CHAPTER 2.1 - Selecting a WET Laboratory

The purpose of this chapter is to provide some guidance and answers to frequently asked questions for permittees who are selecting a new WET lab.

NOTICE: This document is intended solely as guidance, and does not contain any mandatory requirements except where requirements found in statute or administrative rule are referenced. This guidance does not establish or affect legal rights or obligations, and is not finally determinative of any of the issues addressed. This guidance does not create any rights enforceable by any party in litigation with the State of Wisconsin or the Department of Natural Resources. Any regulatory decisions made by the Department of Natural Resources in any matter addressed by this guidance will be made by applying the governing statutes and administrative rules to the relevant facts.

General Information

The first thing that should be done when evaluating a lab for its ability to perform whole effluent toxicity (WET) tests is to find out if it is certified in Wisconsin to perform WET tests (category #20, s. NR 149.22, Wis. Adm. Code). You should ask the lab to provide you with a copy of their certificate or you may contact the Lab Certification Program at the DNR to obtain this information (the Bureau of Integrated Science Services, 101 S. Webster St., Madison, WI 53707, (608) 267-7633). A list of certified laboratories is provided at: http://dnr.wi.gov/topic/wastewater/WETCertified.html

You should ask the lab if they've had an on-site evaluation conducted by the DNR or other accrediting authority. If there has been an on-site evaluation, you may want to see a copy of the audit report and the lab's response to any deficiencies cited in the report. You may also contact the Lab Certification Program and ask to speak to the lab's auditor or go to the Bureau of Integrated Science Services in Madison and review the lab's file.

Sample Handling and Preservation

The "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (Methods Manual) requires that samples arrive at the lab on ice, at $\leq 10^{o}$C, and in $\leq$ 36 hours (see Section 2.4). A sample that arrives at the lab over the prescribed temperature or holding time must be rejected by the lab, as required by the Methods Manual. If a sample must be rejected, another sample will have to be taken that complies with sample holding and use times and the test may have to be restarted (probably at a cost to the permittee). When selecting a new WET lab, you should ask the lab what they do to help make sure that these requirements are met. In some cases, you might want to ask the lab to collect and transport WET samples (although this can significantly increase test costs).

