

Instructions for: *Fathead Minnow Test Code 16/EPA Method 1000 (Cat.# PT-16-WET)*

SCOPE AND APPLICATION

Wibby Environmental's DMRQA Whole Effluent Toxicity (WET) Testing Standard is designed to be used with EPA Test Code 16/Method 1000. 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 20% diluted mineral water (DMW) (20°C test). DMW 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 DMW.
- 2. Storage Requirements.** The concentrated stock solution is to be stored at room temperature, approximately 20 to 25 degrees 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 30 mL of the stock solution to 2.5 L of DMW. Mix well. Bring the final volume to 3 L with DMW. Use 1500 mL of this solution for the 100% effluent concentration.
- 5. Stock II.** 50% effluent concentration. Add 1500 mL of Stock I to 1400 mL of DMW. Mix well. Bring the final volume to 3 L with DMW. Use 1500 mL of this for 50% effluent concentration.
- 6. Stock III.** 25% effluent concentration. Add 1500 mL of Stock II to 1400 mL of DMW. Mix well. Bring the final volume to 3 L with DMW. Use 1500 mL of this for 25% effluent concentration.
- 7. Stock IV.** 12.5% effluent concentration. Add 1500 mL of Stock III to 1400 mL of DMW. Mix well. Bring the final volume to 3 L with DMW. Use 1500 mL of this for 12.5% effluent concentration.
- 8. Stock V.** 6.25% effluent concentration. Add 1500 mL of Stock IV to 1400 mL of DMW. Mix well. Bring the final volume to 3 L with DMW. Use this for the 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 7-day survival and growth test is performed using the test conditions listed in the applicable EPA reference method.
- 11.** Test organism survival in each test chamber is determined daily as described in the method. Growth is determined at the end of the test as described in the method.
- 12.** Record dissolved oxygen (DO), pH, total alkalinity, total hardness, conductivity and temperature as described by the method.

**Instructions for:
Fathead Minnow Test Code 16/EPA Method 1000 continued**

DATA ANALYSIS

1. The endpoints (expressed as percent effluent) listed in the method are determined for:
 - a. NOEC for survival,
 - b. IC25 for growth and
 - c. NOEC for growth.

The IC 25 for growth can be determined manually using the guidelines in the applicable EPA reference method.

REPORTING of DATA

1. Enter NOEC for survival and the IC 25 and NOEC for growth, expressed as percent effluent, on the Data Reporting Sheet provided. Record all results the same as they are recorded for DMR reporting. Do not report endpoints for tests which fail to meet the acceptability criteria listed with the test conditions.
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).