

APPENDIX E

**EXAMPLES OF SELECTED
STATE WET IMPLEMENTATION PROGRAMS**

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EXAMPLES OF SELECTED STATE WET IMPLEMENTATION PROGRAMS

Appendix E contains summaries of approaches that States have taken in implementing their NPDES whole effluent toxicity (WET) programs and efforts instituted to reduce or ensure minimal test variability when conducting WET tests. Preceding the State responses is a matrix (Table E-1) that briefly summarizes the common approaches or program themes for the States that responded. The respondent States are a geographic sampling across the United States. EPA's inclusion of the various State approaches in this document is not an endorsement of their approaches, but a snapshot of additional steps that a permitting authority could consider taking beyond the minimum requirements (i.e., test acceptability criteria) outlined in EPA guidance. This sample of State approaches also responds to recommendations EPA received on the initial draft document to consider and provide reference to other State approaches.¹

¹ Note that the terms, abbreviations, and acronyms used in this appendix may differ from their usage throughout the rest of this document. EPA consciously chose not to edit the State-supplied information so that the actual States' nomenclature and terminology as used in their NPDES programs would be reflected here.

Table E-1. Overview of Selected State WET Implementation Programs

ST	How do you evaluate reference toxicant & effluent test results?	How do you review reference toxicant test data for laboratory performance?	Describe additional QA/QC criteria developed & implemented.
KY	Acute—point-estimation Chronic—linear interpolation	Labs submit annual summary of RTT data, used to determine consistency & conformance with expected values.	1. Monthly acute/chronic RTT within 30 days of each WET test. 2. RTT conducted on each batch of purchased test organisms unless supplier provides information. 3. Culturing & testing in different incubators. 4. Chronic toxicity tests with CV > 40% evaluated on case-by-case basis.
NJ	Acute—point-estimation Chronic—linear interpolation	RTT results reported on standardized form, with UCL & LCL. Control charts submitted annually. RTT data reviewed in on-site audit.	1. <i>C. dubia</i> test: Number of males in surviving organisms over all concentrations $\leq 10\%$; number of males in controls $\leq 20\%$. 2. For all tests, no sporadic mortalities present; $\leq 10\%$ variation per concentration in start count. 3. For tests indicating permit violation, review raw data & test results (data trend, MSD, chain-of-custody, sample handling/holding time).
NC	Acute—point-estimation Chronic—linear interpolation Acute pass/fail, chronic multi-concentration effluent tests—hypothesis tests	RTT data reviewed during annual lab inspection. Lab provides bench sheets, water quality data, calculations, control charts, etc. Information reviewed for test frequency, test conditions, test result validity, & responses to out-of-control events.	1. Dilution water pH 6.5 - 8.5, total hardness 30 - 50 ppm. 2. Biweekly acute RTT or within 7 days of any NPDES test. 3. Test organisms identified to species once/quarter. 4. Culturing & testing in different incubators. 5. Chronic <i>C. dubia</i> test: 3 rd brood neonate production $\geq 80\%$ of control; neonate reproduction from 1 st - 3 rd broods only; % male control organisms $\leq 20\%$; control reproduction CV $\leq 40\%$; solution DO ≥ 5.0 mg/l; exposure duration at least 7 d \pm 2 h. 6. Acute tests terminated w/i 1 h of stated length.
WA	Acute—point estimation Chronic pass/fail, & chronic multi-concentration effluent tests—hypothesis tests	1. Review data in conjunction with effluent tests. 2. Lab provides bench sheets, water quality data, calculations, control charts, etc. Information reviewed for test frequency, test conditions, test result validity, & responses to out-of-control events. 3. If reference CV does not meet certain criteria, test is rejected.	1. Minimum % difference in survival between IWC & control (or NOEC for chronic) that is statistically significant: acute—30%, chronic—40%. 2. Tests failing must be repeated with more replicates. 3. Specific requirements for <i>Ceriodaphnia</i> & bivalve chronic tests.
WI	Acute & chronic—point-estimation Certified labs perform monthly reference toxicant tests	RTT data reviewed by auditor before on-site lab inspections. Lab provides bench sheets, water quality data, control charts, etc. Information reviewed for test frequency, proper test conditions, test result validity, & proper responses to out-of-control events.	1. Testing & culturing in separate rooms or incubators 2. Additional or revised TAC for Static Renewal Acute & Chronic Tests will be included in revised methods.

Abbreviations: CV = coefficient of variation; DO = dissolved oxygen; IWC = instream waste concentration; LCL = lower control limit; MSD = minimum significant difference; NOEC = no observed effect concentration; NPDES = National Pollutant Discharge Elimination System; ppm = parts per million; RTT = reference toxicity test; SETAC = Society of Environmental Toxicology and Chemistry; SOP = standard operating procedures; TAC = technical acceptability criteria; UCL = upper control limit; WET = whole effluent toxicity

Table E-1. Overview of Selected State WET Implementation Programs (continued)

ST	Describe efforts to minimize test method variability.	Explain how you review or conduct lab performance audits.	Describe specific implementation guidance developed for permit writers. How is the guidance available to the public?	Describe how you provide or use toxicity test training.
KY	<ol style="list-style-type: none"> 1. Reporting on standardized form. 2. Labs submit culturing/testing SOP for State review. 3. Tests must comply with all EPA & State test manuals. 4. Dilution water moderately hard-reconstituted water or dilute mineral water. 5. Follow written protocol for splits. 6. Lab audits by State or EPA Region. 	<p>EPA Region or State conducts lab audits, following procedures in EPA inspection manual.</p>	<ol style="list-style-type: none"> 1. Several guidance documents developed by State. 2. Face-to-face training as needed, also available to the public. 3. Some documents available on web or through newsletters. 	<ol style="list-style-type: none"> 1. State communicates program changes & guidance on culturing & testing issues through newsletter & web page. 2. Training sessions for State personnel. 3. Participate in Wastewater Operator's Conference to discuss issues with regulated community & consultants. 4. Teach in SETAC's WET training & statistical analysis courses.
NJ	<ol style="list-style-type: none"> 1. Standardized checklist for screening submitted tests. 2. Lab certification program, on-site audits. 3. Labs report premature cancellation of test & reason. 4. Quarterly meetings of State/lab representatives to discuss current test developments. 	<ol style="list-style-type: none"> 1. Inspections announced or unannounced. Lab SOPs reviewed for adherence to NJ & EPA protocols. Subsets of data reviewed, technician summarizes problems with test reports. 2. Inspections consist of opening conference, lab walk-through, closing conference. SOP review, problematic test results review. Auditor examines equipment, documentation, cultures, lab procedures, chain-of-custody, sample handling. Review of inspection results during closing conference. 	<ol style="list-style-type: none"> 1. Training sessions to permit writers & public. 2. Written guidance is copies of past training sessions on shared drive for permit writers. Generally not available to public. 	<p>Staff attend USEPA or SETAC-sponsored training.</p>

Abbreviations: CV = coefficient of variation; IWC = instream waste concentration; LCL = lower control limit; MSD = minimum significant difference; NOEC = no observed effect concentration; NPDES = National Pollutant Discharge Elimination System; ppm = parts per million; RTT = reference toxicity test; SETAC = Society of Environmental Toxicology and Chemistry; SOP = standard operating procedures; TAC = technical acceptability criteria; UCL = upper control limit; WET = whole effluent toxicity

