

Biological Laboratory Certification Guidance Manual for Effluent Toxicity Testing

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1.5	M. Myers	04/2026	M	Updated dated procedures and formatted for accessibility

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Purpose

The purpose of this document is to aid Biological Testing Laboratories in preparation for certification by the State of North Carolina in the area of toxicity testing. This manual will outline minimum requirements for the establishment of standard operating procedures for aquatic toxicity testing programs. It will also serve as a guide for completion of the Biological Laboratory Certification Application.

Minimum Criteria for Receiving and Maintaining Certification

Minimum criteria for receiving and maintaining certification for aquatic toxicity testing are outlined in North Carolina Administrative Code 15 NCAC 2H .1101-.1110 and in the “North Carolina Biological Laboratory Certification/Criteria Procedures Document.” The following is a discussion of these minimum criteria and what they entail.

An important component of any laboratory operation is its supervisor. This person must have a sufficient combination of academic training and experience to properly implement testing and quality assurance protocols. The minimum requirements are a Bachelor of Science degree from an accredited college or university in a biological science or closely related science curriculum and at least three years full time laboratory experience in aquatic toxicity testing, or a Master of Science degree in a biological or closely related science and at least one year full time of laboratory experience in aquatic toxicity testing. The supervisor is ultimately responsible for the overall performance of the laboratory in its execution and reporting of analyses. North Carolina’s Aquatic Toxicology Branch must be notified within 30 days if the designated laboratory supervisor is replaced. One supervisor may direct no more than two laboratories. Also, a substitute supervisor must be designated to ensure proper execution of laboratory procedures in the event of the supervisor’s absence.

Minimum physical requirements for the toxicity testing laboratory include at least 150 square feet of laboratory space and 20 linear feet of laboratory bench space. The building must also provide adequate lighting, cooling, and heating to maintain appropriate test organism environments. Hot and cold running water must be available for equipment cleaning. Appropriate glassware, chemicals, apparatus, disposable supplies, and equipment necessary to perform any procedure included in the certification application must also be available.

For tests which are included in a requested certification, instrumentation must be accessible for the measurement of dissolved oxygen, temperature, and pH directly from the test vessel. Initial measurements may be taken from surrogate vessels to prevent injury to test organisms if such injury is a concern. Additionally, the capabilities to determine total hardness and detect total residual chlorine at a minimum level of 0.1 mg/l are required.

Viable, reproducing cultures of any cladoceran test organisms included in the certification application must be maintained within the laboratory. However, use of fathead minnow and mysid shrimp test organisms obtained from outside sources will be considered on a case-by-case basis.

The Standard Operating Procedures Document

As part of Certification, the applicant must submit with the completed application all associated fees and a Standard Operating Procedures (SOP) document. This SOP document is to be available to all laboratory employees. The SOP is to contain step-by-step descriptions of equipment cleaning procedures, sample collection, chain-of-custody procedures, and organism culturing techniques. Additionally, stepwise procedures for each test type applied for must be included. Quality assurance programs and responses to problems identified in those quality assurance programs must be detailed for assessing culture health, data analysis, and chemical/physical measurements of water quality. The minimum content necessary in the SOP for each of these areas is discussed in the following sections.

Because maintenance of certification requires adherence to the SOP, this document must be approved prior to certification. All SOP documents must be signed by the laboratory supervisor. Should modification be made at any time, a summary of all changes must be submitted to the Division of Water Quality's Aquatic Toxicology Branch for approval.

Many of these procedures will be based on those presented in the EPA documents *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, Fourth Edition (EPA/600/4-90/027F)* and *Short-Term Method for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Third Edition (EPA/600/4-91/002)*. Hereafter, these documents will be referred to as the "EPA acute book" and the "EPA chronic book", respectively. Whenever disposable supplies or equipment are employed in a procedure, specific brand names and compositions should be noted in the appropriate section of the SOP. Example pages of all logbooks, sample collection form(s), chain-of-custody form(s), and test forms should be included with the appropriate SOP section.

Equipment Cleaning

All glassware and sampling equipment should be cleaned as described in the appropriate EPA methods book. The SOP should include this procedure as it will be employed by the applicant as well as a use description of any special equipment not addressed by the EPA procedure. Specifics to be detailed would include the type of detergent used, specific types and brands of acid and acetone, and sources of tap water and dilution water.

