SUMMARY OF CHANGES
AQUATIC TOXICOLOGY UNIT (ATU) PROCEDURE REVISIONS
DECEMBER 2010

The Aquatic Toxicology Unit (ATU) revised the following four documents in December 2010.

- North Carolina Division of Water Quality Biological Laboratory Certification/Criteria Procedures Document
- North Carolina *Ceriodaphnia* Chronic Effluent Toxicity Procedure
- North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure
- North Carolina Pass/Fail Methodology for Determining Acute Toxicity in a Single Effluent Concentration

These documents were last updated in 1998. Aside from one major procedural change, these revisions represent past and current operating procedures of the laboratory certification program and will require relatively little to no changes in operations by currently certified laboratories.

MAJOR PROCEDURAL CHANGE: 36 Hour Initial Use Holding Time

The only major procedural change involved in the revisions is the implementation of a 36-hour holding time for first use of a whole effluent toxicity (WET) sample for chronic toxicity testing. Previously, the holding time for using chronic WET samples was 72 hours for both first and final use. The change to a 36-hour initial use hold time was requested by EPA after a triennial review of North Carolina's NPDES process. All affected facilities and laboratories were notified by letter of the change to their sampling requirements in November 2010 and had at least 30 days to prepare for the new schedule. Charts 1 and 2 outline the change in sampling and holding time requirements that are being implemented on January 1, 2011. Revisions to the following documents were required due to this change:

- North Carolina Biological Laboratory Certification/Criteria Procedures Document
- North Carolina *Ceriodaphnia* Chronic Effluent Toxicity Procedure
- North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure

CHART 1 **Change to achieve first use within 36 hours of sample collection (begins January 1, 2011)**
Per EPA manual, in static-renewal tests, each grab or composite sample may also be used to prepare solutions for renewal at 24 h, 48 h, and/or 72 h after first use if stored at 0-6ºC, with minimal head space, as described in Subsection 8.5.4 of the chronic manual.

Schedule	Day -2	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	Mon	Tues	Wed
Facility Sampling	Start Comp. 1	End Comp. 1	**Start Comp. 2**	**End Comp. 2**						
Lab Testing			Initiate test with Comp. 1 within 36 hrs of collection		**Renew test with Comp. 2 within 36 hrs of collection**			**Renew test with Comp. 2 within 72 hrs of first use on Friday**		End Test

[1] *USEPA. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th edition. EPA 821/R-02-013. Office of Science and Technology., U.S. Environmental Protection Agency, Washington, D.C. 20460.*

CHART 2 **Current NCDWQ Schedule (ends December 31, 2010)**

Schedule	Day -2	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Mon	Tues	Wed	Thurs	Fri	Sat	Sun	Mon	Tues	Wed
Facility Sampling	Start Comp. 1	End Comp.1		Start Comp.2	End Comp.2					
Lab Testing			Initiate test with Comp.1 within 72 hrs of collection		Renew test with Comp.1 within 72 hrs of collection			Renew test with Comp. 2 within 72 hrs of collection		End Test

OTHER REVISIONS

All other revisions in the documents are language changes that pertain to certified biological laboratory practices and are updates to 1) reflect current laboratory requirements; 2) provide more details; 3) improve the clarity and consistency of the documents. These revisions represent past and current operating procedures of the laboratory certification program and will require relatively little to no changes in operations by currently certified laboratories. These updates are summarized below.

CHANGES AFFECTING ALL FOUR DOCUMENTS

Current EPA Methods Manuals: All three test procedures and the Criteria/Procedures documents now reference the most current editions of the EPA Toxicity Testing Method Manuals. The current EPA editions that are now included in the North Carolina documents are listed below:

- Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition, October 2002, EPA 821-R-02-013.
- Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition, October 2002, EPA 821-R-02-014.
- Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, October 2002, EPA 821-R-02-012.

