JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Rapid Loss of Lampricide from Catfish and Rainbow Trout Following Routine Treatment

VERDEL K. DAWSON,* THERESA M. SCHREIER, MIKE A. BOOGAARD, NANCY J. SPANJERS, AND WILLIAM H. GINGERICH

Upper Midwest Environmental Sciences Center, Biological Resources Division, U.S. Geological Survey, 2630 Fanta Reed Road, La Crosse, Wisconsin 54603

Rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) were exposed to 3-trifluoromethyl-4-nitrophenol (TFM) and Bayluscide (niclosamide) during a sea lamprey control treatment of the Ford River, located in the upper peninsula of Michigan. Caged fish were exposed to a nominal concentration of 0.02 mg/L of niclosamide for a period of approximately 12 h. Samples of fillet tissue were collected from each fish species before treatment and at 6, 12, 18, 24, 48, 96, and 192 h following the arrival of the block of chemical at the exposure site. The fish were dissected, homogenized, extracted, and analyzed by high-performance liquid chromatography. The major residues found in the fillet tissues were TFM and niclosamide. Niclosamide concentrations were highest 12 h after arrival of the chemical block for rainbow trout (0.0395 ± 0.0251 μg/g) and 18 h after arrival of the chemical block for channel catfish (0.0465 ± 0.0212 μg/g). Residues decreased rapidly after the block of lampricide had passed and were below the detection limits in fillets of rainbow trout within 24 h and channel catfish within 96 h after the arrival of the lampricide.

KEYWORDS: Lampricide; Bayluscide; niclosamide; residues; fish

INTRODUCTION

The parasitic sea lamprey (*Petromyzon marinus*) decimated native populations of fish after gaining access to the Great Lakes through navigation projects (*2*). Niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) is a halogenated salicylanilide that has been used successfully for over 25 years by the U.S. Fish and Wildlife Service (FWS) to control sea lamprey populations in the Great Lakes. The mode of action of niclosamide has not been completely delineated, but the selective toxicity of the material toward sea lamprey larvae is probably based on the fact that niclosamide is not efficiently conjugated or eliminated by sea lamprey. This mechanism is similar to that proposed for 3-trifluoromethyl-4-nitrophenol (TFM) by Lech and Statham (*11*). Niclosamide is sold as the 2-aminoethanol salt (Bayer 73) under the commercial name Bayluscide, a formulation that is approximately 70% active ingredient by weight. In addition to being highly toxic to sea lamprey, niclosamide is also toxic to certain nontarget organisms. For this reason, niclosamide is used in combination with TFM to reduce the amount of TFM required for treatment, and in treatments as a granular bottom-release formulation to survey for lamprey ammocoetes in lentic habitats. A combination treatment with TFM is a cost-saving measure usually used when large quantities of TFM are required, such as the treatment of large streams with discharges >100 CFS. When used in combination, the TFM:niclosamide ratio ranges from 98:2 to 99.5:0.5 (*3*).

* Corresponding author [phone (608) 783-6451; fax (608) 783-6066; E-mail vcdawson@aol.com].

The need to conduct a sea lamprey control treatment is determined through stream surveys for sea lamprey larvae. Bayluscide and TFM are metered into a stream simultaneously but independently, in amounts sufficient to achieve the minimum lethal concentration (MLC) for 9 h at the most downstream reach of the segment to be treated. This may require an application of 12 h at the application site (*9*). The application results in a "block" of lampricides that moves downstream. The concentrations of both lampricides in a treatment block are monitored periodically during the treatment, and adjustments are made to maintain the desired concentrations.