The cleaning and maintenance schedule of automated sampling equipment should be kept in a notebook. As each sampler is cleaned, an entry in the notebook should be made which includes an identification of the individual sampler, deviations from standard cleaning procedure, replacement of sampling hose, and any other maintenance performed. This entry should also include the date and signature of the person performing the cleaning and/or maintenance.

Sample Collection

This section should contain toxicity testing sample collection techniques employed by laboratory personnel, client personnel, or both. Sample collection techniques are discussed on pages 39-43 in the EPA acute book and pages 35-43 of the EPA chronic book. For North Carolina clients, the facility's NPDES permit will specify which type of sample is to be collected. Note that North Carolina samples must be between 0.0°C and 6.0°C upon arrival at the laboratory. Samples collected for acute toxicity tests must be used within 36 hours. Samples collected for

chronic toxicity testing must be used within 36 hours of collection and not more than 72 hours after first use of the sample for test renewal. Interpretation of “use” is based upon the time test organisms are introduced. The exception to temperature requirement protocol is the case of an NPDES permit specified grab sample that arrives at the laboratory within three hours of collection covered in ice.

Specifics cited in this section of the SOP would include maximum sample holding times and preservation techniques. The volume and composition of collection containers should be noted as well as those of shipping containers if the samples are shipped in a vessel other than the one in which they were collected. Also, the make and model of automated samplers should be detailed along with operating instructions for collecting 24-hour composite samples. The following sample collection data should be recorded and maintained for a minimum of one year for every sample obtained for toxicity testing:

- Sample name
- Description of sampling site
- NPDES # and pipe number (if applicable)
- County
- Collector (printed)
- Sample type–Grab/Composite (X samples/hr. for Y hrs.)
- Date and time sampling began
- Date and time sampling ended (for composites)
- Was sample collected chilled (for composites)?
- Was sample shipped chilled?
- Method of shipment
- Collector’s signature
- Received in lab by
- Received in lab from
- Time and date sample received in lab
- Temperature of sample when received in lab

It is suggested that this information be recorded on an effluent sample collection form. This form would be filled out by the person or persons collecting the sample and those receiving it upon arrival in the laboratory. An example of this form is to be included in this section or as an appendix of the SOP.

Chain-of-Custody Procedures

When chain-of-custody procedures are used, it is imperative that the possession of the samples be traceable from the time the samples are collected through the termination of any toxicity tests performed. A sample is considered under custody if:

1. It is in the investigator’s actual possession, or
2. It is in the investigator’s view, after being in his/her physical possession, or
3. It was in the investigator’s physical possession and then he/she secured it to prevent tampering, or
4. It is placed in a designated secure area.

This section of the SOP should include procedures which ensure that all chain-of-custody samples remain under custody as defined above throughout sampling and toxicity testing. Sealing the samples inside a plastic bag or cooler with a unique adhesive seal or locking the sample in a padlocked shipping container will satisfy the security requirements of item # 3 above. All chain-of custody forms are to have the following information:

- The name of the facility under evaluation
- The printed name(s) of the person or persons collecting the samples
- A station location and station number for each location sampled
- The time and date that samples were collected from each station
- The number of bottles or containers filled at each station
- The signatures of the person(s) relinquishing and receiving custody of the samples
- The time and date of each transfer of custody
- The signature of the person sealing or locking the samples
- The signature of the person breaking the seal or opening the lock
- The method of sample shipment

An example chain-of-custody form is to be included in this section or as an appendix of the SOP. This form may be combined with the sample collection form.

Sample Receiving Procedures

This section of the SOP should describe each step an effluent sample goes through upon arrival at your laboratory. Information concerning each arriving sample must be recorded in a sample logbook. The temperature and total residual chlorine of the sample must be measured and recorded upon arrival of the sample. Total residual chlorine may be measured again just prior to the sample's use in a toxicity test. A description of the steps that will be taken by your lab when a sample fails to meet the temperature requirements should be included in this section. When a sample is not tested immediately upon arrival, a refrigerator capable of maintaining temperature greater than 0.0°C and less than or equal to 6.0° C is required for storage of samples. Some type of manual or automated temperature recording system should be in place so that it can be shown that the refrigerator consistently maintains temperature greater than 0.0°C and less than or equal to 6.0°C.