Sample Holding Temperature Updated to 6.0ºC: In all documents, the upper limit of the acceptable cold storage sample holding temperature range has been updated from 4.0 to 6.0 º C. The temperature range of 0.0-6.0º C is the current acceptable sample holding temperature for acute and chronic whole effluent toxicity samples in the laboratory certification program and the EPA manuals. This language update will not change any current practices.

Updated Department, Division, and Director Name: Any references to The North Carolina Department of Environment, Health, and Natural Resources, Division of Environmental Management, Water Quality Section have been updated to the North Carolina Department of Environment and Natural Resources, Division of Water Quality. The name of the Director of the Division of Water Quality has been updated to Coleen Sullins.

Updated References: The Reference Sections have been updated.

Version Numbers Added: All Documents revised in 2010 have been given version number 3.0. Previously, none of the documents had a version number. The last revisions were performed in 1992 for the Acute Pass/Fail document and in 1998 for the Biological Laboratory Certification/Criteria Procedures Document and the two Chronic Ceriodaphnia Procedures documents. These last revisions are being considered as version 2.0.

CHANGES AFFECTING THE "BIOLOGICAL LABORATORY CERTIFICATION/CRITERIA PROCEDURES" DOCUMENT

Updated References to North Carolina Administrative Code (NCAC): References to the NCAC were updated in Sections (7) and (9) of the Methods and Procedures Section.

In (7), "Any other toxicological monitoring methods approved by the Director under 15 NCAC 02B.0211" was changed to "Any other toxicological monitoring methods approved by the Director under 15 NCAC 02H.1100."

In (9), 15 NCAC 02B.0212 was changed to 15 NCAC 02B.0220. Historically, 15 NCAC 02B.0212 was the reference for Tidal Salt Water Quality Standards for Class SC Waters, but this was changed in the mid-1990's and 15 NCAC 02B.0212 now refers to Standards for Class WS-I Waters.

Addition of Conductivity and Salinity Measuring Equipment: (1) (h) in Laboratory Facility and Equipment Requirements of the Biological Laboratory Certification/Criteria Procedures document now includes the requirement to have instrumentation for measuring conductivity and salinity (for those laboratories conducting saltwater tests) as a minimum laboratory resource for those laboratories.

Purchased Organism Reference Testing: The following sentence was added to Sections (3) and (4) under Quality Assurance Requirements in the Biological Laboratory Certification/Criteria Procedures document: "For organisms obtained from outside suppliers, a reference test must be performed with each batch of organisms received from the supplier" for both acute toxicity and chronic toxicity testing requirements. This language addition represents a current requirement of the laboratory certification program and will not change current laboratory procedures.

Purchased Organism Taxonomic Identification: The following language was added to Section (7) under the Quality Assurance Requirements in the Biological Laboratory Certification/Criteria Procedures document: "Test organisms obtained from an outside supplier shall be taxonomically identified to the species level at a minimum yearly. All specimens shall be preserved and held for a minimum of one year." This requirement is currently being required through letter by the laboratory certification program and hence will formalize a current requirement.

***Pimephales promelas* Age Range Test Requirement:** Previously, Section (3) under the Procedure Modifications section of the Biological Laboratory Certification/Criteria Procedures document read "Fathead minnows used in acute toxicity tests will be 1 to 14 days in age and 72 hours range in age." Currently, biological certified labs use fathead minnows at 24 hours range in age. The current EPA Acute Methods Manual (EPA-821-R-02-012) requires the fathead minnows to be 24 hours range in age. Because the current laboratory practice is now consistent with the EPA Acute Method manual, this line has been removed as it is no longer a procedure modification.

EPA Method Manual References: Additions and corrections of EPA Method Manual reference sections were made in the Biological Laboratory Certification/Criteria Procedures document. These changes will not have any effect on laboratory procedures. Additions of references include 1.) adding EPA-821-02-014 to Section (5) under Quality Assurance Requirements; 2.) adding the appropriate EPA Acute Manual (EPA-821-R-02-012) reference section to Section (6)(b) under Quality Assurance Requirements; 3.) adding the appropriate EPA Chronic Manual (EPA-821-R-02-013) reference section to Section (4) under Procedure Modifications.

