

1 15A NCAC 02H .1101 is proposed for readoption with amendment as follows:

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3 **15A NCAC 02H .1101 PURPOSE**

4 These Rules shall set forth the requirements for certification of commercial, industrial, and public laboratories to
5 perform biological toxicity testing and aquatic population surveys of water and wastewater as required for National
6 Pollutant Discharge Elimination System (NPDES) permits by G.S. 143-215.3(a)(10) and Environmental
7 Management Commission Rules for Classifications and Water Quality Standards Applicable to the Surface Waters
8 of North Carolina, found in Subchapter 2B of this Chapter, Section .0200, and Rules for Surface Water Monitoring,
9 Reporting, found in Subchapter 2B of this Chapter, Section .0500.

Commented [A1]: Added per RRC review. No effect.

Commented [A2]: Added for clarification. No effect.

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

1 15A NCAC 02H .1102 is proposed for repeal as follows:

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3 **15A NCAC 02H .1102 SCOPE**

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5 *History Note:* Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;

6 *Eff. October 1, 1988.*

1 15A NCAC 02H .1103 is proposed for reoption with amendment as follows:

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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 or

Commented [A1]: Deleted definition of “categories” – unnecessary. No effect.

Commented [A2]: Added for clarification. No effect.

Commented [A3]: Added for clarification. No effect.

Commented [A4]: Clarification. No effect.

Commented [A5]: Deleted “resultant” – unnecessary. No effect.

Commented [A6]: Updated name. No effect.

Commented [A7]: Updated definition to be consistent with definition in O2H.0803

Commented [A8]: Clarifying option for labs. No effect.

Commented [A9]: Clarification per RRC recommendation. No effect.

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.~~

Commented [A10]: Deleted definition of “inaccurate data” – unnecessary. No effect.

(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.

Commented [A11]: Rewording – no effect.

Commented [A12]: Deleted “resultant”– unnecessary. No effect.

(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.~~

Commented [A13]: Clarification. No effect.

Commented [A14]: Deleted definition of “parameters” – unnecessary. No effect.

(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.

Commented [A15]: Deleted “or any other governmental unit”– unnecessary. No effect.

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

Commented [A16]: Deleted definition of “recertification” – unnecessary. No effect.

(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.

Commented [A17]: Clarification. No effect.

(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.

Commented [A18]: Updated name. No effect.

(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.

1 15A NCAC 02H .1104 is proposed for reoption with amendment as follows:

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3 **15A NCAC 02H .1104 FEES ASSOCIATED WITH CERTIFICATION PROGRAM**

4 (a) Certification Fees:

5 (1) Certification Fees shall be a minimum of five hundred dollars per year (\$500.00). The first
6 category ~~will~~ shall be certified at a cost of five hundred dollars (~~(\$500.00)~~ (\$500.00) per year.
7 Additional categories ~~will~~ shall be certified at a cost of four hundred dollars (\$400.00) per year per
8 category. The addition of parameters not included in the original certification ~~will~~ shall be
9 certified at a cost of one hundred dollars (\$100.00) per year per parameter.

Commented [A1]: Clarifications. No effect.

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

12 (b) Renewal or Recertification Fees:

Commented [A2]: Clarification. No effect.

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

Commented [A3]: Clarification. No effect.

16 ~~(2) Recertification fees shall be four hundred dollars (\$400.00) per category recertified.~~

Commented [A4]: Deleted – duplicative of sub-paragraph (b)(1). No effect.

17 ~~(2)~~ (2) Out-of-state laboratories shall reimburse the state for actual travel and subsistence costs incurred
18 in certification, ~~recertification~~ recertification, and maintenance of certification. The certification
19 process requires visual inspection to verify that laboratories meet the requirements established by
20 the Rules of this Section.

Commented [A5]: Clarifying what is involved in cert process. No effect.

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

1 15A NCAC 02H .1105 is proposed for reoption with amendment as follows:

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3 **15A NCAC 02H .1105 CERTIFICATION**

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

Commented [A1]: Deleted – duplicative of definition in 2H .1103. No effect.

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

Commented [A2]: Updated name. No effect.

