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TECHNICAL OPERATING PROCEDURE

PROCEDURE TITLE:

Procedures for Conducting Spectrophotometric Analysis for TFM in Stream Water

APPLICABILITY:

Procedure applies to spectrophotometric analyses conducted in conjunction with lampricide treatments and toxicity tests.

PRINCIPLE:

The waters of streams which receive applications of TFM are analyzed to provide information vital for control and adjustment of lampricide concentrations. Applications are carefully controlled to assure the efficacy and safety of all treatments. Concentrations of TFM are measured with a variety of spectrophotometers (IOP:012.x, IOP:012.xC, IOP:012.xF, IOP:012.xG, and IOP:012.xH). Analyses for TFM conducted during toxicity tests or other activities peripheral to stream treatment utilize similar procedures.

SAMPLE COLLECTION AND PRESERVATION:

Samples of water containing TFM are collected by hand or by automatic water sampler (IOP:002.x). Samples are taken by hand in 250 mL plastic bottles; plastic or glass bottles may be used in automatic water samplers. Water samples are usually analyzed immediately, but if storage is necessary the sample bottles are labeled and refrigerated. Subsequent analysis is completed as soon as possible.

EQUIPMENT REQUIRED:

- I. Sample collection
 - Sampling device for collection of mid-stream water samples
 - Automatic water sampler

- II. TFM analysis
 - Spectrophotometer
 - Ring stand and filter funnel or syringe filter holder
 - Whatman 2v, Whatman 1, or equivalent filters
 - Heating block or water bath for temperature control of samples
 - Calibrated dispenser for addition of sodium tetraborate
 - Plastic sample bottles (about 250 mL)
 - Vacuum filtration system (optional)
 - Millipore filters (45 micron; optional)

POTENTIAL INTERFERENCES:

See IOP:012.x, IOP:012.xC, IOP:012.xF, IOP:012.xG, and IOP:012.xH,

SAFETY:

Standard laboratory safety procedures are followed when handling reagents, otherwise no special precautions are required.

DISPOSAL:

Wastes from field laboratories are collected and emptied into the stream receiving treatment.

REAGENTS:

Sodium tetraborate, saturated solution in deionized water
Sulfuric or hydrochloric acid, 10% solution
Deionized water
TFM field standards; 0.0, 4.0, 8.0, and 12.0 mg/L active ingredient formulated according to TOP:019.x
Stream background absorbance blanks

PROCEDURES:

- I. Gather reagents

- II. Preparation of instruments and essential equipment
 - A. Line power models
 - 1. Spectrophotometer; see IOP:012.xF
 - 2. Associated equipment necessary in procedure
 - a. Filters
 - (1) Whatman filters
 - (a) Set plastic funnels in ring-holders
 - (b) Place a Whatman 2v filter (or equivalent) into each

funnel

- (2) Syringe filters
 - (a) Install 2.5 mm circular filter into body of syringe filter
 - (b) Turn filter onto syringe (with plunger removed)
- b. Sample heater
 - (1) Heating block: Plug in and pre-heat the heating block, or;
 - (2) Water bath: Turn on the constant temperature water bath

B. Battery power models

- 1. Spectrophotometer; see IOP:012.x, IOP:012.xC, IOP:012.xG, and IOP:012.xH
- 2. Associated equipment necessary in procedure
 - a. Filters; syringe filters (see above)
 - b. Sample heater; 12V water bath

III. Sampling

- A. The choice of an appropriate sample site is critical to the collection of accurate analysis data and to the control of lampricide concentrations during a treatment.
 - 1. Ease of access
 - a. Time considerations; a site must be accessible within a reasonable length of time. Excessive driving or walking time is avoided if possible.
 - b. Safety considerations; unsafe walking conditions are avoided. An example of unsafe conditions is a narrow highway bridge with rapidly moving traffic.
 - c. Access permission; if a preferred sampling site is on private property, permission must be secured from the landowner before use.
 - 2. Distance downstream from application site
 - a. A suitable sampling site is located a sufficient distance downstream from an application site to allow mixing of the chemical throughout the water column. Stream morphology is a determining factor for this consideration.
 - b. A suitable site is near enough to an application site to allow timely analysis and adjustment of application rate if needed.
 - 3. Distance from sources of dilution water
 - a. Sites are selected which are not directly downstream of tributaries. Samples are collected from midstream to reduce the influence of tributaries.
 - b. Sites are chosen which are upstream from pools or slow, deep areas.
- B. When information on TFM concentrations is needed to adjust an application, samples are usually collected by hand. Samples are taken at a specified site at time intervals appropriate to need. A one-hour interval between samples is usually sufficient to supply data for adjustment of a lampricide application. Maintenance (boost) applications often require a shorter time interval to assure adequate control of changing concentrations. A single sample is taken at each site after confirmation that the concentrations of lampricides across the stream are homogeneous. Taking several samples across a stream

at one time provides this confirmation.