Hardness/pH Language: Language specifying the hardness requirement for the control solution of 30-50 mg/L $CaCO_3$ and pH requirement of 6.5-8.5 S.U. for freshwater tests has been added as Section (1) to the Procedure Modifications Section of the Biological Laboratory Certification/Criteria Procedures document.

Temperature Requirements: The language of Section (2) under Procedure Modifications has been changed from "Acute toxicity tests using *Ceriodaphnia dubia*, *Daphnia pulex*, and *Pimephales promelas* will be conducted at 25.0 ±1ºC" to "All acute and chronic toxicity tests will be conducted at 25.0 ±1.0℃, except chroni c tests for *Mysidopsis bahia* will be conducted at 26.0±1ºC. Variances may be requested for those species which require alternate temperatures. *(Ref. EPA-821-R-02-012. p. 46. 9.12.1; EPA-821-R-02-013, p.75-76, Table 1, p 164-165, Table 3; EPA-821-R-02-014, pp78-79, Table 3, 178-179; Table 3)."*

Historically, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Pimephales promelas* were the only species typically used in North Carolina acute testing, and *Ceriodaphnia dubia* was virtually the only organism used in North Carolina chronic testing. Over the past decade, other species have begun to be used for saltwater testing and for the second species testing that is now required every five years as part of the permit cycle. Therefore, we expanded the statement to include most test species for both acute and chronic tests. The organisms currently used in North Carolina WET tests require the 25.0 ±1.0℃ te mperature, except for chronic *Mysidopsis bahia*. Additionally, a provision for a temperature variance was included in the event that unusual testing organisms might be used in the future, such as coldwater trout species.

Feeding Requirements: The reference for Section (3) under Procedure Modifications was changed to 9.11.1. This change was made because the North Carolina Procedure modification that acute toxicity testing organisms are fed for at least 2 hrs prior to testing is applicable to all acute testing organisms. Previously, the references included only the *Ceriodaphnia*, *Daphnia*, and *Pimephales promelas* tables. The new reference includes references to all acute testing species tables in the manual.

Physical/Chemical Measurements: The language for Section (4) under Procedure Modifications was modified slightly. The modifications reflect and clarify current requirements and do not change laboratory practices. Salinity was added in the first sentence to indicate salinity is measured and recorded in the undiluted effluent sample. The first sentence was clarified to indicate that the chemical measurements made on the undiluted

effluent samples are measured and recorded "on the day the sample is first used". Previously, the document had said "the day of the test". The words "temperature" and "salinity (for saltwater tests)" were added to the second sentence to indicate that these measurements along with pH and dissolved oxygen are measured in the control and highest toxicant concentration. The references were updated to the correct acute manual reference and to include a chronic manual reference, as these requirements pertain to all acute testing and chronic *Ceriodaphnia* testing.

AT-5 & AT-6 forms: The AT-5 form, entitled "Effluent Aquatic Toxicity Report/Form/Chronic Vertebrate Toxicity Test", and the AT-6 form, entitled "Effluent Toxicity Report Form-Chronic *Mysidopsis bahia* Multi-Concentration Test", have been added to the Form Section of the Biological Laboratory Certification/Criteria Procedures document.

Reporting Units: The definitions for (13) Toxic Unit Acute and (14) Toxic Unit Chronic have been revised to indicate that the calculation involves the reciprocal of the effect concentration times 100, where the effect concentration is expressed as a percentage of whole effluent.