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

- 13 (1) Acute Toxicity Testing/Invertebrate;
- 14 (2) Acute Toxicity Testing/Vertebrate;
- 15 (3) Chronic Toxicity Testing/Invertebrate;
- 16 (4) Chronic Toxicity Testing/Vertebrate;
- 17 (5) ~~Agal~~ Algal and Aquatic Plant Toxicity Testing; and
- 18 (6) Aquatic Population Survey and Analysis.

Commented [A3]: Corrected spelling of algal. No effect.

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

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

Commented [A4]: Deleted – duplicative of language adding to 2H .1110. No effect.

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

1 15A NCAC 02H .1106 is proposed for readoption with amendment as follows:

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3 **15A NCAC 02H .1106 DECERTIFICATION**

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

Commented [A1]: All changes are for clarification. No effect.

Commented [A2]: Updated language for conciseness

- 6 (1) ~~Failing failing~~ to maintain the facilities, records, personnel, ~~equipment equipment~~, or quality
- 7 assurance program as set forth in the application or as required by these Rules; or
- 8 (2) ~~Submitting submitting~~ inaccurate or falsified data reports or other information; or
- 9 (3) ~~Failing failing~~ to pay required fees by the date due.

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

- 11 (1) ~~Obtain obtain~~ acceptable results on two consecutive ~~evaluation sample submittals proficiency~~
12 ~~testing samples~~ from the Division. Acceptable results on ~~performance evaluation proficiency~~
13 ~~testing samples~~ are those that ~~vary by less than two standard deviations of the value established by~~
14 ~~the Division. fall within the specified acceptable range~~ as indicated by the State Laboratory or
15 ~~State Laboratory approved vendor~~. The ~~state laboratory State Laboratory~~ may apply specific
16 variance or statistical limits or performance criteria on performance evaluation samples or split
17 samples for a particular testing procedure, including control population effects and taxonomic
18 identification, as published in ~~the Certification Criteria/Procedures Document; or these Rules;~~
- 19 (2) ~~Obtain obtain~~ acceptable results as set out in Paragraph (1) of this Rule on two consecutive split
20 samples that have also been analyzed by the Division; ~~or~~
- 21 (3) ~~Submit submit~~ a split sample to the Division as requested; ~~or~~
- 22 (4) ~~Use use~~ approved testing techniques; ~~or~~
- 23 (5) ~~Report to the state laboratory report~~ equipment changes that would affect ~~it's the laboratory's~~
24 ability to perform a test category ~~to the State Laboratory~~ within 30 days of such change; ~~or~~
- 25 (6) ~~Report to the state laboratory report~~ analysis of ~~performance evaluation proficiency testing~~
26 samples submitted by the Division ~~to the State Laboratory~~ within required time of completion; ~~or~~
- 27 (7) ~~Maintain maintain~~ records and perform quality controls as set forth by these Rules ~~and the~~
28 ~~Division for a particular category; or Rules;~~
- 29 (8) ~~Maintain maintain~~ equipment required for any certified parameter; ~~or~~
- 30 (9) ~~Implement implement~~ and maintain Quality Control Programs approved in conjunction with
31 certification; or
- 32 (10) ~~Maintain maintain~~ a qualified ~~staff. staff, as specified in Rule .1110 of this Section.~~

Commented [A3]: Redefined acceptable range for proficiency testing samples to allow outside vendors which sometimes use other ranges for pH, conductivity, etc. Less stringent.

Commented [A4]: Changed "performance evaluation sample" to "proficiency testing sample" for consistency with Chemistry Lab Certification rules. No effect.

Commented [A5]: Added reference to Rule 02H.1110 for clarity. No effect.

