

15A NCAC 02H .1111 BIOLOGICAL LABORATORY CERTIFICATION AND QUALITY ASSURANCE

- (a) Aquatic Toxicology Laboratories shall have the following laboratory resources:
- (1) 200 square feet of laboratory space;
 - (2) 20 linear feet of laboratory bench space;
 - (3) one drained sink with hot and cold running water;
 - (4) control of culture environment including lighting, cooling, and heating to maintain organism as set forth in the approved procedures and these Rules;
 - (5) one refrigerator that will maintain sample temperatures between 0.0 degrees Celsius and 6.0 degrees Celsius;
 - (6) current copies of the approved procedures for which the laboratory is requesting certification;
 - (7) glassware, chemicals, supplies, and equipment to perform any procedures included in the requested certification;
 - (8) instrumentation capable of measuring dissolved oxygen, pH, temperature, conductivity, and salinity (for saltwater tests) directly from test vessels of any procedure included in certification application. Equivalent surrogate vessels may be utilized for physical measurements if injury to test organisms may result;
 - (9) instrumentation or analytical capabilities to perform measurements of total residual chlorine to a level at least as low as 0.1 mg/l and total hardness to a level at least as low as 1 mg/l;
 - (10) a dissecting microscope and a compound microscope for those laboratories requesting or maintaining either of the categories of Acute Toxicity Testing/Invertebrate or Chronic Toxicity Testing/Invertebrate. The compound microscope shall have a minimum magnification of 400x and a maximum magnification of greater than or equal to 1,000x;
 - (11) a balance capable of weighting 0.0001g and Class "S" or equivalent reference weights. A balance capable of weighing fish larvae to 0.00001g for those laboratories requesting or maintaining certification for the category Chronic Toxicity Testing/Vertebrate;
 - (12) Cladocerans shall be cultured in-house. All other organisms may be purchased from a supplier;
 - (13) dilution water for use in whole effluent toxicity testing with chemical characteristics such that the pH is between 6.5 S.U. and 8.5 S.U. and total hardness as calcium carbonate is between 30 ppm and 50 ppm for surface water and 80 ppm and 100 ppm for synthetic lab water. If receiving waters have characteristics outside of these stated pH and hardness ranges, then alternate pH and hardness ranges shall be accepted upon demonstration to the State Laboratory that the alternate ranges are better suited to testing objectives, and that quality assurance standards have been met; and
 - (14) chain-of-custody documentation.
- (b) Aquatic Population Survey and Analysis Laboratories shall have the following laboratory resources:
- (1) 150 square feet of laboratory space;
 - (2) eight linear feet of laboratory bench space;
 - (3) binocular dissecting microscopes and compound microscopes suitable for survey type;
 - (4) vials, preservatives, and space to maintain representative sample collections for at least one year after collection;
 - (5) current taxonomic guides and reference materials to support identification;
 - (6) chain-of-custody documentation forms, laboratory records, and seals;
 - (7) sampling equipment to support collection of appropriate biological organisms; and
 - (8) settling tubes and one inverted microscope with a minimum magnification of 300x for those laboratories requesting or maintaining certification for algae.
- (c) All laboratories shall adhere to the following quality assurance requirements:
- (1) instruments used in or associated with toxicity testing, including automatic sampling equipment, pH meter, dissolved oxygen meter, and conductivity meter, shall be calibrated each day before the instrument is used. Calibrations performed shall be recorded;
 - (2) a minimum of five valid reference toxicant tests shall be performed and entered on a control chart for each toxicity test organism and toxicity test type for which a lab is certified. A maximum of 20 data points shall be entered on a control chart;
 - (3) a reference toxicant test shall be performed:
 - (A) every two weeks for each organism used in acute whole effluent toxicity testing; or such that North Carolina National Pollutant Discharge Elimination System (NPDES) acute tests are performed within one week of an acute reference toxicant test for the organism

