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TECHNICAL OPERATING PROCEDURE

PROCEDURE TITLE:

Procedures for Conducting Liquid Chromatographic Analysis for Bayluscide Active Ingredient
(Niclosamide)

APPLICABILITY:

Procedure applies to high performance liquid chromatographic (HPLC) analyses conducted in conjunction with lampricide treatments and toxicity tests. Procedure also applies to the analysis of public water intake samples for niclosamide contamination.

PRINCIPLE:

The waters of streams which receive applications of Bayluscide are analyzed to provide information for adjustment of niclosamide concentrations. Applications are controlled to assure the efficacy and safety of treatments. Concentrations of niclosamide are measured with a Waters Corporation HPLC system (IOP:015.x, IOP:015.xA). Analyses for niclosamide conducted during toxicity tests or while monitoring public water supplies utilize similar procedures.

SAMPLE COLLECTION AND PRESERVATION:

Samples of water containing niclosamide are collected by hand in glass culture tubes or by automatic water sampler (IOP:002.x and IOP:003.x). Glass bottles are used in automatic water samplers used to collect samples for niclosamide analysis. Water samples are usually analyzed immediately, but if storage is necessary the tubes and bottles are properly labeled and refrigerated. Subsequent analysis is completed as soon as possible.

EQUIPMENT REQUIRED:

Sample collection

Sampling device for collection of mid-stream water samples
ISCO brand automatic water sampler (TOP:022.x, IOP:002.x)

Analysis for niclosamide

HPLC; Waters Corporation system
Disposable glass screw-top culture tubes 16X100 mm
Syringe (250 uL) and appropriate glassware for formulation of standard (TOP:019.x)

POTENTIAL INTERFERENCES:

See IOP:015.x

SAFETY:

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

DISPOSAL:

Wastes from the HPLC are collected in a 4-L glass bottle. When filled, the bottle is capped and stored in the chemical storage locker until transported for disposal.

REAGENTS:

High performance liquid chromatography

Methanol, HPLC grade
Distilled water, HPLC grade
Acetic acid, glacial
Sodium acetate
Niclosamide standard, 100 mg/L in dimethyl formamide

PROCEDURES:

- I. Preparation of niclosamide standards: For preparation of standards used for all applications of analytical procedure see TOP:019.x or IOP:015.x.
- II. Preparation of instruments and essential equipment: HPLC, Waters system
 - A. Prepare carrier solution according to procedure in IOP:015.x.
 - B. Turn on system according to IOP:015.x
 - C. Inject a water blank to determine background absorbance.
 - D. Inject samples of standard to determine retention time and adjust flow and carrier ratio if necessary to optimize separation.
 1. Increasing the pump rate or adding methanol to the carrier will decrease the retention time for the niclosamide peak
 2. Decreasing the pump rate or adding buffer to the carrier will increase the retention time for the niclosamide peak

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 at a sufficient distance downstream from an application site to allow mixing of the chemical throughout the water column and across the stream. A sampling site should be far enough downstream to allow the active ingredient to go completely into solution. Stream morphology is a determining factor for this consideration. A second sampling site should be selected downstream, if possible, to confirm analysis results.
 - b. A suitable site is near enough to an application site to allow timely analysis.
 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 lampricide concentrations is needed to adjust application rates of lampricides, 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 lampricide 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 for niclosamide:

- A. Instrumental analyses; see (IOP:015.x)


- V. Adjustment of application rates
 - A. Primary applications
 - 1. Primary applications have no lampricide upstream of the application point, so a single analysis point is required to determine the concentration of lampricide that is being added to the stream.
 - 2. Calculations of new application rates are based on equations in Appendix J.
 - 3. The new rate is given to the applicator and the change is noted on the LAMPRICIDE ANALYSIS data form (Appendix M).
 - 4. 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 niclosamide.
 - B. Maintenance applications
 - 1. Maintenance applications have lampricide downstream and usually upstream of the application, so samples are analyzed from two sites to determine the concentration of lampricide that the application is adding to the stream.
 - 2. Calculations of new application rates are based on equations in Appendix J.
 - 3. The new rate is given to the applicator and the change is noted on the LAMPRICIDE ANALYSIS data form (Appendix M).
 - 4. 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 niclosamide.
 - 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: complete entry in log book
 - B. Data records
 - 1. Stream treatments; concentrations of lampricides are documented on the LAMPRICIDE ANALYSIS data form (Appendix M).
 - 2. Toxicity tests
 - a. Concentrations of niclosamide are recorded on the ANALYSIS data sheet (TOP:010.x).
 - b. Mean concentrations are calculated and recorded on the TOXICITY TEST REPORT data sheet (TOP:010.x).

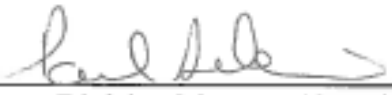
3. Other analyses (research assistance)
 - a. Data are recorded in a convenient, usable form.
 - b. Data are retained with reports which result from or are based on the data.
 - c. Either of the forms used for analyses during stream treatments or toxicity tests may be used.

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 3-24-15
Field Supervisor (U.S.)

REVIEWED/APPROVED  DATE March 25/2015
Division Manager (Canada)