15A NCAC 02H .1101 is proposed for readoption with amendment as follows:

15A NCAC 02H .1101 PURPOSE

These Rules shall 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 for National Pollutant Discharge Elimination System (NPDES) permits by G.S. 143-215.3(a)(10) and Environmental Management Commission Rules for Classifications and Water Quality Standards Applicable to the Surface Waters of North Carolina, found in Subchapter 2B of this Chapter, Section .0200, and Rules for Surface Water Monitoring, Reporting, found in Subchapter 2B of this Chapter, Section .0500.

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.

15A NCAC 02H .1102 is proposed for repeal as follows:

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.

15A NCAC 02H .1103 is proposed for readoption with amendment as follows:

15A NCAC 02H .1103 DEFINITIONS

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

~~(1) Categories are groups of parameters which differ by measured test exposure regimes (chronic and acute) and, in the case of toxicological assay, through the presence or absence of vertebrae in the species of test organisms used or being a member of the plant kingdom. All field population survey techniques are contained within one category.~~

(1) 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(11) for fish, aquatic macroinvertebrates, phytoplankton, and aquatic macrophytes using methods developed in accordance with 15A NCAC 02B .0103(b). Standard operating procedures used by the State are available for review on the Division's website.

(2) Approved Procedure means an analytical procedure developed by the State Laboratory based upon relevant reference methods and approved for use for monitoring subject to G.S. 143-215.1 and G.S. 143-215.63, et seq.

~~(2)~~(3) Certification is a declaration by the Division that personnel, equipment, records, quality control procedures, and methodology cited by the applicant are accurate and that the ~~applicants'~~ applicant's proficiency ~~has been considered and found acceptable.~~ complies with the Rules in this Section.

~~(3)~~(4) Commercial Laboratory means any laboratory, including its employees and agents, ~~which~~ that analyzes, for others, wastewater samples for toxicity measurements or for their ~~resultant~~ impacts on the receiving waters.

~~(4)~~(5) Decertification is the loss of certification.

~~(5)~~(6) Director means the Director of the North Carolina Division of ~~Environmental Management,~~ Water Resources, or his successor.

~~(6)~~(7) Division means the North Carolina Division of ~~Environmental Management,~~ Water Resources, or its successor.

~~(7)~~(8) ~~Evaluation samples are samples submitted~~ Proficiency Testing sample means a performance evaluation sample provided by the State Laboratory or a State Laboratory approved vendor to ~~the~~ a commercial, municipal, industrial, or public laboratory as an unknown toxicant for measurement of ~~toxicity~~ 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.

~~(8)~~(9) Falsified data or information means data or information ~~that~~ that, whether by intent, or reckless disregard for accuracy, has been ~~made untrue by alteration, fabrication, intentional~~ altered, fabricated, or otherwise reported or recorded falsely or mischaracterized by omission, substitution,

~~or mischaracterization.~~ such that the value or information reported is incorrect, incomplete, or inaccurate. ~~The agency need not prove intent to defraud to prove data is falsified.~~

~~(9) Inaccurate data or other information means data or information that is in any way incorrect or mistaken.~~

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

~~(11) Parameters are subgroups of categories. Parameters are unique and separate if they are in separate categories or are performed using different species of test organisms. For the category, Aquatic Population Survey, separate parameters are to be considered fish, macroinvertebrates, algae, aquatic macrophytes, and zooplankton.~~

~~(12)~~(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 ~~or any other governmental unit~~ to analyze samples from its wastewater treatment plant(s) for toxicity measurements or resultant impacts to receiving waters.

~~(13) Recertification is reaffirmation of certification.~~

~~(14)~~(12) Split samples ~~are samples from either a~~ for surface water effluent discharge, surface water, or ~~aquatic biological population survey which are segregated at the point of sampling or in the case of field survey, collected independently and then~~ 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 ~~separately~~ by both the State Laboratory and by the commercial, ~~public~~ public, or industrial laboratory.

~~(15)~~(13) State laboratory means the ~~Environmental~~ Water Sciences ~~Branch~~ Section of the ~~Water Quality Section of the~~ North Carolina Division of ~~Environmental Management~~ Water Resources, or its successor.

~~(16)~~(14) ~~Toxicant Any~~ Toxicant means any specific ~~chemical or compound~~ chemical, compound, or mixture of chemicals or compounds regulated ~~within~~ by an NPDES permit ~~and/or~~ or defined as a toxic substance in Rule .0202 of Subchapter 2B.