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ST	Describe efforts to minimize test method variability.	Explain how you review or conduct lab performance audits.	Describe specific implementation guidance developed for permit writers. How is the guidance available to the public?	Describe how you provide or use toxicity test training.
NC	<ol style="list-style-type: none"> 1. Review submitted test results against TAC. 2. Implement lab certification program. 3. Document investigations of differing test results from splits of effluent samples. 4. Test protocol modifications. 	<ol style="list-style-type: none"> 1. Inspections announced or unannounced. Lab SOPs reviewed for adherence to NJ & EPA protocols. Subsets of data reviewed, technician summarizes problems with test reports. 2. Inspections consist of opening conference, lab walk-through, closing conference. SOP review, problematic test results review. Auditor examines equipment, documentation, cultures, lab procedures, chain-of-custody, sample handling. Review of inspection results during closing conference. 	<ol style="list-style-type: none"> 1. Written guidance established by memo. Face-to-face training sessions as needed. 2. Written guidance available to public upon request, also sent to permit holders with permit & subsequent renewals. Also available on the web. 	<ol style="list-style-type: none"> 1. Participate in aquatic toxicologists group. Communicate program changes & guidance on culturing & testing issues through meetings. 2. Workshops held for Division's regional office personnel 3. Attend SETAC's WET training & statistical analysis courses.
WA	<ol style="list-style-type: none"> 1. Develop, distribute <i>Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria</i>. 2. Review tests for compliance with method & canary book. 3. Fish/mysid growth tests with SD of proportion alive > 0.25 in effluent concentration analyzed for original growth endpoint, not combined endpoint. 4. Permit requirements will lower alpha level for hypothesis testing when differences in test organism response are small. 5. Anomalous test identification criteria established to make WET test results fair & enforceable. 	<ol style="list-style-type: none"> 1. Inspections announced or unannounced. Lab SOPs reviewed for adherence to NJ & EPA protocols. Subsets of data reviewed, technician summarizes problems with test reports. 2. Inspections consist of opening conference, lab walk-through, closing conference. SOP review, problematic test results review. Auditor examines equipment, documentation, cultures, lab procedures, chain-of-custody, sample handling. Review of inspection results during closing conference. 3. Audit report prepared within 30 days of audit 4. Performance audits required twice/year, system audits every three years. 	<p>Develop & update language for use in NPDES permits & fact sheets for POTWs & industry. Language is part of templates for permits & fact sheets that permit writers use as they draft permits. Manual available to the public for cost of printing & also on Web.</p>	<p>Extensive training in all offices early in 1990s. WET test review & technical assistance are centralized functions, permit writing guidance available in <i>Permit Writer's Manual</i> & suggested permit language.</p>

Abbreviations: CV = coefficient of variation; DO = dissolved oxygen; IWC = instream waste concentration; LCL = lower control limit; MSD = minimum significant difference; NOEC = no observed effect concentration; NPDES = National Pollutant Discharge Elimination System; ppm = parts per million; RTT = reference toxicity test; SETAC = Society of Environmental Toxicology and Chemistry; SOP = standard operating procedures; TAC = technical acceptability criteria; UCL = upper control limit; WET = whole effluent toxicity

Table E-1. Overview of Selected State WET Implementation Programs (continued)

ST	Describe efforts to minimize test method variability.	Explain how you review or conduct lab performance audits.	Describe specific implementation guidance developed for permit writers. How is the guidance available to the public?	Describe how you provide or use toxicity test training.
WI	<ol style="list-style-type: none"> 1. Review submitted test results against TAC. 2. Lab certification program 3. Document investigations of differing test results from splits of effluent samples. 4. Strict adherence to clearly specified methods, such as sampling procedures, holding times, test duration. 5. Revising methods to require that labs verify staff training & qualifications. 	<ol style="list-style-type: none"> 1. Inspections announced or unannounced. Auditor reviews laboratory SOPs & recent RTT results for adherence to WDNR protocols. 2. Inspections consist of opening conference, lab walk-through, closing test results review. Auditor examines equipment, documentation, cultures, lab procedures, chain-of-custody, sample handling. Review of inspection results during closing conference. 3. Auditor reviews reference toxicant data after inspection, generates inspection letter. Lab has 60 days to respond. Significant deficiencies may result in decertification. 	<p>Written guidance & clarification on existing rules & methods for State staff, permittees, labs, consultants, others.</p>	<ol style="list-style-type: none"> 1. One-on-one training for State staff & permittees. 2. University lab provides hands-on WET training to State staff, permittees, labs on request. 3. Attend SETAC's WET training & statistical analysis courses.

Abbreviations: **CV** = coefficient of variation; **DO** = dissolved oxygen; **IWC** = instream waste concentration; **LCL** = lower control limit; **MSD** = minimum significant difference; **NOEC** = no observed effect concentration; **NPDES** = National Pollutant Discharge Elimination System; **ppm** = parts per million; **RTT** = reference toxicity test; **SETAC** = Society of Environmental Toxicology and Chemistry; **SOP** = standard operating procedures; **TAC** = technical acceptability criteria; **UCL** = upper control limit; **WET** = whole effluent toxicity

E.1 RESPONSES FROM KENTUCKY DEPARTMENT FOR ENVIRONMENTAL PROTECTION

E.1.1 Describe How Your State Evaluates Reference Toxicant and Effluent Test Results

Acute reference toxicant test and multi-concentration effluent test results are evaluated using the point-estimate (LC50) technique described in the EPA acute testing manual.

Chronic reference toxicant and multi-concentration effluent test results are evaluated using the linear interpolation method (IC25) as described in the EPA chronic manual and using the TOXCALC statistical program software.

E.1.2 Explain How Your State Reviews Reference Toxicant Data for Laboratory Performance

Consulting laboratories that service permittees are required to annually submit to the Bioassay Section a summary of their reference toxicant test data. This information is used to determine consistency and conformance to the expected values. This serves as a review and audit of all consulting laboratories, measures consistency within a laboratory, and provides a level of reliability and accuracy between laboratories.

A letter of request is sent to each laboratory with a standardized response form. The labs provide the requested information, including test date, dilution series, type of control water, organism age, LC50/IC25, 95 percent confidence interval, and average control reproduction/weight. This information is entered into a laboratory QA data base where it is statistically analyzed.

This information is then compiled into an annual summary report. The compiled information includes the lab name, reference toxicant, test species, test type, test duration, number of tests performed, mean, standard deviation (SD), % coefficient of variation (CV), average reproduction, or growth with SD and % CV.

The results are mailed to each participating laboratory. In addition, the summary results are printed in the Kentucky Biomonitoring Newsletter and are presented on the Bioassay Section's web page (<http://water.nr.state.ky.us/wq/bioassay/index.html>).

A control chart is prepared for each reference toxicant and organism combination, and successive toxicity values are plotted and examined to determine if the results are within prescribed limits. A minimum of 30 test results are needed for a reliable mean and upper/lower control chart. If the toxicity value from a given test with the reference toxicant does not fall within the expected range for the test organism when using the standard dilution water, then the sensitivity of the organisms and the overall credibility of the test systems are suspect. In this case the test procedure, control water, and reference toxicant are examined.

Missing and/or out-of-range data must be explained and can result in the invalidation of Kentucky Pollution Discharge Elimination System (KPDES) WET test results.

E.1.3 Describe Any Additional QA/QC Criteria Your State Has Developed and Implemented Within Your State

1. Acute and chronic reference toxicant tests are to be conducted monthly. A reference toxicant test must be conducted within 30 days of each KPDES WET test.
2. If test organisms are purchased from a commercial supplier, a reference toxicant test must be conducted on each batch unless the supplier can provide this information.

