48-HOUR ACUTE TOXICITY TEST USING DAPHNIA MAGNA AND DAPHNIA PULEX

CONTENTS

SUPERCEDES: SOP #2024; Revision 2.0; 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 48-hour (hr) acute toxicity test using Daphnia magna or Daphnia pulex is described below. This test is applicable to leachates, effluents, and liquid phases of sediments.

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 various concentrations of a test media over a 48-hr period. Mortality is the endpoint of the test.

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

2. Dissolved oxygen depletion due to biological oxygen demand, chemical oxygen demand and metabolic wastes also is a potential problem.

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

4. The results of a static toxicity test do not reflect temporal fluctuation in test media toxicity (Peltier and Weber, 1985).

5.0 APPARATUS/EQUIPMENT

5.1 Apparatus

- 60 larval daphnids - acclimated at least 24-hr to dilution water
- 60 exposure chambers - 100 ml volume, labeled
- tray to hold exposure chambers and glass covers
- widebore pipettes - inside diameter 1.5 times the length of the daphnid
- graduated cylinders, 250 mL and 1L
- pipette - 1 mL
- beakers for chemical measurements, 250 mL
- test medium - 1L
- diluent - 3L
- waste containers
- light table - to aid in counting the organisms
- suitable food

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-hr old and from the second to the sixth brood of healthy adults. Populations of healthy daphnids have large individuals, have an absence of floaters, have an absence of ephippia, no parasites, individuals are dark colored and produce large numbers of young (Biesinger, et al. 1987). Place the parent generation into individual cups containing dilution water for a 24-hr prior to the beginning of the test to ensure that less than 24-hr. old daphnids are available.

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 alkalinity and hardness according to a standard method (Standard Methods, 1985).

6.0 REAGENTS

1. Dilution water

Dilution water is reconstituted, deionized water. The water type should be moderately hard 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 Medium

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. The optimum pH range for daphnids is 6.8 - 8.5; therefore, the pH of the dilution water or the concentrations may have to be adjusted prior to the start of the test (Briesinger et al. 1987).

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7.0 PROCEDURES

1. Select a range of concentrations that span those causing zero mortality to those causing complete mortality. 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 also may be used (Sprague, 1973). The example below provides enough test media for five replicates containing 50 mL each and extra for chemical analysis.

Example 1. Test Dilutions

Test Concentrations (% test media)	Diluent	Volume (mL) Test Media
0.0	500.0	0.0
0.1	499.5	0.5
1.0	495.0	5.0
10.0	450.0	50.0
50.0	250.0	250.0
100.0	0.0	500.0

2. Rinse all exposure chambers, except the chamber containing 100% test media, in dilution water.

3. Mix concentrations and pour into each exposure chamber.

4. Measure 0.5 mL of the test media into a beaker and dilute to 500 ml.

5. Using a graduated cylinder, pour out 50 mL into each exposure chamber and pour the rest into a beaker for chemical measurements.

6. Continue these steps for all concentrations. Always work form the lowest concentration to the highest in order to minimize the risk of cross contamination.

7. Using a widebore pipette, randomly select and carefully place ten daphnids into each exposure chamber. Place the pipette tip below the surface and gently expel each daphnid individually into the chamber.

8. The test begins when half of the organisms are in the exposure chambers.

9. Measure and record mortality and survival at one hour and then at 24 and 48 hr.

10. Measure and record temperature, dissolved oxygen, pH, conductivity, alkalinity, and hardness after the test begins and at the completion of the test.

11. The test is complete at the end of 48 hours.

8.0 CALCULATIONS

The methods used to determine the LC_{50} differ depending on the results of the test. If there is no partial mortality in any replicate (i.e. all alive or all dead), then the Moving-Average Method may be used to determine the LC_{50}. If there is partial mortality within a replicate, then the Probit Method should be used to calculate the LC_{50}. Also the Lowest Observable Effect Concentration (LOEC) is recorded and the No Observable Effects 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

Quality control should encompass the following parameters to ensure a valid test. The guidelines in this text and in Table 1 (Appendix A) should be followed to insure adequate QA/QC.

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

10.0 DATA VALIDATION

The following criteria are a basis for rejecting the results of this test:

1. Greater than 10% control mortality
2. Greater than 10% aberrant mortality in concentrations throughout the test range, however, there may be greater than 10% mortality in one replicate if there is 100% survival above that value
3. Temperature variation greater than 2 °C
4. Test media stored more than 72 hours
5. Criterion in Table 1 (Appendix A) are not met

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, refer to US 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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Spraque, J.B. 1973. The ABC's of Pollutant Bioassay using Fish. pp. 6 - 13 in Biological Methods for the Assessment of Water Quality. J. Cairns and K. Dickson (eds.). STP 528. American Society for Testing and Materials, Phila. PA 256 pp.

Standard Methods for the Examination of Water and Wastewater. 1985. American Public Health Association, 16th ed.

APPENDIX A
Table
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48-HOUR ACUTE TOXICITY TEST USING DAPHNIA MAGNA AND DAPHNIA PULEX

TABLE 1. Summary of test conditions for Daphnia magna or Daphnia pulex 48 hour acute toxicity test (based on Peltier and Weber, 1985).

1.	Test type:	Static, daily renewal, 48 hour
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:	50 mL/replicate
8.	Renewal:	None
9.	Age of test organisms:	Less than 24 hours old
10.	Number/container:	10 per exposure chamber
11.	Feeding:	Do not feed during test
12.	Aeration:	None unless dissolved oxygen concentration falls below 40% saturation then less than 100 bubbles per minute
13.	Dilution water:	Moderately hard reconstituted deionized water unless otherwise specified
14.	Test media/leachate concentrations:	Minimum of five and one control
15.	Test duration:	48 hours
16.	Effects measured:	Survival at 1, 24, and 48 hours