TOP:010.8

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and

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Sea Lamprey Control Centre
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TECHNICAL OPERATING PROCEDURE

PROCEDURE TITLE:

Procedures for Conducting On-site Toxicity Tests

APPLICABILITY:

Procedures apply to all toxicity tests conducted in conjunction with stream lampricide treatments and tests conducted on-site during research projects related to lampricides.

PRINCIPLE:

Standard procedures for conducting on-site flow-through toxicity tests with stream water

SAMPLE COLLECTION AND PRESERVATION:

Water samples are collected from the test solutions by hand for lampricide analysis. No samples are preserved.

EQUIPMENT REQUIRED:

Toxicity testing trailer and flow-through testing equipment carried within
Power generator
pH meter
Spectrophotometer
Digital titrator
High performance liquid chromatograph system
Dissolved oxygen meter
Lampricide delivery device

POTENTIAL INTERFERENCES:

See IOPs for instruments

SAFETY:

Standard laboratory safety procedures are followed. No special safety procedures are required.

DISPOSAL:

Effluent from flow-through tests is drained into the stream which is targeted for treatment. If this is not possible, the effluent water is drained through a charcoal filter.

REAGENTS:

pH 4.0, 7.0, and 10.0 buffers (check expiration dates)
Standardized N/50 sulfuric acid solution (check expiration date)
100 mg/L Bayluscide standard
Pre-weighed TFM and Bayluscide stocks
4.0, 8.0, and 12.0 mg/L TFM field standards

PROCEDURES:

Flow-through Toxicity Tests

I. Preparation of mobile laboratory

A. Park the trailer on level ground about 150 feet from the stream.

B. Drop the stabilizers and level the trailer.

C. Pull the generator out of the side compartment.

D. Run an extension cord from the generator to the trailer.

D. Connect the drain hose to the trailer and extended it to the stream, downstream of the water intake hose.

E. Water pump preparation:

1. Place the water pump on level ground upstream of the drain hose.

2. Run an extension cord from the generator to the water pump. Do not plug in to the pump until you are ready to pump water to the trailer.

3. Run a 3/4 inch garden hose from the pump outlet to the trailer intake.

4. Connected the intake hose to the pump.

5. Place the intake basket, connected to the intake hose in the stream.

6. Prime the pump by filling the pump and intake hose with stream water.

F. Water Flow adjustment

1. Ensure water valve on the trailer intake is fully open.

2. Ensure water valve inside the trailer intake is fully open.

3. Adjust the water flow if the water level in the headbox rises above the standpipe.

G. Toxicant pump preparation

1. A materflex pump is used to pump lampricide(s) into the diluter headbox (Attachment 2 and IOP:005.x).

2. Connect the power cord.

3. Intake and delivery tubing is set according to the type of test (TFM or TFM/Bayluscside).

   a. TFM test
      (1) Place the intake end of the tubing at the bottom of a 2000 ml Erlenmeyer flask.
      (2) Place the delivery end of the tubing just above the water line of cell one of the diluter box (Attachments).
   b. TFM/Bayluscide test
      (1) Place the intake end of the tubing (n=2, 1 for each stock solution) at the bottom of the 2000 ml Erlenmeyer flasks of TFM and Baylucide.
      (2) Place the delivery end of each tube just above the water line of cell one of the diluter box (Attachments).

4. Adjust the settings to deliver the desired amount of lampricide(s) (II.B., IOP:005.x).

H. Test Organisms

1. Add 10-20 sea lamprey larvae (60-120 mm preferred) to the wire mesh cages hanging on the side of each tank 24 hours prior to testing.

2. Add non-target animals (generally 5 - 10) to each tank 24 hours prior to testing.

II. Determination of lampricides concentrations and delivery system settings

A. Determine the predicted minimum lethal concentration using the pH/Alkalinity charts (Appendix I).

B. To determine the settings for TFM:

1. Consult the Quick Reference Chart for Estimated Concentration Relationships" or the “Estimated Concentration Spread for Marquette Ten-cell Diluter” (Attachments).

2. Find the predicted MLC in column five (for sea lamprey only tests) or seven for tests with non-targets and sea lampreys) 3. Trace the row that MLC was found to the first column in the chart This is the desired high concentration for the test.