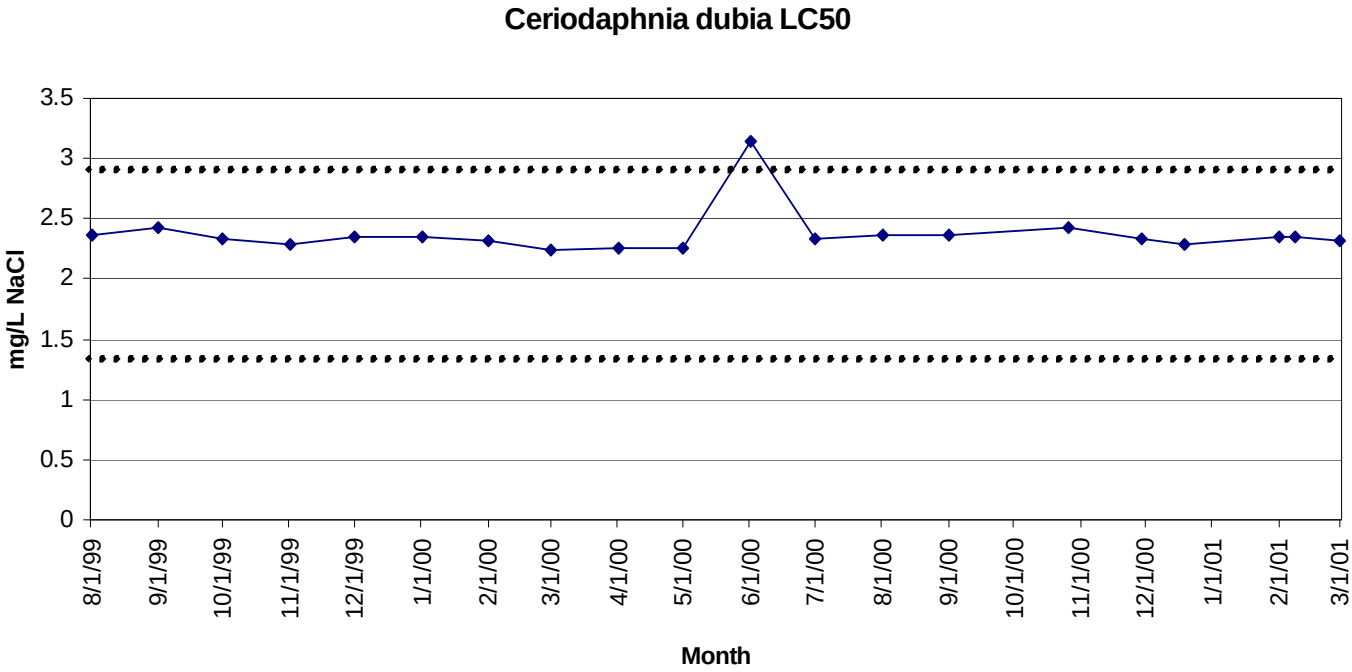
Organism Health And Performance

Lab visit. If visiting the lab, it may be wise to view the organisms used in testing. The organisms should appear active and healthy. If fish appear sluggish, this may be an indication of health problems. *C. dubia* should be free-swimming, not floating or immobile. Aquariums, culturing chambers, and culturing areas should be clean and organized. Culture areas should be separate from testing areas so that cross-contamination can't occur.

Culture Records. The lab should be able to provide records documenting where their organisms came from originally. Species confirmation must be performed annually and the lab should have records on file documenting that they use the correct species. If organisms from an outside supplier are used, ask who the supplier is and have the lab provide the documentation from the supplier regarding the health of the organisms. If the lab performed a reference toxicant test (see below) when the organisms first arrived in the lab, ask for a copy of those results.

Reference Toxicant Testing. Another way to compare laboratories is to look at the reference toxicant tests that have been generated. The Methods Manual requires that each lab demonstrate its ability to obtain consistent,

precise results with reference toxicants before it performs tests with effluents for permit compliance purposes (see Section 3.15). Prior to lab certification or registration, each lab must establish an individual precision benchmark for each species and test type performed. This benchmark is defined as the coefficient of variation (CV) between replicates of the test endpoint (acute = LC_{50}; chronic = IC_{25}) calculated from $\geq$ 5 tests conducted with the reference toxicant sodium chloride (NaCl). These tests are done at the same concentrations, with the same test conditions, and same data analysis methods as effluent tests. Once established, ongoing lab performance must be continually



compared to this benchmark. This ongoing performance is tracked in a "control chart" (see Figure 2.1.1).

Figure 2.1.1 Example control chart showing mean LC_{50} and control limits (mean $\pm$ 2 standard deviations)

A control chart, like the example shown in Figure 2.1.1, is a graphical representation of the mean LC_{50} or IC_{25} and upper and lower control limits (mean $\pm$ 2 standard deviations) of each reference toxicant test using data from the previous 20 months. The size of a control chart's upper and lower control limits is an indication of a lab's capability to reproduce the desired endpoints of a reference toxicity test. Laboratories with very wide control limits, and/or many points outside of the control limits, should be investigating problems related to the quality of the data being produced. A series of exceedances of either the upper or lower control limit after establishment of the control chart should prompt a review of the culture and test systems, as they may cause effluent tests completed during the month(s) of the exceedances to be questioned.

It is important to note that control chart limits are a function of the test species, test type and biological endpoint (survival, growth, etc.) being used. These factors must be considered before drawing conclusions regarding lab performance. Analyst performance should improve with experience, and the control limits for point estimates should gradually narrow. However, control limits of ± 2 S.D., by definition, will be exceeded 5% of the time, regardless of how well a lab performs. Highly proficient laboratories may develop very narrow control limits. A lab that has experienced a test which falls just outside the control limits, but has very narrow control limits, may not warrant the same level of concern as a similar test done at a lab with wider control limits. The width of the control limits should be considered in determining whether or not points outside of the normal range are a signal of poor lab performance.