Culturing Techniques

For each organism specified in the certification application there should be a corresponding section in the SOP describing culture techniques for that organism. The origin of the culture organisms should be discussed thoroughly, including the original source of the organisms and date they were obtained (an approximation will suffice if not known exactly). Any additional sources of organisms and the date they were added to the original culture should also be included.

A discussion of the source and nature of the culture water for the organism should also be included. The name of a natural source employed as well as its location should be listed. Any treatment to which the water is subjected before use should be discussed, as well as any chemical analyses routinely performed. DWQ recommends at a minimum, metals analyses should be performed once per quarter and an organics scan performed annually. If reconstituted water is

utilized, its recipe should be listed as well as any treatments applied before use. Additionally, the distilling or deionizing systems which provide the main ingredient for the reconstituted water should be described thoroughly.

There should be a full description of all of the hardware used in the culture system. This would include the volume and composition of the culture vessels and culture water storage vessels, the type and composition of any water pumping system employed, the type and arrangement of any aeration system utilized, and specific types of lighting and timers associated with the lighting system.

The culturing strategy should be fully discussed. This would include a means for tracking the approximate age of a given group of organisms. If cultures are restarted periodically, the restarting procedure should be discussed, including details such as how often this is done and how many organisms are used to restart an individual culture vessel. Any other population control methodology employed, for example periodic removal of excess organisms with a pipet from a cladoceran culture, should also be discussed. Additionally, this section should detail the procedure for segregating young within the age limits needed for testing. There should be separate aging procedures for each test type as necessary.

All aspects of culture feeding should be discussed in detail. The composition of food and the procedure for its preparation should be clearly delineated. Procedures describing foods prepared for cladoceran cultures should be particularly detailed. A description of Yeast, Cereal leaves (Cerophyll[®]), Trout chow (YCT) preparation as performed in your lab is required if a cladoceran is to be used for certified toxicity tests. Also, Selenastrum capricornutum culture methods, harvest techniques, and cell density calculations are to be detailed. Feeding rates for culture vessels and the weekly feeding schedule should be discussed.

This section should include a taxonomic identification procedure detailing the taxonomic references employed, which laboratory personnel are responsible for taxonomic identification, and the frequency with which identifications are performed. Representative organisms should be identified and preserved on at least an annual basis. The preservation method should also be included in this section. These representative organisms are to be kept on hand for at least one year and should be identifiable for that long.

A description of all culture records should be included, which details the form and nature of all records kept. For example, the records of a fathead minnow culture would include a daily log of all tank maintenance, records of egg production, hatching times, a feeding schedule checklist to insure that the feeding schedule has been followed, records of disease incidence, mortalities and their locations, changes in feeding or culture routine, and note of any transfer or segregation of groups of fish. Additionally, the results of routine chemical analyses should be recorded in an organized manner to facilitate investigation of a culture production, mortality, or sensitivity problem.

Culture Health Documentation

Each culture specified in the application should have its own standard reference toxicant testing program in order to verify its health and sensitivity. Each culture's testing program should be described in a separate section of the SOP. Prior to certification of a particular category/parameter combination, a minimum database of five reference toxicant tests must be

established, each test falling within control limits as described below.

Offspring from in-house cultures should have their acute sensitivity evaluated by performing an acute reference toxicant test. A reference toxicant test should be performed every two weeks for each organism used in acute whole effluent toxicity testing, or alternatively, acute reference toxicant tests may be performed such that NC NPDES acute tests are performed within one week of an acute reference toxicant test for the organism in question. In the case of the latter, to maintain acute certification for an organism, acute reference toxicant tests must be performed, at minimum, on a quarterly frequency.

A reference toxicant test should be performed once per month for each organism used in chronic whole effluent toxicity testing, or alternatively, tests may be performed such that NC NPDES chronic tests are performed within two weeks of a chronic reference toxicant test for the organism in question. In the case of the latter, to maintain chronic certification for an organism, chronic reference toxicant tests must be performed, at minimum, on a quarterly frequency. The minimum dilution factor for acute and chronic tests is ≥ 0.5 .

A control chart, as described on pages 11-19 of the EPA acute book, should be generated and updated with each acute test that is conducted. As each acute reference toxicant test is performed, culture health trends can be analyzed against the control limits of the chart.