- in question. To maintain acute certification for an organism, acute reference toxicant tests shall be performed at least quarterly; and
- (B) once per month for each organism used in chronic whole effluent toxicity testing; or such that North Carolina NPDES chronic tests are performed within two weeks of a chronic reference toxicant test for the organism in question. To maintain chronic certification for an organism, chronic reference toxicant tests shall be performed at least quarterly.
- (4) a reference test shall be performed with each batch of organisms received from an outside supplier;
 - (5) the endpoint for chronic reference toxicant tests shall be the IC25 as determined by the linear interpolation method described in EPA-821-R-02-013 and EPA-821-R-02-014, herein incorporated by reference, including any subsequent amendments or editions. These methods are available free of charge at: <https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods>;
 - (6) acceptable alternative culture media utilized to culture the algae *Selenastrum capricornutum* for use as *Ceriodaphnia* food are as follows:
 - (A) the Marine Biology Laboratory (MBL) medium as described in the Handbook of Phycological Methods Handbook of Phycological Methods: Culture Methods and Growth Measurements. 1973. J. Stein, ed. University Press, Cambridge, MA, available at a cost of sixty eight dollars and eighty five cents (\$68.85), herein incorporated by reference, including subsequent amendments and editions; and
 - (B) additional nutrients for the preparation of algae medium described in Section 13.6.15 of EPA-821-R-02-013 and Appendix A1, Section 3.10.3 of EPA-821-R-02-012. These methods are available free of charge at: <https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods>, herein incorporated by reference, including any subsequent amendments and editions. The volume of nutrient stock solutions found in Table 1 on Page 147 of EPA-821-R-02-013 or Page 133 of EPA-821-R-02-012 may be adjusted so that solutions 1.A, 1.D, and 2 are added at a rate of 2 ml/l, and solutions 1.B and 1.C are added at a rate of 6 ml/l;
 - (7) a representative of each test organism cultured, including those obtained from an outside supplier, shall be taxonomically identified to the species level at least annually. Specimens shall be preserved and held for one additional year;
 - (8) when closed incubators are used for toxicity testing or test organism culturing purposes, culturing and testing activities shall not be contained within the same incubator;
 - (9) effluent samples collected for chronic *Ceriodaphnia dubia* tests shall be used within 36 hours of collection and not more than 72 hours after first use of the sample for test renewal. The beginning of this period is defined as the time of the collection of a grab sample or the time of collection of the last subsample of a composite sample to the time that the organisms are introduced to the test solution; and
 - (10) a record shall be maintained for all samples entering the laboratory that documents the sample identity and includes the following information:
 - (A) the sample number;
 - (B) the sample temperature at receipt;
 - (C) the time and date of sample collection and receipt;
 - (D) the name of person from whom the sample was received; and
 - (E) the name of person who received the sample.
- (d) The following procedure modifications have been approved by the EPA and shall be followed by certified laboratories:
- (1) acute and chronic toxicity tests shall be conducted at 25.0 degrees Celsius plus or minus 1.0 degree Celsius, except that chronic tests for *Mysidopsis bahia* shall be conducted at 26.0 degrees Celsius plus or minus 1.0 degree Celsius. Certified laboratories may request in writing variances from the State Laboratory for species which require alternate temperatures in accordance with EPA procedures;
 - (2) organisms used in acute toxicity tests shall have food made available for a minimum of two hours prior to initiation of testing;
 - (3) for cladoceran species, the feeding amount prior to the acute test shall be at least 0.05 ml of YCT and 0.05 ml of a solution of the algae *Selenastrum capricornutum* with a cell concentration of 1.71×10^7 cells/ ml per 15 ml of culture solution;

- (4) for each sample used in a toxicity test, the following parameters shall be measured and recorded from an undiluted aliquot:
 - (A) pH;
 - (B) specific conductance;
 - (C) total residual chlorine;
 - (D) dissolved oxygen; and
 - (E) salinity (for salt water test);
- (5) for each sample used in a toxicity test, the following parameters shall be measured in the control and the highest toxicant concentration tested at the beginning of the test, prior to renewal, following each renewal, and at the termination of the test:
 - (A) temperature;
 - (B) dissolved oxygen;
 - (C) pH; and
 - (D) salinity (for salt water test);
- (6) Ceriodaphnia dubia used in toxicity tests shall meet the following requirements:
 - (A) be obtained from individual cultures;
 - (B) be obtained from third or subsequent broods of adults not being more than 14 days in age and containing eight or more neonates with an average adult mortality not exceeding 20 percent per culture board;
 - (C) chronic Ceriodaphnia dubia analyses shall have an additional test acceptability criterion of complete third brood neonate production by at least 80 percent of the surviving control organisms;
 - (D) Ceriodaphnia dubia neonate reproduction totals from chronic tests shall include only organisms produced in the first through third broods;
 - (E) the percentage of male Ceriodaphnia dubia control organisms shall not exceed 20 percent in chronic Ceriodaphnia dubia tests; and
 - (F) the Ceriodaphnia dubia control organism reproduction coefficient of variation (CV) shall be less than 40 percent for a chronic Ceriodaphnia dubia test;
- (7) "Observed-effect" in a chronic Ceriodaphnia dubia test shall be defined as:
 - (A) statistical significant decrease in survival of the treatment organism as compared to the control organisms; or
 - (B) 20 percent or greater decrease in treatment organisms as compared to the control organism reproduction that is also determined to be statistically different from the control organism reproduction;
- (8) acute tests shall be terminated within one hour of their stated length;
- (9) the North Carolina Pass/Fail chronic tests and Phase II Ceriodaphnia dubia chronic tests shall meet the following requirements:
 - (A) follow a schedule where the test is started on day zero, renewed on day two and five, and terminated no later than seven days and two hours after the initiation of the test;
 - (B) follow a schedule where each daily feeding shall consist of addition of 0.05 ml of yeast-Cerophyll® -trout chow (YCT) food and 0.05 ml of a solution of the algae Selenastrum capricornutum with a cell concentration of 1.71×10^7 cells/ml per 15 ml of test solution; and
 - (C) the percent reduction for chronic Ceriodaphnia dubia analysis for each treatment shall be calculated by subtracting the mean number of neonates produced by the treatment organisms from the mean number of neonates produced by the control organisms, dividing that number by the mean number of neonates produced by the control organisms, and multiplying by 100 percent;
- (10) the North Carolina Pass/Fail Ceriodaphnia dubia chronic test shall be performed as two treatments exposing 12 test organisms to each treatment. The first treatment shall be considered the control population and shall be exposed at 0 percent effluent and 100 percent dilution water;
- (11) the North Carolina Pass/Fail acute test shall be performed as two treatments with the control population specified as Treatment 1, and the effluent treatment specified as Treatment 2. Each treatment shall be tested using four identical test vessels. Each treatment shall contain 10 test organisms, for a total of 80 test organisms; and

- (12) there shall be no removal of chlorine or any other effluent constituent by either chemical or physical methods prior to testing.

*History Note: Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;
Eff. October 1, 1988;
Readopted Eff. July 1, 2019.*