- C. Automatic water samplers: If the need for information on TFM concentrations is not immediate, automatic water samplers may be used to collect samples. Water collected by the unit is analyzed when sampling is complete. Data from analysis of the samples can be plotted to produce concentration profiles for a lampricide block at the sampling site.

IV. Conducting analyses

A. Analysis for TFM: Line power models

1. Field standards: see TOP:019.x
2. Stream blanks (stream background absorbance)
 - a. Water is collected from all sites on the stream at which analysis for TFM will be conducted. It is desirable to determine stream blank absorbance and B/A ratio values at least one day before treatment.
 - b. Two cuvettes are optically matched (± 0.002 absorbance units) and labelled "Zero" and "Sample."
 - c. The 0.0 mg/L TFM field standard is poured into the "Zero" cuvette. The cuvette is wiped dry and inserted into the spectrophotometer. The absorbance reading is adjusted to 0.000
 - d. Each stream water blank (250 mL) is alkalized by addition of 1.0 mL sodium tetraborate buffer (saturated solution).
 - e. Each alkalized stream water blank is filtered through Whatman 2V paper (or equivalent). If fine particulate matter such as clay is present, the stream water blank is also filtered through a Millipore filter (TOP:020.x).
 - f. The absorbance of each alkalized and filtered stream water blank is measured as follows:
 - (1) The stream blank is heated to a defined temperature (typically 25 °C) in a clean beaker.
 - (2) The "Sample" cuvette is rinsed by filling with the heated stream blank and dumping two or more times.
 - (3) The "Sample" cuvette is filled with the heated stream blank, wiped dry and inserted into the spectrophotometer.
 - (4) The absorbance is measured and recorded on the CHEMICAL ANALYSIS – BLANK VALUES data form (Appendix M).
3. B/A (Base/Acid) ratio
 - a. The sample described in IV.A.2.f.(3) is acidified with 10% sulfuric or hydrochloric acid solution (1 drop/square cuvette or 2 drops/round cuvette). The cuvette is inverted slowly to mix and reinserted into the spectrophotometer. The absorbance is again measured and recorded on the CHEMICAL ANALYSIS – BLANK VALUES data form (Appendix M).
 - b. The absorbance of the alkalized stream blank is divided by the absorbance of the acidified stream blank to produce the B/A ratio for the stream water at that site. The B/A ratio is recorded on the CHEMICAL ANALYSIS – BLANK VALUES data form (Appendix M) and if desired on the computation form (Attachment 1).
 - c. B/A values for sites on a stream should not vary significantly if

