



Instructions for Catalog # WET019  
DMR-QA Test Code 46  
Revision 022217

Organism: Sheepshead minnow (*Cyprinodon variegatus*)

Test Conditions: 48-Hour Acute, Non-renewal, 25°C, Synthetic Seawater (SSW)

Description:

- This reference toxicant is designed for the Sheepshead minnow (*Cyprinodon variegatus*), 48-Hour Acute, Non-renewal, 25°C, Synthetic Seawater (SSW), Toxicity Test (i.e., USEPA Test Code 46, USEPA Method Code 2004.0).
- This reference toxicant is packaged in a 250 mL bottle containing approximately 250 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<sup>®</sup> 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 1.7 L of synthetic seawater (SSW) to a clean, dry, 2.0 Liter, class A volumetric flask.
2. Shake the reference toxicant concentrate bottle prior to opening.
3. Using a clean, dry, 100 mL, class A volumetric flask, carefully transfer 100 mL of the reference toxicant concentrate into the 2.0 L flask.
4. Rinse the 100 mL flask into the 2.0 L flask using SSW.
5. Dilute the 2.0 L flask to final volume using SSW.
6. Cap the flask and mix well.

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

1. Split the 100% effluent sample into two 1.0 L aliquots. The first aliquot is your 100% effluent for testing.
2. Dilute the second aliquot with 1.0 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. If necessary, additional effluent may be prepared using the same reference toxicant concentrate and following instructions 1-6 above.
6. Report your results (expressed as percent effluent) for the 48-Hour LC50 endpoint.

Safety:

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