

Instructions for: *Daphnia Method Test Code 32/EPA Method 2021 (Cat.# PT-32-WET)*

SCOPE AND APPLICATION

Wibby Environmental's DMRQA Whole Effluent Toxicity (WET) Testing Standard is designed to be used with EPA Test Code 32/Method 2021. You should be familiar with the method before analyzing the standard. If you have any questions about the use of these standards, please contact Wibby Environmental Customer service at 1-866-WibbyPT (866-942-2978).

SAMPLE PREPARATION AND ANALYSIS

- 1. Introduction.** The reference toxicant has been supplied as a concentrated stock solution. This stock solution is used to prepare the simulated effluent. Prepare the effluent as described below in Moderately-hard synthetic freshwater (MHSF). MHSF is prepared from Millipore Milli-Q^R (or equivalent) deionized water and reagent grade chemicals as specified in the applicable EPA reference method. Smaller volumes of effluent can be prepared by using proportionally smaller amounts of the stock solution and MHSF.
- 2. Storage Requirements.** The concentrated stock solution is to be stored at room temperature, approximately 20-25°C.
- 3. Preparation of five test dilutions:** The effluent prepared according to these instructions represents the sample ready for testing, 100% effluent, 50% effluent, 25% effluent, 12.5% effluent and 6.25% effluent.
- 4. Stock I.** 100% effluent concentration. Add 5 mL of the stock solution to 900 mL of MHSF (20° test). Mix well. Bring the final volume to one liter with MHSF. Use 500 mL of this solution for the 100% effluent concentration.
- 5. Stock II.** 50% effluent concentration. Add 500 mL of Stock I to 400 mL of MHSF. Mix well. Bring the final volume to 1 L with

MHSF. Use 500 mL of this for 50% effluent concentration.

- 6. Stock III.** 25% effluent concentration. Add 500 mL of Stock II to 400 mL of MHSF. Mix well. Bring the final volume to 1 L with MHSF. Use the 500 mL of this for 25% effluent concentration.
- 7. Stock IV.** 12.5% effluent concentration. Add 500 mL of Stock III to 400 mL of MHSF. Mix well. Bring the final volume to 1 L with MHSF. Use the 500 mL of this for 12.5% effluent concentration.
- 8. Stock V.** 6.25% effluent concentration. Add 500 mL of Stock IV to 400 mL of MHSF. Mix well. Bring the final volume to 1 L with MHSF. Use this for 6.25% effluent concentration.
- 9.** All tests must be conducted according to the standard methods published in the EPA manuals referenced on page 8 of the DMR-QA Study 30 Instructions. A copy of the instructions can be obtained at www.wibby.com. Further method information can be obtained at the following web site (<http://www.epa.gov/waterscience/WET>).
- 10.** The 48-h definitive test is performed using the test conditions listed in the applicable EPA reference method..
- 11.** Biological and physical/chemical measurements are performed and the data recorded as specified in the applicable EPA reference method. Records of these measurements must be maintained on file in the laboratory and made available to USEPA if requested.
- 12.** In the 48-h definitive test, data on test organism survival in each replicate chamber for the controls and effluent concentrations are recorded daily as described in the method.

Instructions for Daphnia Method Test Code 32/Method 2021 (continued)

13. At the beginning of the test, determine dissolved oxygen (DO), pH, total alkalinity total hardness, conductivity and temperature at each concentration of effluent used in the dilution series, and in the control. At the end of the test, or when a replicate sample experiences 100% mortality, determine DO, pH, conductivity, and temperature (please reference method requirements).

DATA ANALYSIS

Use the mortality data (expressed as percent effluent) from the 48th definitive test to determine LC50. Follow the flowchart in the DMR-QA Acute Data Analysis Flow Chart included in the method.

REPORTING of DATA

1. Enter the 48 hour LC 50 (expressed as percent effluent) on the Data Reporting Sheet provided. Do not report LC 50s for tests that fail to meet the acceptability criteria listed with the test conditions. Report results which are below 6.25 as <6.25 and report results which are above 100 as >100.

2. Report all results on line at www.wibby.com. Click on the "Online data entry link" and follow the instructions to enter your results. As an alternative, you may submit your results using the Data Reporting Sheets enclosed with your standards by Fax to 866-283-0269.

3. Wibby Environmental must receive all results prior to the study closing date shown on the Data Reporting Sheets.

SAFETY

The standards are designed for use by laboratory professionals who are familiar with handling environmental reference materials as well as hazardous materials. If you have any questions about the safe handling of these standards or require a MSDS sheet, please contact Wibby Environmental at 1-866-WibbyPT (866-942-2978).

QUESTIONS?

If you have any questions regarding these standards, please call Wibby Environmental at 1-866-WibbyPT (866-942-2978).