposable supplies being used should be recorded. Any changes in cleaning procedures should be noted so that changes in performance might be correlated with these items.

6.3 Manufacturers' operating manuals, standard operating procedures (SOPs) and equipment maintenance log books should be evident to the evaluator, and available and used by the operator/analyst. Balances and other major equipment should be serviced regularly. Lists of reagents and consumable materials are specified in the latest editions of the USEPA acute and chronic toxicity test methods.

6.4 Design, performance or use specifications for selected equipment and supplies for toxicity test methods are provided below:

6.4.1 pH Meter: Scale of 0-14 pH units with accuracy and scale readability to at least $\pm$ 0.1 units. Laboratories are encouraged to purchase meters capable of functioning with specific ion or other electrodes. Units may be line or battery powered.

6.4.2 Dissolved Oxygen Meter: Capable of measurements at 0-100% saturation. Field or laboratory units with accuracy specifications of at least 0.1 mg/L are acceptable.

6.4.3 Analytical Balance: Capable of accurately weighing to 0.01 mg (0.00001 g). The balance must be seated on a steady base to prevent vibration and protected from air currents. Class S certified weights should be available, and the balance must be checked with the weights each time it is used to document acceptable performance of the balance.

6.4.4 Conductivity Meter: Suitable for checking reagent water quality and saline water. Should be readable in ohms or megohms and should have a range from 2 ohms to 20 megohms. Unit may be line or battery operated.

6.4.5 Thermometer: Mercury-filled, centigrade thermometer or digital thermometer with 1°C or finer subdivisions. Continuous recording electronic-chart thermometer or bulb thermographs capable of documenting

a 1°C or less temperature change are acceptable. A certified or National Institute of Standard Technology (NIST) traceable thermometer should be available for calibration checks.

6.4.6 Drying Oven: Gravity or mechanical convection unit with selectable temperature control from room temperature to 180°C.

6.4.7 Refractometer: Hand held, automatic temperature compensated refractometer calibrated for salinity measurements from 0-160 parts per thousand (ppt).

6.4.8 Compound Microscope, Dissecting Microscope, and Magnifying Lens: Hand held or supported, with appropriate light source, for examining small organisms in the test chambers or for examining cells on microscope slides.

6.4.9 Radiometer (light meter): Capable of measuring the intensity of ambient room light over a range of at least 0-200 uE/$m^2$/s (0-1000 ft-candles).

6.4.10 Water Purification System: Consisting of any combination of distillation, reverse osmosis, ion exchange, activated carbon and particle filtration capable of producing nontoxic deionized water of 18 megohms (0.05 umho conductivity) resistivity. Commercially available cartridge systems are preferred (See ASTM, Volume 11.01, D 1193, "Standard Specification for Reagent Water," Type I Reagent Water).

6.4.11 Mechanical Shaker: Variable speed capable of providing orbital motion at a rate of 100 cycles per minute.

6.4.12 Fluorometer or UV-VIS Spectrophotometer: Suitable for measurements of chlorophyll a and performing colorimetric analyses.

6.4.13 Electronic Particle Counter: Coulter counter or equivalent capable of mean cell volume computation (MCV).

6.4.14 Environmental Chamber/Incubator: Capable of maintaining temperatures of 20°C ± 2°C, 24°C ±2°C, and/or 25° ± 1°C

6.4.15 Autoclave: Capable of producing 1.1 Kg. $cm^2$ (15 psi) pressure at 121°C (250°F).

6.4.16 Refrigerator: Explosion proof and capable of maintaining a temperature of 4°C for sample storage; lockable, if for chain-of-custody requirements.

6.4.17 Freezer: Capable of maintaining a temperature of -20°C for storage.

6.4.18 Air Compressor, Air Pumps: capable of producing oil free air.

6.4.19 Standby Generator(110 VAC): For electrical backup in emergency.

6.4.20 Light Box: For illuminating embryos, larvae, and organisms during examination.

6.4.21 Desiccator: For keeping specimens free of moisture.

6.4.22 Amperometric Titrator: For measurement of free, total, and combined chlorine.

6.4.23 Vacuum Pump: Electric powered, capable of providing vacuum in the range of 1-25 Hg.

6.4.24 Counting Chamber: Hemacytometer, Palmer-Maloney, Sedgwick-Rafter, for counting sea urchin gametes and algal cells (Selenastrum capricornutum).

6.4.25 Centrifuge: General purpose bench top or floor model; producing 1000xg; capable of accepting bottles or tubes appropriate for the sample volumes used.

6.4.26 Exhaust Hood: For handling reagents, potentially-toxic samples, and controlling toxic fumes.

6.4.27 Water Bath: For controlling test temperatures of 20-25°C ± 1°C.

6.4.28 Personal Computer (PC): for data analyses.

## SECTION 7

## METHODOLOGY

7.1 One of the most important aspects of the evaluation process is to determine if approved methods are being used by the laboratory. All activities including organism culturing, sample collection, handling, preservation, and preparation, toxicity testing, physical and chemical analyses, and toxicity test data analysis must be covered by written standard operating procedures, protocols or analytical procedures. These written SOP documents must be available to, and understood and used by, the laboratory staff. The evaluator must also determine if these written procedures are consistent with any required (approved) methods and if any significant deviations occur in either the written procedures or in the implementation of the procedures. Any deviations from standard test methods should be documented.

7.2 The evaluator should review the methods used by the laboratory, as stated on the completed pre-survey forms, prior to the on-site visit. The following sources of methods should be available to the laboratory staff:

7.2.1 Standard methods for the examination of water and wastewater. American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C 20005 (latest edition).

7.2.2 Annual Book of ASTM Standards, Vols. 11.01, 11.02, and 11.04. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103 (latest editions).

7.2.3 Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, EPA-600/4-85-013 or latest edition. U.S. Environmental Protection Agency, EMSL-Cincinnati, Cincinnati, OH 45268.

7.2.4 Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, EPA/600/4-89-001 or latest edition. U.S. Environmental Protection Agency, EMSL-Cincinnati, Cincinnati, OH 45268.

7.2.5 Short-term methods for estimating chronic toxicity of effluents and receiving waters to marine and estuarine organisms, EPA/600/4-87-028 or latest edition. U.S. Environmental Protection Agency, EMSL-Cincinnati, Cincinnati, OH 45268

7.2.6 Methods for chemical analysis of water and wastes, EPA/600/4-79-020 or latest edition. U.S. Environmental Protection Agency, EMSL-Cincinnati, Cincinnati, OH 45268.

7.2.7 Handbook for analytical quality control in water and wastewater laboratories, EPA/600/4-79-019 or latest edition. U.S. Environmental Protection Agency, EMSL-Cincinnati, Cincinnati, OH 45268.

7.2.8 Methods for organic chemical analysis of municipal and industrial wastewater, EPA/600/4-82/057 or latest edition. U.S. Environmental Protection Agency, EMSL-Cincinnati, Cincinnati, OH 45268.

7.2.9 Technical support document for water quality-based toxic control. Office of Water Enforcement and Permits, Office of Water Regulations and Standards, EPA/440/4-85-032 or latest edition. U.S. Environmental Protection Agency, Washington, D.C. 20460.

## SECTION 8

## SAMPLE COLLECTION, HANDLING, PRESERVATION, AND PREPARATION[1]

### 8.1 Introduction

8.1.1 Specific requirements for sample collection and handling should be specified in NPDES permits and/or compliance monitoring documents. The evaluator should become familar with the requirements contained in these documents.

8.1.2 The following items are only applicable to laboratories delegated responsibility for sample collection. All laboratories are responsible for items 8.1.5 and 8.1.6.

8.1.3 Sampling frequency must conform to that specified by permits or regulations. Collectors should be trained in sampling procedures and approved by the appropriate regulatory authority or its designated representative.

8.1.4 Applicable state regulations pertaining to chain-of-custody must be followed. An example of chain-of-custody procedures is presented in Appendix A.

8.1.5 The report form must include the date and time of sample arrival at the laboratory and the date and time analysis begins.

8.1.6 Holding/transit time between sampling and analysis must not exceed 36 hours. If the laboratory is required by permits and regulations to examine samples within 36 hours, the laboratory is to indicate that the data may be invalid because of excessive delay before sample processing (see 8.4.1).

### 8.2 Effluent Sampling

8.2.1 The effluent sampling point should be the same as that specified in the NPDES discharge permit (USEPA, 1988a).

8.2.2 Conditions for exception would be the following, but they must be approved by permitting authority:

8.2.2.1 Better access to a sampling point between the final treatment and the discharge outfall.

8.2.2.2 If the processed waste is chlorinated prior to discharge to the receiving waters, it may also be desirable to take samples prior to contact with the chlorine to determine toxicity of the unchlorinated effluent.

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[1]Adapted from: USEPA (1985a), (1988c), and (1989c).

8.2.2.3 In the event there is a desire to evaluate the toxicity of the influent of municipal waste treatment plants or separate wastewater streams in industrial facilities prior to their being combined with other wastewater streams or non-contact cooling water, additional sampling points may be chosen.

8.2.3 The decision on whether to collect grab or composite samples should be specified in the NPDES permit and is based on the objectives of the test and an understanding of the short and long-term operations and schedules of the discharger. If the effluent quality varies considerably with time, which can occur where holding times are short, grab samples may be preferable because of the ease of collection and the potential of observing peaks (spikes) in toxicity. However, the sampling duration of a grab sample is so short that full characterization of an effluent over a 24-h period would require a prohibitive number of separate samples and tests. Collection of a 24-h composite sample, however, may dilute toxicity spiking and averages the quality of the effluent over the sampling period. A lengthy discussion of the advantages and disadvantages of sample types (grab or composite samples) is found in USEPA (1985a) or (1989c). The regulatory program or NPDES permit should dictate sample collection objectives.

8.2.4 Aeration during collection and transfer of effluents should be minimized to reduce the loss of volatile chemicals.

8.2.5 Definitive tests performed for NPDES permit purposes, unless otherwise specified in permit, require daily effluent sample collection and daily renewal of test solutions.

8.3 Receiving Water Sampling

8.3.1 It is common practice to collect grab samples for receiving water toxicity studies.

8.3.2 When non-toxic receiving water is required for a test, it may be collected upstream from the outfall or from other surface water known to be uncontaminated and which has properties similar to the receiving water (see USEPA, 1989c). If the objective of the test is to determine the additive effects of the discharge on receiving water which may be contaminated, the test is performed using dilution water consisting of receiving water collected daily upstream from the outfall.

8.3.3 Dilution water to be taken from the receiving water upstream from the outfall, is collected at a point as close as possible to the outfall, but upstream from or outside of the zone influenced by the effluent.

8.3.4 To determine the extent of the zone of toxicity in the receiving water downstream from the outfall, receiving water samples are collected at several distances downstream from the discharge. The time required for the effluent-receiving-water mixture to travel to sampling points downstream from the outfall, and the rate and degree of mixing, may be difficult to ascertain. It may not be possible to correlate downstream toxicity with effluent toxicity at the discharge point unless a dye study is performed. The

toxicity of receiving water samples from five stations downstream from the discharge point can be evaluated using the same number of test vessels and test organisms as used in one effluent toxicity test with five effluent dilutions.

8.4 Sample Handling, Preservation, and Shipping

8.4.1 If the data from the samples are to be acceptable for use in the NPDES Program, the elapsed time from collection of a grab or composite sample to its first use for initiation of the test, or for test solution renewal, should not exceed 36 h. In no case should a sample be used in a test more than 72 h after removal from the sampling device. Composite samples must be chilled during collection and maintained at 4°C until warmed up for use.

8.4.2 Samples Used in On-Site Tests

8.4.2.1 Samples collected for on-site tests should be used within 24 h.

8.4.3 Samples shipped to Off-Site Facilities

8.4.3.1 Samples collected for off-site toxicity testing should be chilled during collection and maintained at 4°C until used or shipped iced to the central laboratory, and there transferred to a refrigerator (4°C) until used. Every effort must be made to initiate the test with an effluent sample on the day of arrival in the laboratory.