For chronic reference toxicant tests, the endpoint will be the IC25 as determined by the linear interpolation method described in EPA/600/4-91/002. Additionally, a standard procedure for reacting to outliers should be delineated. This procedure would include an immediate retest, notification of the Aquatic Toxicology Branch, notification of clients who have submitted samples for testing since the last satisfactory standard reference toxicant test, and a search for deficiencies in the test procedure or culturing techniques. Also, arrangements should be made for the retesting of effluents tested during the time in which the culture did not produce satisfactory reference toxicant test results when practical. This can be done once the culture begins to return satisfactory reference toxicant test results, or the tests can be subcontracted to another biological laboratory.

The description of each testing program in the SOP should include reference toxicant solution preparation. Also, the source of dilution water should be discussed. This description would follow the same guidelines as that of dilution water discussed in "Testing" above. If the dilution water is the same as that used in the testing of facility effluents, then a reference to that effect will suffice.

Testing Under NPDES Permits

For each parameter endpoint specified in the application, there should be a corresponding section in the SOP delineating the test procedure for that parameter endpoint. The methods published by the EPA or the North Carolina Division of Water Resources should be described as they are implemented by the applicant. Each method explanation should be a step-by-step "cookbook" procedure beginning at the point when the sample is prepared for testing and ending when the test is terminated. Analysis of results will be covered in a separate section.

The following information must be recorded in the lab for each toxicity test performed:

- Test organism
- Test organism age (age from birth or hatching, e.g. 1/1/01-1/2/01 @ 1300-1200)
 - For Ceriodaphnia chronic testing, the brood number from which the neonates were born (i.e. 3rd brood, 4th brood, etc.), age of parent, as well as the date and 8-hour time span for organisms' birth (e.g. organisms born 1/1/01 @ 0900-1700).
- Test organism source (specific tank, board, batch, beaker, etc.)
- Test start date and time
- Test duration or test end date
- Name of the individual who introduced or transferred the organisms
- An indication of what randomization procedures were utilized for each test vessel placement during the test period
- Test chamber numbers for every vessel, which are unique for the period that the test is being conducted
- Toxicant concentrations for every test vessel number
- Total test volume for every test vessel
- The number of organisms introduced into each test vessel at test initiation
- The number of surviving organisms at appropriate points in the test, as dictated by the parameter endpoint type. Depending upon test type, the number of young, length of organism, or organism weight may also need to be recorded.
- As required for reproduction counts, the number of offspring per adult female and average offspring per surviving female. In addition, sufficient information must be available to determine the percentage of control organisms which produced three broods.
- The identification of the specific incubator used for a particular test should be recorded.
- Dilution water used for the test, including source, batch, and treatments. Initial dilution water pH must be between 6.5-8.5 pH units. The total hardness as calcium carbonate must be maintained between 30-50 ppm for surface water and 80-100 ppm for synthetic lab water.
- Temperatures of stock vessel water that test organisms will be transferred from, at least one control vessel, and one high test concentration immediately prior to introduction of the test organisms for each sample or change of sample solution.
- Additionally, temperatures of at least one control and one high test concentration vessel must be taken at termination of the test for each sample or change of sample solution. Only initial temperature measurements may be taken from control and high test concentration surrogate vessels. These surrogates would be prepared identically to the corresponding test vessels but would be intended solely for the measurement of physical/chemical parameters. All temperature and chemical measurements at test termination are to be taken from control and high concentration test vessels only.
- Initial and terminal dissolved oxygen and pH of at least one control vessel and one high concentration toxicant vessel must be recorded for every sample or each change of a sample solution for static renewal tests. Test treatment solutions in Ceriodaphnia chronic toxicity tests must maintain a minimum of 5.0 mg/l dissolved oxygen. A minimum of 4.0 mg/l of dissolved oxygen must be maintained for acute toxicity tests.
- Specific conductance (at 25°C) and total residual chlorine must be determined and recorded for each tested sample.
- Specific conductance (at 25°C), pH, and hardness of the test dilution water must be recorded.
- The signature of analyst(s) that terminated tests and/or determined the endpoint effect

- The initials of analyst(s) performing any necessary feeding of test organisms during tests

The above material can be conveniently placed on a test form. It is suggested that a test form be developed which lists standard conditions such as dilution water source, dilution water treatments, total test volume, and effluent concentrations. Variable data can then be filled in during the preparation and performance of individual tests. An example test form is to be included with the SOP or as an appendix.