3. Culturing and testing activities may not be contained within the same incubator.
4. Chronic toxicity tests where the coefficient of variation (CV) is greater than 40 percent will be evaluated on a case-by-case basis to determine if the results will be considered acceptable.
5. All other QA/QC criteria for culturing and testing, as set forth in the most current editions of the EPA manuals, must be followed.

E.1.4 Describe Any Efforts Your State Has Made to Minimize Test Method Variability

1. All KPDES WET test results are submitted using a standardized report form. Each report is closely reviewed by a member of the Bioassay Section to determine if proper test protocols have been followed.
2. Prior to conducting toxicity test for Kentucky permittees, each laboratory must submit its culturing/testing SOP for review by the Bioassay Section. This insures that proper methods and procedures are being followed.
3. Toxicity tests must comply with all conditions as stated in the EPA testing manuals and in the Kentucky Methods for Culturing and Conducting Toxicity Tests with *Pimephales promelas* and *Ceriodaphnia dubia*. (Fourth Edition, 1996). Special attention is paid to sample holding times and temperatures.
4. Dilution water is to be moderately hard-reconstituted water or moderately hard dilute mineral water.
5. If split samples are going to be used, the Biomonitoring Split-Sample Protocol must be followed. This protocol details sample collection and holding procedures as well as test conditions that must be followed.
6. Laboratories must submit all reference toxicant data for the annual summary. This information assists in determining the quality of information being received from these facilities.
7. Laboratories are audited by Kentucky or EPA Region IV to review testing and culturing procedures.

E.1.5 Explain How Your State Reviews or Conducts Performance Lab Audits

Kentucky has been fortunate in having the expertise of EPA Region IV in performing WET laboratory audits. Their experience has proven beneficial in keeping laboratories compliant with the testing requirements. When the services of EPA are not available, the State will conduct its own lab audits. In either case, the procedures are the same and follow those outlined in the EPA inspection manual.

Inspections are usually announced. If EPA is performing the inspection, a representative from the Bioassay Section will accompany the inspectors. Prior to the inspection, the auditor will review the laboratory's SOP for adherence to Kentucky and EPA protocols. Bioassay Section staff will review test reports to document any problems with the subject lab. In addition, the qualifications of the staff will be reviewed at this time. Generally, three test reports will be chosen for which the laboratory will be required to produce supporting documentation.

The inspection consists of an opening conference, a walk-through of the laboratory, and a closing conference. During the opening conference, the auditor discusses the SOP review and general procedures in the laboratory. In addition, information including culturing records, test data, chain of custody records, reference toxicant data, etc., supporting the three test reports selected prior to the inspection will be reviewed. During the walk-through, the auditor examines equipment, log books, written documentation and laboratory procedures. The closing conference serves as a review of observations and comments during the inspection.

The auditor will generate an inspection response letter detailing any deficiencies noted during the audit. All correspondence is addressed to the permittee, whose test results were used for the inspection. The permittee will have usually 60 days to respond to the deficiencies, noting what actions have been taken by the laboratory to correct them. If significant deficiencies are not addressed, then future data from this laboratory may not be accepted by the State.

E.1.6 Describe Any Specific Implementation Guidance That Your State Has Developed to Assist Permit Writers. How Is the Guidance Available to the Public?

Guidance is provided through several documents developed by the Bioassay Section. This section has developed standardized biomonitoring language, which is provided to the KPDES Permitting Branch. This language is incorporated into each permit with a WET limit or monitoring upon permit issuance or reissuance. In addition, a Standard Test Result Report form is provided to each permit holder with WET. The section has another document: Aquatic Toxicity Testing: Questions and Answers, which is available upon request.

The Bioassay Section provides face-to-face training to the KPDES Branch on an as-needed basis. This training is also available to the public if requested.

Some documents are available on the Bioassay Section's web page or through the Biomonitoring newsletter.

E.1.7 Describe How Your State Provides or Utilizes Any Toxicity Testing Training

The Bioassay Section communicates program changes and specific guidance on culturing and testing issues through the newsletter and the web page. The section has held several training sessions for State personnel since the inception of the program. In addition, the section participates in the State's annual Wastewater Operator's Conference to discuss issues with the regulated community and consultants.

Section members have attended and participated as instructors in the Society for Environmental Toxicology and Chemistry's two-day WET training course and statistical analysis course.

E.2 RESPONSES FROM NEW JERSEY DEPARTMENT OF ENVIRONMENTAL PROTECTION

E.2.1 Describe How Your State Evaluates Reference Toxicant and Effluent Test Results

Acute effluent tests are evaluated using the point estimate techniques described in the USEPA acute methods document. New Jersey also uses the NOAEC endpoint set equal to 100 percent effluent when an evaluation of no acute toxicity is required. The hypothesis testing techniques contained in the USEPA manual are used in that case.

Requests have been received from certified laboratories and from permittees that the point estimate techniques be further standardized. Using one version of Probit versus another can result in a different value, sometimes making a difference whether a facility passes or fails.

Chronic effluent and reference toxicant test results are evaluated using the linear interpolation method originally provided by Teresa Norberg King (July 1993). A p value of 25 is selected for all permits and for reference toxicant recording.

E.2.2 Explain How Your State Reviews Reference Toxicant Data For Laboratory Performance

New Jersey Pollution Discharge Elimination System (NJPDES) permits require that in order for chronic toxicity test results to be considered acceptable, there must be an acceptable Standard Reference Toxicant (SRT) result conducted within 30 days of the compliance test result, for the test species and reference toxicant in question. The States standardized report form requires the reporting of the applicable SRT result directly on the compliance test report, along with the applicable upper and lower control limits. Missing or out of range data can result in the invalidation of test results.

Control charts are forwarded to the Department on an annual basis, on the anniversary of the approval for the test species. Many labs have chosen to include copies of applicable control charts with the submittal of compliance test results. SRT data is also reviewed as part of an on-site audit, including a review of procedures, raw data, and data analysis any excluded results.

State methods governing laboratories also require that if a lab produces any SRT test result which is outside the established upper and lower control limits for a test species at a frequency greater than one test in any ten tests, a report shall be forwarded to the Department. That report shall include any identified problem which caused the values to fall outside the expected range and the corresponding actions that have been taken by the laboratory. If a laboratory produces two consecutive SRT test results or three out of any ten test results, which are outside the established upper and lower control limits for a specific test species, the laboratory shall be unapproved to conduct testing. Reapproval is contingent upon the laboratory producing SRT test results within the established upper and lower limits.

The laboratory selects the reference toxicant used. However, the Department recommends using KCl.

E.2.3 Describe Any Additional QA/QC Criteria Your State Has Developed and Implemented With Your State

For Ceriodaphnia testing:

- Number of males in surviving organisms overall concentration ≤ 10 percent [(no. males / total no. surv) x 100].
- Number of males in controls ≤ 20 percent (no. males / total no. organisms in controls).

All test species

- No sporadic mortalities present (Deaths that are not related to sample toxicity, confined to a few test chambers and scattered throughout the test).
- Variation in start count must be ≤ 10 percent per concentration (animals lost or killed by accident).

These items are specifically included on standardized review sheets.

For any tests that would result in the collection of penalties based on violation of an effective toxicity limit, a detailed review of the raw data and test results are conducted, including review of the data trend, minimum significant difference, chain-of-custody, sampling handling, and holding times.