8.4.3.2 Samples may be shipped in 4-L (1-gal) CUBITAINERS$^{R}$, or new plastic "milk" jugs. All sample containers should be rinsed with source water before being filled with sample. CUBITAINERS$^{R}$ and plastic jugs are not to be reused. CUBITAINERS$^{R}$ and plastic jugs used for effluents or toxic surface water samples should be punctured after use to prevent reuse.

8.4.3.3 Several sample shipping options are available, including Express Mail, air express, bus, and courier service. Express Mail is delivered seven days a week. Shipping and receiving schedules of private carriers on weekends vary with the carrier.

8.5 Sample Preparation

8.5.1 With Ceriodaphnia dubia or other cladoceran invertebrate and fish tests, effluents and surface waters must be filtered through a (60 um) plankton net to remove indigenous organisms that may attack or be confused with the test organisms (see Ceriodaphnia dubia test methods, USEPA, 1989c, for details). Surface waters used in algal toxicity tests must be filtered through a 0.45 um pore diameter filter before use. It may be necessary to first coarse-filter the dilution and/or waste water through a nylon sieve having 2-4 mm holes to remove debris and/or break up large floating or suspended solids. Caution: filtration may remove toxicity.

8.5.2 The dissolved oxygen (DO) concentration in the dilution water should be

near saturation prior to use. Aeration will bring the DO and other gases into equilibrium with air, minimize oxygen demand, and stabilize the pH.

8.5.3 If the dilution water and effluent must be warmed to bring them to the prescribed test temperature, supersaturation of the dissolved gases may become a problem. To prevent this problem, the effluent and dilution water are checked for dissolved oxygen (DO) with a probe after heating to 25°C. If the DO is greater than 100% saturation (8.5 mg/L) or lower than 40% saturation, the solutions are aerated moderately with a pipet tip for a few minutes until the DO is within the prescribed range.

## SECTION 9

## QUALITY ASSURANCE[1] AND DATA HANDLING

### 9.1 Quality Assurance/Quality Control Definitions[2]

9.1.1 There is often confusion about the specific definitions of the terms "Quality Assurance" and "Quality Control". While many people recognize a distinction, the terms are often used interchangeably.

9.1.2 Quality Assurance is the total program for assuring the reliability of monitoring data.

9.1.3 Quality Control is the routine application of procedures for controlling the measurement process.

9.2 Each laboratory shall have a written quality assurance (QA) plan. A routine, on-going, quality assurance (QA) program and quality assurance plans are necessary to insure and document the quality of the data produced. Quality assurance/quality control (QA/QC) should be a continous process implemented throughout the entire culturing and toxicity testing program.

9.2.1 Three guidance documents are available from USEPA to assist in preparation of the QA plan. USEPA (1980b) describes management policies, organization, objectives, principles, and general procedures to establish how data of known and acceptable quality should be produced. USEPA (1980c) is a general guidance document, and USEPA (1984a) is a more detailed guidance document that combines a work plan with the QA project plan.

9.2.3 The QA program should cover all aspects of the biological testing activity, including sampling and sample handling, test conditions, equipment, methodology, record keeping and data evaluation, which are subject to QA/QC procedures. The data quality indicators (e.g, the desired precision in controls and in reference toxicants) and objectives developed with this QA program including overall precision and accuracy should accompany all data produced by the laboratory.

9.2.4 The determination of data quality is accomplished through the development of data quality objectives (DQOs) by the interaction of decision-makers and the technical staff (see USEPA, 1984b; 1989a; 1989b).

9.2.4.1 DQOs are qualitative and quantitative statements developed by data users to specify the quality of data needed to support specific decisions or regulatory actions.

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[1]Adapted from: USEPA (1978c), (1985a), (1988c), (1989c), and (1979c).
[2]USEPA (1978a). Newsletter Quality Assurance. Environmental Monitoring and Support Laboratory - Cincinnati.

9.2.5 For general guidance on good laboratory practices related to toxicity testing, see: FDA (1978), USEPA (1979a, 1980a, 1980e, 1988b), and DeWoskin (1984).

9.3 Appropriate sampling, sample handling and preservation should include considerations for selection of the sampling locations and number of samples necessary to adequately represent the source. Additional considerations concern the use of composite or grab samplers, sample containers, volume of sample required, collection of appropriate dilution waters, preservation at 4°C if samples are held for more than 24 hours. Location, date, and time of sampling must be recorded as well as the date and time that analysis was initiated. If necessary, chain-of-custody procedures should be used.

9.4 The test organisms must be in good health, be disease-free, show minimum mortality in holding tanks, and demonstrate "acceptable" performance in control test solutions. Additionally, the health of the organism should be documented by periodic use of reference toxicant testing.

9.5 Documentation of instrument and equipment performance, calibration, and maintenance must be available for review. Balances and other major equipment should be serviced annually.

9.6 Methods and procedures used should be well documented and available to the staff and the evaluator. Any deviations from the methods as written, or options used, should be noted.

9.7 Field and laboratory/bench data should be kept in bound notebooks. Additional data such as bench sheets, sample receipt forms or chain-of-custody records should be referenced and noted. Use of electronic (computer) data bases is acceptable if adequate security and backup are maintained.

9.8 Data evaluation procedures should be reviewed to determine if adequate statistical support is available and used. Data from toxicity tests should be plotted as a preliminary step to aid in interpretation of results and to help detect problems and expected trends or patterns in the responses. Recommended statistical programs should be used to calculate results.

9.9 Reference toxicants and control charts should be used to document (1) the health of the organisms used, (2) data quality, and (3) the overall laboratory performance. Control charts or acceptance ranges should be constructed or calculated and routine QC data plotted or compared to these pre-established criteria. The laboratory should demonstrate that the QC data generated is used to document data quality.

9.10 Proper documentation of the entire biological testing process is critical in supporting the validity of the data produced. Bound notebooks or secure computer data bases should be used to maintain detailed records of all data required by the methods and covered in this manual. Annotations to the record should be made as soon as possible (at least daily) to minimize or prevent any loss of information. These records should be made available to the evaluator during the on-site visit. The record should be complete enough

to follow a sample chronologically through the entire collection, measurement, data collection and sample disposal process. Data reports should include the DQOs and data quality indicators developed by the laboratory's quality assurance program as an aid to the ultimate data user.

9.11 Procedures for confirming data validity (e.g., checking final reports to laboratory bench sheets) should be performed.

9.12 All bound notebooks should have consecutively numbered pages and no pages should be missing. Pages with errors should be corrected near where the error occurs and initialed and dated by the person making the correction. Pages with multiple errors or with major problems such as spills or illegible ink marking should not be discarded, but should remain in the bound notebook. The next page or a page nearby should be used to re-enter information. Waterproof plastic-coated paper notebooks are available.

9.13 All information reported on the pre-survey information forms and checklists should be confirmed and possibly expanded upon during the on-site visit.

9.14 Data validation may include a comparison of final reports with lab bench sheet data.

## SECTION 10

## SAFETY AND GENERAL PRACTICES

### 10.1 Introduction

10.1.1 While safety is not an integral part of laboratory evaluation, the evaluator should be aware of unsafe conditions and lack of, or weaknesses in a, formal safety program. This program should begin with management support and include the assignment of health and safety responsibility, maintenance of safe working conditions, establishment of safety training, accident reporting, medical and first aid treatment and acceptance of the program by the staff. Written safety policies should be available to the laboratory staff and to the evaluator for review.

10.1.2 Basic good housekeeping practice should also be evident in a neat and orderly laboratory and office environment. These practices must be adequate to protect the staff from physical injury and exposure to hazardous or toxic substances, to avoid interference with laboratory operations, and to assure the production of valid data of known quality (see USEPA, 1985a; 1988c; 1989c).

10.1.3 Training on the use of safety equipment, first aid and medical emergency treatment should be documented.

### 10.2 Safety Equipment and Supplies

10.2.1 Necessary and appropriate safety apparel such as aprons, lab coats, respirators, gloves, safety glasses and shoes, and hard hats should be available.

10.2.2 First aid kits, fire extinguishers and blankets, safety showers and emergency spill kits should be available as well as a record of their maintenance and inspection.

10.2.3 Mobile and remote locations should be equipped with a communication system to summon help in case of an emergency. It is recommended that personnel not work alone in the field.

10.2.4 Facilities should be available for soap and water cleaning of exposed body parts that may be contaminated by effluent samples.

### 10.3 Safety Practices

10.3.1 All personnel handling environmental samples known or suspected to contain human waste should be immunized against disease causing agents.

10.3.2 The exterior of all sample containers should be protected from contamination during sample collection and handling. If the outside of the container is contaminated, it should be decontaminated to prevent human

exposure. Sample spills must be cleaned up and/or neutralized (all acids), as required, to prevent human exposure.

10.3.3 Sample labels and identification should be adequate to alert personnel of potential or known hazards.

10.3.4 Material safety data sheets should be available for all chemical preservatives and reagents used.

10.3.5 Work with and disposal of effluent samples should be performed in compliance with current federal, state, and local rules pertaining to hazardous materials.

10.3.6 All electrical equipment should be properly grounded. Ground fault interrupters should be used in all wet lab areas.

## SECTION 11

## EVALUATION REPORT

11.1 Prior to termination of the on-site visit, the evaluator should have an exit debriefing meeting with the laboratory senior staff. At this debriefing the following findings should be discussed:

11.1.1 Any deviations or inadequacies in procedures, documentation, and/or records.

11.1.2 Recommendations concerning equipment and supply needs, staffing, and facility improvements.

11.1.3 Areas in which the evaluating authority can provide technical assistance.

11.1.4 Performance rating of the laboratory: acceptable, minimally acceptable, or unacceptable.

11.1.5 During this open discussion it is important to provide the laboratory with all of the findings, to rate the performance of the laboratory, and to provide recommendations for correction and improvement. In some instances all the findings that are discussed will be incorporated into a draft report. This will avoid any misunderstandings and allow time for the laboratory to take appropriate corrective actions prior to preparation and distribution of the final report. It should be stressed that the report is intended to provide positive information on how the laboratory may improve performance and not intended to be a negative or punitive device. It should be determined and agreed upon at this meeting what the distribution of the final report shall be.

11.2 After completion of the on-site visit, the evaluator prepares a draft narrative report that contains all of the information pertinent to the evaluation that was discussed at the debriefing and actions taken as a result of the evaluation. The draft report should be sent to the laboratory for review, comment and action prior to any outside distribution. Additional follow-up visits may be required to verify corrections of serious problems. From this draft report and review comments the final report is prepared that includes the general headings and information listed below:

11.3 Title Page

11.3.1 This page should include the following: Name of laboratory, address, date of the on-site visit, name, title, address, and signature of the evaluator.

11.4 Commendations

11.4.1 Point out those areas where the laboratory exceeds the minimum standards of performance.

11.5 Deviations

11.5.1 List each deviation and describe it in detail. Provide recommendations or appropriate corrective actions. Point out specifically those areas where deviations occurred previously and where recommended corrective actions were not taken.

11.6 Remarks

11.6.1 Recommend improvements which, while not affecting the evaluation status, would improve laboratory operations.

11.7 List of Personnel

11.7.1 List names and title of all technical personnel along with the functions that each one normally performs. Also identify the critical laboratory personnel that could influence the performance rating of the laboratory if their job functions were changed. Note in this section that the evaluating authority must be notified if any of the critical personnel are involved in changes of job function and how the critical functions will be performed in the future.

11.8 Signatures

11.8.1 The evaluation report should be signed by the evaluator and any accompanying letter or action memorandum should state the performance rating granted and be signed by the evaluating authority.