It has been shown that the toxicity of the lampricide TFM is dependent on the chemical and physical properties of water (*2*). Le Maire (*17*) first identified pH as one of the factors affecting the toxicity of TFM to rainbow trout and sea lampreys. Laboratory studies showed that the toxicity of the lampricide to both species was significantly reduced at alkaline pHs. Marking and Olson (*12*) reported that the lethal concentration of TFM to fingerling rainbow trout (*Oncorhynchus mykiss*) decreased as the pH of the treatment water decreased. The resulting correlation between pH and toxicity suggests that the bioavailable form of TFM is the lipid-soluble free phenol. This observation is also supported by the findings of Hunn and Allen (*8*), who reported that residues of TFM in channel catfish (*Ictalurus punctatus*) muscle tissue decreased with increasing pH. TFM has an ionizable proton with a pK_a of approximately 6.07 (*15*). When TFM is added to water, an equilibrium between the protonated and unprotonated forms is rapidly established.

10.1021/jf020443h
Published on Web 10/04/2002

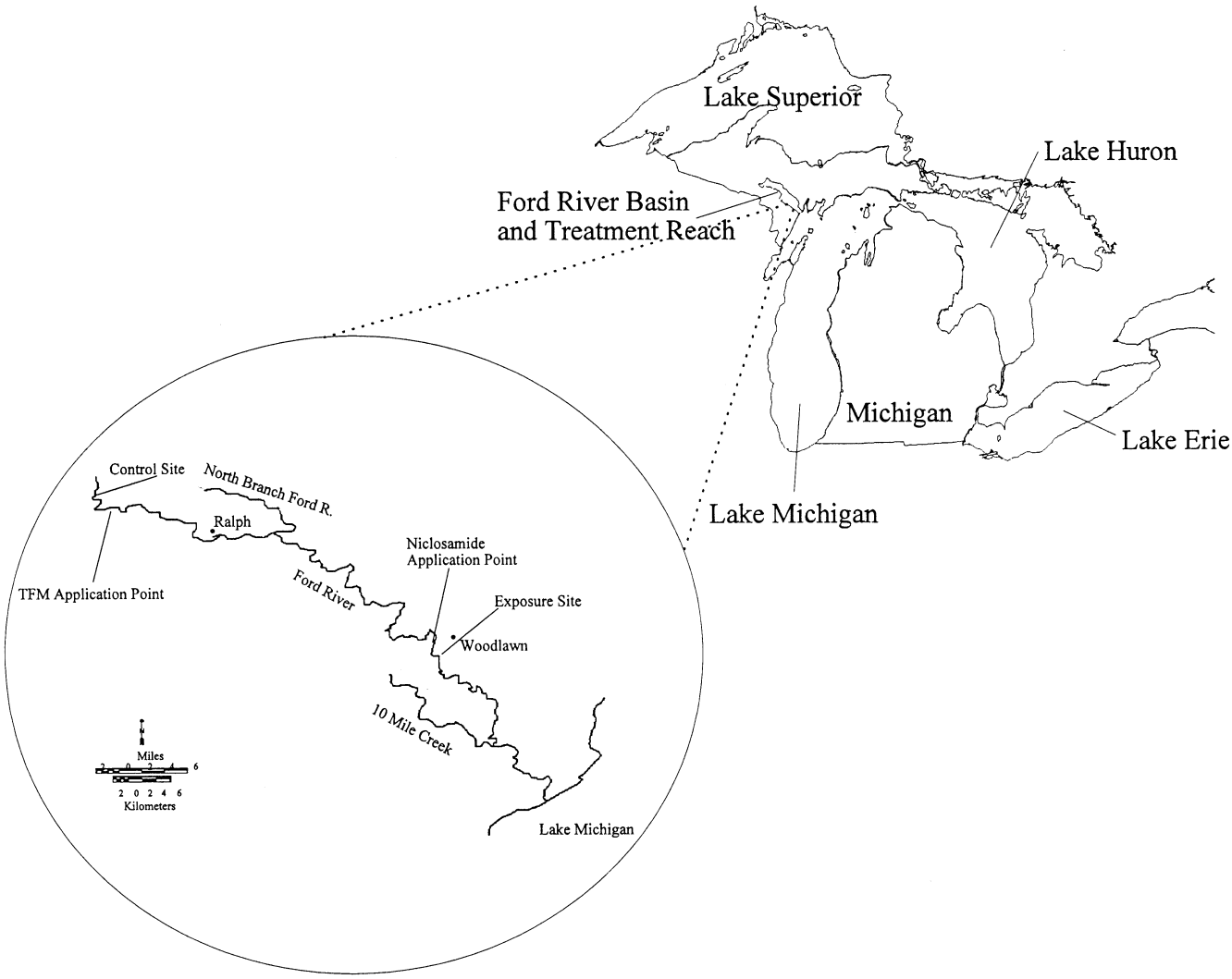

Figure 1. Map of Michigan, showing the location of the Ford River with a detailed map showing the treatment application and fish exposure sites.

Niclosamide also has an ionizable proton with a pK_a of approximately 6.25, and therefore a similar result is expected. The equilibrium concentrations of ionized and un-ionized forms depends on the pH of the water but is independent of the form of niclosamide added. Thus, there is no difference in the toxicity or efficacy of niclosamide to the lamprey between the free base and the 2-aminoethanol salt; the bioavailability of niclosamide is the same. For this study, the 2-aminoethanol salt (Bayluscide, 70% wettable powder) was used as the test material.

The study was intended to meet the objectives of fish residue studies in the residue chemistry guidelines of the U.S. Environmental Protection Agency (EPA) so that the data could be submitted to the EPA to support reregistration (*16*). The study was designed (1) to determine the concentration of niclosamide and potential niclosamide metabolites in rainbow trout and channel catfish exposed to Bayluscide under field conditions and (2) to show the effect of time on residue levels.

MATERIALS AND METHODS