E.2.4 Describe Any Efforts Your State Has Made To Minimize Test Method Variability

Each test that is submitted receives at least a screening using a standardized check list, anywhere from 30 to 40 questions depending upon the test species, dealing with all aspects of the test.

New Jersey maintains a laboratory certification program for toxicity testing, including on-site audits.

A laboratory who cancels a test prior to the scheduled ending time/date must report that cancelled test, including the reason for the cancellation, to the Department. This allows the Department to track a laboratory's ability to run a test to completion. Tests that do not meet USEPA's test acceptability criteria are not submitted to the Department since they are not valid. This way the frequency that this is occurring at a laboratory can be tracked. Frequent test cancellations are addressed during an on-site audit.

New Jersey has a Bioassay Subcommittee that is a subset of the State's Laboratory Advisory Committee. This committee meets quarterly and consists of State and laboratory representatives. The committee discusses problems with the tests, certification, updates from USEPA, SETAC, NELAC, or anything else applicable to toxicity testing. This gives the laboratories and the State an opportunity to discuss either deficiencies that are occurring at laboratories and are showing up in the test data, problems the laboratories are having with regard to any of the methods, and any improvements to the program that should be easily implemented.

E.2.5 Explain How Your State Reviews Or Conducts Performance Lab Audits

Inspections can be announced or unannounced, although generally time is not adequate to perform unannounced inspections. Prior to the inspection, the auditor will review the laboratory's SOPs for adherence to New Jersey and EPA protocols. Subsets of data will also be reviewed and the technician responsible for day to day screening using the standardized check list is asked to summarize any problems with the review of toxicity test reports.

The actual inspections consist of an opening conference, a walk-through of the lab facility, and a closing conference. During the opening conference, the auditor discusses the SOP review and general procedures in the lab. In addition she will request and review supporting information associated with the any test reports identified prior to the inspection as a concern. During the walk-through, the auditor examines equipment, written documentation, cultures, laboratory procedures, chain-of-custody, and sample handling. The closing conference serves as a review of observations and comments during the inspection.

E.2.6 Describe Any Specific Implementation Guidance That Your State Has Developed To Assist Permit Writers. How Is The Guidance Available To The Public?

The Office of Quality assurance provides training sessions to the permit writer and the public upon request. Written guidance consists of copies of past training sessions, located on the share drive for permit writers. This guidance is not generally available to the public.

E.2.7 Describe How Your State Provides Or Utilizes Any Toxicity Testing Training

When possible, staff will attend any USEPA- or SETAC-sponsored training on the topic.

E.3 RESPONSES FROM NORTH CAROLINA DEPARTMENT OF ENVIRONMENT AND NATURAL RESOURCES

E.3.1 Describe How Your State Evaluates Reference Toxicant and Effluent Test Results

Acute reference toxicant test and multi-concentration effluent test results are evaluated using the point-estimation techniques described in the EPA manual.

Acute pass/fail, chronic pass/fail, and chronic multi-concentration effluent test results are evaluated using hypothesis tests as described in the EPA manuals.

Chronic reference toxicant test results are evaluated using the linear interpolation method (IC_p, where p=25) described in the EPA manual.

For both types of chronic *Ceriodaphnia* effluent tests, a reproductive effect is defined by both a statistically significant difference between the treatment and the control and a 20 percent reduction in neonate reproduction of the treatment organisms as compared to the controls. Hypothesis tests for both acute and chronic pass/fail tests are performed at an alpha level of 0.01.

E.3.2 Explain How Your State Reviews Reference Toxicant Data for Laboratory Performance

The data is reviewed in conjunction with the laboratory's annual laboratory inspection. The laboratory provides copies of bench sheets, water quality data, and calculations or printouts from the data analysis for each reference toxicant test performed since the last laboratory inspection:

In addition, the lab submits the current control chart (with data listing) and any explanations of out-of-range test results for each test type and organism combination.

The materials are reviewed for appropriate test frequency, proper test conditions, test result validity, and proper responses to out-of-range events.

Missing or out-of-range data can result in the invalidation of NPDES test results.

E.3.3 Describe Any Additional QA/QC Criteria Your State Has Developed and Implemented Within Your State

- Laboratories must use dilution water in whole effluent toxicity testing with chemical characteristics such that the pH is between 6.5 and 8.5 and total hardness as calcium carbonate is between 30 and 50 µg/l as calcium carbonate.
- Acute and chronic reference toxicant tests must be performed once every two weeks or within one week of any NPDES tests.
- A representative of each test organism cultured shall be taxonomically identified to the species level at a minimum frequency of once per quarter.
- If closed incubators (refrigerator-sized) are utilized for toxicity testing and/or test organism culturing purposes, culturing and testing activities may not be contained within the same incubator.
- Chronic *Ceriodaphnia dubia* analyses will have an additional test acceptability criterion of complete third brood neonate production by at least 80 percent of the control organisms.

- *Ceriodaphnia dubia* neonate reproduction totals from chronic tests shall include only organisms produced in the first through third broods.
- The percentage of male *Ceriodaphnia* control organisms may not exceed 20 percent in chronic *Ceriodaphnia* tests.
- The *Ceriodaphnia* control organism reproduction coefficient of variation (CV) must be less than 40 percent for a chronic *Ceriodaphnia* test to be considered acceptable.
- *Ceriodaphnia* chronic test solutions must maintain dissolved oxygen levels greater than or equal to 5.0 mg/l.
- *Ceriodaphnia* chronic test exposure duration will be no greater than seven days \pm 2 hours regardless of control organism reproductive success.
- Acute tests will be terminated within one hour of their stated length.

E.3.4 Describe Any Efforts Your State Has Made to Minimize Test Method Variability

1. Close review of each test result submitted with consistent adherence to test protocol test acceptability criteria.
2. Implementation of a biological laboratory certification program.
3. Paper trail investigations of test results from disagreeing “split” effluent sample analyses.
4. Test protocol modifications.

EPA methods allow for a relatively wide window for termination of the chronic *Ceriodaphnia* test. Tests may be terminated as soon as 60 percent of the control organisms produce three broods of young or as late as eight days after test initiation. Logically, narrowing the termination window will reduce variability and improve precision of test results. The North Carolina Division of Water Quality (NC DWQ) has narrowed the window available for the termination of the chronic *Ceriodaphnia* test by:

- Placing a shorter limit on the exposure period (seven days + two hours)
- Requiring that at least 80 percent of the control organisms produce a third brood prior to test termination

Analysis of a data base of NC chronic *Ceriodaphnia* test results has shown that reducing control organism reproduction variability improves the sensitivity of the reproduction analysis. Logically, holding all labs to a common precision standard with respect to control organism reproduction should reduce between-lab test result variability. The Division has reduced variability of control organism reproduction by:

- Implementing a test acceptability criterion limiting the control organism reproduction coefficient of variation to less than 40 percent
- Requiring that at least 80 percent of the control organisms produce a third brood prior to test termination
- Excluding fourth and subsequent brood neonates from the reproduction effects analysis

DWQ's experience has shown that high quality laboratories can produce extremely sensitive tests that can detect quite small differences between treatment and control reproduction. Unfortunately, this can be a disincentive for laboratories to produce high quality tests, since experience has shown that some clients gravitate toward laboratories that produce compliant test results. Less sensitive tests will be more likely to produce compliant results. Analysis of reproduction data from the same data base described above indicated that tests performed by NC certified labs could routinely detect a difference between the control and a treatment when there was a 20 percent reduction in neonate reproduction by the treatment organisms compared to the controls. Based on this data, NC DWQ has placed a second data evaluation criterion on the *Ceriodaphnia* chronic reproduction analysis. Specifically, for an effluent treatment to be considered producing an effect, the reproduction mean must be both statistically significantly lower than the control mean **and** represent at least a 20 percent reduction from that mean. In effect, this sets a lower limit on test sensitivity and also reduces within-laboratory and between-laboratory test result variability.