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APPENDIX A

CHAIN-OF-CUSTODY GUIDELINES[1]

1. Introduction

1.1 Written procedures for sample handling should be available and followed whenever samples are collected, transferred, stored, analyzed, or destroyed. For the purposes of litigation, it is necessary to have accurate records which can be used to trace the possession and handling of samples from the moment of collection through analysis. Secure, computer based data management systems may be appropriate. The procedures defined here represent a means to satisfy this requirement.

1.1.1 A sample is in someone's "custody" if:

it is in one's actual physical possession;

it is in one's view, after being in one's physical possession;

it is in one's physical possession and then locked up so that no one can tamper with it, and it is kept in a secured area, restricted to authorized personnel only.

2. Sampling Collection, Handling, and Identification

2.1 It is important that a minimum number of persons be involved in sample collection and handling. Guidelines established in standard manuals for sample collection, preservation, and handling should be used (e.g., USEPA, NPDES Compliance Inspection Manual EN-338 (1988a); Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (1985a) or latest edition, Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600-4-85-013.

2.2 Field records should be completed at the time the sample is collected and should be signed or initialed, including the date and time, by the sample collector(s).

2.3 Field records should contain the following information:

a. unique sample number;
b. date and time;
c. type of sampler;

---

[1]Adapted from: USEPA (1982a) and (1990a).

d. source of sample (facility name, location, and sample type);
e. preservative used (if preserved);
f. analyses required;
g. name of collector(s);
h. pertinent field data (pH, DO, Cl residual, etc.);
i. serial number on seals and transportation cases.

2.4 Each sample is identified by affixing a pressure sensitive gummed label or standardized tag on the container(s) (Figure 1). This label should contain the sample number, source of sample, preservative used, and the collector(s) initials. Analysis required should be identified. Where a label is not available, the same information should be written on the sample container with an indelible marking pen.

2.5 The sample container should then be placed in a transportation case along with the chain-of-custody record form (Figure 2.), pertinent field records, and analysis request form. The transportation case should then be sealed and labeled. All records should be filled out legibly in pen. The use of locked or sealed chests will eliminate the need for close control of individual sample containers. However, there will undoubtedly be occasions when the use of a chest will be inconvenient. On these occasions, the collector should place a seal around the cap of the individual sample container which would indicate tampering if removed.

3. Transfer of Custody and Shipment

3.1 When transferring the possession of the samples, the transferee must sign and record the date and time on the chain-of-custody record (Figure 2). Custody transfers, if made to a sample custodian in the field, should account for each individual sample, although samples may be transferred as a group. Every person who takes custody must fill in the appropriate section of the chain-of-custody record.

3.2 The field custodian (or field collector if a custodian has not been assigned) is responsible for properly packaging and dispatching samples to the appropriate laboratory for analysis. This responsibility includes filling out, dating, and signing the appropriate portion of the chain-of-custody record.

3.3 All packages sent to the laboratory should be accompanied by the chain-of-custody record and other pertinent forms. A copy of these forms should be retained by the field custodian (either carbon or photocopy).

3.4 Mailed packages can be registered with return receipt requested. If packages are sent by common carrier, receipts should be retained as part of the permanent chain-of-custody documentation.

3.5 Samples to be transported must be packed to prevent breakage. If samples are shipped by mail or by other common carrier, the shipper must comply with any applicable Department of Transportation regulations. The package must be sealed or locked to prevent tampering. Any evidence of tampering should be readily detected if adequate sealing devices are used.

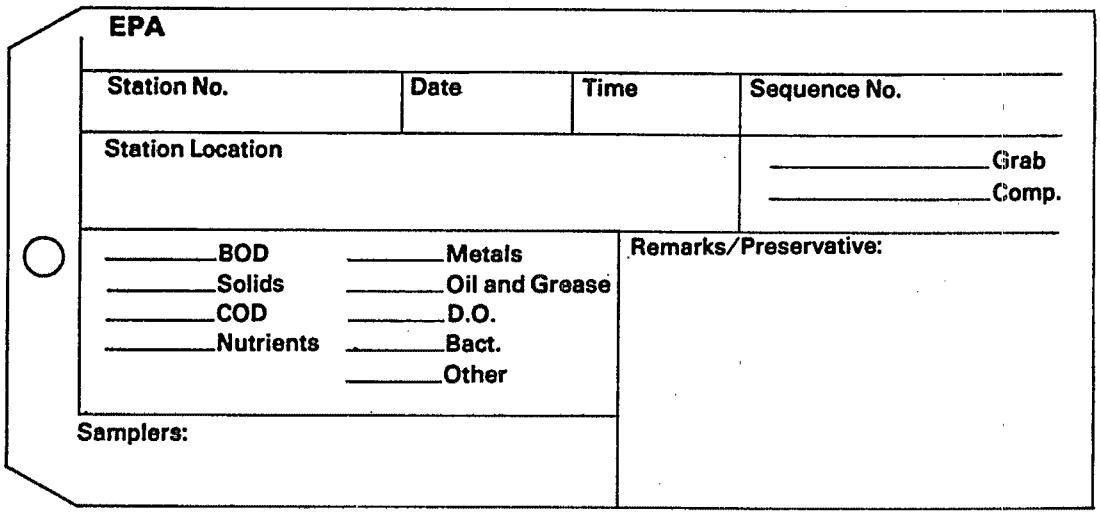


Figure 1. Example of Sample Identification Tag.

| Survey | | | | Collectors: Signature | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Station Number | Station Location | Date | Time | Sample Type Water Comp. | Water Grab | Air | Seq. No. | No. of Containers | Analysis Required |
| | | | | | | | | | |
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| | | |
|---|---|---|
| Relinquished by: *Signature* | Received by: *Signature* | Date/Time |
| Relinquished by: *Signature* | Received by: *Signature* | Date/Time |
| Relinquished by: *Signature* | Received by: *Signature* | Date/Time |
| Relinquished by: *Signature* | Received by Mobile Laboratory for Field analysis: *Signature* | Date/Time |

| | | | |
|---|---|---|---|
| Dispatched by: *Signature* | Date/Time | Received for Laboratory by: | Date/Time |

Method of Shipment:

Distribution: Orig.—Accompany Shipment
1 Copy—Survey Coordinator Field Files

Figure 2. Example of Chain-of-Custody Record.

3.6 If the field collector delivers samples to the laboratory, custody may be relinquished to laboratory personnel. If appropriate personnel are not present to receive the samples, the samples shall be locked in a designated area of the laboratory to prevent tampering. Tampering with field samples is prohibited. The person delivering the samples should make a log entry stating where and how the samples were delivered and secured. Laboratory personnel may then receive custody by noting in a log the absence of evidence of tampering, unlocking the secured area, and signing the custody sheet.

4. Laboratory Sample Control Procedures

4.1 Sample control procedures are necessary in the laboratory from the time of sample receipt to the time the sample is discarded. The following procedures are recommended for the laboratory:

4.1.1 There must be a designated custodian and an alternate person to act in his or her absence. All incoming samples must be received by the custodian, who must indicate receipt by signing the accompanying custody/control forms and who must retain the signed forms as permanent records.

4.1.2 The custodian must maintain a permanent log book to record, for each sample, the person delivering the sample, the person receiving the sample, date and time received, source of sample, date the sample was taken, sample identification or log number, how transmitted to the laboratory, and condition received (sealed, unsealed, broken container, or other pertinent remarks). This log should also show the movement of each sample within the laboratory; i.e., who removed the sample from the custody area, when it was removed, when it was returned, and when it was destroyed. A standardized format should be established for log entries.

4.1.3 A clean, dry, isolated room, building, and/or refrigerated space that can be securely locked from the outside must be designated as a "custody room."

4.1.4 The custodian must ensure that heat-sensitive samples, light-sensitive samples, radioactive samples, or other sample materials having unusual physical characteristics, or requiring special handling, are properly stored and maintained prior to analysis.

4.1.5 Distribution of samples to the analyst performing the analysis must be made by the custodian.

4.1.6 The laboratory area must be maintained as a secured area, restricted to authorized personnel only.

4.1.7 Laboratory personnel are responsible for the care and custody of the sample once it is received by them and must be prepared to testify that the sample was in their possession and view or secured in the laboratory at all times from the moment it was received from the custodian until the time the analyses are completed.

4.1.8 Once the sample analyses are completed, the unused portion of the sample, together with all identifying labels, must be returned to the custodian. The returned tagged sample must be retained in the custody room until permission to destroy the sample is received by the custodian.

4.1.9 Samples will be destroyed only upon the order of the responsible laboratory official when it is certain that the information is no longer required or the samples have deteriorated. The same procedure is true for sample tags. The log should show when each sample was discarded or if any sample tag was destroyed.

4.1.10 Procedures must be established for audits of sample control information. Records should be examined to determine traceability, completeness, and accuracy.

APPENDIX B

## Quality Control Checklist for Self-Biomonitoring Toxicity Tests[1]

Permit No. _____
Facility Name _____
Facility Location _____
Test Date _____
Reviewer _____
Date Reviewed _____
Permit Requirement _____

_____

| ITEM | (CHECK ONE) | YES | NO |
|---|---|---|---|
| Report Format meets EPA Methods requirement? (see Weber, et al., 1988, 1989.) | | ____ | ____ |
| Were EPA test Methods followed? | | ____ | ____ |
| Test Evaluation: | | | |
| A. Effluent Sampling | | | |
| 1. Grab or Composite (compare to permit) | | ____ | ____ |
| 2. Was holding time met? (36 hrs. or 72 hrs.) | | ____ | ____ |
| B. Dilution Water | | | |
| 1. Source _____ | | | |
| 2. Meets EPA requirements | | ____ | ____ |
| C. Test Type | | | |
| 1. Acute _____ Chronic _____ | | | |
| 2. Test duration _____ | | | |
| 3. Static _____, Static renewal _____, Flow-through _____ | | | |
| 4. Chronic renewal_____, Chronic Flow-through _____ | | | |
| D. Test Organisms | | | |
| 1. Permit requirement (List species) _____ | | | |
| _____ | | | |
| 2. Age of Test Organisms _____ | | | |
| _____ | | | |
| _____ | | | |
| 3. Source of Test Organisms _____ | | | |
| _____ | | | |

[1]Adapted from: William Peltier, USEPA, Environmental Services Division, Region IV.

Quality Control Checklist for Self-Biomonitoring Toxicity Tests (Continued)

| ITEM | (CHECK ONE) | YES | NO |
|---|---|---|---|
| E. Test Temperature: (for each day of test) | | | |
| 1. Acute $\pm 2^{o}C$ | | ____ | ____ |
| 2. Chronic $\pm 1^{o}C$ | | ____ | ____ |
| F. Dissolved Oxygen $\geq$ 40% Saturation | | ____ | ____ |
| G. Total Residual Chlorine (TRC) Measurements | | | |
| 1. Before chlorination | | ____ | ____ |
| 2. After dechlorination | | ____ | ____ |
| H. Test Results* | | | |
| 1. Controls | | | |
| a. Acute Tests $\leq$ 10% mortality after 48 hours | | ____ | ____ |
| b. Chronic Tests $\leq$ 20% mortality after 7 days | | ____ | ____ |
| 2. Test acceptability for Ceriodaphnia chronic test | | | |
| a. $\geq$ 15 young/♀ and 60% of controls have three broods | | ____ | ____ |
| 3. Effluent Effect | | | |
| a. Species | Species | | |
| b. % mortality in | | | |
| c. 100% effluent | | | |
| d. LC50/EC50 | | | |
| e. NOEC | | | |
| f. LOEC | | | |
| 4. Raw data included for check on errors/inconsistencies | | ____ | ____ |
| I. Quality Assurance Test Results | | | |
| 1. Type Reference Toxicants | | | |
| 2. 24 hrs. LC50/EC50 | 48 hrs. LC50/EC50 | | |
| 3. Acceptable EPA range | | | |
| 4. Test conducted on species within 30 days of reference toxicant test | | ____ | ____ |
| | | ____ | ____ |
| J. Is Self-Biomonitoring Test acceptable to Regulatory Agency? | | ____ | ____ |
| K. Does Test have to be repeated? | | ____ | ____ |

*Note: For other test species use same format but substitute test acceptability criteria of organisms in Appendix G.

