## SECTION .1100 - BIOLOGICAL LABORATORY CERTIFICATION

### 15A NCAC 02H .1101 PURPOSE

These Rules set forth the requirements for certification of commercial, industrial, and public laboratories to perform biological toxicity testing and aquatic population surveys of water and wastewater as required by G.S. 143-215.3(a) and 15A NCAC 02B .0200 and .0500. These Rules establish an EPA-designated program for the State to implement the Clean Water Act, as set forth in 33 U.S.C. 1318 and 1319.

*History Note:* *Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215(c); 143-215.66;*
*Eff. October 1, 1988;*
*Amended Eff. March 1, 1993;*
*Readopted Eff. July 1, 2019.*

**15A NCAC 02H .1102 SCOPE**

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*Repealed Eff.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

**15A NCAC 02H .1103 DEFINITIONS**

The following terms as used in this Section shall have the assigned meaning:

(1) "Approved Procedure" means an analytical procedure developed by the State Laboratory based upon 40 CFR 136.3 and subject to G.S. 143, Article 21, Part 1. A link to the approved procedures can be found at https://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-sciences-home-page/aquatic-toxicology-branch/downloads.

(2) "Aquatic population survey and analysis" means field sampling, laboratory identification, analysis, and metric derivation for determining biological integrity, as defined in 15A NCAC 02B .0202 for fish, aquatic macroinvertebrates, phytoplankton, and aquatic macrophytes using methods developed in accordance with 15A NCAC 02B .0103(b).

(3) "Certification" means a declaration by the Division that personnel, equipment, records, quality control procedures, and methodology cited by the applicant complies with the rules in this Section.

(4) "Commercial Laboratory" means any laboratory, including its employees and agents, that analyzes, for others, wastewater samples for toxicity measurements or for their impacts on the receiving waters.

(5) "Decertification" means the loss of certification.

(6) "Director" means the Director of the North Carolina Division of Water Resources.

(7) "Division" means the North Carolina Division of Water Resources.

(8) "Falsified data or information" means data or information that, whether by intent, or disregard for accuracy, has been altered, fabricated, recorded falsely or mischaracterized by omission or substitution.

(9) "Industrial Laboratory" means a laboratory, including its employees and agents, operated by an industrial facility to analyze samples from its wastewater treatment plants for toxicity measurements or impacts to receiving waters or to conduct aquatic population surveys and analysis.

(10) "Proficiency Testing sample" means a performance evaluation sample provided by the State Laboratory or a State Laboratory-approved vendor as defined in 15A NCAC 02H .0803(38), located at https://nelac-institute.org/content/NEPTP/ptproviders.php to a commercial, industrial, or public laboratory as an unknown toxicant for measurement of toxicity, as an unknown analyte for measurement by laboratory equipment or wet chemistry methods, or as an unknown set of preserved organisms for identification to specified levels of taxonomic classification.

(11) "Public Laboratory" means a laboratory, including its employees and agents, operated by a municipality, county, water and sewer authority, sanitary district, metropolitan sewerage district, or State or federal installation to analyze samples from its wastewater treatment plant(s) for toxicity measurements or resultant impacts to receiving waters.

(12) "Split samples" for surface water effluent discharge, surface water, or phytoplankton means two or more representative portions taken from a single sampling device. For aquatic macrophytes or macroinvertebrates, split sample means a single sample that is analyzed by both the State Laboratory and by the commercial, public, or industrial laboratory.

(13) "State laboratory" means the Water Sciences Section of the North Carolina Division of Water Resources.

(14) "Toxicant" means any specific chemical, compound, or mixture of chemicals or compounds regulated by an NPDES permit or defined as a toxic substance in 15A NCAC 02B .0202.

*History Note:* *Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;*
*Eff. October 1, 1988;*
*Amended Eff. April 1, 1993;*
*Readopted Eff. July 1, 2019.*

**15A NCAC 02H .1104 FEES ASSOCIATED WITH CERTIFICATION PROGRAM**

(a) Certification Fees:

(1) The first category, as set forth in Rule .1105 of this Section, shall be certified at a cost of five hundred dollars ($500.00) per year. Additional categories, shall be certified at a cost of four hundred dollars ($400.00) per year per category. The addition of parameters not included in the original certification shall be certified at a cost of one hundred dollars ($100.00) per year per parameter.

(2) Certification fees are due upon application and no later than 45 days prior to the requested certification date.

(b) Renewal Fees:

(1) The certified laboratory shall pay the State a four hundred dollar ($400.00) per year renewal fee for each category of certification or the minimum fee of five hundred dollars ($500.00) per year if only one category is certified. Renewal certification fees are due by November 1 annually.

(2) Out-of-state laboratories shall reimburse the State for actual travel and subsistence costs incurred in certification, recertification, and maintenance of certification.

