CHAPTER 2.8 - The CO_2 Entrapment Method

The purpose of this chapter is to provide some explanation of the reasoning behind the CO_2 entrapment method required by the Methods Manual, and how it controls pH and eliminates artifactual ammonia toxicity.

Methods Manual CO2 Requirements

The "*State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd Edition*" (Methods Manual) requires acute tests be conducted in a 2.5% CO_2 atmosphere as follows:

4.15.7.5 All static-renewal acute tests must be conducted in a CO_2 atmosphere (a 2.5% mixture, or an equivalent mixture shown to work successfully in the lab) or under flow-through conditions that maintain the pH at a level no lower than the measured effluent pH at the time of discharge. Static and static-renewal chronic tests are not required to be conducted in a CO_2 atmosphere or under flow-through conditions, but if pH control measures are used, the pH shall be maintained at a level no lower than the receiving water pH…"

What is Artifactual Toxicity?

The Methods Manual requires acute tests be conducted in a 2.5% carbon dioxide (CO_2) atmosphere to maintain pH at a level that is more representative of discharge conditions, in order to avoid "artifactual" toxicity. "Artifactual" is usually meant to refer to something that is created due to an outside, unnatural (i.e., human-influenced) factor having been introduced. In other words, it is something that would not exist under "normal" or natural conditions. In this case, it is referring to the toxicity that occurs when a pH drifts unnaturally high in a toxicity test.

During the conduct of static or static-renewal WET tests, the pH in test containers can change from the initial pH value. This "pH drift" can be upwards or downwards, depending on test conditions and sample characteristics, but usually is an upward drift. For instance, the addition of food substances such as algae may cause a decrease in pH, while the loss of CO_2 from supersaturated effluent samples often causes an increase in pH.

The most common example of artifactual toxicity occurs when ammonia is present in an effluent sample. Most municipal effluents have pH values in the 7.0 to 7.5 range when discharged, but the pH of highly organic effluents may drift to pH 8.5 and above under static test conditions. The toxicity of ammonia can increase by an order of magnitude between pH 7 and pH 9. Concentrations of ammonia common to many municipal and industrial effluents may cause toxicity in standard effluent toxicity tests when effluent pH level rises, yet show no toxicity at lower pH levels. Ammonia toxicity caused by this abnormal pH drift during a WET test is what is often referred to as "artifactual ammonia toxicity", because it would not be expected to occur under real-world discharge conditions.

Any degree of pH drift may interfere with test results if the sample contains a compound with toxicity that is pH-dependent at a concentration that is near the toxicity threshold. Compounds with pH-dependent toxicity are those with chemical characteristics that allow sufficient differences in dissociation, solubility, or speciation to occur within a certain pH range. It is a common assumption and misconception that only ammonia is affected by pH drift in WET tests - other examples of pH-dependent toxicants include metals, hydrogen sulfide, cyanide, and ionizable organics.

As pH increases, the toxicity of ammonia also increases, so upward pH drift may increase sample toxicity. For metals, toxicity may increase or decrease with increasing pH. Lead and copper have been shown to be more toxic at a lower pH, while nickel and zinc are more toxic at a higher pH. A change in pH during testing means that an effluent sample might be tested for toxicity at a pH different than what is actually present at the point of discharge. Under certain circumstances, this pH drift could influence sample toxicity and be considered a test interference. For these reasons, pH control measures are required in all acute tests and recommended in most chronic tests completed for WPDES permit compliance, as specified in the Methods Manual (Section 4.15.7.5).

The CO_2 Method and pH Control

So, what good does the CO_2 method do? Artifactual toxicity caused by a shift in pH during testing can be reduced or eliminated by exposing the test chambers to a CO_2 controlled atmosphere. Advantages of using CO_2 include less alteration of normal test solution chemistry and use of a natural buffer system to achieve ongoing pH control. An alternate, but much more costly, approach would be to conduct onsite flow-through toxicity tests with a turnover rate in the test chamber which maintains the pH of the test solution to that of the effluent.

In most natural waters and many effluents, pH is controlled by the carbonate buffer system. In this buffer system, carbon dioxide and water combine to form carbonic acid and carbonate salts. When a solution contains a weak acid (such as carbonic acid) and a salt of that acid (such as carbonate salt), the solution is referred to as a buffered solution. In a buffered solution, small additions of acid or base will produce very little change in pH. In pure water, pH is controlled by the partial pressure of carbon dioxide in the atmosphere. In many effluents, high partial pressures of CO_2 may be present, due to high biological activity, causing pHs to drift upwards when placed in static WET tests. Abnormal pH drift can be controlled in static toxicity tests by introducing more CO_2 into the atmosphere over the test chambers. Introducing more CO_2 will encourage the interaction of CO_2 and water to form the buffer system mentioned above.

By controlling the CO_2 present in the atmosphere over the test chambers in a static test, the lab can stop the pH from drifting upwards. Drift in pH can be very important, as changes in pH can alter the toxicity of many common components of effluents. ***Since this method is not thought to alter the effluent and more closely maintains the effluent's pH as it was discharged, the Methods Manual requires the CO_2 entrapment method be used for all acute tests and recommends it's use during chronic tests.*** Effective control of test pH may also be critical when determining the potential toxic effects of materials other than ammonia whose toxicity is pH sensitive, such as certain heavy metals, sulfide, cyanide, and others. By using the CO_2 entrapment method, the effluent can be tested for toxic effects without any interference from the artifactual toxicity caused by pH drift. This method should not be used to artificially produce a certain pH, but to maintain the pH of the effluent at a level which is comparable to the pH at the time the sample arrived at the laboratory.

The CO_2 Method - A Cost Savings?

You may think that the CO_2 method is just another reason to increase the costs of effluent testing. In fact, acute tests run using this method may be slightly higher priced than an acute test completed without CO_2 addition (some labs have estimated an increase of about $25.00/test). However, in most cases when a WET test fails, the permittee is required by their permit to perform two or more additional tests, in order to determine whether the toxicity is a persistent, repetitive problem or a one-time event. Through the use of the CO_2 method, permittees may be able to avoid toxicity retests. For example, imagine the following scenario: the original test was done without using the CO_2 method, ammonia levels were moderately high, and the test failed. The permittee and their lab suspect that the failure is due to artifactual ammonia toxicity. The permittee would have to complete retests demonstrating to the

WDNR that it was indeed artifactual ammonia toxicity that caused the problem (i.e. retests using the CO_2 method). By determining the level of ammonia present and controlling pH drift in the first test, the two retests could have been avoided. (That's a savings of thousands of dollars at current test prices.)