33 (c) Decertification Requirements:

- 34 (1) A laboratory ~~is not to shall not~~ analyze samples for parameters in decertified categories for
35 programs ~~described in Rule .1102 governed by Rules~~ of this Section.
- 36 (2) A decertified commercial laboratory ~~must shall~~ notify any clients affected by the ~~laboratory's~~
37 decertification ~~of such~~ and supply the ~~state laboratory State Laboratory~~ with a list of those clients

1 affected and a written certification that those clients have been notified. ~~Should~~ If the decertified
2 laboratory ~~arrange~~ arranges for a certified laboratory to perform analyses during the period of
3 decertification, the decertified laboratory ~~must~~ shall supply the Division with the name of the
4 replacement laboratory and the ~~client(s)~~ clients involved. The name of the certified laboratory's
5 ~~name which~~ laboratory that performs analyses must appear on all data submitted to the Division.
6

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

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1 15A NCAC 02H .1107 is proposed for readoption with amendment as follows:
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3 **15A NCAC 02H .1107 RECERTIFICATION**

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

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

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

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

Commented [A1]: All changes are for clarification. No effect.

1 15A NCAC 02H .1108 is proposed for readoption with amendment as follows:
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3 **15A NCAC 02H .1108 RECIPROCITY**

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

7 (b) Laboratories certified on the basis of program equivalency shall pay all fees specified by these Rules.
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9 *History Note:* Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;

10 *Eff. October 1, 1988;*

11 *Amended Eff. March 1, 1993.*

Commented [A1]: Changes for clarification. No effect.

1 15A NCAC 02H .1109 is proposed for readoption with amendment as follows:

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3 **15A NCAC 02H .1109 ADMINISTRATION**

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

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

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

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11 *History Note: Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;*

12 *Eff. October 1, 1988;*

13 *Amended Eff. March 1, 1993.*

1 15A NCAC 02H .1110 is proposed for reoption with amendment as follows:

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3 **15A NCAC 02H .1110 IMPLEMENTATION**

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

10 (b) Analytical methods, sample preservation, sample ~~containers~~ containers, and sample holding times shall conform
11 to the methodologies specified in the Certification/Criteria Procedures Document. Deviations from these methods
12 are acceptable only upon prior written approval from the state laboratory, in:

13 (1) 40 CFR Part 136, hereby incorporated by reference and including subsequent amendments and
14 editions. Copies of the Code of Federal Regulations, 40 CFR Part 136, may be obtained from the
15 Superintendent of Documents, U.S. Government Printing Office (GPO), Superintendent of Public
16 Documents, Washington, D.C. 20402 and free of charge on the Internet at <http://www.ecfr.gov>;
17 and

18 (2) Rule .1111 of this Section.

19 (c) The State Laboratory may develop Approved Procedures for Biological Procedures based upon the methods
20 contained in 40 CFR Part 136 and Rule .1111 of this Section. The State Laboratory Approved Procedures for
21 Biological Procedures document shall be available for inspection at the State Laboratory, 4401 Reedy Creek Road,
22 Raleigh, North Carolina, 27607 or may be obtained free of charge on the State Laboratory Certification website at
23 [https://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-sciences-home-page/aquatic-](https://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-sciences-home-page/aquatic-toxicology-branch)
24 [toxicology-branch](https://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-sciences-home-page/aquatic-toxicology-branch).

25 (d) The Director, or assigned delegate, may approve other analytical procedures, parameters, or parameter methods
26 that have been demonstrated to produce verifiable and repeatable results and that have a widespread acceptance in the
27 scientific community.

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

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

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

Commented [A1]: Procedure modifications moved to 2H .1111. No effect.

Commented [A2]: Added to be consistent with DWR Wastewater/Groundwater Certification Rules 02H.0805

Commented [A3]: Changing "by" to "to" allows the DWR to provide options for vendors to the laboratories. This provides more options for labs. Less stringent.

Commented [A4]: Changed to reflect more accurate terminology. No effect.

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

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

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

Commented [A5]: Re-worded for clarity. No effect.

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

Commented [A6]: Conductivity added for consistency with existing EPA methods. No effect.

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

24 (6) Supporting records shall be maintained for five years as evidence that these practices are being
25 effectively carried out and shall be available to the ~~state laboratory~~ State Laboratory upon request.

Commented [A7]: Clarifying that records must be maintained for five years. Less stringent.

