



Instructions for Catalog # WET002
DMR-QA Test Code 13
Revision 040307

Organism: Fathead minnow (*Pimephales promelas*)

Test Conditions: 48-Hour, Acute, Non-renewal, 25°C, Moderately-Hard Synthetic Freshwater (MHSF)

Description:

- This reference toxicant is designed for the Fathead minnow (*Pimephales promelas*), 48-Hour, Acute, Non-renewal, 25°C, Moderately-Hard Synthetic Freshwater (MHSF), Toxicity Test (i.e., USEPA Test Code 13, USEPA Method Code 2000.0).
- This reference toxicant is packaged in a 125 mL bottle containing approximately 125 mL of standard concentrate.
- The 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. 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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