APPENDIX C

Example of Standard Operating Procedures (SOPs) Format

This outline should be used as a guideline in the preparation of the written Standard Operating Procedures (SOPs). Each SOP should be a separate document covering each toxicity test organism or group of similar test organisms. All quality assurance (QA) and quality control (QC) procedures must be included in the SOPs. SOPs must be prepared for each of the following activities preformed by the laboratory:

SOP Document: _____

I. Sample Collection, Handling, Preservation, and Preparation

II. Chain-of-Custody Procedures

III. Equipment/Glassware Cleaning

IV. Culturing and Holding Methods (Each test species is a separate SOP.)

A. Ceriodaphnia dubia
B. Daphnia pulex, D. magna
C. Fathead minnow (Pimephales promelas)
D. Rainbow trout (Oncorhynchus mykiss)
E. Selenastrum capricornutum
F. Lemna sp.
G. Sheepshead minnow (Cyprinodon variegatus)
H. Inland silverside (Menidia beryllina)
I. Mysidopsis bahia
J. Arbacia punctulata
K. Champia parvula
L. Other

V. Culture Health

A. Ceriodaphnia dubia
B. Daphnia pulex, D. magna
C. Fathead minnow (Pimephales promelas)
D. Rainbow Trout (Oncorhynchun mykiss)
E. Selenastrum capricornutum
F. Lemna sp.
G. Sheepshead minnow (Cyprinodon variegatus)
H. Inland silverside (Menidia beryllina)
I. Mysidopsis bahia
J. Arbacia punctulata
K. Champia parvula
L. Other

APPENDIX C

Example of Standard Operating Procedures (SOPs) Format (Continued)

IV. Testing Methods

A. Freshwater Acute Toxicity Tests

1. *Ceriodaphnia dubia*
2. *Daphnia pulex*, *D magna*
3. Fathead minnow (*Pimephales promelas*)
4. Rainbow trout (*Oncorhynchus mykiss*)
5. Other

B. Estuarine and Marine Acute Toxicity Tests

1. Sheepshead minnow (*Cyprinodon variegatus*)
2. Inland silverside (*Menidia beryllina*)
3. Mysid (*Mysidopsis bahia*)
4. Other

C. Freshwater Short-Term Toxicity Tests

1. Fathead minnow (*Pimephales promelas*) larval survival and growth test
2. Fathead minnow (*Pimephales promelas*) embryo/larval survival and teratogenicity test
3. *Ceriodaphnia dubia* survival and reproduction test
4. *Selenastrum capricornutum* growth test
5. Other

D. Estuarine and Marine Short-Term Toxicity Tests

1. Sheepshead minnow (*Cyprinodon variegatus* larval survival and growth test
2. Sheepshead minnow (*Cyprinodon variegatus* embryo/larval survival and teratogenicity test.
3. Inland silverside (*Menidia beryllina*) larval survival and growth test
4. Mysid (*Mysidopsis bahia*) survival, growth, and fecundity test
5. Sea urchin (*Arbicia punctulata*) fertilization test
6. *Champia parvula* reproduction test
7. Other

VII. Equipment/Chemical Analyses and Calibration of Instruments

A. Dissolved oxygen meters
B. pH meter
C. Conductivity meter
D. Total residual chlorine analysis
E. Hardness analysis
F. Refractometer

APPENDIX C

Example of Standard Operating Procedures (SOP) Format (Continued)

VII. Data Analysis

A. $LC_{50}$ calculation
B. Ceriodaphnia dubia Pass/Fail Results Analysis
C. Dunnetts Procedures
D. Probit Analysis
E. Linear Interpolation Method
F. Other

IX. References

APPENDIX D

Example of Culturing Criteria SOP Format[1]

Ceriodaphnia dubia

I. Mandatory Requirements

1. Fifteen - twenty young/female in first three broods.
2. Eighty percent survival of brood animals.
3. Testing is delayed if 1 and 2 above are not met.
4. Brood animals of known age.
5. Brood animals replaced on a specified schedule.
6. Culture water replaced on a specified schedule.
7. Equipment (droppers, reservoir, food containers) sterilized on a schedule.
8. Chronic reference toxicant tests completed at least quarterly.
9. Coefficient of variation of reference toxicant tests should be $\leq$ 50% for endpoints LC50 and IC50. (NaCl suggested Reference Toxicant).

II. Important Practices

1. Feeding on weekends and holidays.
2. New food prepared at least biweekly.
3. Broods of eight or more used for testing.
4. Age of test animals not more than 24 hours old; all released within an 8 hour period.
5. Brood animals for a test cultured in water of same hardness, pH, and conductivity as dilution water for testing.

III. Recommended Practices of High Quality Labs

1. Suspended solids of YCT food measured and feeding level adjusted.
2. Algae included in diet.
3. Algal quantity fed is measured (# cells or optical density).
4. Algal cultures harvested at known age.
5. Standard procedures for cleaning equipment.
6. Males are recognized and counted.
7. Blocking of young from females in test.

---

[1]Adapted from: Donald I. Mount and Teresa J. Norberg, USEPA, Environmental Research Laboratory - Duluth.

# APPENDIX E

## PRE-SURVEY FORMS: TOXICITY TEST LABORATORY EVALUATION

APPENDIX E

PRE-SURVEY FORM: TOXICITY TEST LABORATORY EVALUATION

Laboratory Name _____

Address _____

City _____ State _____ Zip Code _____

Proposed Evaluation Date _____

Current Federal/State Certification _____

Laboratory Director _____

Parent Company/Organization _____

Toxicity Program Supervisor/Manager _____

Evaluation Coordinator _____

Phone _____

Individual(s) Filling Out Pre-Survey Forms _____

_____

PLEASE RETURN PRE-SURVEY FORMS BY _____ TO:
DATE

Evaluator(s) _____

Organization _____

Address _____

City _____ State _____ Zip Code _____

Phone _____

APPENDIX E

PRE-SURVEY FORM: TOXICITY TEST LABORATORY EVALUATION

| CLIENTS SERVED (NPDES PERMITTEES) | NUMBER OF TOXICITY TESTS PER YEAR |
|---|---|
| Industrial | |
| Municipal | |
| Federal | |
| Interstate | |
| State | |
| Private Sector | |
| Others | |

MATERIALS TESTED

| Items | Yes | No | Comments |
|---|---|---|---|
| Effluents | | | |
| Industrial | | | |
| Municipal | | | |
| Sediments | | | |
| Solid Phase | | | |
| Suspended Solids | | | |
| Leachate | | | |
| Pure Compounds | | | |
| Criteria Development | | | |
| Hazard Assessment | | | |
| Drilling Fluids | | | |
| Surface Waters | | | |
| Hazardous Wastes | | | |

APPENDIX E

PRE-SURVEY FORM: STAFF RESUME

Submit a resume for each staff member in the toxicity testing program which includes the following:

Name: _____

Address: _____

_____

Job Title: _____

Phone Number: _____

Education: (Degrees, Dates, Institutions Conferring Degree, Major and Minor Fields)

_____

_____

Toxicity Testing
Formal Training: _____

_____

_____

On-the-Job Training: _____

_____

_____

_____

Professional
Experience: _____

_____

_____

_____

_____

_____

_____

_____

Professional Organizations/
Honor Societies: _____

_____

_____

Publications: _____

_____

_____

## APPENDIX E

### PRE-SURVEY FORM: LABORATORY STAFF FOR AQUATIC TOXICOLOGY

| Position | Name | Degree/Diploma | | | | | Major Field | Present Assignment | Years Experience | Years in Present Job |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PhD | MS | BS | ASSOC | HS | | | | |
| Manager/ Supervisor | | | | | | | | | | |
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| Professional Staff | | | | | | | | | | |
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| Technicians | | | | | | | | | | |
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APPENDIX E

PRE-SURVEY FORM: FACILITY INFORMATION

FIXED LABORATORY

Total space _____ $ft^2$ Culture _____ $ft^2$ Testing _____ $ft^2$

Office _____ $ft^2$

Separate culturing and testing areas Y _____ N _____

Temperature control system: Manual _____ Automatic _____

Daily temperature range _____Recording device available: Yes____ No____

Ventilation: Recirculating _____ Once through _____

Lighting: Type _____ Photoperiod: _____h light; _____ h dark

Backup emergency power available: Yes ____ No ____

Water supply: Source _____

Lab Treatment _____

_____

MOBILE LABORATORY

Type _____ Self-propelled _____ Trailer _____

Length _____ ft Width _____ ft

Total space _____ $ft^2$ Culture _____ $ft^2$ Testing _____ $ft^2$

Temperature control system: Manual _____ Automatic _____

Daily range _____ Recording device available Yes ___ No ___

Ventilation: Recirculating _____ Once through _____

Lighting: Type _____ photoperiod: _____h Light; _____ h dark

Backup emergency power available: Yes ____ No ____

Water supply: Source _____

Lab Treatment _____

_____

## APPENDIX E

### PRE-SURVEY FORM: LABORATORY EQUIPMENT

| EQUIPMENT TYPE | MODEL NO. | AGE | INSTRUCTION MANUAL AVAILABLE | SOP AVAILABLE | MAINTENANCE LOG BOOK | SERVICED SCHEDULE | CALIBRATION PERFORMANCE CHECK | COMMENTS |
|---|---|---|---|---|---|---|---|---|
| pH Meter | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| DO Meter | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| Conductivity Meter | | | | | | | | |
| | | | | | | | | |
| Refractometer | | | | | | | | |
| | | | | | | | | |
| Thermometer | | | | | | | | |
| | | | | | | | | |
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| Fluorometer | | | | | | | | |
| Radiometer | | | | | | | | |
| Photometer | | | | | | | | |

APPENDIX E

PRE-SURVEY FORM: LABORATORY EQUIPMENT (Continued)

| EQUIPMENT TYPE | MODEL NO. | AGE | INSTRUCTION MANUAL AVAILABLE | SOP AVAILABLE | MAINTENANCE LOG BOOK | SERVICED SCHEDULE | CALIBRATION PERFORMANCE CHECK | COMMENTS |
|---|---|---|---|---|---|---|---|---|
| Particle Counter | | | | | | | | |
| Compound Microscope | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| Dissecting Microscope | | | | | | | | |
| | | | | | | | | |
| Balance | | | | | | | | |
| | | | | | | | | |
| Drying Oven | | | | | | | | |
| | | | | | | | | |
| Autoclave | | | | | | | | |
| Refrigerator | | | | | | | | |
| Freezer | | | | | | | | |
| Mechanical Shaker | | | | | | | | |
| Environmental Chamber | | | | | | | | |
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APPENDIX E

PRE-SURVEY FORM: LABORATORY EQUIPMENT (Continued)

| EQUIPMENT TYPE | MODEL NO. | AGE | INSTRUCTION MANUAL AVAILABLE | SOP AVAILABLE | MAINTENANCE LOG BOOK | SERVICED SCHEDULE | CALIBRATION PERFORMANCE CHECK | COMMENTS |
|---|---|---|---|---|---|---|---|---|
| Incubator | | | | | | | | |
| | | | | | | | | |
| Spectrophotometer | | | | | | | | |
| Centrifuge | | | | | | | | |
| | | | | | | | | |
| Water Bath | | | | | | | | |
| | | | | | | | | |
| Water Purification System | | | | | | | | |
| | | | | | | | | |
| Air Compressor | | | | | | | | |
| | | | | | | | | |
| Aquarium Pump | | | | | | | | |
| | | | | | | | | |
| Vacuum Pump | | | | | | | | |
| | | | | | | | | |
| Standby Generator | | | | | | | | |

