QUESTIONS TO ASK OF LABS WHEN CONTRACTING FOR WET TESTS

1) What specific decision-criteria, other than the statistical tests described in the WET test manuals, does the lab use to identify anomalous data and particularly Type-I errors (false positives)?

2) What specific decision-criteria does the lab use to confirm the presence or absence of a concentration-response relationship (a.k.a. dose-response curve) in before concluding that a sample is toxic?

3) EPA states that the interlaboratory precision for WET tests indicates a 40-60% coefficient-of-variation and that simultaneous tests of identical effluent samples should produce results that are plus or minus one concentration interval in the dilution series from the median. How does the lab use this information to estimate the probable range of error around TUa and TUc estimates?

4) What is the probability that the true IC-25 is actually higher than the reported estimate using the Linear Interpolation technique? Lower?

5) Occasionally, the bootstrapping function of the Linear Interpolation software fails to generate confidence intervals around an IC-25 estimate. Why does this happen? What are the implications for assessing the stability and certainty of the IC-25 estimate? How does it effect a compliance determination (toxic or non-toxic?)

6) If a WET test indicates no significant difference in reproduction at the 6.25%, 25% & 50% concentrations but, shows a significant difference in reproduction at the 12.5% and 100% concentrations, what is the reported NOEC and LOEC? If the permitted instream waste concentration (IWC) is also 12.5%, did the discharger pass or fail the WET test?

7) What is the labs average coefficient-of-variation between replicates in the control groups of WET tests? What is the labs average coefficient-of-variation between control group means of different tests?

8) Please provide copies of:
   a) Statistical process control charts for reference toxicant tests
   b) Statistical process control charts for performance control organisms
   c) Statistical process control charts for Minimum Significant Difference
   d) Previous five years DMR-QA results.
TECHNICAL AGREEMENT FOR ANALYTICAL SERVICES
RELATED TO NPDES WHOLE EFFLUENT TOXICITY MONITORING

DISCHARGER NAME (hereinafter “the CLIENT”) is required to conduct periodic whole effluent toxicity testing (WET). The testing and monitoring requirements are set forth in the NPDES permit issued by state and federal authorities (PERMIT #).

The CLIENT desires to contract with ___________________________________________ (hereinafter: “the Lab,” to conduct the required tests. As these tests will be used to establish compliance with conditions in the NPDES permit, they must meet certain specifications.

This document is intended to provide detailed descriptions of the work to be performed, the manner in which it is to be performed, and the procedures for reporting results.

1. PRE-REQUISITE QUALIFICATIONS

1. ELAP Certification

The lab must be certified and registered as an environmental testing laboratory pursuant to the provisions of the LABORATORY CERTIFICATION REGULATION to perform all analyses listed in Section II of this agreement. The Lab shall provide a copy of their current ELAP certificate to the CLIENT. The Lab shall also provide a copy of their renewal certificate when it is reissued.

Alternatively, the lab must be approved by the PERMITTING AUTHORITY if no ELAP certification is available. Regulatory approvals can be coordinated through CLIENT.

2. DMR and WP Studies

The Lab shall participate in QA/QC performance studies for WET testing when requested by the CLIENT. The Lab shall notify the CLIENT whenever such studies are planned or proposed by the EPA OR STATE PERMITTING AUTHORITY. The Lab shall submit a copy of all study results to the CLIENT within 15 working days of receipt of those results.
3. Guidance Document

The Lab shall maintain complete copies of:

- a) The CLIENT NPDES permit including the monitoring and reporting program (93-45). CLIENT will provide copies of these documents.
- b) Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; Second Edition; March, 1989 (EPA 600-4-89-001)
- c) Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms; Third Edition; March, 1985 (EPA 600-4-85-013)
- e) 40 CFR 136 and the related appendices (methods 1000.0, 1002.0, & 1003.0 including updated revisions to the toxicity test protocols)

4. Qualified Technicians and Analysts

All of the laboratory work, including the statistical analyses, conducted at the request of the CLIENT shall be performed by qualified and experienced technicians and analysts. The educational qualifications and work experience of all technicians and analysts performing work for the CLIENT shall be available for review at the request of the CLIENT.