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.

15A NCAC 02H .1104 is proposed for readoption with amendment as follows:

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

(a) Certification Fees:

(1) Certification Fees shall be a minimum of five hundred dollars per year ($500.00). The first category ~~will~~ shall be certified at a cost of five hundred dollars ~~($500.00).~~ ($500.00) per year. Additional categories ~~will~~ 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 ~~will~~ 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 or Recertification Fees:

(1) The certified laboratory ~~will~~ 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) Recertification fees shall be four hundred dollars ($400.00) per category recertified.~~

~~(3)~~(2) Out-of-state laboratories shall reimburse the state for actual travel and subsistence costs incurred in certification, ~~recertification~~ recertification, and maintenance of certification. The certification process requires visual inspection to verify that laboratories meet the requirements established by the Rules 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.

15A NCAC 02H .1105 is proposed for readoption with amendment as follows:

15A NCAC 02H .1105 CERTIFICATION

~~(a) Certification is affirmation by the Director or his delegate that the requirements specified by these rules have been met for specific categories and parameters and that all fees associated with certification have been received.~~

~~(b)~~(a) Commercial, public and industrial laboratories ~~must~~ shall obtain certification from the Division of ~~Environmental Management~~ Water Resources ~~only~~ for biological parameters ~~which will be~~ that are required to be reported pursuant to ~~comply with the rules and requirements as stated in~~ an administrative letter, permit condition, permit limit, special order by consent, judicial order, or the biological monitoring requirements established by the Division.

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

(1) Acute Toxicity Testing/Invertebrate;

(2) Acute Toxicity Testing/Vertebrate;

(3) Chronic Toxicity Testing/Invertebrate;

(4) Chronic Toxicity Testing/Vertebrate;

(5) ~~Agal~~ Algal and Aquatic Plant Toxicity Testing; and

(6) Aquatic Population Survey and Analysis.

~~(d)~~(c) All certifications ~~are~~ shall be designated for the period of one year after initial certification.

~~(e) Protocol Documents considered as standard methodology and facilities and equipment requirements considered as minimum acceptable resources will be listed in the Certification Criteria/Procedures Document.~~

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

15A NCAC 02H .1106 is proposed for readoption with amendment as follows:

15A NCAC 02H .1106 DECERTIFICATION

(a) ~~A laboratory certification may be revoked for all categories for:~~ The Director or the Director's designee shall consider revoking a laboratory certification for a parameter for:

(1) ~~Failing~~ failing to maintain the facilities, records, personnel, ~~equipment~~ equipment, or quality assurance program as set forth in the application or as required by these Rules; or

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

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

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

(1) ~~Obtain~~ obtain acceptable results on two consecutive ~~evaluation sample submittals~~ proficiency testing samples from the Division. Acceptable results on ~~performance evaluation~~ proficiency testing samples are those that ~~vary by less than two standard deviations of the value established by the Division.~~ fall within the specified acceptable range as indicated by the State Laboratory or State Laboratory approved vendor. The ~~state laboratory~~ 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 ~~the Certification Criteria/Procedures Document; or~~ these Rules;

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

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

(4) ~~Use~~ use approved testing techniques; ~~or~~

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

(6) ~~Report to the state laboratory~~ report analysis of ~~performance evaluation~~ proficiency testing samples submitted by the Division to the State Laboratory within required time of completion; ~~or~~

(7) ~~Maintain~~ maintain records and perform quality controls as set forth by these ~~Rules and the Division for a particular category; or~~ Rules;

(8) ~~Maintain~~ maintain equipment required for any certified parameter; ~~or~~

(9) ~~Implement~~ implement and maintain Quality Control Programs approved in conjunction with certification; or

(10) ~~Maintain~~ maintain a qualified ~~staff.~~ staff, as specified in Rule .1110 of this Section.

(c) Decertification Requirements:

(1) A laboratory ~~is not to~~ shall not analyze samples for parameters in decertified categories for programs ~~described in Rule .1102~~ governed by Rules of this Section.

(2) A decertified commercial laboratory ~~must~~ shall notify any clients affected by the laboratory's decertification ~~of such~~ and supply the ~~state laboratory~~ State Laboratory with a list of those clients

affected and a written certification that those clients have been notified. ~~Should~~ If the decertified laboratory ~~arrange~~ arranges for a certified laboratory to perform analyses during the period of decertification, the decertified laboratory ~~must~~ shall supply the Division with the name of the replacement laboratory and the ~~client(s)~~ clients involved. The name of the certified ~~laboratory's name which~~ laboratory that performs analyses must appear on all data submitted to the Division.