APPENDIX E

PRE-SURVEY FORM: LABORATORY EQUIPMENT (Continued)

| EQUIPMENT TYPE | MODEL NO. | AGE | INSTRUCTION MANUAL AVAILABLE | SOP AVAILABLE | MAINTENANCE LOG BOOK | SERVICED SCHEDULE | CALIBRATION PERFORMANCE CHECK | COMMENTS |
|---|---|---|---|---|---|---|---|---|
| Fume Hood | | | | | | | | |
| | | | | | | | | |
| Desiccator | | | | | | | | |
| | | | | | | | | |
| Light Box | | | | | | | | |
| | | | | | | | | |
| Amperometric Titrator | | | | | | | | |
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APPENDIX E

PRE-SURVEY FORM: LABORATORY EQUIPMENT (Continued)

| EQUIPMENT TYPE | MODEL NO. | AGE | INSTRUCTION MANUAL AVAILABLE | SOP AVAILABLE | MAINTENANCE LOG BOOK | SERVICED SCHEDULE | CALIBRATION PERFORMANCE CHECK | COMMENTS |
|---|---|---|---|---|---|---|---|---|
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APPENDIX E

PRE-SURVEY FORM: LABORATORY EQUIPMENT (Continued)

| EQUIPMENT TYPE | MODEL NO. | AGE | INSTRUCTION MANUAL AVAILABLE | SOP AVAILABLE | MAINTENANCE LOG BOOK | SERVICED SCHEDULE | CALIBRATION PERFORMANCE CHECK | COMMENTS |
|---|---|---|---|---|---|---|---|---|
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APPENDIX E

PRE-SURVEY FORM: LABORATORY EQUIPMENT (Continued)

| EQUIPMENT TYPE | MODEL NO. | AGE | INSTRUCTION MANUAL AVAILABLE | SOP AVAILABLE | MAINTENANCE LOG BOOK | SERVICED SCHEDULE | CALIBRATION PERFORMANCE CHECK | COMMENTS |
|---|---|---|---|---|---|---|---|---|
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APPENDIX E

PRE-SURVEY FORM: TOXICITY TEST METHODOLOGY

| TEST PERFORMED | METHOD USED | COPY AVAILABLE | DEVIATIONS USED | SOPS | | COMMENTS |
|---|---|---|---|---|---|---|
| | | | | YES | NO | |
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APPENDIX E

PRE-SURVEY FORM: ACUTE TOXICITY TEST PROGRAM

| ORGANISM | CULTURE YES OR NO HOW LONG | TOX TEST YES OR NO HOW LONG | SOPS | | NO OF TEST PER YEAR ACUTE | | SAMPLE TYPE | METHODOLOGY | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | YES | NO | STATIC | RENEWAL | | EPA | ASTM | SM | OTHER |
| Freshwater | | | | | | | | | | | |
| C. dubia | | | | | | | | | | | |
| D. pulex | | | | | | | | | | | |
| D. magna | | | | | | | | | | | |
| S. capricornutum | | | | | | | | | | | |
| P. promelas | | | | | | | | | | | |
| O. mykiss | | | | | | | | | | | |
| | | | | | | | | | | | |
| | | | | | | | | | | | |
| Estuarine/Marine | | | | | | | | | | | |
| M. bahia | | | | | | | | | | | |
| C. variegatus | | | | | | | | | | | |
| M. beryllina | | | | | | | | | | | |
| M. menidia | | | | | | | | | | | |
| M. peninsulae | | | | | | | | | | | |
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APPENDIX E

PRE-SURVEY FORM: CHRONIC TOXICITY TEST PROGRAM

| ORGANISM | CULTURE YES OR NO HOW LONG | TOX TEST YES OR NO HOW LONG | SOPS | | NO OF TEST PER YEAR CHRONIC | | SAMPLE TYPE | METHODOLOGY | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | YES | NO | RENEWAL | FLOW-THROUGH | | EPA | ASTM | SM | OTHER |
| Freshwater | | | | | | | | | | | |
| C. dubia | | | | | | | | | | | |
| P. promelas | | | | | | | | | | | |
| S. capricornutum | | | | | | | | | | | |
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| | | | | | | | | | | | |
| Estuarine/Marine | | | | | | | | | | | |
| C. parvula | | | | | | | | | | | |
| A. punctulata | | | | | | | | | | | |
| M. bahia | | | | | | | | | | | |
| C. variegatus | | | | | | | | | | | |
| M. beryllina | | | | | | | | | | | |
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## APPENDIX E

### PRE-SURVEY FORM: QUALITY ASSURANCE

| QUALITY ASSURANCE ITEMS | AVAILABLE AND/OR USED | COMMENTS |
|---|---|---|
| QA Program Plan | | |
| QA/QC Project Plan(s) | | |
| QA/QC Records Calibrations/Log Books | | |
| In House QA/QC Audits | | |
| Water Supply | | |
| Culture SOPs Documentation | | |
| Toxicity Tests SOPs Documentation | | |
| Monitoring/Culturing Test Conditions | | |
| Other SOPs | | |
| Reference Toxicants | | |
| Reference Evaluation Sample | | |
| Chain-of-Custody Documentation | | |
| Analytical Support | | |
| Analytical Support Documentation | | |
| Data Analyses Capabilities | | |
| Safety Program Documentation | | |
| | | |

# APPENDIX F

## ON-SITE LABORATORY EVALUATION FORMS AND CHECKLISTS

The laboratory must have an informal meeting to introduce the technical staff and other personnel of the toxicity test program to the evaluator. The laboratory must present and discuss aspects of the laboratory QA and toxicity test program. The evaluator must explain the audit or evaluation process if it is not clear to the laboratory.

The completed pre-survey forms (Appendix E) should be used by the evaluator as supplemental on-site checklists with Appendix F.

The following items are covered in this appendix:

I. On-Site informal Laboratory Presentation

II. Organization History

III. Laboratory Staff

IV. Facilities and General Equipment

V. Test Equipment, Instruments, and Supplies

VI. Test Organisms

VII. Documentation

VIII. Toxicity Test Methodology

IX. Quality Assurance and Quality Control

X. Data Handling

XI. Summary

APPENDIX F

INFORMAL LABORATORY MEETING AND INTRODUCTION
OF THE TECHNICAL STAFF

I. Organizational Information

Facility Description: _____

Name/Affiliation: _____

Address: _____

_____

_____

_____

Phone Number: _____

Laboratory Director/Manager: _____

Type of Evaluation: _____

Informal meeting and introduction of laboratory staff: Y ____ N ____

Laboratory Personnel Contacted

| Name | Title |
|---|---|
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APPENDIX F

ON-SITE LABORATORY FORMS AND CHECKLISTS

Note: The completed pre-survey forms (Appendix E) should be used by the audit or evaluator to supplement the on-site forms and checklists.

II. Organization History

| Item | Yes | No | Comments |
|---|---|---|---|
| Laboratory On-Site Introduction/ Meeting with staff | | | |
| Laboratory demonstrates active toxicity test program | | | |
| Performance reference toxicant data available and checked | | | |
| Organization chart provided | | | |
| | | | |

III. Laboratory Staff

| Item | Yes | No | Comments |
|---|---|---|---|
| Pre-survey forms verification | | | |
| Appropriate educational background experience/toxicity testing | | | |
| Laboratory adequately staffed | | | |
| All technical staff available during evaluation | | | |
| QA officer report to senior management | | | |
| Director/Supervisor/ Manager available during evaluation | | | |
| QA officer available during evaluation | | | |
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IV. Facilities and General Equipment

| Item | Yes | No | Comments |
|---|---|---|---|
| Pre-survey forms verification | | | |
| Tour lab | | | |

IV. Facilities and General Equipment (Continued)

| Item | Yes | No | Comments |
|---|---|---|---|
| Tour mobile lab, if available | | | |
| Lab work space adequate | | | |
| Culture space adequate | | | |
| Toxicity Test space adequate | | | |
| Lab has distilled/ demineralized water. | | | |
| Lab has distilled demineralized water checked/recorded. | | | |
| Analytical balance/ calibrated yearly | | | |
| Balance routinely checked/ class S weights/recorded logbook | | | |
| Exhaust hoods provided | | | |
| Refrigerator/freezer adequate, etc. | | | |
| Lab maintained in clean/ organized manner | | | |
| Contamination-free work areas available for handling test materials | | | |
| Culture and test areas separated | | | |
| Adequate storage areas available | | | |
| Temperature of lab adequate | | | |
| Lighting adequate | | | |
| Air condition/ventilation adequate | | | |
| Chemical waste disposal policies/ SOPs available | | | |
| Lab secure | | | |

V. Test Equipment, Instruments, and Supplies

| Item | Yes | No | Comments |
|---|---|---|---|
| Pre-survey forms verification | | | |

V. Test Equipment, Instruments, and Supplies (Continued)

| Item | Yes | No | Comments |
|---|---|---|---|
| SOP(s) verification | | | |
| Calibration checks/log books pre-survey forms | | | |
| Manual available to operator | | | |
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VI. Test Organisms

| Item | Yes | No | Comments |
|---|---|---|---|
| Pre-survey forms verification | | | |
| Culture Maintenance SOP(s) available | | | |
| Disease control/treatment protocols documented | | | |
| Holding/acclimation facilities adequate | | | |
| Source of test organisms documented | | | |
| Food and feeding program documented | | | |
| Freshwater supply/source/quantity used/quality documented | | | |
| Estuarine/marine water supply/ source/quantity used/quality/ documented | | | |
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VII. Documentation

| Item | Yes | No | Comments |
|---|---|---|---|
| Pre-survey forms verification | | | |

VII. Documentation (Continued)

| Item | Yes | No | Comments |
|---|---|---|---|
| Sample custodian designated | | | |
| Sample procedures/ responsibilities documented | | | |
| Written SOPs available for receipt of samples | | | |
| QA procedures documented/ available to staff | | | |
| Written SOPs developed for compiling/maintaining sample document files | | | |
| Written SOPs for samples preservation, storage/ are maintained. | | | |
| Written SOPs for culture/ test methods | | | |
| Daily activities/toxicity test documented | | | |
| Bound logbooks available/ general chemistry (pH,DO,etc.) | | | |
| Bound logbooks used, pages numbered consecutively | | | |
| Type of work clearly displayed on logbooks | | | |
| Logbooks maintained in legible manner | | | |
| Are anomalies recorded routinely | | | |
| Are inserts permanently affixed and signed. | | | |
| Supervisor inspects notebooks/ for appropriate documentation | | | |
| | | | |

VIII. Toxicity Test Methodology (Recommended Toxicity Test Conditions and Test Acceptability Criteria: On-Site Checklists, see Appendix G.)

| Item | Yes | No | Comments |
|---|---|---|---|
| Pre-survey forms verification | | | |
| Required methods used | | | |
| Any unauthorized deviations | | | |
| Are written SOPs provided | | | |

VIII. Toxicity Test Methodology (Recommended Toxicity Test Conditions and Test Acceptability Criteria: On-Site Checklists, see Appendix G.) (Continued)

| Item | Yes | No | Comments |
|---|---|---|---|
| Biologist/technician record bench data in neat accurate manner | | | |
| Appropriate instrumentation used with each toxicity test | | | |
| | | | |
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IX. Quality Assurance/Quality Control (QA/QC)

| Item | Yes | No | Comments |
|---|---|---|---|
| Pre-survey forms verification | | | |
| Lab maintains QA/QC manual | | | |
| Manual addresses elements of QA program, including the following: | | | |
| a. Personnel | | | |
| b. Facilities and equipment | | | |
| c. Operation of instruments | | | |
| d. Documentation of SOPs | | | |
| e. Procurement and inventory practices | | | |
| f. Project plans/ Data quality objectives | | | |
| g. Reliability of data | | | |
| h. Data validation | | | |
| i. Feedback and corrective action | | | |
| j. Instrument calibration | | | |