5. Supervision

All of the work performed by the Lab for the benefit of the CLIENT shall occur under the general supervision and control of ________________________________. The Lab must notify the CLIENT in the event the person named above is no longer able to supervise the conduct of tests performed for the CLIENT.

6. Subcontractors

No analytical services, requested by the CLIENT, may be subcontracted to another laboratory, person or firm without prior written consent by the CLIENT. Where consent is given, the Lab shall attach complete copies of the subcontractor’s report to their own final report. The subcontractor’s report must be submitted on the subcontractor’s own letterhead. Subcontractor services will be billed through the standard contract agreement between the Lab and the CLIENT. All subcontractors must agree to certify the test results in the same manner as the Lab.
7. Laboratory Conditions

All of the testing and analyses performed for the benefit of the CLIENT shall be conducted in clean laboratory conditions. Clean conditions means there is no potential for test contamination by toxics in toxic amounts from sources other than the effluent sample as received by the Lab from the CLIENT.

8. Reference Toxicant Tests

The Lab shall conduct reference toxicant tests for all species and protocols used to analyze the effluent at least once each month. The Lab shall maintain historical performance charts for the results of all reference toxicant tests run in the preceding twelve months or in the twenty most recent tests performed. The charts must record the results from each reference toxicity test, the mean for all reference toxicity tests, and the upper and lower 95% confidence limits for the preceding twelve months or twenty tests. The charts shall be updated and attached to each WET report the Lab submits to the CLIENT.

9. Control Charts

The Lab shall maintain historical performance charts documenting results from all control groups evaluated during the four weeks. The charts must record the average result for each control group, the date of that test, the mean result for all controls, and the 95% upper and lower control limits for preceding four weeks. The charts shall be updated and attached to each WET report the Lab submits to the CLIENT.

10. Notifications

The Lab shall notify the CLIENT of any change in the laboratory operation that impacts ELAP certification, such as: revocation, suspension or non-renewal of certification, transfer of ownership, change of laboratory director, change in location, major changes in instrumentation, or structural alterations that have an effect on the quality of analyses performed. A copy of any required notices submitted to the ELAP program shall also be sent to the CLIENT.

The Lab shall also notify the CLIENT if data from control charts indicate that test organisms may not be able to meet EPA’s minimum control performance criteria for test acceptability. With such notification, the Lab shall suggest an alternate sampling period when test organisms are more likely to meet performance specifications.
WORK TO BE PERFORMED

1. Regular Acute Toxicity Testing

Once each month, the Lab shall conduct a whole effluent acute toxicity test, using Ceriodaphnia dubia, and the protocols specified in EPA document #600-4-85-013. The older protocol is specifically required in the CLIENT permit. CLIENT may require more recent protocols if, and when, the NPDES permit is updated.

2. Regular Chronic Toxicity Testing

Once each month, the Lab shall conduct a whole effluent chronic toxicity test, using Ceriodaphnia dubia, and the protocols specified in EPA document #600-4-89-001. The older protocol is specifically required in the CLIENT permit. CLIENT may require more recent protocols if, and when, the NPDES permit is updated.

3. Annual Species Sensitivity Testing

Once each year, the Lab shall conduct acute and chronic whole effluent toxicity tests using Fathead minnows, Ceriodaphnia dubia and Selenastrum Capricornutum.

4. Retesting

In the event that any toxicity test fails to meet EPA’s recommended test acceptance criteria, then the Lab shall notify the CLIENT within 24 hours. The lab shall be responsible to conduct a new test, at their own expense, when new sample water is received from CLIENT. CLIENT shall provide additional effluent samples to the Lab at no expense to the Lab.

5. Accelerated Testing

In the event that any toxicity test shows a statistically-significant reduction in measured biological endpoints, the Lab shall notify CLIENT within 24 hours. CLIENT may be required to run additional toxicity tests when previous failures are recorded. The lab shall coordinate with CLIENT to run the extra tests at the earliest available opportunity. The accelerated tests shall be conducted at the expense of CLIENT.

6. Audit Testing

At the request of CLIENT, the Lab may be asked to perform other whole effluent toxicity tests for quality assurance purposes. Such audits shall be conducted at the expense of the CLIENT.
EXPERIMENTAL TEST DESIGN

1. Dilution Series

All whole effluent toxicity tests performed on behalf of the CLIENT shall be conducted using a dilution series containing the following concentrations: 0%, 12.5%, 25%, 50%, 75%, & 100% effluent.