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

28
29 *History Note:* Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;
30 Eff. October 1, 1988;
31 Amended Eff. October 1, 1993.
32

1 15A NCAC 02H .1111 is proposed for reoption with amendment as follows:

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3 **15A NCAC 02H .1111 BIOLOGICAL LABORATORY CERT/CRITERIA PROCEDURES DOCUMENT**
4 **BIOLOGICAL LABORATORY CERTIFICATION AND QUALITY ASSURANCE**

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

Commented [A1]: Deleted – unnecessary. No effect.

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

Commented [A2]: Moved req't for Director approval to 02H .1110. No effect.

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

Commented [A3]: Updated how labs can access the Document/manual via Internet in 02H .1110. No effect.

12 (a) To be considered for certification and to maintain certification, Aquatic Toxicology Laboratories shall have
13 the following laboratory resources:

14 (1) 200 square feet of laboratory space;

15 (2) 20 linear feet of laboratory bench space;

16 (3) one drained sink with hot and cold running water;

17 (4) adequate control of culture environment including lighting, cooling, and heating to maintain
18 appropriate organism requirements;

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

21 (6) current copies of the approved methods and procedures for which the laboratory is requesting
22 certification;

23 (7) glassware, chemicals, supplies, and equipment to perform any procedures included in the
24 requested certification;

25 (8) instrumentation capable of measuring dissolved oxygen, pH, temperature, conductivity, and
26 salinity (for saltwater tests) directly from test vessels of any procedure included in certification
27 application. Equivalent surrogate vessels may be utilized for physical measurements if injury to
28 test organisms may result;

29 (9) instrumentation or analytical capabilities to perform measurements of total residual chlorine to a
30 level at least as low as 0.1 mg/l and total hardness to a level at least as low as 1 mg/l;

31 (10) a dissecting microscope and a compound microscope for those laboratories requesting or
32 maintaining either of the categories of Acute Toxicity Testing/Invertebrate or Chronic Toxicity
33 Testing/Invertebrate. The compound microscope shall have a minimum magnification of 400x and
34 a maximum magnification of greater than or equal to 1,000x;

35 (11) a balance capable of accurately weighting 0.0001g and Class "S" or equivalent reference weights.
36 A balance capable of accurately weighing fish larvae to 0.00001g for those laboratories requesting
37 or maintaining certification for the category Chronic Toxicity Testing/Vertebrate.

- 1 (12) Daphniid need to be cultured in house. All other organisms can be purchased from a supplier.
- 2 (13) appropriate dilution water for use in whole effluent toxicity testing with chemical characteristics
3 such that the pH is between 6.5 S.U. and 8.5 S.U. and total hardness as calcium carbonate is
4 between 30 ppm and 50 ppm for surface water and 80 ppm and 100 ppm for synthetic lab water.
5 If receiving waters have characteristics outside of these stated pH and hardness ranges, then
6 alternate pH and hardness ranges shall be accepted upon demonstration to the State Laboratory
7 that the alternate ranges are better suited to testing objectives, and that quality assurance standards
8 have been met; and
- 9 (14) chain-of-custody documentation forms.
- 10 (b) To be considered for certification and to maintain certification, Aquatic Population Survey and Analysis
11 Laboratories shall have the following laboratory resources:
- 12 (1) 150 square feet of laboratory space;
13 (2) 8 linear feet of laboratory bench space;
14 (3) binocular dissecting microscopes and compound microscopes suitable for survey type;
15 (4) vials, preservatives, and space to maintain representative sample collections for at least one year
16 after collection;
17 (5) current taxonomic guides and reference materials to support identification;
18 (6) chain-of-custody documentation forms, laboratory records, and seals;
19 (7) sampling equipment to support collection of appropriate biological organisms; and
20 (8) settling tubes and one inverted microscope with a minimum magnification of 300x for those
21 laboratories requesting or maintaining certification for the parameter Algae.
- 22 (c) To be considered for certification and to maintain certification, laboratories shall adhere to the following
23 quality assurance requirements:
- 24 (1) instruments used in or associated with toxicity testing, including, but not limited to, automatic
25 sampling equipment, pH meter, dissolved oxygen meter, and conductivity meter, shall be
26 calibrated daily or with each use if instruments are used more than once daily. Calibrations
27 performed shall be recorded in a designated notebook;
- 28 (2) a minimum of 5 valid reference toxicant tests shall be performed and entered on a control chart for
29 each organism and test type for which a lab is certified. A maximum of 20 data points shall be
30 entered on a control chart;
- 31 (3) a reference toxicant test shall be performed:
- 32 (A) every two weeks for each organism used in acute whole effluent toxicity testing; or such
33 that North Carolina National Pollutant Discharge Elimination System (NPDES) acute
34 tests are performed within one week of an acute reference toxicant test for the organism
35 in question. To maintain acute certification for an organism, acute reference toxicant
36 tests shall be performed at least quarterly; and
- 37 (B) once per month for each organism used in chronic whole effluent toxicity testing; or