IX. Quality Assurance and Quality Control (Continued)

| Item | Yes | No | Comments |
|---|---|---|---|
| k. Recordkeeping | | | |
| l. Internal QA/QC audits | | | |
| m. QC responsibilities/ reporting clearly defined | | | |
| n. QC charts maintained for routine analysis | | | |
| o. QC records show corrective action to meet QC criteria | | | |
| p. Supervisory personnel review data and QC results | | | |
| Chain-of-custody maintained | | | |
| Record keeping adequate | | | |
| Instrument Calibration/ logbooks maintained | | | |
| Reference toxicant evaluations used | | | |
| Analytical support/ inorganic analyses | | | |
| Analytical support/ organic analyses | | | |
| | | | |
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X. Data Handling

| Item | Yes | No | Comments |
|---|---|---|---|
| Recommended statistical programs used | | | |
| Data calculations check/ second person | | | |
| Data calculations documented | | | |
| Data analyses capabilities available | | | |
| Data and records retained | | | |
| PC computer(s) available | | | |
| | | | |

XI. Summary.

| Item | Yes | No | Comments |
|---|---|---|---|
| Evaluation criteria met | | | |
| Response to evaluation indicates an awareness to QA | | | |
| Staff place positive emphasis on QA/QC? | | | |
| Responses to QA/QC open and direct | | | |
| Cooperative attitude displayed by staff | | | |
| Lab places proper emphasis on QA/QC | | | |
| QA/QC deficiencies discussed during debriefing | | | |
| Overall QA/QC adequate to accomplish objectives | | | |
| Corrective actions during previous evaluations implemented | | | |
| Corrective actions result from this evaluation | | | |
| Technical assistance needed | | | |
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APPENDIX G

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA:

ON-SITE CHECKLISTS[1]

Freshwater, Marine, and Estuarine Acute Toxicity Tests

Freshwater Short-Term Tests

Marine and Estuarine Short-Term Tests

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[1]Adapted from: USEPA (1985a), USEPA, (1988c), and USEPA, (1989c) or latest editions.

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR
CERIODAPHNIA DUBIA SCREENING AND DEFINITIVE ACUTE TESTS

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Static | | |
| 2. | Test duration: | 48 h | | |
| 3. | Temperature: | 20 ± 2°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h darkness | | |
| 7. | Test chamber size: | 30 mL | | |
| 8. | Test solution volume: | 25 mL | | |
| 9. | Renewal of test solution: | None | | |
| 10. | Age of test organisms: | Less than 24-h old | | |
| 11. | No. organisms per test chamber: | 10 | | |
| 12. | No. replicate chambers per concentration: | 2 | | |
| 13. | No. organisms per concentration: | 20 | | |
| 14. | Feeding regime: | Not fed during test; fed while holding prior to use in the test | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR *CERIODAPHNIA DUBIA* SCREENING AND DEFINITIVE ACUTE TESTS (Continued)

| Test | Conditions | Recommended | Used | Comments |
|---|---|---|---|---|
| 15. | Cleaning: | Cleaning not required | | |
| 16. | Aeration: | None, unless DO concentration falls below 40% saturation; rate should not exceed 100 bubbles/min. | | |
| 17. | Dilution water: | Moderately hard synthetic water is prepared using MILLIPORE MILLI-Q$^{R}$ or equivalent deionized water and reagent grade chemicals or 20% DMW | | |
| 18. | Test concentrations: | 1 effluent concentration and a control for screening tests<br>5 effluent concentrations and a control for definitive tests | | |
| 19. | Dilution factor: | 0.5 or 0.3 (definitive tests) | | |
| 20. | Endpoint: | Survival (LC50) | | |
| 21. | Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device. | | |
| 22. | Sample volume required: | 1 L | | |
| 23. | Test acceptability criterion; | 90% or greater survival in controls | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR *DAPHNIA PULEX* AND *D. MAGNA* SCREENING AND DEFINITIVE ACUTE TESTS

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 1. Test type: | Static | | |
| 2. Test duration: | 24 h - Screening test<br>48 h - Definitive test | | |
| 3. Temperature: | 20 ± 2°C | | |
| 4. Light quality: | Ambient laboratory illumination | | |
| 5. Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c)<br>(ambient laboratory levels) | | |
| 6. Photoperiod: | 16 h light, 8 h darkness | | |
| 7. Test chamber size: | 100 mL | | |
| 8. Test solution volume: | 50 mL | | |
| 9. Renewal of test solution: | None | | |
| 10. Age of test organisms: | Less than 24-h old (neonates) | | |
| 11. No. organisms per test chamber: | 10 | | |
| 12. No. replicate chambers per concentration: | 2 | | |
| 13. No. organisms per concentration: | 20 | | |
| 14. Feeding regime: | Not fed during test; fed while holding prior to use in the test | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR DAPHNIA PULEX AND D. MAGNA SCREENING AND DEFINITIVE ACUTE TESTS (Continued)

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 15. | Cleaning: | Cleaning not required | | |
| 16. | Aeration: | None, unless DO concentration falls below 40% saturation; rate should not exceed 100 bubbles/min. | | |
| 17. | Dilution water: | Hard water for Daphnia magna; moderately hard or /soft water for D. pulex | | |
| | | Synthetic water is prepared using MILLIPORE MILLI-Q$^{R}$ or equivalent deionized water and reagent grade chemicals or 20% DMW | | |
| 18. | Test concentrations: | 1 effluent concentration and a control for screening tests | | |
| | | 5 effluent concentrations and a control for definitive tests | | |
| 19. | Dilution factor: | 0.5 or 0.3 (definitive tests) | | |
| 20. | Endpoint: | Survival (LC50) | | |
| 21. | Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device. | | |
| 22. | Sample volume required: | 1 L | | |
| 23. | Test acceptability criterion: | 90% or greater survival in controls | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*) SCREENING AND DEFINITIVE ACUTE TESTS

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Static | | |
| 2. | Test duration: | 24 h - Screening test<br>48 h - Definitive test<br>48 - 96 h - Definitive test (flow thru) | | |
| 3. | Temperature: | 20 ± 2°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c)<br>(ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h darkness | | |
| 7. | Test chamber size: | 1 L | | |
| 8. | Test solution volume: | 750 mL | | |
| 9. | Renewal of test solution: | None | | |
| 10. | Age of test organisms: | 1-90 days<br>1 - 14 days ± 24 h (proposed change) | | |
| 11. | No. organisms per test chamber: | 10 | | |
| 12. | No. replicate chambers per concentration: | 2 | | |
| 13. | No. organisms per concentration: | 20 | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FATHEAD MINNOW (PIMEPHALES PROMELAS) SCREENING AND DEFINITIVE ACUTE TESTS (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 14. Feeding regime: | Not fed during test; fed while holding prior to use in the test | | |
| 15. Cleaning: | Cleaning not required | | |
| 16. Aeration: | None, unless DO concentration falls below 40% saturation; rate should not exceed 100 bubbles/min. | | |
| 17. Dilution water: | Moderately hard synthetic water is prepared using MILLIPORE MILLI-Q$^{R}$ or equivalent deionized water and reagent grade chemicals or 20% DMW | | |
| 18. Test concentrations: | 1 effluent concentration and a control for screening tests | | |
| | 5 effluent concentrations and a control for definitive tests | | |
| 19. Dilution factor: | 0.5 or 0.3 (definitive tests) | | |
| 20. Endpoint: | Survival (LC50) | | |
| 21. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device. | | |
| 22. Sample volume required: | 9 L (2.5 gal cubitainer) | | |
| 23. Test acceptability criterion: | 90% or greater survival in controls | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE RAINBOW TROUT (ONCORHYNCHUS MYKISS) SCREENING AND DEFINITIVE ACUTE TESTS

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Static | | |
| 2. | Test duration: | 48 h | | |
| 3. | Temperature: | 12 ± 2°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h darkness. In the morning, light intensity should be raised gradually over a 15 minute period, using a dimmer switch or other suitable control device. | | |
| 7. | Test chamber size: | 5 L (Test chamber should be covered to prevent fish from jumping out) | | |
| 8. | Test solution volume: | 4 L | | |
| 9. | Renewal of test solution: | None | | |
| 10. | Age of test organisms: | 15 - 30 days, ± 24 h (after yolk sac is absorbed) | | |
| 11. | No. organisms per test chamber: | 10 | | |
| 12. | No. replicate chambers per concentration: | 4 for screening tests<br>2 for definitive tests | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE RAINBOW TROUT (ONCORHYNCHUS MYKISS) SCREENING AND DEFINITIVE ACUTE TESTS (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 13. No. organisms per concentration: | 40 for screening tests<br>20 for definitive tests | _____<br>_____ | _____<br>_____ |
| 14. Feeding regime: | Feeding not required | _____ | _____ |
| 15. Cleaning: | Cleaning not required | _____ | _____ |
| 16. Aeration: | None, unless DO concentration falls, below 60% saturation; rate should not exceed 100 bubbles/min. | _____ | _____ |
| 17. Dilution water: | Moderately hard synthetic water prepared using MILLIPORE MILLI-Q$^{R}$ or equivalent deionized water and reagent grade chemicals or 20% DMW | _____ | _____ |
| 18. Test concentrations: | 1 effluent concentration and a control for screening tests<br>5 effluent concentrations and a control for definitive tests | _____<br>_____ | _____<br>_____ |
| 19. Dilution factor: | 0.5 or 0.3 (definitive tests) | _____ | _____ |
| 20. Endpoint: | Survival (LC50) | _____ | _____ |
| 21. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device. | _____ | _____ |
| 22. Sample volume required: | 20 L (5 gal cubitainer) | _____ | _____ |
| 23. Test acceptability criterion: | 90% or greater survival in controls | _____ | _____ |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) SCREENING AND DEFINITIVE ACUTE TESTS

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Static | | |
| 2. | Test duration: | 48 h | | |
| 3. | Temperature: | 20 ± 2°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h darkness | | |
| 7. | Test chamber size: | 1 L | | |
| 8. | Test solution volume: | 750 mL | | |
| 9. | Renewal of test solution: | None | | |
| 10. | Age of test organisms: | 1-14 days, ± 24 h | | |
| 11. | No. organisms per test chamber: | 10 | | |
| 12. | No. replicate chambers per concentration: | 2 | | |
| 13. | No. organisms per concentration: | 20 | | |
| 14. | Feeding regime: | Not fed during the test; fed while holding prior to use in the test. | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) SCREENING AND DEFINITIVE ACUTE TESTS (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 15. Cleaning: | Cleaning not required | ____ | ____ |
| 16. Aeration: | None, unless DO concentration falls below 40% saturation; rate should not exceed 100 bubbles/min. | ____ | ____ |
| 17. Dilution water: | 25°/oo Forty Fathoms® artificial seawater prepared with MILLI-Q® or equivalent deionized water | ____ | ____ |
| 18. Test concentrations: | 1 effluent concentration and a control for screening tests | ____ | ____ |
| | 5 effluent concentrations and a control for definitive tests | ____ | ____ |
| 19. Dilution factor: | 0.5 or 0.3 (definitive tests) | | |
| 20. Endpoint: | Survival (LC50) | | |
| 21. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device. | ____ | ____ |
| 22. Sample volume required: | 9 L (2.5 gal cubitainer) | ____ | ____ |
| 23. Test acceptability criterion: | 90% or greater survival in controls | ____ | ____ |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SILVERSIDES (MENIDIA BERYLLINA, M. MENIDIA, M. PENINSULAE) SCREENING AND DEFINITIVE ACUTE TESTS