*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.*

**15A NCAC 02H .1105 CERTIFICATION**

(a) Commercial, public, and industrial laboratories shall obtain certification from the Division for biological parameters that are required to be reported pursuant to G.S. 143, Article 21, Part 1.

(b) For the purposes of certification and setting fees, parameters shall be grouped in the following categories:

(1) Acute Toxicity Testing/Invertebrate;
(2) Acute Toxicity Testing/Vertebrate;
(3) Chronic Toxicity Testing/Invertebrate;
(4) Chronic Toxicity Testing/Vertebrate;
(5) Algal and Aquatic Plant Toxicity Testing; and
(6) Aquatic Population Survey and Analysis.

(c) All certifications shall be in effect for one year and may be renewed for additional one-year periods as set forth in Rule .1104 of this Section.

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

**15A NCAC 02H .1106 DECERTIFICATION**

(a) The Director or the Director's designee may revoke the entire laboratory certification for:

(1) failing to maintain the facilities, records, personnel, equipment, or a quality assurance program as required by these Rules;

(2) submitting inaccurate or falsified data reports or other information; or

(3) failing to pay required fees by the date due.

(b) A laboratory certification may be revoked for a category for failure to:

(1) obtain acceptable results on two consecutive proficiency testing samples. Acceptable results on proficiency testing samples are those that fall within the specified acceptable range as indicated by the State Laboratory or State Laboratory-approved vendor. The State Laboratory may apply specific variance or statistical limits or performance criteria on performance evaluation samples or split samples for a particular testing procedure, including control population effects and taxonomic identification, as published in these Rules;

(2) obtain acceptable results as set out in Subparagraph (b)(1) of this Paragraph on two consecutive split samples that have also been analyzed by the Division;

(3) submit a split sample to the Division as requested;

(4) use approved procedures as defined in Rule .1103 of this Section;

(5) report equipment changes that would affect the laboratory's ability to perform a test category to the State Laboratory within 30 days of the change;

(6) report results of proficiency testing to the State Laboratory within the requirements that are set forth by the proficiency study;

(7) maintain records and perform quality controls as set forth by these Rules;

(8) maintain equipment required for any certified parameter;

(9) implement and maintain quality control programs approved in conjunction with certification; or

(10) maintain a qualified staff, as specified in Rule .1110(f)(1) and (2) of this Section.

(c) Requirements for Laboratories following Decertification:

(1) A laboratory shall not analyze samples for parameters in decertified categories for programs governed by rules of this Section.

(2) A decertified commercial laboratory shall notify any clients affected by the laboratory's decertification and supply the State Laboratory with a list of those clients affected and a written certification that those clients have been notified. If the decertified laboratory arranges for a certified laboratory to perform analyses during the period of decertification, the decertified laboratory shall supply the Division with the name of the replacement laboratory and the clients involved. The name of the certified laboratory that performs analyses shall appear on all data submitted to the Division.

*History Note:* *Authority G.S. 143-215.3(a)(1); 143-215.3(a)(4); 143-215.3(a)(10); 143-215.66;*
*Eff. October 1, 1988;*
*Amended Eff. March 1, 1993;*
*Readopted Eff. July 1, 2019.*

**15A NCAC 02H .1107 RECERTIFICATION**

(a) A laboratory decertified for any reason other than the submittal of falsified data reports or other information shall be recertified after 30 days upon demonstrating to the State Laboratory that all deficiencies have been corrected.

(b) In the case of a laboratory decertified for submitting falsified data reports or other information, recertification shall not occur prior to 12 months after the decertification and then only at such time as the laboratory has demonstrated to the Director, or their delegate, that the standards for initial certification have been met.

(c) If a laboratory that was decertified due to either failure of proficiency testing samples or split samples seeks recertification, the laboratory shall submit a written request to the State Laboratory requesting evaluations for the category pursuant to Rule .1106(b) of this Section for which the laboratory was decertified. Two consecutive samples shall have acceptable results as set forth in Rule .1106 of this Section to achieve recertification. The first of these samples for recertification shall be submitted or arranged by the Division no later than 30 days after receipt of the written request. The second shall be submitted or arranged no later than 30 days after the first.

*History Note:* *Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;*
*Eff. October 1, 1988;*
*Amended Eff. March 1, 1993;*
*Readopted Eff. July 1, 2019.*

**15A NCAC 02H .1108 RECIPROCITY**

(a) Laboratories certified by other states or federal programs shall be given reciprocal certification if the programs meet the requirements of these Rules. In requesting certification through reciprocity, laboratories shall include with the application a copy of their certification and the rules of the original certifying agency.

(b) Laboratories certified pursuant to this Rule shall pay all applicable fees set forth in Rule .1104 of this Section.