1 such that North Carolina NPDES chronic tests are performed within two weeks of a
2 chronic reference toxicant test for the organism in question. To maintain chronic
3 certification for an organism, chronic reference toxicant tests shall be performed at least
4 quarterly.

5 (4) a reference test shall be performed with each batch of organisms received from an outside
6 supplier;

7 (5) the endpoint for chronic reference toxicant tests shall be the IC25 as determined by the linear
8 interpolation method described in EPA-821-R-02-013 and EPA-821-R-02-014, herein incorporated
9 by reference, including any subsequent amendments or editions. These methods are available at:
10 <https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods>

11 (6) acceptable alternative culture media utilized to culture the algae *Selenastrum capricornutum* for
12 use as *Ceriodaphnia* food are as follows:

13 (A) the MBL medium as described in the Handbook of Phycological Methods Handbook of
14 Phycological Methods: Culture Methods and Growth Measurements, 1973, J.Stein, ed.
15 University Press, Cambridge, MA, herein incorporated by reference, including
16 subsequent amendments and editions; and

17 (B) additional nutrients for the preparation of algae medium described in Section 13.6.15 of
18 EPA-821-R-02-013 and Appendix A1, Section 3.10.3 of EPA-821-R-02-012, herein
19 incorporated by reference, including any subsequent amendments and editions. The
20 volume of nutrient stock solutions found in Table 1 on Page 147 of EPA-821-R-02-013 or
21 Page 133 of EPA-821-R-02-012 may be adjusted so that solutions 1.A, 1.D, and 2 are
22 added at a rate of 2 ml/l, and solutions 1.B and 1.C are added at a rate of 6 ml/l.

23 (7) a representative of each test organism cultured, including those obtained from an outside supplier,
24 shall be taxonomically identified to the species level at least annually. Specimens shall be
25 preserved and held for one additional year;

26 (8) when closed incubators are used for toxicity testing or test organism culturing purposes, culturing
27 and testing activities shall not be contained within the same incubator;

28 (9) effluent samples collected for chronic *Ceriodaphnia dubia* tests shall be used within 36 hours of
29 collection and not more than 72 hours after first use of the sample for test renewal. The beginning
30 of this period is defined as the time of the collection of a grab sample or the time of collection of
31 the last subsample of a composite sample to the time that the organisms are introduced to the test
32 solution; and

33 (10) a record shall be maintained for all samples entering the laboratory that documents the sample
34 identity and includes the following information:

35 (A) sample number;

36 (B) sample temperature at receipt;