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.

15A NCAC 02H .1107 is proposed for readoption with amendment as follows:

15A NCAC 02H .1107 RECERTIFICATION

(a) A laboratory decertified for any ~~reason,~~ reason other than the submittal of falsified data reports or other ~~information, may~~ information shall be recertified after 30 ~~days,~~ days upon ~~satisfactory demonstration~~ demonstrating to the ~~state laboratory~~ 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 ~~until at least~~ prior to12 months after the decertification and then only at such time as the laboratory has ~~satisfactorily~~ demonstrated to the ~~Director~~ Director, or their delegate, that the standards for initial certification have been met.

(c) ~~Should decertification occur due to either failure of performance samples or split samples,~~ 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 ~~must be made to the state laboratory~~ to the State Laboratory requesting evaluations ~~similar to~~ for the parameters for which the laboratory was decertified. Two consecutive samples ~~must~~ shall be successfully evaluated to achieve recertification. The first of these samples for recertification ~~will~~ shall be submitted or arranged by the Division no later than 30 days after receipt of the written request. The second ~~will~~ 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.

15A NCAC 02H .1108 is proposed for readoption with amendment as follows:

15A NCAC 02H .1108 RECIPROCITY

(a) Laboratories certified by other states or federal programs ~~may~~ shall be given reciprocal certification ~~where~~ if such 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 on the basis of program equivalency shall pay all fees specified by these Rules.

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.

15A NCAC 02H .1109 is proposed for readoption with amendment as follows:

15A NCAC 02H .1109 ADMINISTRATION

~~The Director of the Division of Environmental Management, Department of Environment, Health, and Natural Resources, or his delegate, is delegated authority to issue certification, to reject applications for certification, to renew certification, to issue recertification, to issue decertification, and to issue reciprocity certification.~~

(a) Appeals. If the Director of their delegate denies certification, or decertifies a laboratory, the laboratory may appeal to the N.C. Office of Administrative Hearings in accordance with Chapter 150B of the General Statutes.

(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); 143-215.3(a)(10); 143-215.66;*
Eff. October 1, 1988;
Amended Eff. March 1, 1993.

15A NCAC 02H .1110 is proposed for readoption with amendment as follows:

15A NCAC 02H .1110 IMPLEMENTATION

(a) Each laboratory requesting state ~~certification or~~ certification, certification ~~renewal~~ renewal, or recertification shall submit an application ~~in duplicate~~ to the Division. Each application ~~will~~ shall be reviewed to determine the adequacy of personnel, equipment, records, quality control ~~procedures~~ procedures, and methodology. After receiving a completed application and prior to issuing certification, a representative of the Division ~~may visit~~ shall inspect each laboratory to verify the information in the application and the adequacy of the ~~laboratory.~~ laboratory pursuant to these Rules.

(b) Analytical methods, sample preservation, sample ~~containers~~ containers, and sample holding times shall conform to the methodologies specified ~~in the Certification/Criteria Procedures Document. Deviations from these methods are acceptable only upon prior written approval from the state laboratory.~~ 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) The State Laboratory may develop Approved Procedures for Biological Procedures based upon the methods contained in 40 CFR Part 136 and Rule .1111 of this Section. The State Laboratory 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) The Director, or assigned delegate, shall approve other analytical procedures, parameters, or parameter methods that have been demonstrated to produce verifiable and repeatable results and that have a widespread acceptance in the scientific community.

(e) In order to maintain certification, each laboratory ~~will~~ shall demonstrate satisfactory performance on ~~evaluation~~ proficiency testing samples submitted ~~by~~ to the Division. ~~These will be~~ Demonstration of satisfactory performance by certified laboratories shall be required no more than three times annually ~~of certified laboratories~~ for each parameter certified.

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

(1) The supervisor of an aquatic toxicology or biological survey laboratory ~~must~~ shall have a ~~minimum of a B.S.~~ Bachelor's degree from an accredited college or university in a biological science or ~~closely related~~ closely-related science curriculum and ~~at least~~ three years of cumulative laboratory experience in aquatic toxicity testing or aquatic ~~biological survey,~~ population surveying, as appropriate, or a ~~M.S.~~ Master's degree in a biological or ~~closely related~~ closely-

related science and ~~at least~~ one year of cumulative laboratory experience in aquatic toxicity testing or aquatic ~~biological survey,~~ population surveying, as appropriate.

