Toxicity Testing FAQ

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What kind of lab is Marinco Bioassay Laboratory?

Marinco Bioassay Laboratory, Inc. performs whole effluent toxicity testing (environmental toxicology) on industrial and municipal wastewater (effluent) discharges that are regulated by NPDES permits. Our clients include wastewater treatment facilities, industrial dischargers, engineering firms, consultants, and laboratories.

What is effluent (wastewater) toxicity testing?

Whole effluent toxicity (WET) tests were developed as a tool for evaluating the potential harmful effects of effluents discharged into surface waters.

Although quantities of pollutants can be analytically determined in samples, these measurements may fall short of actually identifying toxic discharges. Aquatic toxicity tests offer a more direct measure. They measure an observable toxic response to the interaction of toxicants and other constituents within the sample. Biological availability and interactions are inherently included.

In these tests, carefully chosen indicator organisms are exposed to whole effluent and/or effluent dilutions for a pre-determined time period in order to observe the effluent's effect on the organisms, and thereby, approximate it's potential to effect organisms within the receiving water.

Thousands of NPDES permittees around the country are required to run these "bioassays" to ensure compliance with environmental regulations.

What is a bioassay?

Bioassay is simply a more general term that applies to this type of test. A bioassay test determines the relative strength of a substance by comparing its effect on a test organism with that of a standard preparation.

What is being tested?

Most commonly, effluent from industrial or municipal wastewater discharges are tested. WET tests are a method of biomonitoring wastewater toxicity.

What are the differences between acute WET tests and chronic WET tests?

Acute tests measure how well organisms survive, while chronic tests measure survival and sub-lethal effects, such as a sample's effect on organism growth, reproduction, or fecundity (WET test methods).

What are the common causes of toxicity?

Toxicity can be caused by chemical, physical, or biological factors or a combination thereof. Chlorine, a chemical used for disinfection, and ammonia, a by-product of waste, are common causes of toxicity. Solids, pH and issues with dissolved oxygen or ion imbalance may also contribute to toxic responses, as can invertebrates, such as ciliates, and bacteria, especially if they also exacerbate issues with dissolved oxygen.

Further, breakdowns in the treatment process may also allow household and organic chemicals to enter the discharge at toxic levels.

In some cases, the toxicants or cause of toxicity can be found occurring naturally within the environment. It may be the concentration or synergistic effect that makes these factors exceed the tolerance of the test organisms.

Toxic response, notably when accompanied by erratic or inverted data, may also be a by-product of poor test organism health or poor laboratory techniques. These concerns are the motivating factors

behind our extensive in-house culturing program and the significant investment our laboratory puts into employee training and quality control.

Also see *What causes whole effluent toxicity test variability?* below.

What organisms are used in WET testing?

The organisms used in testing vary by region and test type. For aquatic toxicity studies in the Southeast US, the following are the most common species for acute and chronic tests:

- *Ceriodaphnia dubia* (water flea)
- *Pimephales promelas* (fathead minnow)
- *Cyprinella leedsi* (bannerfin shiner)
- *Mysidopsis bahia* (mysid shrimp)
- *Menidia beryllina* (tidewater silverside)

What is the holding time for NPDES whole effluent toxicity samples?

The holding time is thirty-six (36) hours.

When twenty-four (24) hour composite samples are collected, the sample holding time starts at the time the compositing of the sample has been completed.

In cases where NPDES permits require a series of four grab samples to be collected every six (6) hours over twenty-four (24) hours, the sample holding time starts at the time the last sample is collected.

What preservatives are used for WET samples?

Samples should be kept refrigerated at 4° Celsius and shipped in coolers packed with wet ice. Ice should cover the top of the samples so that the samples arrive at the testing laboratory cold and within the temperature constraints (usually 0 to 4°C). Blue ice packs DO NOT keep the sample temperature low enough and should not be used.

How long do the tests run?

Acute tests are run for twenty-four (24) hours, forty-eight (48) hours, or most commonly in Florida for ninety-six (96) hours.

Short-term chronic tests run for about seven (7) days. Tests with larval fish or *Mysidopsis* require an additional couple of days of further analysis to determine and analyze the growth parameter of the test.

What are the advantages of using in-house organisms in WET tests?