2. Replicates

All whole effluent toxicity tests performed on behalf of the CLIENT shall be initiated with the minimum number of replicates specified in the following table:

<table>
<thead>
<tr>
<th>Species</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenastrum Capricornutum</td>
<td>n/a</td>
<td>4</td>
</tr>
</tbody>
</table>


3. Selection of Test Organisms

All organisms used in whole effluent toxicity testing must be selected in accordance with the procedures specified by EPA. Specifically, Fathead minnow larvae, used in the chronic test procedure, must be less than 48 hours old (<24 hrs. if in-house cultures are used) and all hatched within 24 hours of one another. Ceriodaphnia dubia, used in the chronic test procedure, must be less than 24 hours old and all within 8 hours of the same age to begin the test. To qualify for use in chronic testing, neonate Ceriodaphnia may only be taken from adults that have eight or more young in their third or subsequent broods and the adult brood stock must be less than 14 days old (see section 12.2.3 of EPA protocol for Ceriodaphnia).

4. Randomization

All test organisms must be placed in test cells using randomization procedures specified by EPA. The Ceriodaphnia test must also use the “blocking” methods described in section 12.2.4 of EPA’s chronic protocol for Ceriodaphnia.
5. Dilution Water

Water used to dilute effluent or serve as a test control shall conform to the recipe for “moderately hard” water as described in Section 7 of EPA’s chronic test manual (Tables 1 & 2 on page 26). No other formulation may be substituted without prior written authorization from the CLIENT. And, the Lab must certify that the dilution water is “free from toxics in toxic amounts” in the final report submitted to the CLIENT.

6. Deviation

Any deviation from the experimental design prescribed by EPA’s official guidance documents must be identified and justified in the Lab’s final report to CLIENT. In addition, such deviations must be highlighted in a transmittal letter which accompanies the final report.

RECEIPT OF SAMPLES

1. Sampling Containers

The Lab shall supply clean, unused cubitainers to be used in gathering effluent samples of effluent for WET testing.

2. Receiving

The Lab shall assure that qualified personnel are available to receive effluent samples when they are scheduled to arrive.

3. Chain-of-Custody Forms

The Lab shall record the date and time of receipt, and temperature of each water sample upon arrival, on the chain-of-custody form which accompanies each effluent sample. Copies of the chain-of-custody forms shall be included with each test report submitted to the CLIENT.

4. Non-Receipt of Scheduled Samples

The Lab shall immediately notify the CLIENT in the event that a scheduled sample is not received by 3pm on the date expected. Such notification must be by both phone and fax to the following persons and locations (in the ascribed order):

(a)

(b)

(c)
1. Required Analyses

The Lab shall analyze each effluent sample for the following constituents/parameters:

(a) Temperature  
(b) pH  
(c) Alkalinity  
(d) Hardness  
(e) Conductivity  
(f) Dissolved Oxygen  
(g) Total Residual Chlorine  
(h) Ammonia

2. Special pH Recording

The Lab shall report the average pH of each test concentration before and after each renewal. The average pH may be measured by pooling the “used” water from all replicates, in each treatment group, after organisms are moved to replacement water. Alternatively, the lab may elect to measure the pH of each and every replicate before and after sample water is replaced.

3. Reporting Chemical Results

The Lab shall include the results of all chemical analyses in the written report summarizing each whole effluent toxicity test series. Where chemical analyses are performed by a subcontractor, the results may be submitted as an appendices to the lab’s final report.

4. Reporting Exceptions

Where one or more chemical parameters is believed to be outside acceptable limits, as defined in EPA’s protocols, the Lab shall note the exception in their final report. The Lab shall also provide describe the impact of any deviation on test acceptability in their written report (see section 4.9.2 of EPA chronic protocol).

5. Special Conditions for Chlorine

If chemistry analysis indicates that chlorine appears to be present, the Lab shall continue to run the WET test without dechlorinating the sample unless specific written instructions to the contrary accompany the Chain-of-Custody forms. The Lab should record the chlorine results, including the detection limit for the analytical method used, in their final report.
6. Physical Inspection of Samples

The Lab shall visually inspect each effluent sample when it is opened for testing. The samples should be clear of debris and free of odors. Any unusual conditions shall be noted in the Lab’s written report to the CLIENT.