You should ask the lab to provide a copy of their reference toxicant results and control charts. Reference toxicant testing is required by the Methods Manual in order to determine the sensitivity of test organisms over time and to assess the lab's ability to obtain consistent test results using a known toxicant. Results can be used to identify potential sources of variability, such as test organism health, difference among batches of organisms, changes in lab water or food quality, and performance by lab technicians. Performance of reference toxicity tests as recorded by control charts is a criterion that may be used by a permittee when selecting which laboratory to use for WET tests. Charting the performance of a lab's controls relative to its reference toxicity test results can also be a good way to track the lab's performance and to identify when the lab's performance is not acceptable.

Lab and Individual Analyst Experience and Qualifications

A number of factors are important in reducing test variability and improving test quality. Some of the most important of these factors are careful adherence to test guidelines, adequate analyst expertise, and conscientious selection of contract laboratories. Analyst experience and organism health are probably the two most important aspects of any successful WET test. Wisconsin's WET laboratory certification program requires that each lab meet certain experience and qualification requirements for their staff (see Section 3.17 of the Methods Manual).

The ability to successfully complete toxicity tests is a direct function of the training and expertise that an analyst has accumulated. If laboratories have staff who are responsible for the toxicity testing program but have no training in the biological sciences or little practical experience in WET testing, this can be a major source of test variability. The DNR has written and codified the Methods Manual, in order to set Wisconsin-specific test methods and test acceptability criteria. The Methods Manual also contains specific requirements for lab analyst training and expertise (see Section 3.17). Section 3.17.2 of the Methods Manual says that "*These methods are restricted to use by or under the supervision of analysts experienced in the use or conduct of aquatic toxicity testing and the interpretation of data from aquatic toxicity testing. Each analyst must demonstrate the ability to generate acceptable test results with these methods using the procedures described in this methods manual*". One would not consider chemical analysis of water samples, including effluents, without adequate analyst expertise and proper quality assurance/control (QA/QC). Since WET test results are as important as chemical analyses in most regulatory decisions, it seems only appropriate to have the same high standards for these test methods.

Permittees should ask to see the training files of lab staff and insure that each lab technician has the necessary combination of experience and education to adequately demonstrate a specific knowledge of their particular function and a general knowledge of lab operations, analytical test methods, QA/QC procedures, and records management. Lab management should be able to certify that personnel with appropriate educational and/or technical background perform all tests for which the lab is accredited.

Lab Selection Based on Quality, Not Cost

Because lab selection can be such an important factor in test results, it is important that the experience of the analysts be carefully considered. The educational qualifications and experience of the lab individuals who will actually perform the tests, as well as the qualifications of the supervisory staff, should be reviewed prior to lab selection. The toxicity testing lab should demonstrate a serious commitment to a QA/QC program that extends beyond analyst experience. Considerations such as an ongoing reference toxicant program, a two-tiered review process for all toxicity test data and summary reports, a good sample custody tracking system that is always used, proper equipment maintenance, dilution water quality monitoring, facility maintenance, and attention to test organism health are all characteristics of a lab that is committed to generating quality data.

The costs associated with more experienced and better qualified laboratories can be higher than those of the less

qualified laboratories, and many entities are constrained by existing procurement regulations that require the selection of the least expensive (and potentially least qualified) bidder. Perhaps one way to improve this situation is to convince the individuals responsible for making procurement decisions that WET testing is a professional service (much like engineering and chemical analyses services), which may give more latitude in selecting better qualified laboratories, rather than simply those that charge the least. A more direct approach is to better define lab acceptance criteria in the specifications released for bid. Only those labs which can meet the specifications would be considered responsive, and only the lowest bid among those responsive would be considered for contract.

Probably the best lab-selecting tool is obtaining recommendations from others who have the expertise to critique lab performance. Since WET testing is required for many reasons, one can always find several individuals or firms who have been required to perform compliance toxicity tests, and it is very easy and straightforward to obtain information from them on how well (or poorly) their WET lab met their needs. The regulated community has every reason to be honest in their assessments (in fact, there is a real incentive not to be dishonest if they value their relationship with the person asking the question), and as a consequence, this is probably the best source of information currently available for making a decision regarding selection of a WET lab.