24-HOUR RANGE FINDING TEST USING DAPHNIA MAGNA AND DAPHNIA PULEX

CONTENTS

SUPERCEDES: SOP #2021; Revision 1.0; 09/24/90; U.S. EPA Contract EP-W-09-031.

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1.0 SCOPE AND APPLICATION

The procedure for conducting a 24-hour rangefinding toxicity test using Daphnia magna or D. pulex is described below. This test is applicable to leachates, effluents, and liquid phases of sediments. The selections of concentrations to use in a definitive toxicity test are based on the results of the rangefinder.

These are standard (i.e., typically applicable) operating procedures which may be varied or changed as required, dependent on site conditions, equipment limitations or limitations imposed by the procedure or other procedure limitations. In all instances, the ultimate procedures employed should be documented and associated with the final report.

Mention of trade names or commercial products does not constitute U.S. Environmental Protection Agency (U.S. EPA) endorsement or recommendation for use.

2.0 METHOD SUMMARY

Larval daphnids are placed in individual containers and exposed to a wide range of test media concentrations. No replicates are needed and only a few concentrations (i.e. 0, 1, 10 and 100%) are used.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

The selected environmental matrix will be sampled utilizing the methodology detailed in ERT/SERAS SOPs #2012, Soil Sampling; #2013, Surface Water Sampling; #2016, Sediment Sampling; and any other procedure applicable for the media sampled.

Once collected, the samples will be placed in containers constructed from materials suitable for the suspected contaminants. Because surrogate test species will be exposed to varying concentrations of the sample material, no chemical preservatives are to be used. The preservation and storage protocol is therefore limited to holding the samples on ice at 4°C for the holding time specified by the analytical method. Prior to shipping, the laboratory performing the toxicity tests will be notified of any potential hazards that may be associated with the samples.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

1. The results of a static toxicity test do not reflect temporal fluctuation in effluent toxicity (Peltier and Weber, 1985). This is a preliminary test which provides an estimate of toxicity and the results are viewed as such.

2. Non-target chemicals (i.e. residual chlorine) cause adverse effects to the organisms giving false results.

3. Dissolved oxygen depletion due to biological oxygen demand, chemical oxygen demand or metabolic wastes is a potential problem.

4. Loss of a toxicant through volatilization and adsorption to exposure chambers also may occur (Peltier and Weber, 1985).

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5.0 EQUIPMENT

5.1 Apparatus

- 25 larval daphnids - acclimated 24 hours to dilution water
- 4 exposure chambers - 100 mL/chamber rinsed in dilution water
- tray to hold exposure chambers and glass covers
- wide bore pipettes - 1.5 times the size of the daphnid
- graduated cylinders, 250 mL
- beakers for chemical measurements
- suitable food
- test media - 150 mL
- diluent - 300 mL
- pipette - 1 mL

5.2 Test Organisms

Test organisms may be reared in-house or obtained from an outside source. Positive identification of the species is required before beginning testing. Daphnids to be used must be less than 24 hours old and from the second to the sixth brood of a healthy adult. Populations of healthy daphnids have large individuals, an absence of floaters, an absence of ephippia, no parasites, and individuals are dark colored and produce large numbers of young (Biesinger, et al. 1987).

5.3 Equipment for Chemical Analysis

Meters are needed to measure dissolved oxygen, temperature, pH and conductivity. Calibrate the meters according to the manufacturers' instructions. Measure and record alkalinity and hardness according to a standard method (Standard Methods, 1985).

6.0 REAGENTS

1. Dilution Water

Dilution water is moderately hard, reconstituted deionized water unless otherwise specified. See Horning and Weber (1985) for the preparation of synthetic fresh water. The dilution water for a test is the same as the water used to culture daphnids and the water used to acclimate daphnids before the beginning of the test.

2. Test Media

If the test medium is a liquid, dilutions may be made directly for the required concentrations. If the test medium is a sediment, preliminary filtration and dilutions are required to produce a liquid phase.