(2) All laboratory supervisors ~~are~~ shall be subject to review by the Division. One person ~~may~~ shall not serve as supervisor of ~~no~~ more than two laboratories. The supervisor ~~is to~~ shall provide direct supervision and evaluation of all technical personnel and ~~is~~ shall be responsible for the proper performance and reporting of all analyses. Upon absence, the supervisor shall arrange for a suitable substitute who meets the requirements of Sub-Paragraph (f)(1) of this Rule and is capable of insuring the proper performance of all laboratory procedures. Existing laboratory supervisors who do not meet the minimum requirements ~~may~~ shall be accepted after review by the Division if they meet all other certification requirements and previous performance is deemed adequate.

(3) All applications and fees ~~are~~ shall be due ~~45 days prior to the requested certification date.~~ pursuant to Rule .1104 of this Section. Upon the State establishing compliance with the requirements of this Section, certification shall be issued within 45 days of receipt of the fees for certification. ~~Problems identified with the applying laboratory and resolution of these problems may extend the requested 45 day period from application to certification.~~

(4) Each laboratory shall develop and maintain a document outlining quality control procedures for testing of all parameters in their certification and dissolved oxygen, temperature, conductivity, and pH. All aquatic toxicology laboratories ~~must~~ shall also develop and maintain a document outlining quality control procedures for testing of total hardness and total residual chlorine. These documents ~~are to~~ 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 are being effectively carried out and shall be available to the ~~state laboratory~~ State Laboratory upon request.

(7) The quality control program ~~is to~~ shall be approved in conjunction with certification by the ~~Director.~~ Director or their delegate.

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

15A NCAC 02H .1111 is proposed for readoption with amendment as follows:

15A NCAC 02H .1111 ~~BIOLOGICAL LABORATORY CERT/CRITERIA PROCEDURES DOCUMENT~~
BIOLOGICAL LABORATORY CERTIFICATION AND QUALITY ASSURANCE

~~The Biological Laboratory Certification/Criteria Procedures Document describes specific scientific reporting units, forms, test methods and procedures pertaining to certification.~~

~~The manual, and any addition thereto, shall be approved by the director before it is released to the public. The manual shall be mailed to all certified biological laboratories and to any persons on the mailing list. To be placed on the mailing list, a letter must be sent to the director.~~

~~If the manual is revised at any time, all changes shall be sent to the certified biological laboratories and those persons on the mailing list.~~

(a) To be considered for certification and to maintain certification, 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) adequate control of culture environment including lighting, cooling, and heating to maintain appropriate organism requirements;

(5) one refrigerator of adequate size which will maintain sample temperatures between 0.0 degrees Celsius and 6.0 degrees Celsius;

(6) current copies of the approved methods and 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 accurately weighting 0.0001g and Class “S” or equivalent reference weights. A balance capable of accurately weighing fish larvae to 0.00001g for those laboratories requesting or maintaining certification for the category Chronic Toxicity Testing/Vertebrate.

(12) Daphniid need to be cultured in house. All other organisms can be purchased from a supplier.

(13) appropriate 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 forms.

(b) To be considered for certification and to maintain certification, Aquatic Population Survey and Analysis Laboratories shall have the following laboratory resources:

(1) 150 square feet of laboratory space;

(2) 8 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 the parameter Algae.

(c) To be considered for certification and to maintain certification, laboratories shall adhere to the following quality assurance requirements:

(1) instruments used in or associated with toxicity testing, including, but not limited to, automatic sampling equipment, pH meter, dissolved oxygen meter, and conductivity meter, shall be calibrated daily or with each use if instruments are used more than once daily. Calibrations performed shall be recorded in a designated notebook;

(2) a minimum of 5 valid reference toxicant tests shall be performed and entered on a control chart for each organism and 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 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 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, 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, 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) sample number;

(B) sample temperature at receipt;

(C) time and date of sample collection and receipt;

(D) name of person from which sample was received; and

(E) 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 variances 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 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 on the day the sample is first used:

(A) pH;

(B) specific conductance; and

(C) total residual chlorine;

(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; and

(C) pH;

(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 which 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 0, renewed on day 2 and 5, and terminated no later than 7 days and 2 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%;

(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% effluent and 100% 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.