VI. TEST ACCEPTABILITY

1. Minimum Control Performance Criteria

All whole effluent toxicity tests must meet EPA’s recommended minimum control performance criteria (shown in the table below). Failure to meet the minimum criteria constitutes a breach of quality assurance and makes the data “unacceptable” for use in assessing NPDES permit monitoring and compliance.

<table>
<thead>
<tr>
<th>Control Organisms</th>
<th>Acute Tests</th>
<th>Chronic Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fathead minnow</td>
<td>&gt;90% survival</td>
<td>&gt;80% survival and ≥0.25 mg average</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight per fish</td>
</tr>
<tr>
<td>Ceriodaphnia dubia</td>
<td>&gt;90% survival</td>
<td>&gt;80% survival and ≥15 offspring per</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surviving female invertebrate</td>
</tr>
<tr>
<td>Selenastrum Capricornutum</td>
<td>n/a</td>
<td>≥200,000 cells/ml</td>
</tr>
</tbody>
</table>

2. Notification for Failed QA/QC

If a test fails to meet EPA’s minimum control performance criteria, the Lab shall notify the CLIENT within 24 hours of test termination. Such notification shall be by phone, by fax, and by pager to the following persons and numbers:

a) 

b) 

c) 

3. Retesting for Failed QA/QC

If a test fails to meet EPA’s recommended minimum control performance criteria, the Lab must initiate a new test at their own expense. The CLIENT shall provide additional effluent samples at no expense to the Lab.
4. Data Submission for Failed QA/QC

The Lab must submit copies of all benchsheet data from any test which fails to meet EPA’s recommended minimum control performance criteria to the CLIENT. No additional statistical analysis is required or expected when data otherwise fails to meet QA/QC criteria.

5. Control Group Specification for Assessing QA/QC

Control performance shall be assessed based on the results from the dilution control group only. Under no circumstances shall alternative test data, from other control groups, be substituted for the dilution control group without prior written authorization from the CLIENT.

VII. DATA ANALYSIS

1. Selection of Statistical Methods

The Lab shall use EPA’s recommended flowcharts to conduct all statistical analyses of whole effluent toxicity test data (see Section 12.3; figure 8 of EPA’s chronic procedures for Fathead minnows and Section 13.2, figure 4 and Section 13.5, figure 5 of EPA’s chronic procedures for Ceriodaphnia dubia).

2. Acute Test Metrics

The Lab shall report the percent survival for each effluent concentration and calculate the LC50 for each acute toxicity test.

3. Chronic Test Metrics

The Lab shall calculate and report the highest No Observed Effect Concentration (NOEC) for all biological endpoints (lethal and sublethal) in each chronic toxicity test. The Lab shall also record and note where the results for any effluent concentration are significantly less than control performance. The threshold for statistical significance shall be set so that the risk of Type-I inferential error is less than or equal to 5% (p<.05).

4. Calculating TUC for Sub-Lethal Endpoints

The lab shall assess all sub-lethal endpoints using the NOEC methodology. The lab shall also calculate the IC25 using the Inhibition Concentration methodology where recommended in EPA’s flowchart. However, only the NOEC shall be used to calculate and report the estimated TUC value for reproduction, growth or cell density. The IC25 shall not be used to assess the “pass/fail” status of any toxicity test.
5. Computer Printouts

The Lab shall provide copies of all printouts (text and graphics) from any computer programs used to analyze whole effluent toxicity data in their final written report to CLIENT.

6. Minimum Significant Difference Calculations

The Lab shall calculate and report the Minimum Significant Difference (MSD) for each biological endpoint (lethal and sublethal) in the chronic toxicity tests. The MSD shall be reported as the percent reduction from the mean of control performance which would be statistically-significant (95% confidence).

7. Reporting Brood-level Data

For all chronic toxicity tests performed using Ceriodaphnia dubia, the Lab shall report the percentage of control replicates which produced at least three broods prior to test termination. The Lab shall also record and report the percentage of replicates which produced at least three broods for each and every effluent concentration.