CHANGES AFFECTING THE "NORTH CAROLINA CERIODAPHNIA CHRONIC WHOLE EFFLUENT TOXICITY PROCEDURE" AND THE "NORTH CAROLINA PHASE II CHRONIC WHOLE EFFLUENT TOXICITY TEST PROCEDURE"

Updated References to North Carolina Administrative Code (NCAC): Page 2 of each document had stated that the procedures had "been approved by the Director of the North Carolina Division of Water Quality under the Fresh Surface Water Classification Standards (15A.NCAC.02B.0211) as suitable for evaluation of the effects of toxic substances to sensitive aquatic species. This statement has been changed to "This test procedure has been approved by the Director of the North Carolina Division of Water Quality under the Certification/Criteria Procedures document described in the Biological Laboratory Certification Rules (15A.NCAC.02H.1100) as a standard procedure for evaluation of the effects of toxic substances to sensitive aquatic species."

Test Acceptability Criterion of Third Brood Production by 80% Controls for Chronic *Ceriodaphnia* Tests: The word "surviving" has been added to all references to the chronic *Ceriodaphnia* test acceptability criterion of complete third brood production by 80% of controls. This language addition represents a current requirement and will not change current laboratory practices.

Clarity and Consistency: Language modifications and additions have been made to improve the clarity, consistency, and amount of detail of these two documents. These include:

- The "Effluent Sampling and Handling" Section of the "North Carolina *Ceriodaphnia* Chronic Effluent Bioassay Procedure" has been expanded to include the same level of detail that is provided in the "North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure".
- Small changes were made in the structure of the document to improve clarity. For instance, the "Table", "Appendix", and "Quality Assurance Checklist" that occur at the end of the document were made into "Appendix 1, Appendix 2, and Appendix 3", respectively.
- The term "chemical/physical" measurements was replaced with "temperature, dissolved oxygen, and pH" measurements throughout the documents.
- The title of the "North Carolina *Ceriodaphnia* Toxicity Procedure" was expanded to "North Carolina *Ceriodaphnia* Chronic Whole Effluent Toxicity Procedure".

CHANGES AFFECTING THE "PASS/FAIL METHODOLOGY FOR DETERMINING ACUTE TOXICITY IN A SINGLE EFFLUENT CONCENTRATION" PROCEDURE

Test Species: The language in the acute manual has been modified so that the pass/fail methodology is not specific to *Ceriodaphnia dubia*, *Daphnia pulex*, and *Pimephales promelas*. Historically, these were the testing organisms listed in permits and as such were specifically listed in the procedure. Over the past decade, some additional species have been used for saltwater testing and for second species testing. Therefore, the document was modified to make the procedure applicable to all acute testing organisms. The language now provides a list of some commonly used test organisms, but does not limit the procedure to specific organisms. Additionally, the

language was modified to make the acceptable temperature range of 25.0±1℃ applicable to all acute tes ts. Previously, only *Ceriodaphnia dubia*, *Daphnia pulex*, and *Pimephales promelas* were listed. It is also specified that variances may be requested for species which require alternate temperatures, such as trout species. These species have not historically been used in North Carolina permits, but the language was included to provide flexibility to the document.

Hardness/pH Language: Language has been added in the Methodology Section of the Pass/Fail Methodology for Determining Acute Toxicity in a Single Effluent Concentration that specifies the hardness and pH requirements for the control/dilution water at 30-50 mg/L $CaCO_3$ and pH requirement of 6.5-8.5 S.U. for freshwater tests.

Sampling Paragraph in the Methodology Section of the Acute Document: The following paragraph regarding effluent sampling was added to the Methodology section of the Pass/Fail Methodology for Determining Acute Toxicity in a Single Effluent Concentration document to make it consistent with the chronic documents. This is a current laboratory requirement and will not affect current practices. "Sampling must be performed below the last waste treatment process, including disinfection. There may be no removal of chlorine or any other effluent constituent by either chemical or physical methods prior to testing with the exceptions of allowable filtration of the effluent through 60 um nylon screen or plankton netting and the reduction of excess dissolved oxygen to the saturation level, as per EPA methods."

Acute Pass/Fail Toxicity Test Data Analysis: The language in the Data Analysis section of the Pass/Fail Methodology for Determining Acute Toxicity in a Single Effluent Concentration document has been revised for clarity and correctness. Additional details have been added. The language changes do not affect current laboratory requirements.