*History Note:* *Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;*
*Eff. October 1, 1988;*
*Amended Eff. March 1, 1993;*
*Readopted Eff. July 1, 2019.*

**15A NCAC 02H .1109 ADMINISTRATION**

(a) Appeals. If the Director or the Director's delegate denies certification, or decertifies a laboratory, the laboratory may appeal pursuant to G.S. 150B, Article 3.

(b) The State Laboratory shall maintain a current list of certified commercial, industrial, or public laboratories.

*History Note:* *Authority G.S. 143-215.3(a)(1); 143-215(a)(4); 143-215.3(a)(10); 143-215.66;*
*Eff. October 1, 1988;*
*Amended Eff. March 1, 1993;*
*Readopted Eff. July 1, 2019.*

**15A NCAC 02H .1110 IMPLEMENTATION**

(a) Each laboratory requesting State certification, certification renewal, or recertification shall apply to the Division. Each application shall be reviewed to determine if personnel, equipment, records, quality control procedures, and methodology meet the requirements pursuant to 40 CFR 136.3 and these Rules. After receiving a completed application and prior to issuing certification, a representative of the Division shall inspect each laboratory to verify the information in the application and if the laboratory meets requirements pursuant to these Rules.

(b) Analytical methods, sample preservation, sample containers, and sample holding times shall conform to the methodologies specified in:

(1) 40 CFR Part 136, hereby incorporated by reference and including subsequent amendments and editions. Copies of the Code of Federal Regulations, 40 CFR Part 136, may be obtained from the Superintendent of Documents, U.S. Government Printing Office (GPO), Superintendent of Public Documents, Washington, D.C. 20402 and free of charge on the Internet at http://www.ecfr.gov; and

(2) Rule .1111 of this Section.

(c) Pursuant to G.S. 143B-282, the Environmental Management Commission or designated delegate, shall approve the State Laboratory to develop Approved Procedures for Biological Procedures based upon the methods contained in 40 CFR Part 136 and Rule .1111 of this Section. Approved Procedures for Biological Procedures document shall be available for inspection at the State Laboratory, 4401 Reedy Creek Road, Raleigh, North Carolina, 27607 or may be obtained free of charge on the State Laboratory Certification website at https://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-sciences-home-page/aquatic-toxicology-branch.

(d) Pursuant to G.S. 143B-282, the Environmental Management Commission or designated delegate, may approve other analytical procedures, parameters, or parameter methods that have been demonstrated to produce verifiable and repeatable results.

(e) In order to maintain certification, each laboratory shall meet the requirements of this Section for proficiency testing samples submitted to the Division. Proficiency testing by certified laboratories shall be required no more than three times annually for each category certified.

(f) In order to receive and maintain certification, the following criteria shall be met:

(1) The supervisor of an aquatic toxicology or biological survey laboratory shall have a Bachelor's degree from an accredited college as defined in 34 CFR 602 or university in a biological science or related science curriculum and three years of cumulative laboratory experience in aquatic toxicity testing or aquatic population surveying, or a Master's degree in a biological or related science and one year of cumulative laboratory experience in aquatic toxicity testing or aquatic population surveying.

(2) All laboratory supervisors shall be subject to review by the Division. One person shall not serve as supervisor of more than two laboratories. The supervisor shall provide direct supervision and evaluation of all technical personnel and shall be responsible for the performance and reporting of all analyses. Upon absence, the supervisor shall arrange for a suitable substitute who meets the requirements of Subparagraph (f)(1) of this Rule and is capable of insuring the performance as set forth by these Rules of all laboratory procedures. Existing laboratory supervisors who do not meet the minimum requirements shall be accepted after review by the Division if they meet all other certification requirements and previous performance has met the requirements of these Rules.

(3) All applications and fees shall be due pursuant to Rule .1104 of this Section. Upon the Division establishing compliance with the requirements of this Section, certification shall be issued by the Director or Director's delegate within 45 days of receipt of the fees for certification.

(4) Each laboratory shall develop and maintain a document outlining quality control procedures for testing of all approved procedures in their certification and dissolved oxygen, temperature, conductivity, and pH. All aquatic toxicology laboratories shall also develop and maintain a document outlining quality control procedures for testing of total hardness and total residual chlorine. These documents shall be included with submittal of the application.

(5) Each laboratory certified for the category of Aquatic Population Survey and Analysis shall develop and maintain a document outlining quality control procedures for taxonomic identifications and life-stage determinations.

(6) Supporting records shall be maintained for five years as evidence that these practices have met the requirements of these Rules and are being carried out and shall be available to the State Laboratory upon request.

(7) The quality control program shall be approved in conjunction with certification by the Director or the Director's delegate.

*History Note:* *Authority G.S. 143-215.3(a)(1); 143-215.3(a)(4); 143-215.3(a)(10); 143-215.66;*
*Eff. October 1, 1988;*
*Amended Eff. October 1, 1993;*
*Readopted Eff. July 1, 2019.*

**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 X $10^7$ 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 $1.71 \times 10^{7}$ 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.*