7.0 PROCEDURES

1. Choose a wide range of concentrations to estimate the toxicity of the test media. The concentrations

cited below are used as an example and may be adjusted to meet the criteria of the specific situation. A geometric or logarithmic range of concentrations may be used (Sprague, 1973).

2. The example below provides enough test media for three test chambers containing 80 mL each. In addition, 100 mL each of dilution water and test media are required for chemical analyses. Temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness should be measured prior to the start of the test.

Example 1. Test Dilution

Test Media Concentrations (% test media)	Diluent	Volume (mL) Test Media
0	100	0
1	99	1
10	90	10
100	0	100

3. Label clean exposure chambers and rinse in dilution water, except for the chamber containing 100% test media.

4. To prepare test solutions, measure 1.0 mL of the test media into a beaker and dilute to 100 mL with dilution water.

5. Using a graduated cylinder, pour 80 mL into the exposure chamber. Continue these steps for all concentrations. Always work from the lowest concentrations to the highest.

6. Using a wide bore pipette, randomly select a daphnid, place the pipette below the surface of the test solution and gently expel each daphnid individually into the exposure chamber.

7. The test begins when half of the organisms have been placed into exposure chambers. Mortality should be determined at one hour and again at 24 hours.

8.0 CALCULATIONS

The methods used to determine the LC_{50} differ depending on the results of the test. The Moving-Average Method is used to determine the LC_{50} when there is no partial mortality in any replicate (i.e. all alive or all dead). If there is partial mortality, the Probit Method is used to calculate the LC_{50}. The Lowest Observable Effect Concentration (LOEC) is recorded and the No Observable Effect Concentration (NOEC) is recorded (Peltier and Weber, 1985). Since this is a simple acute test, only mortality is recorded. Other methods of estimating the LC_{50} may be used if justified and an accepted reference is cited (Biesinger, et al. 1987).

9.0 QUALITY ASSURANCE/QUALITY CONTROL

Follow the guidelines below and in Table 1 (Appendix A) to ensure a valid test and to meet quality assurance/quality control standards.

1. Effluent sampling
2. Test organisms
3. Facilities equipment
4. Effluent/leachate preparation
5. Dilution water
6. Test conditions
7. Standard reference toxicant

10.0 DATA VALIDATION

The following criteria will be used as a basis to reject test results:

1. Greater than 10% control mortality
2. Greater than 10% aberrant mortality in concentrations
3. Temperature variation greater than 2 °C
4. Effluent stored more than 72 hours
5. Criterion in Table 1 (Appendix A) not met

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, follow U.S. EPA, OSHA and corporate health and safety procedures.

12.0 REFERENCES

Biesinger, K.E., L.R. Williams, and W.H. van der Schalie. 1987. Procedures for Conducting Daphnia magna Toxicity Bioassays. EPA/600/8 - 87/011. Environmental Monitoring and Support Laboratory. Cincinnati, OH. 57 pp.

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APPENDIX A
Table
SOP #2021
October 1994

24-HOUR RANGE FINDING TEST USING DAPHNIA MAGNA AND DAPHNIA PULEX

TABLE 1. Summary of test conditions for Daphnia magna and D. pulex 24 hour rangefinding test (Based on Peltier and Weber, 1975).

1.	Test type:	Static, 24 hours
2.	Temperature:	20.0 ± 2°C
3.	Light quality:	Ambient laboratory illumination
4.	Light intensity:	50-100 foot-candles
5.	Photoperiod:	16 hours light, 8 hours dark
6.	Test chamber size:	100-mL containers
7.	Test solution volume:	80-mL/replicate
8.	Age of test organisms:	Larval daphnids, less than 24 hours old and within four hours of each other
9.	Number/container:	10 per container
10.	Feeding:	Do not feed during the test
11.	Aeration:	None unless DO concentration falls below 40% saturation then <100 bubbles per minute
12.	Dilution water:	Moderately hard reconstituted deionized water, unless otherwise specified
13.	Effluent/leachate concentrations:	Three and one control