There are several. First, organisms that are cultured and used in-house have not undergone the stress of shipment. Factors such as fluctuating temperatures, harsh physical movements, photoperiod alterations, and the build up of metabolic wastes during transportation stress the organisms and, depending on their severity, alter organism sensitivity and health.

Secondly, in-house organisms are immediately available. This is advantageous for receiving samples on short notice and also because laboratory analysts can evaluate the condition of a particular batch of organisms with recourse. Unsatisfactory organisms can be disposed of and replaced with a better batch.

What causes whole effluent toxicity test variability?

Clearly there are many factors that can affect inter-laboratory and intra-test variability. A few of the greatest concern include the nature of the sample and sampling, the physical and chemical testing conditions, the test protocol, technician expertise, and test organism health, age, and handling.

Grab samples, particularly low-volume ones, are more likely to catch spikes or ebbs in effluent toxicity. Sample toxicity may be reduced with increased holding time and sample disturbance. Therefore, if samples are handled and shipped differently, response variations are likely to occur. Highly toxic samples or samples with sharp toxicity response curves can produce data that is less suitable for statistical analysis. Samples may also have toxicants that are strongly affected by exposure time (volatile samples) and test chamber conditions.

Samples are sometimes manipulated prior to testing, such as when the sample's salinity or pH is adjusted to meet testing parameters. Differences in adjustment methods and reagents can affect inter-laboratory variability, while the constituents within individual samples may react in distinctive ways to such modifications.

Abiotic testing conditions, such as alkalinity, hardness, temperature, and especially dissolved oxygen and pH, can dramatically influence test variability. In general, these factors are standardized in the test procedures and controlled during the test period. Organisms experiencing low levels of dissolved oxygen are under significance stress, affecting both toxicant responses and chance deaths. Such fluctuations can easily occur in individual test chambers, while not present in other test replicates. pH and hardness levels may also affect the bioavailability and toxicity of chemical components of the test medium.

The test procedure itself, as well as the implementation of the testing guidelines, influences organism response and data quality. Longer tests are somewhat more likely to find toxic responses, encounter chance organism deaths, and require test chamber adjustments, such as aeration. The number and concentration of effluent dilutions and test replicates affect the quality of resulting data. In general, more assessable data are obtained with a greater number of dilutions, test organisms, and test replicates. Although these test parameters are addressed within the test protocols, some flexibility is provided to laboratories, creating opportunities for inter-laboratory variation.

Despite the rigorous requirements of laboratory accreditation and testing protocols, the skill and care of the technicians performing the tests can play a considerable role in variation. Technicians must handle the organisms carefully during test initiation and during test chamber manipulations, such as during routine chemical analyses, feeding, and cleaning. The analysts must also recognize and properly react to adverse test conditions, such as dropping dissolved oxygen levels. The difficulty in counting live organisms, some smaller than a pencil head, and assessing conditions such as fecundity further affect the reliability and consistency of test data, especially with inexperienced or unskilled technicians.

Perhaps more than any other single factor, the condition of test organisms can affect observed responses and data quality. Age specifications allow technicians to use different age organisms from test to test and lab to lab. Younger organisms are in general thought to be more sensitive. Organisms must also be acclimated to testing conditions prior to test initiation. Changes in temperature, salinity, and physical disturbances affect the sensitivity and overall health of test organisms. Many laboratories purchase test organisms from outside sources that must be shipped-in and acclimated just prior to testing, while some laboratories maintain in-house cultures that do not undergo these stresses before use. Further, the condition and procedures used in culturing the organisms may vary from facility to facility and even from batch to batch, resulting in significantly different development, size, and health of the organisms.

Where can I learn more about WET Bioassays and Aquatic Toxicology?

- EPA WET Methods Index
- EPA Whole Effluent (WET) Control Policy
- Ecotox Database System
- Whole Effluent Toxicity Basics (PowerPoint Viewer)
- SETAC's Whole Effluent Toxicity Experts Advisory Panel
- NTIS Product Search Page
- Toxicity.com
- FLDEP SOPs

FREE PERMIT ANALYSIS

For a complimentary permit evaluation and quotation, please contact Jason Weeks, Laboratory Director, at 1-800-889-0384 or weeks@biologylab.com.

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