3. Determine pump settings for the lampricide delivery system (IOP:005.x).

C. To determine the settings for Bayluscide:

1. Multiply the high concentration of TFM by the percentage of Bayluscide active ingredient desired for the test, e.g.:

   a. 5.0 mg/L TFM X 0.01 (1.0%) = .050 mg/L (50 ug/L)
   b. 5.0 mg/L TFM X 0.005 (0.5%) = .025 mg/L (25 ug/L)

2. Determine the pump settings following IOP:005.x).

D. Determine the concentrations for the other tanks.

1. Refer to "Quick Reference Chart for Estimated Concentration Relationships" or the “Estimated Concentration Spread for Marquette Ten-cell Diluter” (Attachments). Find the desired high concentration in the first column. The remaining concentrations are listed in the same row, or

2. Approximate the concentrations by multiplying the high concentration by 0.8 (80% dilution system), then multiplying that result and subsequent results by 0.8 until the series is complete.

III. Preparation of toxicant

A. TFM

1. Determine the volume of lampricide necessary for the test.
   a. Masterflex LS pump:
      (1) Determine the delivery rate in mL/min.
      (2) Multiply the delivery rate by 720 to determine the total volume of stock that will be used during the test.
2. Pour a pre-weighed (50.0g) amount of TFM into a 1000 mL volumetric flask. Rinse the cap and bottle into the flask with deionized water until the rinsate is clear. Fill the volumetric flask to the 1000 mL mark with deionized water.

3. Pour the contents of the flask into a 2000 mL polypropylene Erlenmeyer flask (lampricide reservoir). Repeat if more than 1000 mL stock solution is needed.

B. Bayluscide

1. Pour a pre-weighed (0.24 g liquid bayluscide) amount of Bayluscide EC into a 1000 mL volumetric flask.

2. Rinse the culture tube and cap into the flask with methanol, add methanol up to 500 ml in the flask, mix thoroughly, fill the flask up to the 1000 mL mark with deionized water.

3. Mix well by inversion or with a stir bar and let stand overnight.

4. Pour the contents of the flask into a 2000 mL polypropylene Erlenmeyer flask (lampricide reservoir). Repeat if more than 1000 mL stock solution is needed for the test.

5. See Attachment for DFO procedures.

IV. Toxicity testing

A. Prepare data forms for the test (Appendix M).

B. Draw down the water level in all tanks to the minimum level (about 10 L).

C. Collect Time 0 water chemistry measurements (dissolved oxygen, pH, alkalinity, temperature).

D. Inspect each test chamber for dead or stressed animals **and for clogs in the drain pipe**.

E. Check the over-flow tube in the diluter for proper flow.

F. Turn on the pump and inspect the delivery tube to assure proper delivery of lampricide (s) to the diluter.

G. Record the time that the test began.

H. About fifteen minutes after the beginning of lampricide delivery, return the stand pipes in the test chambers to the vertical position or insert the stand pipes into the drains (depending on drain system type) to reestablish the water level at full volume.

Peripheral Procedures

I. Care of test animals

A. Collected and transported organisms with minimal stress. Stress factors include:

1. Temperature: Avoid rapid changes in temperature; limit changes during acclimation to no more than 3 °C over a 24-h period.

2. Alkalinity: Significant changes in total alkalinity may cause physiological stress which may influence the tolerance of the test animals.

3. Oxygen: Change or aerate transport water to avoid overloading. Maintain dissolved oxygen concentration at greater than 5 mg/L or 60% of saturation.

4. Mechanical: Strong agitation during transport or mishandling during transfer will stress, injure, or kill test animals.

5. Disease: Other forms of stress may encourage the outbreak of disease. Transport also may bring out signs of otherwise asymptomatic disease among the test animals.

B. Hold test organisms with care until testing.

1. Hold test organisms in the test stream or in coolers filled with water from the test stream. Shade the cooler and adequately aerated water in the coolers.

2. Provide substrate (clean sand if possible) for sea lamprey larvae to minimize stress associated with continuous swimming.

3. Transfer sea lamprey larvae and non-target organisms to test tanks at least 24 h before exposure.

C. Record information about the test organisms on the Test Animal Source Information sheet (Appendix M).

II. Data collection and documentation

A. Water chemistry

1. pH (TOP:006.x)

a. Standardize the pH meter (at a minimum) each day before measuring pH and during the test and a standardization check is completed at the end of the day.
b. Take hourly pH measurements in all test chambers; Take the first reading just prior to the addition of test animals.
c. Recorded pH to 0.01 on the *Supplementary Chemical Parameters Format* data sheet (Appendix M).

2. Total alkalinity (TOP:005.x)

   a. Measure total alkalinity every four hours (0, 4, 8, and 12 h). .
   b. Record total alkalinity on the *Supplementary Chemical Parameters Format* data sheet (Appendix M).

3. Temperature

   a. Measure temperature hourly in tanks 1 and 10. Take the first measurement immediately prior to the addition of test animals.
   b. Record temperature (C°) on the *Supplementary Chemical Parameters Format* data sheet (Appendix M).