- background absorbance for water at that site is > 0.020 .
- d. The B/A ratio using millipored samples will often be different than a B/A ratio obtained from samples that are not millipored. On river systems where milliporing is anticipated, the B/A ratio should be obtained from millipored samples prior to commencing the treatment.
4. Standard response curve
- a. TFM field standards (0.0, 4.0, 8.0, and 12.0 mg/L) are opened. New sets of field standards are used each time an analysis trailer is prepared for a new treatment. TFM field standard codes are recorded in the instrument log book.
- b. Two cuvettes are optically matched (± 0.002 absorbance units) and labelled "Zero" and "Sample."
- c. The 0.0 mg/L TFM field standard is poured into the "Zero" cuvette. The cuvette is wiped dry and inserted into the spectrophotometer.
- d. The absorbance reading is adjusted to 0.000
- e. The absorbance of the 4.0, 8.0, and 12.0 mg/L TFM field standards are measured sequentially as follows:
- (1) The standard is heated to a defined temperature (typically 25 °C) in a clean beaker.
 - (2) The "Sample" cuvette is rinsed by filling with the heated standard and dumping two or more times.
 - (3) The "Sample" cuvette is filled with the heated standard, wiped dry and inserted into the spectrophotometer.
 - (4) The absorbance is measured and recorded in the instrument log book.
- f. Each standard is acidified with 10% sulfuric or hydrochloric acid solution (1 drop/square cuvette or 2 drops / round cuvette) and the absorbance is again measured and recorded. The results of these measurements determine the residual absorbance for TFM.
- (1) A linear plot is made from the absorbance of the acidified standards.
 - (2) The values described by this plot are used to correct the absorbance of the acidified stream water sample.
- g. The instrument response is plotted on graph paper, or the slope of the response curve is calculated.
- (1) Graph: Plot mg/L TFM on the horizontal axis; plot absorbance on the vertical axis. The response curve is linear. The response of unknowns is read directly from the graph.
 - (2) Slope calculation: Divide the absorbance of each standard by the mg/L TFM in the standard. Average the results and record on the LAMPRICIDE ANALYSIS DATA FORM (Appendix M) under the column heading 'SLOPE.' The response of unknowns is divided by the average response of the standards to provide the mg/L TFM in the unknown.
5. Conducting analyses
- a. The absorbance of an alkalinized and filtered stream water blank is measured and recorded (see section IV.A.2)
- b. Samples of stream water are collected according to section III. Each water sample is buffered, filtered through a Whatman 2v filter (or equivalent), and heated to a standard temperature (generally 25 °C).
- c. Each sample is poured into the "Sample" cuvette (two rinses) and inserted into the spectrophotometer. The absorbance is measured and

- recorded on the LAMPRICIDE ANALYSIS DATA FORM under the column heading 'SPEC ABS.'
- d. The absorbance of the stream water blank (section IV.A.5.a.) is subtracted from the sample absorbance and the difference is recorded on the LAMPRICIDE ANALYSIS DATA FORM (Appendix M) under the column heading 'ABS COR.'
- e. The TFM concentration is interpolated from the graphic response curve or calculated by dividing the measured absorbance by the average slope of the response (see section IV.A.4.g). The resulting concentration is recorded on the LAMPRICIDE ANALYSIS DATA FORM (Appendix M) under the column heading 'TFM mg/L.'
6. Checks and corrections to the absorbance blank
- a. The absorbance blank is checked at each analysis site prior to conducting analysis for TFM. The computation form (Attachment 1) may serve as an aid in the following calculations.
- (1) A buffered, filtered sample of stream water is heated to 25 °C, and the absorbance is measured.
 - (2) 10% sulfuric or hydrochloric acid (1 drop/square cuvette or 2 drops / round cuvette) is added to the cuvette + sample, the cuvette is again inserted into the spectrophotometer, and the absorbance is measured.
 - (3) The absorbance of the acidified sample is multiplied by the B/A ratio previously determined for that analysis site or by a new B/A ratio if the presence of TFM is not suspected (see (b) below). This calculated absorbance is the blank value for the analysis site. A difference from the original indicates that the background absorbance has changed and/or that TFM is present.
 - (a) Change in background absorbance; the calculated blank value is different from the recorded value, but the same as the absorbance value of the alkalized stream water sample.
 - (b) Presence of TFM; the calculated blank value is the same as the recorded value, but less than the absorbance value of the alkalized stream water sample.
 - (c) Change in background absorbance and presence of TFM; the calculated blank value is different from the recorded value, and is less than the absorbance value of the alkalized stream water sample.
 - (4) If TFM is present, the absorbencies of the acidified samples are corrected for residual absorbance (section IV.A.4.f).
 - (5) Concentrations of TFM are calculated or graphically determined on the basis of absorbance corrected for calculated blank values.
- b. Additional checks are routinely conducted at 4 to 6-hour intervals to document that the background absorbance is stable and correct.
- c. Conditions which may cause changes in the background absorbance such as rain, require more frequent checks. If a change in absorbance is detected, the analysis of each sample includes a calculated correction.
- d. A re-calculated blank absorbance value must pass a "common sense

check.” If a significant (more than .002 to .003) change is calculated, and there is no apparent reason for a change, another sample should be analyzed and the background absorbance again calculated.