8. Independent Data Review

The Lab’s Study Director shall conduct an independent review of all procedures, data and statistical analyses for whole effluent toxicity tests conducted using effluent. The Study Director shall signify such review has occurred by initialing every page of the final report submitted to CLIENT.

VIII. REPORTING

1. Urgent Results

The Lab shall notify the CLIENT of any test result which appears to indicate the presence of toxicity (TUc>1 or TUa>1) within 24 hours of test completion. Such notification shall be by phone, by fax, and by pager to the following persons and numbers:

(a)
(b)
(c)
2. Normal Reporting

The Lab shall provide a complete written report summarizing test methods, procedures, results and analyses to the CLIENT within fourteen calendar days of test completion.

3. Transmittal Letter

The Lab shall provide a cover letter to their final written report for each whole effluent toxicity test conducted on behalf of the CLIENT. The transmittal letter must include all of the following specific information:

(a) Whether controls met EPA’s minimum performance requirement for each test.
(b) Whether a statistically-significant reduction in survival, growth or reproduction was observed when comparing controls organisms to organisms exposed to undiluted effluent.
(c) Any exceptions to EPA methods and procedures must be specifically identified.

4. Certification Statement

The Lab must certify the results of their testing procedures in accordance with 40 CFR 122.22. Therefore, a formal certification statement must be attached to the final written report submitted. The NPDES permit, issued to CLIENT, requires the study director must sign and date the following specific certification statement:

“I certify that all laboratory reports were prepared under my direction or supervision, and that all analyses were performed in accordance with a system designed to assure that qualified personnel perform the analysis, use the specified EPA-approved methods, and review the data before it is reported. Based on my inquiry of the person or persons who manage the system, or those directly responsible for gathering the information, the information reported is, to the best of my knowledge, true, complete and accurate.”

5. Benchesheets

The Lab must include copies of all laboratory benchesheets with their final written reports. Benchsheets should clearly indicate daily measurements of all relevant chemical and biological data. Benchsheets must also distinguish between dead and missing (lost) organisms.

Errors should be corrected on the benchesheets by crossing out the wrong information and adding the correct information. Erasure or “white-out” are unacceptable methods for correcting errors. The previous incorrect data must remain legible even after correction. All error corrections must be initialed by the person making the correction.
6. Other Appendices

The Lab shall attach copies of all other data and information relevant to reviewing and interpreting the results of each whole effluent toxicity test as an appendix to their final report.

IX. SUPPORT SERVICES

1. Customer Service Representative

A customer service representative will be assigned to work directly with CLIENT staff. An alternate customer service representative shall also be designated in the event that the primary contact staff person is not available.

2. Direct Access

The CLIENT staff and their designated technical consultants shall have direct access to the QA/QC manager, laboratory director and section supervisors for issues which cannot be resolved by the customer service representatives.

3. Supplementary Written Documentation

The Lab shall provide written clarifications and responses to technical questions when specifically requested by the CLIENT. Such services may result in additional cost to the CLIENT.

X. NOTIFICATIONS

1. Official Communications

The written, verbal and facsimile (fax) notifications required in this technical agreement should be made to the persons, addresses and telephone numbers given below. Any changes to points-of-contact for the Lab should be submitted to the CLIENT within 7 days of the effective date of change.

2. Contact Logs

The Lab should maintain a log of all written and verbal communications between themselves and representatives of the CLIENT. The log should show the date, time, persons, and purpose of each communication. Copies of the log must be provided to the CLIENT upon request.
3. Points-of-Contact for the Lab

Name: __________________________________________
Laboratory: __________________________________________
Address __________________________________________
City, State, ZIP __________________________________________
Telephone: __________________________________________
Fax Number: __________________________________________
Pager Number __________________________________________

4. Points-of-Contact for CLIENT:

Name: __________________________________________
Laboratory: __________________________________________
Address __________________________________________
City, State, ZIP __________________________________________
Telephone: __________________________________________
Fax Number: __________________________________________
Pager Number __________________________________________

5. Points-of-Contact for PERMITTING AUTHORITY:

Name: __________________________________________
Laboratory: __________________________________________
Address __________________________________________
City, State, ZIP __________________________________________
Telephone: __________________________________________
Fax Number: __________________________________________
Pager Number __________________________________________