E.3.5 Explain How Your State Reviews or Conducts Performance Lab Audits

Inspections may be announced or unannounced. Prior to the inspection, the auditor will review the laboratory's SOP for adherence to North Carolina and EPA protocols. The Aquatic Toxicology Unit member responsible for reviewing test report submittals will be requested to summarize any recurring problems with the target laboratory regarding data submission. Three test reports will be chosen for which laboratory personnel will be asked to produce supporting documentation.

The actual inspection consists of an opening conference, a walk-through of the laboratory facilities, and a closing conference. During the opening conference, the auditor discusses the SOP review and general procedures in the laboratory. In addition he/she will request and review supporting information associated with the three test reports selected prior to the inspection. During the walk-through, the auditor examines equipment, written documentation, cultures, and laboratory procedures. The closing conference serves as a review of observations and comments during the inspection.

The auditor will review reference toxicant data (see question 2 above) after the inspection. Within two weeks, the auditor will generate an inspection response letter, to which the laboratory will be given 60 days to respond. If there are significant deficiencies discovered during the inspection, a laboratory or categorical decertification may occur.

E.3.6 Describe Any Specific Implementation Guidance That Your State Has Developed to Assist Permit Writers. How Is the Guidance Available to the Public?

Written guidance is established by memo from the Water Quality Section Chief to the NPDES Permitting Unit and other affected Water Quality Section Units. The Aquatic Toxicology Unit provides face-to-face training sessions to the NPDES Unit on an as-needed basis.

The written guidance in memo form is available to the public upon request. Parts of the guidance are included in a document called "Aquatic Toxicity Testing: Understanding and Implementing Your Testing Requirement," that is disseminated to each permit holder with a WET limit or monitoring requirement upon permit issuance and subsequent renewals. The document is also available at the Aquatic Toxicology Unit web page, <http://www.esb.enr.state.nc.us/ATUwww.default.html>.

E.3.7 Describe How Your State Provides or Utilizes Any Toxicity Testing Training

NC DWQ actively participates in the Carolinas Area Aquatic Toxicologists group (CAAT). The Aquatic Toxicology Unit utilizes the meetings of this group to communicate program changes and specific guidance on culturing and testing issues. Additionally, the Unit has held two workshops for the Division's regional office personnel since the inception of the aquatic toxicity testing program. Unit members have

attended The Society of Environmental Toxicology and Chemistry's two-day WET course and statistical analysis course.

E.4 RESPONSES FROM WASHINGTON DEPARTMENT OF ECOLOGY

E.4.1 Describe How Your State Evaluates Reference Toxicant and Effluent Test Results

The State of Washington Department of Ecology reviews every WET test report for compliance with the test method and instructions in the permit. Permit instructions include reference to a document called "Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria" that provides the lab with standard testing instructions and provides the basis for test report review. Reference toxicant tests are not evaluated separately but are evaluated as a part of the review of WET test reports. The Department of Ecology also maintains a data base of WET test raw data and statistical results in order to have comprehensive records for each discharger and to enhance our ability to learn from experience and improve our WET program.

E.4.2 Explain How Your State Reviews Reference Toxicant Data for Laboratory Performance

The minimum reference toxicant testing needed to meet our interpretation of the requirements in the EPA manuals (both sections 4.7 and 4.16) is one per month for every acute and 7-day (short-term) chronic test species used routinely (more than once per month). Because an acute test result can be determined during a 7-day chronic test, acute and chronic reference toxicant testing for a fish or mysid can be combined. If a lab has difficulty establishing a concentration series that produces good results for both a lethal and sublethal endpoint, the lab may focus on lethality, as long as the sublethal endpoint is not completely abandoned in the conduct and analysis of the test.

In addition to the nonroutine tests (test performed once per month or less), all tests conducted with plants are required to have concurrent reference toxicant testing. In addition, brood stock can vary in condition, and the concurrent check on test organism sensitivity is a good precaution. Algal toxicity tests must have concurrent reference toxicant tests for similar reasons. Concurrent reference toxicant testing is also required when test organisms (or the brood stock used to produce the test organisms) have been collected from the wild. Increases in test costs, especially the cost of 7-day chronic tests, are to be avoided if possible. The alternative to concurrent reference toxicant testing in section 4.7 for labs getting test organisms from an outside supplier is reference toxicant testing by the organism supplier, and this alternative seems to be generally believed by testing labs as well as the Department of Ecology to be inferior to monthly reference toxicant testing by the testing lab. We do not accept the use by labs of reference toxicant tests performed by organism suppliers, and apparently labs agree because the vast majority have, to their credit, continued to conduct their own reference toxicant testing. Labs, however, should use organism suppliers that routinely conduct reference toxicant testing and control charting because, as noted in the table below, this information can be useful when deciding the consequences of lab conducted reference toxicant testing.

All labs must conduct ongoing control charting based on reference toxicant testing and report the results, acceptable or unacceptable, of the control charting in the report for each effluent or ambient water test. Acceptability is based on the standard test acceptability criteria for the test and on control charting with the upper and lower control limits set at twice the standard deviation (95 percent confidence) of the point estimates (LC_{50} , EC_{50} , IC_{25} , etc.) accumulated from the last 20 reference toxicant tests. At least five reference toxicant tests are needed to establish a minimally effective control chart for new tests. The reference toxicant test data must be presented with the report for each associated test.

Any reference toxicant test determined to be unacceptable must be repeated either until an acceptable result is obtained or until there have been three consecutive unacceptable test results (the initial unacceptable test plus two repeats). Because about 1/20 reference toxicant test results will fall outside of control limits

due to chance alone, it is necessary to repeat unacceptable reference toxicant tests in order to reduce the role of chance. Assuming no unusual problems with test organisms or lab performance, there is only a 1/400 chance of two unacceptable reference toxicant test results in a row and only a 1/8,000 chance of three unacceptable results in a row. If a lab has no unusual problems, repeating an unacceptable reference toxicant test should quickly produce an acceptable result. If a lab repeatedly produces unacceptable reference toxicant test results, it will give confidence to the conclusion that the lab has problems with test organisms or testing technique.

When the reference toxicant test result is within the 95 percent confidence limits, then the test report must state this fact and present the reference toxicant data at the end of the report. When the reference toxicant test result is outside the 95 percent confidence limits, then the test report must state this fact and present the reference toxicant data at the end of the report. The lab should not delay test reports while waiting for the results of reference toxicant test repeats. The results from the first repeated test might be available in time for inclusion in the test report. If begun promptly, the results of all of the reference toxicant testing in response to an unacceptable reference toxicant test result will be available in time for the review of the test report. The WET Coordinator will contact the lab during the test review for any additional reference toxicant test data not contained in the test report.

When a reference toxicant test result falls outside of the 95 percent confidence limits, a lab must qualify the associated test result for an effluent or ambient water sample by a statement in the test report that the reference toxicant test result was outside control limits. The Department of Ecology WET Coordinator will decide whether these tests are acceptable based on the degree of departure from control limits and the frequency of occurrence. Because it is expected that an average of one out of 20 tests will fall outside of the control limits due to chance alone, the degree of departure from the control limits and frequency of occurrence will be considered before rejecting toxicity tests. Because control limits narrow as laboratory performance improves, the width of the control limits will also be considered before rejecting toxicity test results when the associated reference toxicant test results are just outside the limits.

