

Instructions for: *Sheepshead Minnow Test Code 47/EPA Method 1004 (Cat.# PT-47-WET)*

SCOPE AND APPLICATION

Wibby Environmental's DMRQA Whole Effluent Toxicity (WET) Testing Standard is designed to be used with EPA Test Code 47/Method 1004. 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 40 fathoms artificial seawater. 40 fathoms artificial seawater with a salinity of 25 ppt is prepared from Millipore Milli-Q (or equivalent) deionized water, 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 40 fathoms of artificial seawater.
- 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 45 mL of the stock solution to 2500 mL of 40 fathoms of artificial seawater, with a salinity of 25 ppt, used as dilution water. Mix well. Bring the final volume to 3 liters with 40 fathoms of artificial seawater. Use 1500 mL of this solution for the 100% effluent concentration.
- 5. Stock II.** 50% effluent concentration. Add 1500 mL of Stock I to 1000 mL of 40 fathoms of artificial seawater. Mix well. Bring the final volume to 3 L with 40 fathoms of artificial seawater. Use 1500 mL of this for 50% effluent concentration.
- 6. Stock III.** 25% effluent concentration. Add 1500 mL of Stock II to 1000 mL of 40 fathoms of artificial seawater. Mix well. Bring the final volume to 3 L with 40 fathoms of artificial seawater. Use the 1500 mL of this for 25% effluent concentration.
- 7. Stock IV.** 12.5% effluent concentration. Add 1500 mL of Stock III to 1000 mL of 40 fathoms of artificial seawater. Mix well. Bring the final volume to 3 L with 40 fathoms of artificial seawater. Use the 1500 mL of this for 12.5% effluent concentration.
- 8. Stock V.** 6.25% effluent concentration. Add 1500 mL of Stock IV to 1000 mL of 40 fathoms of artificial seawater. Mix well. Bring the final volume to 3 L with 40 fathoms of artificial seawater. 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 7-day survival and growth test is performed using the test conditions listed in the applicable EPA reference method.

Instructions for: *Sheepshead minnow Test Code 47/Method 1004 (continued)*

11. Biological and physical/chemical measurements are performed and the data recorded as specified in the EPA method. Records of these measurements must be maintained on file in the laboratory and made available to USEPA if requested.
12. Data on test organism survival and reproduction are recorded daily for each test chamber as described in the method.
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).

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).

DATA ANALYSIS

The endpoints (expressed as percent effluent) are determined from the test data according to the method.

REPORTING of DATA

1. Enter NOEC for survival and the IC25 & NOEC for reproduction, expressed as percent effluent on the data report sheet. Record all results the same as they are recorded for DMR reporting. NOTE: Do not report endpoints for tests that 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.