This section should describe automated or manual methods for recording temperatures in incubators employed in the test method. Temperature in the incubator should be recorded at a minimum of once per day when tests are in progress. Also, when light intensity and photoperiod are specified for each procedure, the mechanism for controlling the photoperiod should be explained as well.

To minimize effects of varying light intensity and temperature on a group of test vessels, particularly when an incubator is employed, a procedure for the random placement of test vessels is required and should be described in this section. The appropriate test type for North Carolina dischargers can be found in their NPDES permit. If this is not available, or further explanation is required, the Aquatic Toxicology Branch should be contacted.

Data Analysis

There should be a section in the SOP for each type of data analysis performed. These may or may not apply to more than one parameter test endpoint. For instance, a method for determining LC50 values may apply to 48-hour static Ceriodaphnia tests, 96-hour Pimephales promelas flow throughs, and seven-day full range Ceriodaphnia static replacement tests. Some tests may require more than one statistical analysis. For instance, a Ceriodaphnia pass/fail toxicity test may require a check for significant mortality, a test for normality of reproduction data, a test for homogeneity of variances between the control and treatment, and a t test for a significant difference in reproduction between the control and treatment. Each section should begin by listing the parameter endpoints, which are determined using the procedure described. The procedure should then be presented step-by-step.

Any computers and computer programs employed should be noted. All data entry and manual calculations should be checked by at least one person other than the person who initially performed the data entry and calculations.

Special Equipment and Physical/Chemical Analyses

Many of the instruments, special equipment, and chemical analyses employed in toxicity testing will require their own section in the SOP. Sections on instrumentation, such as D.O. meters, pH meters and conductivity meters will need a description of their calibration and a step-by-step description of their use. These descriptions must include the makes and models of all instruments which will be used in toxicity testing. If calibration methods differ between instruments measuring the same parameters, each calibration procedure and the specific instrument(s) to which it applies should be clearly stated. Also, the sources of reagents should be documented. If the reagents are prepared in the lab, the preparation procedure should be presented. The calibrations will need to be documented in calibration books, formatted appropriately for each meter or instrument. For instance, a calibration book for a D.O. meter routinely calibrated by the

Winkler method would require pages with blanks for date, time, initial reading, results of Winkler titration A, results of Winkler titration B, adjusted meter reading, maintenance performed (changed membrane, replaced probe, changed batteries, etc.), and initials of the analyst. An example page of the calibration record book should be included in this section or as an appendix to the SOP. Calibrations for all instruments used in measuring water quality parameters for toxicity testing must be performed at minimum each day the instrument is used.

Special equipment such as a serial diluter or programmable solution dispenser would require step-by-step instructions for their use and calibration. Again, calibration records would be required, and an example calibration sheet included in the SOP document. Procedures for chemical analyses such as hardness and total residual chlorine should be written in detail. Also, a description of calibration and quality assurance measures (blanks or blind samples) should be included. As with the instruments above, the sources or preparation of reagents and standards need to be stated.

Example Outline

To make the general structure of the SOP document clear, the following outline of a theoretical SOP document is presented.

Standard Operating Procedures Outline for SomeLab Laboratories, Inc.

- I. Equipment Cleaning
- II. Sample Collection
- III. Chain-of-Custody Procedures
 - a. Sample Receiving Procedures
 - b. Culturing Techniques
 - i. Ceriodaphnia dubia
 - ii. Pimephales promelas
- IV. Testing
 - a. 48-hour Ceriodaphnia Static Acute
 - b. 96-hour Pimephales promelas Flowthrough
 - c. Three Brood 168-hour Ceriodaphnia Chronic Pass/Fail
 - d. Three Brood 168-hour Ceriodaphnia Phase II
- V. Culture Health Documentation
 - a. Ceriodaphnia dubia
 - b. Pimephales promelas
- VI. Data Analysis
 - a. LC50 Calculation
 - b. Ceriodaphnia Pass/Fail Results Analysis
 - c. Multiple Concentration Chronic Results Analysis

- VII. Equipment/Chemical Analyses
 - a. Dissolved Oxygen Meters
 - b. pH Meters
 - c. Conductivity Meters
 - d. Mount–Brungs Serial Diluter
 - e. Compulab™ Programmable Solution Dispenser
 - f. Total Residual Chlorine Analysis
 - g. Hardness Analysis

