10-DAY CHRONIC TOXICITY TEST USING DAPHNIA MAGNA OR DAPHNIA PULEX

CONTENTS

SUPERCEDES: SOP #2028; Revision 2.0; 09/24/90; U.S. EPA Contract EP-W-09-031.

10-DAY CHRONIC TOXICITY TEST USING DAPHNIA MAGNA OR DAPHNIA PULEX

1.0 SCOPE AND APPLICATION

The procedure for conducting a 10-day chronic toxicity test using Daphnia magna or Daphnia pulex is described below. This test is applicable to leachates, effluents, and liquid phases of sediments. Mortality, reproduction and growth are used to assess the toxicity of the test media.

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. EPA endorsement or recommendation for use.

2.0 METHOD SUMMARY

Larval daphnids are placed in individual containers and exposed to different concentrations of a test media over a 10-day period. Concentrations are renewed every other day and mortality, reproduction and growth are recorded.

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 preservative are to be used. The preservation and storage protocol is therefore limited to holding the samples on ice at 4^{o}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 also 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). Also the effect of the toxicant is organism dependent.

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
- wide bore pipettes - inside diameter 1.5 times the length of the daphnid
- graduated cylinders - 250 mL and 1L
- pipette - 1 mL
- beakers - 250 mL
- volumetric flasks - 500 mL
- test media - 1 L/day
- diluent - 3 L/day
- waste containers
- light table - to aid in counting the organisms
- suitable food

5.2 Washing Procedure

1. Wash with warm water and detergent.
2. Rinse with tap water.
3. Rinse with 10% nitric acid solution.
4. Rinse with deionized water.
5. Rinse with 100% acetone.
6. Rinse with deionized water.
7. Final rinse with dilution water.

5.3 Test Organisms

Test organisms may be reared inhouse or obtained from an outside source. Positive identification of the species is required before beginning the test. Daphnids to be used must be less than 24 hours old and from the second to the sixth brood of an healthy adult. 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. 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).

5.4 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 (APHA, 1985).

6.0 REAGENTS

1. Dilution water

Dilution water is reconstituted deionized water unless otherwise specified. See Horning and Weber (1985) for the preparation of synthetic fresh water. Set up a laboratory or standard dilution water control when reconstituted deionized water is used as the dilution 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 media is a liquid, dilutions may be made directly for the required concentrations. If the test media is a sediment, preliminary filtration and dilutions are required to produce a liquid phase.

7.0 PROCEDURES

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

Destroy all test organisms at the completion of the test.

1. Choose 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).

2. The example below provides enough test media for five replicates containing 80 mL each and extra for chemical analysis. Other ranges may be used according to needs of the analyses.

Example 1. Test Dilutions

Test Concentration (% Test Media)	Dilution water	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

3. Rinse all exposure chambers, except the chamber containing 100% test media, in dilution water before the start of the test.

4. Pipette 0.5 mL of the test media into a volumetric flask and dilute to 500 mL. Using a graduated cylinder, pour 80 mL into each exposure chamber and pour the rest into a beaker for chemical measurements.

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

6. Using a wide bore pipette, randomly select and carefully place one daphnid into each exposure chamber.

7. Place the pipette tip below the surface and gently expel the daphnid into the chamber.

8. Concentrations are renewed every other day for the duration of the test. However, if the test begins on a Monday, then renewals may be done on Wednesday, Friday and the following Monday and Wednesday.

9. Measure and record mortality and survival at one hour and then when test concentrations are renewed.

10. Count the number of live or dead young produced by each female.

11. Temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness should be measured on all new concentrations. Conduct these measurements on old test concentrations at least three times during the test.

12. Prepare test media concentrations as done previously. Pour the concentrations into new exposure chambers, reserving extra for chemical analyses.

13. Count the number of live or dead adults and young, using the light table if necessary.

14. Record these results and then carefully transfer the adult daphnid into the new concentrations.

15. Using a suitable food, feed daphnids once daily during the test.

16. After feeding the daphnids, cover the exposure chamber to reduce evaporation of the test concentrations.

8.0 CALCULATIONS

The methods used to determine the EC_{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 EC_{50}. If there is partial mortality within a replicate, then the Probit Method should be used to calculate the EC_{50}. Also, the Lowest Observable Effect Concentration (LOEC) is recorded and the No Observable Effects Concentration (NOEC) is recorded (Peltier and Weber,1985). Dunnett's many-one t procedure or Bonferroni t procedure (Miller, 1966) may be used to determine comparisons between the concentrations response to the test media as compared to the control. Other methods of estimating the response values 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

Test data is invalidated for the following reasons:

1. Greater than 20% control mortality.
2. Standard reference toxicant results greater than 2 standard deviations from an accepted value (APHA, 1985).
3. Greater than 20% aberrant mortality in concentrations.
4. Temperature variation greater than 2 °C.
5. Test media stored more than 72 hours.
6. 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

APHA. 1985. Standard Methods for the Examination of Water and Wastewater. 1971. American Public Health Association, 16th ed.

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.

Horning, W.B. and C. Weber. 1985. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, Cincinnati, OH. 162 pp.

Huston, Mark, SOP.I, 10 Day Chronic Toxicity Test Using Daphnia Magna or Daphnia Pulex, U.S. EPA Environmental Response Team - Technical Assistance Team TDD: 11871206.

Miller, R.G. 1966. Simultaneous Statistical Inference. McGraw-Hill Book Company, New York, N.Y.

Peltier, William H. and Cornelius Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Cincinnati, OH. 216 pp.

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.

APPENDIX A
Table
SOP #2028
November 1994

TABLE 1 Summary of test conditions for Daphnia magna or Daphnia pulex 10 day chronic toxicity test (Horning and Weber, 1975).

1.	Test type:	Static, renewal, 10 day
2.	Temperature:	25.0 ± 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.	Renewal:	Every other day
9.	Age of test organisms:	Less than 24 hours old
10.	Number/container:	One per exposure chamber
11.	Feeding:	Feed on day of renewal
12.	Aeration:	None unless DO concentration falls below 40% saturation then <100 bubbles per minute
13.	Dilution Water:	Moderately hard reconstituted deionized water
14.	Test media/leachate concentrations:	Minimum of five and one control
15.	Test duration:	10 days
16.	Effects measured:	Survival, growth and reproduction