

Instructions for Catalog # WET004

DMR-QA Test Code 15

Revision 022217

Organism: Fathead minnow (Pimephales promelas)

Test Conditions: 7-day Chronic, Daily Renewal, Moderately-Hard Synthetic Freshwater (MHSF)

Description:

- This reference toxicant is designed for the Fathead minnow (*Pimephales promelas*), 7-day Chronic, Daily Renewal, Moderately-Hard Synthetic Freshwater (MHSF), Survival and Growth Test (i.e., USEPA Test Code 15, USEPA Method Code 1000.0).
- This reference toxicant is packaged in a 500 mL bottle containing approximately 500 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 moderately-hard synthetic freshwater (MHSF) prepared from Millipore Milli-Q[®] deionized water (or equivalent) and reagent grade chemicals, 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 moderately-hard synthetic freshwater (MHSF) 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, 50 mL, class A volumetric flask, carefully transfer 50 mL of the reference toxicant concentrate into the 2.0 L flask.
- 4. Rinse the 50 mL flask into the 2.0 L flask using MHSF.
- 5. Dilute the 2.0 L flask to final volume using MHSF.
- 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 MHSF and mix. This is your 50% effluent sample.
- 3. Continue diluting half of each sample with the same volume of MHSF 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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