The Biomonitoring Science Advisory Board (BSAB) criteria for acceptable intralaboratory variability provide values that are useful for considering the width of control limits while deciding whether to reject toxicity tests on the basis of reference toxicant test results. If the coefficient of variation (standard deviation mean toxicity value) from the reference toxicant test data used in control charting falls into the excellent (< 0.35) or good (0.35 to 0.60) range established by the BSAB, then a higher confidence in the test results is justified. If the reference toxicant test data coefficient of variation for the lab falls into the acceptable range (0.61 to 0.85), then a smaller amount of confidence should be applied. If the reference toxicant test data coefficient of variation for the lab falls into the unacceptable range (> 0.85), then none of the lab's test results are acceptable. Labs must report the coefficient of variation for the last 20 reference toxicant tests in every report for the same test conducted on an effluent or environmental sample. (Reference: Biomonitoring Science Advisory Board. BSAB Report #1, *Criteria for Acceptable Variability of Marine Chronic Toxicity Test Methods*. Washington Dept. of Ecology. February 1994.) Effluent or ambient water toxicity test results will be accepted or rejected based on the following table. Rejection will occur when any condition in the appropriate "Test Accepted" box was not met or when any condition in the appropriate "Test Rejected" box was met.

Effluent tests and their associated (initial) reference toxicant tests must have start dates separated in time by no more than 18 days. Labs typically take about two weeks to produce a test report. From the point of view of practicality and the most meaningful control charting, it makes sense for a reference toxicant test result to be used retroactively about two weeks. The reference toxicant test result will then be used for control charting for the balance of the monthly time period. A grace period of 7 days will be added to the 18 days for tests begun from December 1st to the following January 10th. Acute tests will be allowed a grace period of 4 days over the 18 day maximum.

Table for Determining Test Rejection Based on Reference Toxicant Test Results

Unacceptable Reftox Tests	Test Accepted	Test Rejected
Only the original reftox test result was outside of control limits (the first repeat reftox test result fell within control limits)	If the organism supplier reftox results were within control limits, and the coefficient of variation for the last 20 reftox tests is ≤ 0.85	If there are notable reporting errors or deviations from test protocol, or if the reftox test result fell outside of control limits to the more sensitive side (point estimate was too low) by 3 or more standard deviations and the effluent test showed toxicity at levels of regulatory concern
Both the original and the first repeat reftox test results were outside of control limits (the second repeat reftox test result fell within control limits)	If the 95 percent confidence interval for the point estimate used in control charting can be calculated and in both failing reftox tests overlapped the control limits in the control chart, organism supplier reftox results were within control limits, and the coefficient of variation for the last 20 reftox tests is ≤ 0.60	If there are notable reporting errors or deviations from test protocol, or if any reftox test result fell outside of control limits to the more sensitive side (point estimate was too low) and the effluent test showed toxicity at levels of regulatory concern
All three reftox tests were outside of control limits	Never	Always
Coefficient of variation for the last 20 reftox tests > 0.85	Never	Always

Because point estimates provide the best basis for control charting, all labs must control chart using point estimates. Point estimates require fewer replicates than NOECs and reference toxicant testing may be done using the minimum number of replicates allowed by the test method.

Another Ecology staff person with primary responsibility for reference toxicant testing requirements is the Advisory Laboratorian in the Quality Assurance Section, who reviews standard operating procedures (SOPs) for toxicity tests and accredits labs. For bioassay labs to maintain Department of Ecology laboratory accreditation, the QA section has begun to require participation in a round-robin test (such as the DMR-QA) or the performance of one reference toxicant test at least once every six months. In the event that a lab does not conduct any tests on environmental samples using a particular species/method within a six-month period, it must perform a reference toxicant or round-robin test. In the event that a lab does not conduct any tests by a particular method within a one-year period, it must do two reference toxicant or round-robin tests for that year. Further, these tests must be done at least four months apart. This is to assure that the labs maintain proficiency with the species and methods for which they are accredited. The Quality Assurance Section can efficiently enforce good reference toxicant testing requirements because it has direct authority over labs to approve SOPs and conduct routine onsite audits.

E.4.3 Describe Any Additional QA/QC Criteria Your State Has Developed and Implemented Within Your State

- Sometimes variability across replicates will prevent a large difference in response (in other words, a toxic effluent) from being detected as statistically significant. False negatives can happen when the number of replicates is low. The acute statistical power standard says that acute toxicity tests must be able to detect a minimum of a 30 percent difference in survival between the IWC and a control as statistically significant. The chronic statistical power standard says that chronic toxicity tests must be able to detect a minimum of a 40 percent difference in response between the IWC (the NOEC if the IWC is unknown) and a control as

statistically significant. Tests which fail to meet the power standard must be repeated with an increased number of replicates.

***Ceriodaphnia* Chronic Test**

- ≤ 10 percent males in the surviving test organisms over all test concentrations.
- ≤ 20 percent males in the surviving test organisms in the IWC or LOEC.
- All surviving *Ceriodaphnia* producing no neonates in the test must be examined to determine gender, and the results of the determination reported. It is not necessary to identify gender when reproduction has been nearly eliminated in any test concentration when this fits an expected concentration-response relationship. It is understood that very young *Ceriodaphnia* can be difficult to sex, and any *Ceriodaphnia* that dies in the first two days of the test may be excluded from calculations for reproduction if gender is difficult to determine and it is one of no more than two mortalities in a concentration. Otherwise, difficult to sex young *Ceriodaphnia* must be considered to be female and included in all calculations.

E.4.4 Describe Any Efforts Your State Has Made to Minimize Test Method Variability

1. Development and distribution to all labs of a document called “Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria” (*canary book*) that lets them know our expectations for an acceptable toxicity test. The *canary book* also narrows testing choices and provides for more consistent testing between labs.
2. Test reviews for compliance with the test method and canary book.
3. Fish or mysid growth tests that have a standard deviation for proportion alive above 0.25 in any effluent concentration (unless the partial mortality occurs at the threshold of toxicity in a good concentration-response relationship) are analyzed for the original growth endpoint instead of the combined (“biomass”) endpoint.
4. To reduce the opportunity for WET limit violations due to statistically significant differences in response that are type I errors, permit requirements will lower the *alpha* level for hypothesis testing when differences in test organism response are small. To prevent excessive type I errors, eliminate some interrupted concentration-response relationships, and have more fair and enforceable test results, we will set *alpha* = 0.01 for small differences in response. If the difference in survival between the control and the IWC in an acute test is less than 10 percent, the level of significance will be lowered from 0.05 to 0.01. If the difference in test organism response between the control and the IWC in a chronic test is less than 20 percent, the level of significance will be lowered from 0.05 to 0.01.
5. The identification of anomalous tests is a valuable tool for reducing false positives. A concentration-response relationship where response increases with concentration is a good identifier of toxicity as opposed to other sources of organism stress such as disease. Test method variability or lab error will also very rarely produce a good concentration-response relationship. Identifying a test as anomalous does not necessarily mean rejection of the test and a requirement to repeat. If a test result meets one of the criteria for anomalous test identification but has no statistically significant toxicity at concentrations of regulatory concern (IWC), then the test need not be repeated unless other factors contribute to a decision to reject the test.

The anomalous test definitions below must be considered in light of the expectations for the different toxicity tests and endpoints.

