



Instructions for Catalog # WET020
DMR-QA Test Code 47
Revision 022217

Organism: Sheepshead minnow (*Cyprinodon variegatus*)

Test Conditions: 7-Day Chronic, Daily Renewal, Synthetic Seawater (SSW)

Description:

- This reference toxicant is designed for the Sheepshead minnow (*Cyprinodon variegatus*), 7-Day Chronic, Daily Renewal, Synthetic Seawater (SSW), Survival and Growth Test (i.e., USEPA Test Code 47, USEPA Method Code 1004.0).
- This reference toxicant is packaged in a 1.0 L bottle containing approximately 1000 mL of standard concentrate.
- This concentrate can be stored at room temperature.

Before you begin:

- This reference toxicant has been prepared as a concentrate and must be diluted prior to analysis to prepare a “simulated” effluent (hereafter referred to as the effluent).
- This reference toxicant must be diluted with synthetic seawater (SSW), with a salinity of 25 ‰, prepared from Millipore Milli-Q[®] deionized water (or equivalent) and artificial sea salts, as specified in the current version of the USEPA methods manual.
- The diluted effluent should be utilized as soon as possible after preparation.

Instructions:

1. Add approximately 1.8 Liters of synthetic seawater (SSW) to each of two clean, dry, 2.0 Liter, class A volumetric flasks.
2. Shake the reference toxicant concentrate bottle prior to opening.
3. Using a clean, dry, 50 mL, class A volumetric flask, carefully transfer 50 mL of the reference toxicant concentrate into each 2.0 L flask.
4. Rinse the 50 mL flasks into the 2.0 L flasks using SSW.
5. Dilute each 2.0 L flask to final volume using SSW.
6. Cap each flask and mix well.
7. The total effluent volume is 4.0 Liters.

The effluent prepared according to these instructions represents the 100% effluent. See below for secondary dilution instructions:

1. The first 2 L flask is your 100% effluent for testing.
2. Dilute the second flask with 2.0 L of SSW and mix. This is your 50% effluent sample.
3. Continue diluting half of each sample with the same volume of SSW to make your 25%, 12.5% and 6.25% effluent dilutions, which represent all five test dilutions.
4. You are now ready to proceed with the test following your normal procedures.
5. All five test dilutions must be freshly prepared each day of the testing period.
6. Report your results (expressed as percent effluent) for the following test endpoints: NOEC survival, IC25 (ON) growth, and NOEC (ON) growth. (ON – original number)

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