24-HOUR RANGE FINDING TEST USING LARVAL PIMEPHALES PROMELAS

CONTENTS

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1.0   SCOPE AND APPLICATION

The procedure for conducting a 24-hour range finding test using larval Pimephales promelas (fathead minnow) is described below. This test is used as a preliminary guide when testing an effluent, leachate, or liquid phase of a sediment with an unknown toxicity. The results of this test are used to determine the concentration range of a definitive toxicity test.

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 (EPA) endorsement or recommendation for use.

2.0   METHOD SUMMARY

Larval fathead minnows are exposed to various concentrations of a test media over a 24-hour period. Survival and mortality data are used to determine the definitive concentration range to be used in a static or flow-through toxicity 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 preservative 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 changes in effluent toxicity (Peltier and Weber, 1985). This method is less sensitive than a flow-through toxicity test and the sensitivity is dependent on the accuracy of the dilutions.

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

3.   Dissolved oxygen depletion due to biological oxygen demand, chemical oxygen demand or metabolic

24-HOUR RANGE FINDING TEST USING LARVAL PIMEPHALES PROMELAS

wastes also is a potential problem.

4.   Loss of a toxicant through volatilization and adsorption to exposure chambers may occur.

5.0   APPARATUS/EQUIPMENT

5.1   Apparatus

- 40 fathead minnows - less than 30 days old
- 4 small cups - 50 mL
- 4 exposure chambers - 1L glass or plastic, labeled graduated cylinders - 1L and 10 mL
- mixing bucket - 1L or larger
- plastic tubing - 3/8" outside diameter
- plastic screening
- dilution water - 3L
- test media - 1.5L
- wide bore pipettes - 1.5 times the length of the organism
- waste containers
- brine shrimp or other suitable food

5.2   Test Organisms

Test organisms may be reared in-house or received from an outside source. Positive identification of the test organisms must be made prior to starting the test. The fish to be used for a rangefinding test must be the same age (less than 30 days old), in the same condition, and come from the same culture as those to be used for the definitive test. Place fish into a holding tank and slowly drip the dilution water into the tank over a 24 hour period. Then leave the fish in this water for another 24 hours so that the fish become acclimated to the dilution water. Use populations of fish that have less than 5% mortality and that are healthy. For more detail and information including culturing, care, and handling, and disease prevention of Pimephales promelas see Peltier and Weber (1985) and Denny (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. Use a standard method to measure alkalinity and hardness (Standard Methods, 1985). Record all measurements on data sheets.

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 acclimate the fish before the beginning of the test.

24-HOUR RANGE FINDING TEST USING LARVAL PIMEPHALES PROMELAS

2.   Test Medium

If the test medium is a liquid, dilutions may be made directly for the required concentrations. If the test media is a liquid phase of a soil, preliminary filtration and dilutions are required.

7.0   PROCEDURES

1.   In order to determine the range of concentrations to be used for a definitive toxicity test, a preliminary rangefinding test is conducted. Ten fish are placed into exposure chambers with a broad range of concentrations (0, 1, 10, and 100% test media).

2.   Survival and mortality are recorded after one and 24 hours and the results are used to determine definitive test concentrations.

3.   Replicates are not necessary for this test.

4.   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). Other ranges may be used according to the needs of the specific situation.

Example 1. Test Dilutions

| Test Concentrations | Volume (mL) | |
(% test media)	Diluent	Test Media
0	750.0	0
1	742.5	7.5
10	675.0	75.0
100	0	750.0

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

6.   Measure 750 mL of dilution water and pour into the control exposure chamber.

7.   Measure 7.5 mL of test media and dilute to 750 mL with dilution waer. Pour this mixture into the exposure chamber. Continue this procedure until all the concentrations are prepared. Always go from the lowest concentration to the highest in order to minimize the risk of cross contamination.

8.   Using a wide bore pipette, randomly select one fish at a time into a small cup, placing ten (10) fish into each cup. After all the fish have been selected, pour into the exposure chambers. Gently submerse the cup below the water surface and pour the fish out.

24-HOUR RANGE FINDING TEST USING LARVAL PIMEPHALES PROMELAS

9.   Measure and record temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness at the beginning of the test after the fish have been added to the exposure chamber.

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} (Peltier and Weber, 1985). Since the results of this test are only preliminary, exact calculations need not be made. An estimate of the LC_{50} is needed to determine the range of concentrations to be used for the definitive test. Other methods to determine the LC_{50} of the test media may be used if justified and the appropriate reference cited.

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 preparation
5.   Dilution water
6.   Test conditions
7.   Standard reference toxicant

10.0   DATA VALIDATION

The following criteria is a basis for rejecting the results generated under this test:

1.   Greater than 10% control mortality
2.   Criterion in Table 1 (Appendix A) not met

Note: Since this is only a preliminary test, the strict guidelines used for the definitive test need not be adhered to. However, this test should be run according to standard laboratory guidelines.

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

24-HOUR RANGE FINDING TEST USING LARVAL PIMEPHALES PROMELAS

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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.

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24-HOUR RANGE FINDING TEST USING LARVAL PIMEPHALES PROMELAS

APPENDIX A
Table
SOP #2023
October 1994

24-HOUR RANGE FINDING TEST USING LARVAL PIMEPHALES PROMELAS

TABLE 1.   Summary of test conditions for 24-hour range finding toxicity test using fathead minnow (Pimephales promelas) rangefinding test (based on Peltier and Weber, 1985).

#	Condition	Value
1.	Test type:	Static
2.	Temperature:	$25.0 \pm 2°C$
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:	1-L containers
7.	Test solution volume:	750-mL
8.	Renewal:	None
9.	Age of test organisms:	Newly hatched larva (less than 24 hours old)
10.	Larvae/chamber:	10 per chamber
11.	Feeding:	None
12.	Washing:	N/A
13.	Aeration:	None unless dissolved oxygen concentration falls below 40% saturation then less than 100 bubbles per minute
14.	Dilution water:	Moderately hard reconstituted deionized water unless otherwise specified
15.	Test media/leachate concentrations:	Minimum of three and one control
16.	Test duration:	24 hours