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

- 1 (D) name of person from which sample was received; and
2 (E) name of person who received the sample.
- 3 (d) The following procedure modifications have been approved by the EPA and shall be followed by certified
4 laboratories:
- 5 (1) acute and chronic toxicity tests shall be conducted at 25.0 degrees Celsius plus or minus 1.0
6 degree Celsius, except that chronic tests for *Mysidopsis bahia* shall be conducted at 26.0 degrees
7 Celsius plus or minus 1.0 degree Celsius. Certified laboratories may request variances for species
8 which require alternate temperatures in accordance with EPA procedures;
- 9 (2) organisms used in acute toxicity tests shall have food made available for a minimum of two hours
10 prior to initiation of testing;
- 11 (3) for cladoceran species, the feeding amount shall be at least 0.05 ml of YCT and 0.05 ml of a
12 solution of the algae *Selenastrum capricornutum* with a cell concentration of 1.71×10^7 cells/ ml
13 per 15 ml of culture solution;
- 14 (4) for each sample used in a toxicity test, the following parameters shall be measured and recorded
15 from an undiluted aliquot on the day the sample is first used:
- 16 (A) pH;
- 17 (B) specific conductance; and
- 18 (C) total residual chlorine;
- 19 (5) for each sample used in a toxicity test, the following parameters shall be measured in the control
20 and the highest toxicant concentration tested at the beginning of the test, prior to renewal,
21 following each renewal, and at the termination of the test:
- 22 (A) temperature;
- 23 (B) dissolved oxygen; and
- 24 (C) pH;
- 25 (6) *Ceriodaphnia dubia* used in toxicity tests shall meet the following requirements:
- 26 (A) be obtained from individual cultures;
- 27 (B) be obtained from third or subsequent broods of adults not being more than 14 days in age
28 and containing eight or more neonates with an average adult mortality not exceeding 20
29 percent per culture board;
- 30 (C) chronic *Ceriodaphnia dubia* analyses shall have an additional test acceptability criterion
31 of complete third brood neonate production by at least 80 percent of the surviving control
32 organisms;
- 33 (D) *Ceriodaphnia dubia* neonate reproduction totals from chronic tests shall include only
34 organisms produced in the first through third broods;
- 35 (E) the percentage of male *Ceriodaphnia dubia* control organisms shall not exceed 20
36 percent in chronic *Ceriodaphnia dubia* tests; and

- 1 (F) the *Ceriodaphnia dubia* control organism reproduction coefficient of variation (CV) shall
2 be less than 40 percent for a chronic *Ceriodaphnia dubia* test;
- 3 (7) “Observed-effect” in a chronic *Ceriodaphnia dubia* test shall be defined as:
- 4 (A) statistical significant decrease in survival of the treatment organism as compared to the
5 control organisms; or
- 6 (B) 20 percent or greater decrease in treatment organisms as compared to the control
7 organism reproduction which is also determined to be statistically different from the
8 control organism reproduction;
- 9 (8) acute tests shall be terminated within one hour of their stated length;
- 10 (9) the North Carolina Pass/Fail chronic tests and Phase II *Ceriodaphnia dubia* chronic tests shall
11 meet the following requirements:
- 12 (A) follow a schedule where the test is started on day 0, renewed on day 2 and 5, and
13 terminated no later than 7 days and 2 hours after the initiation of the test;
- 14 (B) follow a schedule where each daily feeding shall consist of addition of 0.05 ml of yeast-
15 *Cerophyll®* -trout chow (YCT) food and 0.05 ml of a solution of the algae *Selenastrum*
16 *capricornutum* with a cell concentration of 1.71×10^7 cells/ml per 15 ml of test solution;
17 and
- 18 (C) The percent reduction for chronic *Ceriodaphnia dubia* analysis for each treatment shall
19 be calculated by subtracting the mean number of neonates produced by the treatment
20 organisms from the mean number of neonates produced by the control organisms,
21 dividing that number by the mean number of neonates produced by the control
22 organisms, and multiplying by 100%;
- 23 (10) the North Carolina Pass/Fail *Ceriodaphnia dubia* chronic test shall be performed as two treatments
24 exposing 12 test organisms to each treatment. The first treatment shall be considered the control
25 population and shall be exposed at 0% effluent and 100% dilution water;
- 26 (11) the North Carolina Pass/Fail acute test shall be performed as two treatments with the control
27 population specified as Treatment 1, and the effluent treatment specified as Treatment 2. Each
28 treatment shall be tested using four identical test vessels. Each treatment shall contain 10 test
29 organisms, for a total of 80 test organisms; and
- 30 (12) there shall be no removal of chlorine or any other effluent constituent by either chemical or
31 physical methods prior to testing.

32
33 *History Note: Authority G.S. 143-215.3(a)(1); 143-215.3(a)(10); 143-215.66;*
34 *Eff. October 1, 1988.*