



## Instructions for Catalog # AQC010 USEPA Test Code 21

Revision 090119

**Organism:** *Ceriodaphnia dubia*

**Test Conditions:** 3-Brood Chronic, Daily Renewal, Moderately-Hard Synthetic Freshwater (MHSF)

### Description:

- This reference toxicant is designed for the *Ceriodaphnia dubia*, 3-Brood Chronic, Daily Renewal, 25°C, Moderately-Hard Synthetic Freshwater (MHSF), Survival and Reproduction Test (i.e., USEPA Test Code 21, USEPA Method Code 1002.0).
- 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.
- This product is intended to be used as a quality control check of the entire analytical process for the analytes/matrix included in the standard.
- The dilution instructions below represent the minimum suggested sample size for this product. Using a smaller sample size may invalidate the assigned value and/or uncertainty shown on the certificate of analysis.
- The certified values apply to the diluted sample after following the stated dilution instructions.

### Helpful Hints:

- 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 800 mL of moderately-hard synthetic freshwater (MHSF) to a clean, dry, 1.0 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 1.0 L flask.
4. Rinse the 50 mL flask into the 1.0 L flask using MHSF.
5. Dilute the 1.0 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 0.5 L aliquots. The first aliquot is your 100% effluent for testing.
2. Dilute the second aliquot with 0.5 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.

**Safety:**

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