4. Dissolved oxygen (TOP:008.x)

   a. Measurements are conducted using a dissolved oxygen meter.
   b. Measure dissolved oxygen in in all test containers prior to addition of test animals.
   c. Measure dissolved oxygen every 1-4 hours during testing.
   d. Record dissolved oxygen to the nearest 0.1 mg/L on the *Supplementary Chemical Parameters Format* data sheet (Appendix M).

B. Mortality

1. Record mortality of all test organisms beginning at hour 2. Record mortality each hour for twelve hours during exposure and again at 12 hour post-exposure.

2. Record mortality on the *Toxicity Test Mortality* data sheet (Appendix M).

3. Criteria for death
   a. Sea lamprey larvae
      (1) Immobility and lack of movement in the tail
      (2) Lack of response to an external stimulus
   b. Nontarget species
      (1) Fish: immobility, lack of gill action and lack of response to an outside stimulus
      (2) Invertebrates: immobility and lack of response to an electrical stimulus (9-volt dry cell with wire electrodes)

C. Concentrations of TFM and Bayluscide

1. Concentrations of TFM are measured hourly in each Tank (1-9).

2. Concentrations of Bayluscide are measured (TOP:021.x) every two hours (minimum) in each Tank (1-9).

3. Concentrations of TFM and Bayluscide are recorded on the *Toxicity Test Lampricide Analysis* data sheet (Appendix M).

III. Reporting of data

A. Data from each toxicity test are recorded on one of five forms. The data on these forms are retained as a hard copy record of the test (Appendix M).

1. Test Animal Source Information sheet

2. *Toxicity Test Mortality*

3. Toxicity Test Lampricide Analysis

4. Supplementary Chemical Parameters Format

B. Data are tabulated on two summary forms.

1. Toxicity Test Summary (Appendix M; for treatment supervisor): This sheet provides a summary of toxicity test data for several concentrations of TFM. The responses of sea lamprey larvae to concentrations near the MLC or the responses of nontarget species are detailed on this form.

C. The person in charge of conducting the toxicity test prepares a short paragraph describing problems or unusual conditions encountered during the test, and what was done to compensate. Unusual test results and any observations made during the test are noted. This information is forwarded to the toxicity test coordinator upon return to the station.

IV. Interpretation of data

A. Sea lamprey larvae

1. Empirical method

a. The pattern of mortality for concentrations of lampricides which produce mortality is examined.
b. The minimum concentrations of lampricides which produced 100% mortality in 9 and 12 hours are estimated (MLC).
c. The estimated MLC is compared with the MLC from the appropriate pH/alkalinity prediction model.

2. Litchfield-Wilcoxon method

An application which automates the following procedure is available at: https://jvadams.shinyapps.io/LW1949demo

a. The 24-h mortality data (12-h post exposure) are plotted on logarithmic-probability graph paper and a line is drawn which fits the arrangement of points.
b. The fit of the line is determined statistically with the Litchfield-Wilcoxon data reduction program.
c. The intersections of the mortality curve with the 99.9% lines is the SMLC.

B. Nontarget species

1. Litchfield-Wilcoxon method

An application which automates the following procedure is available at: https://jvadams.shinyapps.io/LW1949demo

a. The 24-h mortality data (12-h post exposure) are plotted on logarithmic-probability graph paper and a line is drawn which fits the arrangement of points.
b. The fit of the line is determined statistically with the Litchfield-Wilcoxon data reduction program.
c. The intersections of the mortality curve with the 25% and 50% lines are the estimated LC25 and LC50.
d. If brown trout is the nontarget species, the estimated LC25 is compared with that listed on the appropriate pH/alkalinity prediction chart.

2. Empirical method

a. This method is used to estimate "no kill" concentrations of lampricides for nontarget species.
b. The pattern of mortality for concentrations of lampricides is examined.
c. The maximum concentrations which produced no mortality in 24 hours are the "no kill" concentrations.

REFERENCES:

Instrument operating manuals:
pH meter instruction booklets
Waters HPLC operator manuals
Guide to Successful Operation of Your LC System
Spectrophotometer Operator Reference Manuals
Dissolved Oxygen Meter Instruction Manuals
Digital Titrator Operating Instructions

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99-113.

This procedure has been reviewed and approved by the undersigned representatives of the U.S. Fish and Wildlife Service and Fisheries and Oceans Canada.

REVIEWED/APPROVED_____ DATE 2/13/2017
Field Supervisor (U.S.)

REVIEWED/APPROVED_____ DATE 2/8/2017
Division Manager (Canada)