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Static | | |
| 2. | Test duration: | 24 h - Screening test | | |
| | | 48 h - Definitive test | | |
| | | 48 - 96 h Definitive test (flow thru) | | |
| 3. | Temperature: | 20 ± 2°C (northern latitude) | | |
| | | 25 ± 2°C (southern latitude) | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h darkness | | |
| 7. | Test chamber size: | 1 L | | |
| 8. | Test solution volume: | 750 mL | | |
| 9. | Renewal of test solution: | None | | |
| 10. | Age of test organisms: | 1 to 90 days | | |
| | | 7-14 days, ± 24 h (proposed change) | | |
| 11. | No. organisms per test chamber: | 10 | | |
| 12. | No. replicate chambers per concentration: | 2 | | |
| 13. | No. organisms per concentration | 20 | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SILVERSIDES MENIDIA BERYLLINA, M. MENIDIA, M. PENINSULAE) SCREENING AND DEFINITIVE ACUTE TESTS (Continued)

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 15. | Feeding regime: | Not fed during the test; fed while holding prior to use in the test | | |
| 16. | Cleaning: | Cleaning not required | | |
| 17. | Aeration: | None, unless DO concentration falls below 40% saturation; rate should not exceed 100 bubbles/min. | | |
| 18. | Dilution water: | 25°/oo Forty Fathoms$^{R}$ artificial seawater prepared with MILLI-Q$^{R}$ or equivalent deionized water | | |
| 19. | Test concentrations: | 1 effluent concentration and a control for screening tests | | |
| | | 5 effluent concentrations and a control for definitive tests | | |
| 20. | Dilution factor: | 0.5 or 0.3 (definitive tests) | | |
| 21. | Endpoint: | Survival (LC50) | | |
| 22. | Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device. | | |
| 23. | Sample volume required: | 9 L (2.5 gal cubitainer) | | |
| 24. | Test acceptability criterion: | 90% or greater survival in controls | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSIDOPSIS BAHIA SCREENING AND DEFINITIVE ACUTE TESTS

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Static | | |
| 2. | Test duration: | 24 h - Screening test | | |
| | | 48 h - Definitive test | | |
| | | 48 - 96 h Definitive test (flow thur) | | |
| 3. | Temperature: | 20 ± 2°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h darkness | | |
| 7. | Test chamber size: | 250 mL | | |
| 8. | Test solution volume: | 200 mL | | |
| 9. | Renewal of test solution: | None | | |
| 10. | Age of test organisms: | 1 - 5 days, ± 24 h | | |
| 11. | No. organisms per test chamber: | 10 | | |
| 12. | No. replicate chambers per concentration: | 2 | | |
| 13. | No. organisms per concentration: | 20 | | |
| 14. | Feeding regime: | Two drops of concentrated brine shrimp nauplii suspension twice daily (approx. 100 nauplii/mysid) | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSIDOPSIS BAHIA SCREENING AND DEFINITIVE ACUTE TESTS (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 15. Cleaning: | Cleaning required | | |
| 16. Aeration: | None, unless DO concentration falls below 40% saturation; rate should not exceed 100 bubbles/min. | | |
| 17. Dilution water: | 25°/oo Forty Fathoms® artificial seawater prepared with MILLI-Q® or equivalent deionized water | | |
| 18. Test concentrations: | 1 effluent concentration and a control for screening tests | | |
| | 5 effluent concentrations and a control for definitive tests | | |
| 19. Dilution factor: | 0.5 or 0.3 (definitive tests) | | |
| 20. Endpoint: | Survival (LC50) | | |
| 21. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device. | | |
| 22. Sample volume required: | 4 L (1 gal cubitainer) | | |
| 23. Test acceptability criterion: | 90% or greater survival in controls | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE *CERIODAPHNIA DUBIA* SURVIVAL AND REPRODUCTION TEST

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Renewal | | |
| 2. | Test duration: | Until 60% of control females have three broods (may require more or less than 7 days). | | |
| 3. | Temperature: | 25 ± 1°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h dark | | |
| 7. | Test chamber size: | 30 mL | | |
| 8. | Test solution volume: | 15 mL | | |
| 9. | Renewal of test solution: | Daily | | |
| 10. | Age of test organisms: | Less than 24 h; and all released within a 8 h period | | |
| 11. | No. neonates per test chambers: | 1 | | |
| 12. | No. replicate test chambers per concentration | 10 | | |
| 13. | No. neonates per test concentration | 10 | | |
| 14. | Feeding regime | Feed 0.1 mL each of YCT and 0.1 mL of algal suspension (3.0-3.5 x$10^7$ cells/mL) per test chamber daily | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 15. Aeration: | None | | |
| 16. Dilution water: | Moderately hard synthetic water is prepared using MILLIPORE MILLI-Q$^R$ or equivalent deionized water and reagent grade chemicals or 20% DMW | | |
| 17. Effluent concentrations: | Minimum of 5 effluent concentrations and a control | | |
| 18. Dilution factor[1] | Approximately 0.3 or 0.5 | | |
| 19. Endpoints: | Survival and reproduction | | |
| 20. Sampling requirements: | For on-site tests, samples are collected daily, and used within 24 h of the time they are removed from the sampling device. For off-site tests, a minimum of three samples are collected, and used as described in Paragraph 12.6.1, Weber, et al., 1989 | | |
| 21. Sample volume required: | 1 L | | |
| 22. Test acceptability: | 80% or greater survival and an average of 15 or more young/surviving females in the control solutions. At least 60% of surviving females in controls should have produced their third brood. | | |

[1]Surface water test samples are used undiluted.

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FATHEAD MINNOW (PIMEPHALES PROMELAS) LARVAL SURVIVAL AND GROWTH TEST

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Renewal | | |
| 2. | Test duration: | 7 days | | |
| 3. | Temperature: | 25 ± 1°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (Ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h darkness | | |
| 7. | Test chamber size: | 500 mL | | |
| 8. | Test solution volume: | 250 mL/replicate | | |
| 9. | Renewal of test solution: | Daily | | |
| 10. | Age of test organisms: | Newly hatched larvae less than 24 h old | | |
| 11. | No. larvae per test chamber: | 15 (minimum of 10) | | |
| 12. | No. replicate chambers per concentrations: | 4 (minimum of 3) | | |
| 13. | No. larvae per concentration: | 60 (minimum of 30) | | |
| 14. | Feeding regime: | Feed 0.1 mL newly hatched (less than 24 h old) brine shrimp nauplii three times daily at 4 h intervals or, as a minimum, 0.15 mL twice daily, 6 h between feedings (at the beginning of | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FATHEAD MINNOW (PIMEPHALES PROMELAS) LARVAL SURVIVAL AND GROWTH TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| | the work day following renewal). Sufficient larvae are added to provide an excess. Larvae are not fed during the final 12 h of the test | | |
| 15. Cleaning: | Siphon daily, immediately before test solution renewal | | |
| 16. Aeration: | None, unless DO concentration falls below 60% saturation. Rate should not exceed 100 bubbles/min | | |
| 17. Dilution water: | Moderately hard synthetic water is prepared using MILLIPORE MILLI-Q$^{R}$ or equivalent deionized water and reagent grade chemicals or 20% DMW (see Section 7, Weber, et al., 1989) | | |
| 18. Effluent concentrations: | Minimum of 5 and a control | | |
| 19. Dilution factor:[1] | Approximately 0.3 or 0.5 | | |
| 20. Endpoints: | Survival and growth (weight) | | |
| 21. Sampling requirement: | For on-site tests, samples are collected daily, and used within 24 h of the time they are removed from the sampling device. For off-site tests, a minimum of three samples are collected, and used as described in Paragraph 11.7.1, Weber, et al., 1989 | | |
| 22. Sample volume required: | 2.5 L/day | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FATHEAD MINNOW (PIMEPHALES PROMELAS) LARVAL SURVIVAL AND GROWTH TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 23. Test acceptability: | 80% or greater survival in controls: Average dry weight of surviving controls equal or exceeds 0.25 mg | _____ | _____ |

[1]Surface water test samples are used as collected (undiluted).

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FATHEAD MINNOW (PIMEPHALES PROMELAS) EMBRYO-LARVAL SURVIVAL AND TERATOGENICITY TEST

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Renewal | | |
| 2. | Test duration | 7 days | | |
| 3. | Temperature: | 25 ± 1°C | | |
| 4. | Light quality: | Ambient laboratory illumination | | |
| 5. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (Ambient laboratory levels) | | |
| 6. | Photoperiod: | 16 h light, 8 h dark | | |
| 7. | Test chamber size: | 150-500 mL | | |
| 8. | Test solution volume: | 70-200 mL | | |
| 9. | Renewal of test solution: | Daily | | |
| 10. | Age of test organisms: | Less than 36 h old embryos | | |
| 11. | No. embryos per test chamber: | 15 (minimum of 10) | | |
| 12. | No. replicate test chambers per concentration: | 4 (minimum of 3) | | |
| 13. | No. embryos per concentration: | 60 (minimum of 30) | | |
| 14. | Feeding regime: | Feeding not required | | |
| 15. | Aeration: | None unless DO falls below 60% saturation. Rate should not exceed 100 bubbles/min. | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FATHEAD MINNOW (PIMEPHALES PROMELAS) EMBRYO-LARVAL SURVIVAL AND TERATOGENICITY TEST (Continued)

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 16. | Dilution water: | Moderately hard synthetic water is prepared using MILLIPORE MILLI-Q$^R$ or equivalent deionized water and reagent grade chemicals or 20% DMW (Section 7, Weber, et al., 1989). The hardness of the test solutions must equal or exceed 25 mg/L ($CaCO_3$) to ensure hatching. | | |
| 17. | Effluent test concentrations: | 5 and a control | | |
| 18. | Dilution factor:[1] | Approximately 0.3 or 0.5 | | |
| 19. | Endpoint: | Combined mortality (dead and deformed organisms) | | |
| 20. | Sampling requirement: | For on-site tests, samples are collected daily and used within 24 h of the time they are removed from the sampling device. For off-site tests a minimum of three samples are collected and used as described in Paragraph 11.7.1, Weber, et al., 1989. | | |
| 21. | Sample volume required: | 2.5 L/day | | |
| 22. | Test acceptability: | 80% or greater survival in controls | | |

[1]Surface water test samples are used as collected (undiluted).

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE ALGAL (SELENASTRUM CAPRICORNUTUM) GROWTH TEST

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Static | | |
| 2. | Test duration: | 96 h | | |
| 3. | Temperature: | 25 ± 1°C | | |
| 4. | Light quality: | "Cool white" fluorescent lighting | | |
| 5. | Light intensity: | 86 ± 8.6 uE/$m^2$/s (400 ± 40 ft-c) | | |
| 6. | Photoperiod: | Continuous illumination | | |
| 7. | Test chamber size: | 125 mL or 100 mL | | |
| 8. | Test solution volume: | 50 mL or 100 mL | | |
| 9. | Renewal of test solution: | None | | |
| 10. | Age of test organisms: | 4 to 7 days | | |
| 11. | Initial cell density in test chambers: | 10,000 cells/mL | | |
| 12. | No. replicate chambers per concentration: | 3 | | |
| 13. | Shaking rate: | 100 cpm continuous, or twice daily by hand | | |
| 14. | Dilution water: | Algal stock culture medium without EDTA or enriched surface water | | |
| 15. | Test concentrations: | Minimum of 5 and a control | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE ALGAL (SELENASTRUM CAPRICORNUTUM) GROWTH TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 16. Dilution factor[1]: | Approximately 0.3 or 0.5 | | |
| 17. Endpoint: | Growth (cell counts, chlorophyll fluorescence, absorbance, biomass | | |
| 18. Sample volume required: | 1 L (one sample for test initiation) | | |
| 19. Test acceptability: | 2 X $10^5$ cell counts, chlorophyll Variability of controls should not exceed 20%. | | |

[1]Surface water samples for toxicity tests are used undiluted.