Criteria for Identifying Anomalous Test Results

- A WET test result is anomalous if it shows a statistically significant difference in response between the control and the IWC, but no statistically significant difference in response at one or more higher effluent concentrations. The lack of statistical significance must be associated with a lower toxic effect at the higher effluent concentration. Any higher effluent concentration used in this determination must be a part of a dilution series. Labs should not cluster test concentrations just above the IWC in order to increase the opportunity for an anomalous test result.

- A WET test is anomalous if there is a statistically significant difference in response between the control and the IWC which together with other nearby concentrations of effluent, have a zero slope and appear to be nontoxic (performance is typical of healthy test organisms). Another description of this criterion is a test with a control that seems not to belong to the concentration-response relationship because of exceptionally good performance.

- A WET test is anomalous if the overall slope of the line fitted to the concentration-response plot is opposite of normal expectations and there is a statistically significant difference in response at the IWC. A test might be considered acceptable if the slope is opposite over only part of the concentration series.

- A WET test is anomalous if the standard deviation for proportion alive equals or exceeds 0.3 in any test concentration unless the partial mortality fits a good concentration-response relationship. A WET test is anomalous if mortalities occur in any test concentration in excess of the control performance criterion for survival when the concentration-response relationship indicates that the effluent concentration is nontoxic (sporadic mortalities).

E.4.5 Explain How Your State Reviews or Conducts Performance Lab Audits

The Department of Ecology manages an Environmental Laboratory Accreditation Program designed to assure that accredited labs have the capability to provide reliable and accurate environmental data to the department. Applicant labs apply for accreditation for specific parameters and methods. An applicable parameter/method pair for WET testing would be “*Pimephales promelas* by EPA Method 1001.0.”

Concurrent with submission of the initial application, the lab submits a quality assurance manual that is given a thorough review by Ecology staff. If there are reasonably-available performance evaluation (also known as “proficiency testing”) samples available for the requested tests, the lab is required to submit one set of such PE results for initial accreditation. This is referred to in our program as a “performance audit.” There are no PE samples we consider to be “reasonably available” for WET testing.

Following review of the lab’s QA manual and PE study results and successful resolution of any noted problems, Ecology and the lab schedule a mutually agreeable date for an on-site, or system, audit. (Although this survey asks about “performance” audits, which could be construed as being synonymous with our required PE studies, we think it rather is synonymous with what we call the on-site, or system, audit). For initial system audits, depending on the scope of tests done by the lab, checksheets may be sent to the lab to be completed and returned to the auditor prior to the audit. The auditor studies the checksheet responses and verifies accuracy of the response during the audit. For subsequent audits, which are routinely scheduled every three years but may be conducted at any time there is a need, the auditor may choose to send checksheets in time for them to be completed by the lab or take them to be filled in during the audit.

The actual audit, if for WET testing only, would involve one auditor and last one or two days depending on the scope of tests done in the lab. If the lab does other testing, the audit team may involve as many as five, and the audit may last as many as three days (or longer if required, but none have to date). The audit consists of an in-briefing, a thorough audit of personnel qualifications and equipment/supplies status (which were reported as part of the application), facility adequacy, sample management, records keeping/data management, performance evaluation study data (if applicable), the overall quality assurance program, status of quality control testing results (to see if the lab is meeting data quality objectives which were approved in the QA manual), and a check to see that current methods/SOPs are readily available and being followed. An out-briefing follows the audit during which the audit team informally summarizes major findings, both good and bad.

Following the audit, our program allows us 30 calendar days to prepare a written report. Depending on the scope of testing, this report, which addresses each of the factors discussed above, may be only 3 or 4 pages, or many more, and might include several attachments providing guidance or assistance to the lab. The secondary objective of our program as specified in the code is to assist labs in achieving the ability to meet required standards of performance, a perhaps novel but very effective approach to achieving desired capability in accredited labs. Historically, we have been deficient in meeting the 30-day report requirement, which has caused us to change our accreditation strategy. Using a fixed-price contract to encourage prompt reporting, we now contract out the audit task to a highly-qualified auditor whose last audit report was delivered within 10 days of the audit.

Performance audits (PE studies) are required in our program twice each year, and system audits are preferably conducted every three years with the code allowing four years for documented cause. At this time, we see no need to exceed three years for future audits of WET testing labs.

E.4.6 Describe Any Specific Implementation Guidance That Your State Has Developed to Assist Permit Writers. How Is the Guidance Available to the Public?

We have developed and kept updated suggested language for use in NPDES permits and fact sheets for POTWs and industries. The suggested language is a part of templates (“shells”) for permits and fact sheets that permit writers use as they draft a permit. We also have a “Permit Writer’s Manual” (USEPA 1996a) which addresses species choice, WET monitoring frequency, recommendations for number of test concentrations, etc. The “Permit Writer’s Manual” was developed with public input/review and is available to the public for the cost of printing.

E.4.7 Describe How Your State Provides or Utilizes Any Toxicity Testing Training

We had extensive training in all of our offices at the beginning of our use of WET testing in water quality-based permitting early in the 1990s. Because of budget constraints, because WET test review and technical assistance are centralized functions, and because of the availability of permit writing guidance in the “Permit Writer’s Manual” and suggested permit language, we no longer hold WET training sessions.

E.5 RESPONSES FROM WISCONSIN DEPARTMENT OF NATURAL RESOURCES

E.5.1 Describe How Your State Evaluates Reference Toxicant and Effluent Test Results

Reference toxicant and effluent test data is sent directly to the Biomonitoring Coordinator in Madison (central office). Certified labs are required to perform reference toxicant tests (using NaCl, specified dilutions and dilution water) on a monthly basis. Acute and chronic reference toxicant results are evaluated using the point-estimation techniques described in the EPA manual (LC₅₀, IC₂₅). Control charts (graphical and tabular) representing the mean LC₅₀ or IC₂₅ and upper and lower control limits (mean \pm 2 standard deviations) are established for each species, using data from the previous 20 months. Any exceedance of

either the upper or lower control limit after establishment of the control chart requires a review of the culture and test systems. Missing or out-of-range data must be explained (if possible) and may result in invalidation of Washington Pollution Discharge Elimination System (WPDES) test results conducted during the same period.

Each test report for all effluent tests is reviewed by the Biomonitoring Coordinator for completeness, adherence to QA and test acceptability requirements, and for compliance with the WPDES permit. Deviations from permit requirements, test acceptability criteria, or other factors may cause tests to be repeated.

E.5.2 Explain How Your State Reviews Reference Toxicant Data for Laboratory Performance

(See above.)

In addition to the regular review by the Biomonitoring Coordinator, reference toxicant data is reviewed by the Department's WET Laboratory Auditor prior to on-site laboratory inspections. The laboratory provides copies of bench sheets, water quality data, current control chart data, and any explanations of out-of-range test results for each test type and organism combination. The materials are reviewed for appropriate test frequency, proper test conditions, test result validity, and proper responses to out-of-range events.