7. Quality assurance steps

- a. Instrument log books
 - (1) The log book designated for each instrument is stored with the instrument.
 - (2) Appropriate entries are made in the log book each time the instrument is used or maintenance is conducted on the instrument.
- b. Data record sheets; completed for all analyses
 - (1) CHEMICAL ANALYSIS - BLANK VALUES (Appendix M)
 - (2) LAMPRICIDE ANALYSIS DATA FORM (Appendix M)
- c. Handling of standards; see TOP:019.x
- d. Training; see AOP:004.x
- e. Periodic standard and background absorbance checks (TOP:019x)

B. Analysis for TFM: Battery power models

- 1. See IOP:012.x, IOP:012.xC, IOP:012.xD, or IOP:012.xG for instrument operating procedures.
- 2. Basic theory and procedures for the sampling and analysis of water samples are the same as for the A/C powered spectrophotometers.

V. Adjustment of application rates

A. Primary applications

- 1. Primary applications have no lampricide upstream of the application point, so a single analysis site is required to determine the concentration of lampricide that is being added to the stream.
- 2. During the first several sampling collections at a site, a spread of two or more samples is collected across the stream at the analysis point to confirm that the mixture of lampricide and water is homogeneous. If the concentrations are within 10 percent, a mean concentration or weighted mean concentration is calculated.
- 3. If the concentrations of multiple samples from a site are not within 10 percent;
 - a. An alternate site is found downstream if reasonable access is available
 - b. Application equipment is relocated to provide a better mix of lampricide
 - c. If no other options exist, more samples are collected across the stream to provide a more representative mean concentration for the site.

4. The first set of samples is collected when the lampricide is expected to be present. Subsequent samples are collected at minimal time intervals until the application rate has been adjusted and the desired concentration of TFM is being maintained.
5. After the desired concentration is reached the time between sample collections may be increased to a reasonable, workable interval (usually one hour).
6. Corrections for the application rate are calculated by proportions. A new application rate is determined by dividing the existing application rate by the concentration of TFM the rate produces, and multiplying that number by the desired concentration.

$$\text{new rate} = \frac{\text{existing rate}}{\text{existing conc.}} \times \text{desired conc.}$$

7. The new rate is given to the applicator, and the change is noted on the LAMPRICIDE ANALYSIS DATA FORM (Appendix M).
8. Additional adjustments to the application rate are not made until sufficient time has elapsed for the change in application rate to appear as a stabilized change in concentration of TFM.

B. Maintenance applications

1. Maintenance applications have lampricide both downstream and upstream of the application point, so samples are analyzed from two sites to determine the concentration of lampricide that the application is adding to the stream.
2. The upstream sampling site may be located immediately upstream of the location where lampricide is being applied. A downstream site is established according to criteria described in section III.
3. During each sample collection, water first is taken at the upstream site. After a time interval roughly equal to the flow time to the downstream site, a sample is collected at the second site. The samples are analyzed for concentration of TFM.
4. The concentration of TFM upstream of the application is subtracted from the concentration downstream. This provides the concentration added to the stream. An estimate of the relationship between volume of lampricide added and concentration added is then calculated by dividing the concentration added by the application rate. A new application rate is calculated with proportions and given to the lampricide applicator. The concentrations of lampricide at each site and any application change are recorded on the LAMPRICIDE ANALYSIS DATA FORM (Appendix M).
5. The concentration reaching the application point is usually changing, so samples are collected at a minimal time interval to provide rapid updates of application rates. The minimum interval may be limited by the time necessary to conduct

an application rate change.

VI. Documentation

A. Quality assurance steps

1. Standards: see TOP:019.x
2. Instrument: Spectrophotometer; complete entries in log book including absorbencies of standards.


B. Data records

1. Stream treatments; concentrations of lampricides and other information pertaining to the application are documented on the LAMPRICIDE ANALYSIS DATA FORM (Appendix M).
2. Toxicity tests
 - a. Concentrations of TFM are recorded on the ANALYSIS data sheet (TOP:010.x).
 - b. Mean concentrations are calculated and recorded on the TOXICITY TEST REPORT (Appendix M) data sheet (TOP:010.x).
3. Other analyses (research assistance)
 - a. Data are recorded in a convenient, usable form.
 - b. Data are retained with reports which result from or are based on the data.
 - c. Either of the forms used for analyses during stream treatments or toxicity tests may be used.

REFERENCES:

None

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 2-12-14
Field Supervisor (U.S.)

REVIEWED/APPROVED  DATE 2-12-14
Division Manager (Canada)