**Date:** July 20, 2018

**To:** Surface Water Ambient Monitoring Program (SWAMP) Round Table

**From:** SWAMP Toxicity Work Group

**Subject:** Use of Additional Controls in SWAMP Toxicity Tests

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SWAMP toxicity test Measurement Quality Objectives (MQOs) are based on U.S. EPA protocols. As such, preparation and use of control waters for the various water column toxicity tests are thoroughly described in the method manuals (U.S. EPA, 1995, 2002a, b, c). These protocols include appropriate control waters for the dilution of effluent samples (dilution control) and the culturing of laboratory organisms (culture control). A dilution control consists of the water used to dilute samples to specified test concentrations, whereas the culture control uses the same water that the organisms were raised in; these two controls often use the same water. There is also guidance for the use of an additional control when a sample has been adjusted (e.g., brine control), and the protocols provide additional language for statistical comparisons based on these controls. Because the goal of SWAMP is to test the absolute toxicity of ambient samples, sample dilution is generally not recommended, and statistical comparisons are most often conducted between the organism response in the sample, and the response in a primary control.

A primary control water is "appropriate for the objectives of the test; supports adequate performance of the test organisms with respect to survival, growth, reproduction, or other responses that may be measured in the test (i.e., consistently meets test acceptability criteria [TAC] for control responses); is consistent in quality; and does not contain contaminants that could produce toxicity. Receiving waters, synthetic waters, or synthetic waters adjusted to approximate receiving water characteristics may be used for dilution provided that the water meets the above listed qualifications for an acceptable dilution water" (U.S. EPA, 2002a).

There are two occasions when use of a secondary control is warranted. Situations where a sample is adjusted for parameters such as salinity or pH, require an additional control to account for the alteration. For example, if the salinity of a brackish sample is increased with artificial salts in order to test with a marine organism, a salinity control must be prepared and tested in the same manner. This control must meet TAC in order for the test to be valid. Sometimes a control is added to a sample batch when one of the parameters in a sample is near the limit of the organism's tolerance. For example, a sample with low conductivity might affect the reproduction of *Ceriodaphnia dubia*. The addition of a low conductivity tolerance control could allow for a better interpretation of the test results. A toxic response occurring at the edge of the tolerance range of the organism could be accounted for through the testing of this type of control, which could be described as a form of toxicity identification evaluation. This type of control does not need to meet TAC unless it is to be used for statistical comparisons.

The primary control is always used to determine TAC and is generally compared to the sample response for the calculation and reporting of test results. The secondary control can play a different statistical role depending on which type it is (see Table 1 below). In the case of a control used for salinity adjustment, TAC must be met in both controls, but if the salinity control differs statistically from the primary control (based on a t-test), the salinity control should be used for statistical comparisons. In the case of a control that is added to account for an organism response at the edge of its tolerance range (e.g., conductivity control), the secondary control does not need to meet TAC unless it is to be used for statistical comparisons.

Table 1. Examples of secondary controls and requirements for statistical comparisons.

| Control Type | Example | Reason for Use | Requirement |
|---|---|---|---|
| Adjustment Control | Salinity Control | Sample salinity has been altered to fit within the tolerance range of the organism, and method of adjustment needs to be controlled for. | Must meet TAC. Can be used for statistical comparisons if significantly different from primary control. |
| Tolerance Control | Conductivity Control | Sample conductivity is near the edge of the tolerance range of the organism, and a laboratory wants to account for potential effects of sample conductivity. | Must meet TAC to be used for statistical comparisons. Does not have to be significantly different from control for statistical comparisons. |

Previously, SWAMP provided a recommendation on the statistical use of additional controls that would account for conductivity or salinity outside the optimal range of the organism. That recommendation allowed the use of the additional control for a statistical comparison as long as the response in the additional control met TAC, and that response was significantly different from the response in the culture control. This recommendation was based, in part, on U.S. EPA (2000) and has been slightly modified (Table 1).

Previous SWAMP guidance did not provide specific thresholds for which control to use for statistical comparisons. Because of the wide range of organism culture conditions, specific recommendations for statistical analyses are impossible to make, and the final decision should be made by the testing laboratory or based on an individual Quality Assurance Project Plan. Recommended conductivity and salinity ranges are included in organism-specific MQOs, but the decision to use additional controls in test batches with unadjusted samples will be left to the discretion of the laboratory or the project. Additional controls for adjusted samples are required as per U.S. EPA methods.

If there is concern about an organism having a negative response to a non-anthropogenic stressor, such as conductivity, care should be taken when choosing organisms for testing. SWAMP provides recommendations in some MQOs, but discretion ultimately lies with the laboratory and project. For example, the recommended conductivity range for the *C. dubia* chronic test is 100 – 1900 µS/cm. Some laboratory cultures will be tolerant outside of this range, and the project will have to decide, with the

help of the laboratory, if this organism is appropriate. SWAMP recommends using *Hyalella azteca* for water samples greater than 2500 µS/cm.

## References


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