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE *MYSIDOPSIS BAHIA* SEVEN DAY SURVIVAL, GROWTH, AND FECUNDITY TEST

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Renewal | | |
| 2. | Test duration: | 7 days | | |
| 3. | Salinity: | 20 °/oo to 30 °/oo ± 2 °/oo | | |
| 4. | Temperature: | 26 - 27 °C | | |
| 5. | Photoperiod: | 16 h light, 8 h dark, with phase in/out period | | |
| 6. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (Ambient laboratory levels) | | |
| 7. | Test chamber: | 8 oz plastic disposable cups, or 400 mL glass beakers | | |
| 8. | Test solution volume: | 150 mL per replicate cup | | |
| 9. | Renewal of test solution: | Daily | | |
| 10. | Age of test organisms: | 7 days | | |
| 11. | No. of treatments per study: | Minimum of 5 treatments and a control | | |
| 12. | No. of organisms per test chamber: | 5 | | |
| 13. | No. of replicate chambers per treatment: | 8 | | |
| 14. | Source of food: | *Artemia* nauplii | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE MYSIDOPSIS BAHIA SEVEN DAY SURVIVAL, GROWTH, AND FECUNDITY TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 15. Feeding regime: | Feed 150 24 h old nauplii per mysid daily, half after test solution renewal and half after 8 - 12 h. | | |
| 16. Cleaning: | Pipette excess food from cups daily | | |
| 17. Aeration: | None unless DO falls below 60% Saturation, than gently aerate in all cups | | |
| 18. Dilution water: | Natural sea water or hypersaline brine | | |
| 19. Test concentrations: | Minimum of 5 concentrations and a control | | |
| 20. Dilution factor: | Approximately 0.3 or 0.5 | | |
| 21 Endpoints: | Survival, growth, and egg development | | |
| 22. Sampling requirement: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device | | |
| 22. Sample volume required: | 1200 mL | | |
| 23. Test acceptability: | 80% survival and an average weight of at least 0.20 mg/mysid controls. If fecundity in controls is adequate (egg production by 50% of females), fecundity should be used as an effect in addition to survival and growth. | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) LARVAL SURVIVAL AND GROWTH TEST

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 1. Test type: | Renewal | | |
| 2. Test duration | 7 days | | |
| 3. Salinity: | 20 °/oo to 32 °/oo ± 2 °/oo | | |
| 4. Temperature: | 25 ± 2 °C | | |
| 5. Light quality: | Ambient laboratory illumination | | |
| 6. Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 7. Photoperiod: | 14 h light, 10 h darkness | | |
| 8. Test chamber size: | 300 mL - 1 L beakers or equivalent | | |
| 9. Test solution volume: | 250 - 750 mL/replicate (loading and DO restrictions must be met) | | |
| 10. Renewal of test solution: | Daily | | |
| 11. Age of test organisms: | Newly hatched larvae (less than 24 h olds) | | |
| 12. No. larvae per test chamber: | 15 (minimum of 10) | | |
| 13. No. replicate chambers per concentration: | 4 (minimum of 3) | | |
| 14. Source of food: | Newly hatched Artemia nauplii (less than 24 h old) | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SHEEPSHEAD MINNOW (CRYPRINODON VARIEGATUS) LARVAL SURVIVAL AND GROWTH TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 15. Feeding regime: | Feed once a day 0.10 g wet weight Artemia nauplii per replicate on Days 0-2; feed 0.15 g wet weight Artemia nauplii per replicate on Days 3-6 | | |
| 16. Cleaning: | Siphon daily, immediately before test solution renewal | | |
| 17. Aeration: | None, unless DO falls below 60% saturation, then aerate all chambers. Rate should be less than 100 bubbles/min. | | |
| 18. Dilution water: | Uncontaminated source of natural seawater, or hypersaline brine or artificial seawater mixed with deionized water | | |
| 19. Test concentration: | 5 concentrations and a control | | |
| 20. Dilution factor: | Approximately 0.3 or 0.5 | | |
| 21. Endpoints: | Survival and growth (weight) | | |
| 22. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device | | |
| 23. Sample volume required: | 5 L | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SHEEPSHEAD MINNOW (CRYPRINODON VARIEGATUS) LARVAL SURVIVAL AND GROWTH TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 24. Test Acceptability: | 80% or greater survival in controls; average dry weight of unpreserved control larvae is equal to or greater than 0.60 mg, or the average dry weight of preserved control larvae is equal to or greater than 0.50 mg | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) EMBRYO LARVAL SURVIVAL AND TERATOGENICITY TEST

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Renewal | | |
| 2. | Test duration: | 9 days | | |
| 3. | Salinity: | 5 °/oo to 32 °/oo ± °/oo | | |
| 4. | Temperature: | 25 ± 2°C | | |
| 5. | Light quality: | Ambient laboratory light | | |
| 6. | Light intensity: | 10-20 uE/$m^2$/s, (50-100 ft-c) (ambient laboratory levels) | | |
| 7. | Photoperiod: | 14 h light, 10 h dark | | |
| 8. | Test chamber size: | 500 mL | | |
| 9. | Test solution volume: | 400 mL (minimum 250 mL) | | |
| 10. | Renewal of test solution: | Daily | | |
| 11. | Age of test organisms: | less than 24 h old | | |
| 12. | No. of embryos/chamber: | 15 (minimum of 10) | | |
| 13. | Relicate test chambers per concentration: | 4 (minimum of 3) | | |
| 14. | Embryos per concentration: | 60 (minimum of 30) | | |
| 15. | Feeding regime: | Feeding not required | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) EMBRYO LARVAL SURVIVAL AND TERATOGENICITY TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 16. Aeration: | None unless DO falls below 60% saturation, than aerate all chambers. Rate should be less than 100 bubbles/min. | | |
| 17. Dilution water: | Uncontaminated source of sea water deionized water mixed with artificial sea salts or hypersaline brine | | |
| 18. Test concentrations: | 5 and a control | | |
| 19. Dilution factor: | Approximately 0.3 or 0.5 | | |
| 20. Endpoints: | Percent hatch; percent larvae dead or with debilitating morphological and/or behavior abnormalities such as: gross deformities; curved spine; disoriented, abnormal swimming behavior; surviving normal larvae from original embryos | | |
| 21. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device | | |
| 22. Sample volume required: | 4 L | | |
| 23. Test acceptability: | 80% or greater survival in controls | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE INLAND SILVERSIDE (MENIDIA BERYLLINA) LARVAL SURVIVAL AND GROWTH TEST

| Test Conditions | | Recommended | Used | Comments |
|---|---|---|---|---|
| 1. | Test type: | Renewal | | |
| 2. | Test duration: | 7 days | | |
| 3. | Salinity: | 5 °/oo to 32 °/oo ± 2 °/oo of the selected test salinity | | |
| 4. | Temperature: | 25 ± 2°C | | |
| 5. | Light quality: | Ambient laboratory illumination | | |
| 6. | Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (ambient laboratory levels) | | |
| 7. | Photoperiod: | 14 h light, 10 h darkness | | |
| 8. | Test chamber size: | 300 mL - 1 L containers | | |
| 9. | Test solution volume: | 250-750 mL/replicate (loading and DO restrictions must be met) | | |
| 10. | Renewal of test solution: | Daily | | |
| 11. | Age of test organisms: | 7 - 11 days post hatch | | |
| 12. | No. larval per test chamber: | 15 (minimum of 10) | | |
| 13. | No. replicate chambers per concentration: | 4 (minimum of 3) | | |
| 14. | Source of feed: | Feed 0.10 g wet weight Artemia nauplii per replicate on days 0-2; Feed 0.15 g wet weight Artemia nauplii per replicate on days 3-6 | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE INLAND SILVERSIDE (MENIDIA BERYLLINA) LARVAL SURVIVAL AND GROWTH TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 15. Cleaning: | Siphon daily, immediately before test solution renewal and feeding | | |
| 16. Aeration: | None, unless DO concentration falls below 60% of saturation, then aerate all chambers. Rate should be less than 100 bubbles min. | | |
| 17. Dilution water: | Uncontaminated source of sea water or deionized water mixed with hypersaline brine | | |
| 18. Test concentrations: | 5 and a control | | |
| 19. Dilution factor: | Approximately 0.3 and 0.5 | | |
| 20. Endpoints: | Survival and growth (weight) | | |
| 21. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device | | |
| 22. Sample volume required: | 5 L | | |
| 23. Test acceptability: | 80% or greater survival in controls; or where test starts with 7 day old larvae, the average dry weight of control larvae, when dried immediately after test termination, is 0.50 mg or greater, or average dry weight of control larvae preserved in 4% formalin or 70% ethanol is 0.43 mg or greater | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SEA URCHIN (ARBACIA PUNCTULATA) FERTILIZATION TEST

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 1. Test type: | Static | | |
| 2. Test duration: | 1 h and 20 min. | | |
| 3. Salinity: | 30 o/oo ± 2 o/oo | | |
| 4. Temperature: | 20 + 1°C | | |
| 5. Light quality: | Ambient laboratory light during test preparation | | |
| 6. Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) (Ambient laboratory levels) | | |
| 7. Test vessel size: | Disposable (glass) liquid scintillation vials (20 mL capacity), not pre-cleaned | | |
| 8. Test solution volume: | 5 mL | | |
| 9. No. of sea urchins: | Pooled sperm from four males and pooled eggs from four females are used per test | | |
| 10. No. of egg and sperm cells per chamber: | About 2000 eggs and 5,000,000 sperm cells per vial | | |
| 11. No. of replicate chambers per concentration: | 4 (minimum of 3) | | |
| 12. Dilution water: | Uncontaminated source of natural seawater; deionized water mixed with hypersaline brine or artificial sea salts | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SEA URCHIN (ARBACIA PUNCTULATA) FERTILIZATION TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 13. Dilution factor: | Approximately 0.3 or 0.5 | | |
| 14. Endpoints: | Fertilization of sea urchin eggs | | |
| 15. Test concentrations: | 5 and a control | | |
| 16. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device | | |
| 17. Sample volume required: | 2 L | | |
| 18. Test acceptability: | 70% to 90% fertilization of eggs in controls | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE ALGAL (CHAMPIA PARVULA) SEXUAL REPRODUCTION TEST

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 1. Test type: | Static | | |
| 2. Test duration: | 2-days exposure to effluent, followed by 5- to 7 day recovery period in control medium for cystocarp development | | |
| 3. Salinity: | 30 °/oo ± 2 °/oo | | |
| 4. Temperature: | 22 - 24 °C | | |
| 5. Photoperiod: | 16 h light, 8 h dark | | |
| 6. Light intensity: | 100 uE/$m^2$/s (500 ft-c) | | |
| 7. Light source: | Cool-white fluorescent lights | | |
| 8. Test chamber: | 200 mL polystyrene cups or 250 mL Erlenmeyer flasks | | |
| 9. Test solution volume: | 100 mL | | |
| 10. Dilution water: | 30 °/oo salinity natural seawater or a combination of 50% 30°/oo salinity natural seawater and 50% 30 °/oo salinity artificial seawater | | |
| 11. Dilution factor: | Approximately 0.3 or 0.5 | | |
| 12. Test concentrations: | 5 and a control | | |
| 13. No. of replicate chambers per concentration: | 4 (minimum of 3) | | |

RECOMMENDED TOXICITY TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AGAL (*CHAMPIA PARVULA*) SEXUAL REPRODUCTION TEST (Continued)

| Test Conditions | Recommended | Used | Comments |
|---|---|---|---|
| 14. No. of organisms per concentration: | 5 female branch tips and 1 male plant | | |
| 15. Endpoints: | Reduction in cystocarp production compared to controls | | |
| 16. Sampling requirements: | Grab or composite samples are used within 36 h of the time they are removed from the sampling device | | |
| 17. Sample volume required: | 1.2 L | | |
| 18. Test acceptability: | Exceeds 20% mortality in controls plants fragment in controls or lower concentrations, may indicate they are under stress, or plants should average 10 or more cystocarps in controls | | |



*U.S. Government Printing Office: 1981 — 548-187/20535