- VIII. Appendices
 - a. Sample Collection Form
 - b. Chain-of-Custody Form
 - c. Example Page from Sample Logbook
 - d. Example Page from Ceriodaphnia dubia Culture Logbook
 - e. Example Page from Pimephales promelas Culture Logbook
 - f. Test Form for 168-hour Ceriodaphnia Chronic Pass/Fail
 - g. Test Form for 48-hour Ceriodaphnia Static Acute
 - h. Test Form for 96-hour Pimephales promelas Flowthrough
 - i. Test Form for 168-hour Ceriodaphnia Phase II
 - j. Test Form for 24-hour Ceriodaphnia Acute Quality Assurance test
 - k. Test Form for 24-hour Pimephales promelas Acute Quality Assurance test
 - l. Test Form for 168-hour Ceriodaphnia Chronic Quality Assurance test Standard Operating Procedures Outline for SomeLab Laboratories, Inc.
 - m. Example Page from Buffers/Standards logbook
 - n. Example of LC50 calculation
 - o. Example of analysis of Ceriodaphnia Chronic test data
 - p. Example Page from D.O. meter calibration logbook
 - q. Example Page from pH meter calibration logbook
 - r. Example Page from conductivity meter calibration logbook
 - s. Example Page from Mount–Brungs Serial Diluter calibration logbook
 - t. Example Page from Compulab™ Programmable Solution Dispenser calibration logbook
 - u. Example Page from equipment cleaning logbook

References Used in Aquatic Toxicity Testing Associated with the NPDES Program

Acute Tests; Freshwater and Estuarine

Methods for Measuring the Acute Toxicity of Whole Effluents to Freshwater and Marine Organisms, Fourth Edition. EPA/600/4-90/027 F, September 1991.

“North Carolina Pass/Fail Methodology for Determining Acute Toxicity in a Single Effluent Concentration.” North Carolina Department of Environment, and Natural Resources, DWQ, Water Quality Section. Revised July 1992.

Short-Term Chronic Tests

Freshwater

Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. EPA/600/4-91/002. July 1994.

“North Carolina Ceriodaphnia Chronic Effluent Bioassay Procedure.” North Carolina Department of Environment, and Natural Resources, DWQ, Water Quality Section. Revised February 1998.

“North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure.” North Carolina Department of Environment, and Natural Resources, DWQ, Water Quality Section-Revised February 1998.

Marine/Estuarine

Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA/600/4-91/003. July 1994.

Toxicity Reduction Evaluation Methods/ Effluent Fractionation Toxicity Testing

Methods for Aquatic Toxicity Identification Evaluations, Phase I: Toxicity Characterization Procedures. Second Edition. EPA/600/6-91/003. February 1991. Environmental Research Laboratory, Duluth, MN.

Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I. EPA/600/6-91/005F. May 1992. Environmental Research Laboratory, Duluth, MN.

EPA 600/R-92-080

Methods for Aquatic Toxicity Identification Evaluations, Phase II: Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. September 1993. (NTIS / PB94-114907)

EPA 600/R-92-081

Methods for Aquatic Toxicity Identification Evaluations, Phase III: Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. September 1993. (NTIS / PB94-123833)

EPA 625/R-96-054

Marine Toxicity Identification Evaluation (TIE): Phase I Guidance Document. September 1996 (NSCEP)

Toxicity Reduction Evaluation Guidance For Municipal Wastewater Treatment Plants. EPA/83313-99/002. August 1999, EPA Risk Reduction Engineering Laboratory, Cincinnati, OH.

Generalized Methodology for Conducting Industrial Toxicity Reduction Evaluations (TREs). EPA 600/2-88/070. 1989, EPA Water Engineering Research Laboratory, Cincinnati, OH.

To Obtain: Permits Division (EN-336), Office of Water Enforcement and Permits, 401 M St. SW, Washington, D.C. 20460.

Contacts for Questions:

Department of Environmental Quality
Division of Water Resources
Water Sciences Section
Aquatic Toxicology Branch

Aquatic Toxicology Branch website:

<https://www.deq.nc.gov/about/divisions/water-resources/water-sciences-section/aquatic-toxicology-branch-atb>

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