E.5.3 Describe Any Additional QA/QC Criteria Your State Has Developed and Implemented Within Your State

Test acceptability requirements, based on current "State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, Edition 1":

Testing must be separated from culturing activities (separate rooms with separate ventilation systems; if closed incubators are used, culturing & testing may not be contained within the same incubator)

For Static Renewal Acute Tests:

Pretest Requirements (Requirements For Culture Acceptability)

— C. dubia:

- Average Number Of Neonates In 3 Broods ≥ 15
- Mean Survival ≥ 80 percent
- Number Of Neonates In Each Brood ≥ 8
- Age Of Organism ≤ 24 -H

— Fathead Minnows:

- Age Of Organism 1- 14 Days
- Sample Requirements
- Holding Time ≤ 36 -H
- Temperature During Collection & Prior To Shipping ≤ 4 °C
- Temperature Upon Arrival At The Laboratory ≤ 10 °C

Test Requirements (Requirements For Test Acceptability)

- Temperature 20 ° ± 1 °C
- Dissolved Oxygen > 40 percent and < 100 percent saturation
- Effluent - pH ≥ 6.0 and ≤ 9.0 .
- Control Survival ≥ 90 percent

For Static Renewal Chronic Tests:

Pretest Requirements (Requirements For Culture Acceptability)

— *C. dubia*:

- Average Number Of Neonates ≥ 20
- Mean Survival ≥ 80 percent
- Neonates Used In Test Must Be From 3rd Or Subsequent Brood
- Number Of Neonates In 3rd Or Subsequent Brood ≥ 8
- Age Of Organism ≤ 24 -H; Released Within Same 8-H Window

— Fathead Minnows:

- Age Of Larvae ≤ 24 -H
- Sample Requirements
- Holding Time ≤ 36 -H
- Temperature During Collection & Prior To Shipping ≤ 4 °C
- Temperature Upon Arrival At The Laboratory ≤ 10 °C

Test Requirements (Requirements For Test Acceptability)

- Temperature 25 ° ± 1 °C
- Dissolved Oxygen > 40 percent and < 100 percent saturation
- Effluent - pH ≥ 6.0 and ≤ 9.0
- Control Survival ≥ 80 percent
- *C. dubia* Mean Control Reproduction ≥ 15 Neo./Adult; > 60 percent produce 3 broods
- Fathead Minnow Mean Control Biomass ≥ 0.25 mg/individual

The Wisconsin Department of Natural Resources (WDNR) is in the process of updating its WET Methods Manual. Future methods (2nd Edition expected in 2001) will include *additional* or *revised* test acceptability criteria:

For Static Renewal Acute Tests:

Pretest Requirements (Requirements For Culture Acceptability)

— Fathead Minnows:

- Age Of Organism 4 - 14 Days
- Sample Requirements
- Temperature Upon Arrival At The Laboratory ≤ 6 °C

Test Requirements (Requirements For Test Acceptability)

- Control Variability CV < 40 percent

For Static and Static Renewal Chronic Tests:

Sample Requirements

- Temperature Upon Arrival At The Laboratory ≤ 6 °C

Test Requirements (Requirements For Test Acceptability)

- Control Variability - Fathead Minnow & *C. dubia* CV < 40 percent
- Control Variability - *R. subcapitata* CV < 20 percent
- *C. dubia* Male Production < 20 percent in controls & < 20 percent all concentrations
- *C. dubia* Mean Control Reproduction > 80 percent produce 3 broods
- *R. subcapitata* Control Performance Cell Density $> 1 \times 10^6$ cells/ml at end of test

E.5.4 Describe Any Efforts Your State Has Made to Minimize Test Method Variability

1. Close review of each test result submitted with consistent adherence to test protocol test acceptability criteria.
2. Investigations of test results from disagreeing “split” effluent sample analyses.

3. State specific methods: In order to limit the variability that may occur when different procedures are used by different labs, WDNR requires strict adherence to clearly specified methods, regarding: (a) sampling procedures (volume, type, storage conditions, etc.); (b) holding times; (c) test duration; (d) deviations in feeding & environmental conditions (light, pH, temperature, DO, etc.); (e) dilution water; (f) number of concentrations and replicates tested; and (g) number of organisms per replicate.

Each of these is addressed in EPA methods, but flexibility is allowed so labs can make tests fit in specific situations. The more flexibility allowed in test methods, the higher the chance that tests will be done differently between labs or between tests, resulting in increased WET variability. In order to control WET variability and improve the consistency of methods used by Wisconsin labs and permittees, WDNR created the “State of Wisconsin Aquatic Life Toxicity Testing Methods Manual,” Edition 1 (PUBL-WW-033-96) (Methods Manual) and incorporated it by reference into NR 149.22 and NR 219.04, Wis. Adm. Code, in 1996. The Methods Manual contains specific procedures regarding testing and sampling procedures, types of tests, quality control/quality assurance procedures, test acceptability criteria (see above), etc., that labs must follow when performing WET tests for permit compliance.

4. Implementation of a WET Laboratory Certification program. In order to insure labs are of the highest quality and are able to demonstrate a serious commitment to a quality assurance/control program, WDNR, under State statutes, certifies labs to perform WET tests. In order for a lab to apply for certification for WET testing, the lab must submit a completed application and a quality assurance plan to the lab certification program and pass an on-site evaluation. WET labs must have an ongoing reference toxicant program, a review process for all test data and reporting, a good sample custody system, proper equipment maintenance, dilution water quality monitoring, facility maintenance, and attention to test organism health, and make other demonstrations of good lab practices in order to pass an audit.
5. The WDNR's WET Team strives to continually improve the WET program. The WET Team is now revising the Methods Manual to require that labs verify the training and qualifications of their staff, to include test acceptability criteria related to variability, and other changes to further improve WET test quality and reduce variability (see above).

E.5.5 Explain How Your State Reviews or Conducts Performance Lab Audits

Inspections may be announced or unannounced. Prior to the inspection, the auditor reviews laboratory SOPs and recent reference toxicant results for adherence to WDNR protocols. The actual inspection consists of an opening conference, a walk-through of the laboratory facilities, and a closing conference. During the opening conference, the auditor discusses the SOP review and general procedures in the laboratory. During the walk-through, the auditor examines equipment, written documentation, cultures, and laboratory procedures. He/she will also interview lab personnel to insure that they understand lab quality assurance and methods requirements. The closing conference serves as a review of observations and comments during the inspection. After the inspection, the auditor generates an inspection report, to which the laboratory will be given 60 days to respond. If there are significant deficiencies discovered during the inspection, and the laboratory fails to fix those deficiencies satisfactorily within the allotted time, the laboratory's certification may be revoked.

E.5.6 Describe Any Specific Implementation Guidance That Your State Has Developed to Assist Permit Writers. How Is the Guidance Available to the Public?

The WDNR created the “WET Program Guidance Document” in 1996, as a companion document to the “State of Wisconsin Aquatic Life Toxicity Testing Methods Manual,” in order to provide guidance and

clarification of existing rules, for WDNR staff, permittees, labs, consultants, and others. The WET Guidance Document is updated as program needs dictate, at least once yearly, and can be obtained by contacting the Biomonitoring Coordinator at: WDNR, Bureau of Watershed Management, P.O. Box 7921, 101 S. Webster St., Madison, WI, 53707-7921; email: flemik@dnr.state.wi.us; or at <http://www.dnr.state.wi.us/org/water/wm/ww/biomon/biomon.htm>.

E.5.7 Describe How Your State Provides or Utilizes Any Toxicity Testing Training

The Biomonitoring Coordinator provides one-on-one training, as needed, for WDNR staff and permittees (usually as permits are reissued with new WET requirements). The University of Wisconsin-Madison State Lab of Hygiene (who provides WET testing and research services to WDNR) can provide hands-on WET training to WDNR staff, permittees, and/or new staff at contract laboratories, at their request. WDNR staff, permittees, and contract lab staff have also attended The Society of Environmental Toxicology and Chemistry's two-day WET course and statistical analysis course.

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