Test and Reference Substances. The test material (70% wettable powder formulation of 2′,5-dichloro-4′-nitrosalicylanilide ethanolamine salt) was supplied by Bayer Corp., Kansas City, MO. Niclosamide analytical standard (Lot No. 82H0011) was purchased from Sigma Chemical Co. The sulfate ester of niclosamide (Lot No. DS-20809-4) was synthesized for Upper Midwest Environmental Sciences Center (UMESC) by Derse and Schroeder Associates, Ltd., Madison, WI. The structure of the sulfate ester was confirmed by mass spectrometry and infrared and UV spectroscopy. The purity reported by Sigma Chemical Co. for the niclosamide analytical standard was 99%. The purity of the sulfate ester was reported to be 99+%. The purities of the test chemical and analytical standards were confirmed by reverse-phase high-performance liquid chromatography (HPLC). The percent active ingredient content of the Bayluscide wettable powder formulation was determined to be 74.8 ± 0.42%. For the analytical standards, HPLC analysis indicated that niclosamide had a purity of 100 ± 0.001%, and the sulfate ester had a purity of 99.5 ± 0.64%.

Rainbow trout and channel catfish were exposed to niclosamide in the laboratory to obtain glucuronide conjugate reference material. The fish were euthanized by electrocution, and the bile was extracted from the gall bladder. The major component of the bile was identified as the glucuronide conjugate of niclosamide using the β-glucuronidase hydrolysis procedure described by Lech (*10*). This reference material was used to confirm the absence of niclosamide glucuronide in the acetone extracts of rainbow trout and channel catfish fillet tissue.

Test Animals. Rainbow trout were used as a representative cold water species, and channel catfish were used as a representative warm water species. Both species are found in lamprey-producing streams. Rainbow trout (total weight 200−400 g; age about 1.5 years) were reared from eyed eggs in the UMESC fish culture facility. Channel catfish (total weight 200−400 g; age about 3 years) were obtained from Osage Catfish Farms in Pulaksi County, Osage, MO. The fish were identified to genus and species according to Eddy and Underhill (*6*).

A total of 61 fish of both species were housed in stainless steel cages in the stream; 40 were exposed to the lampricides and 21 served as

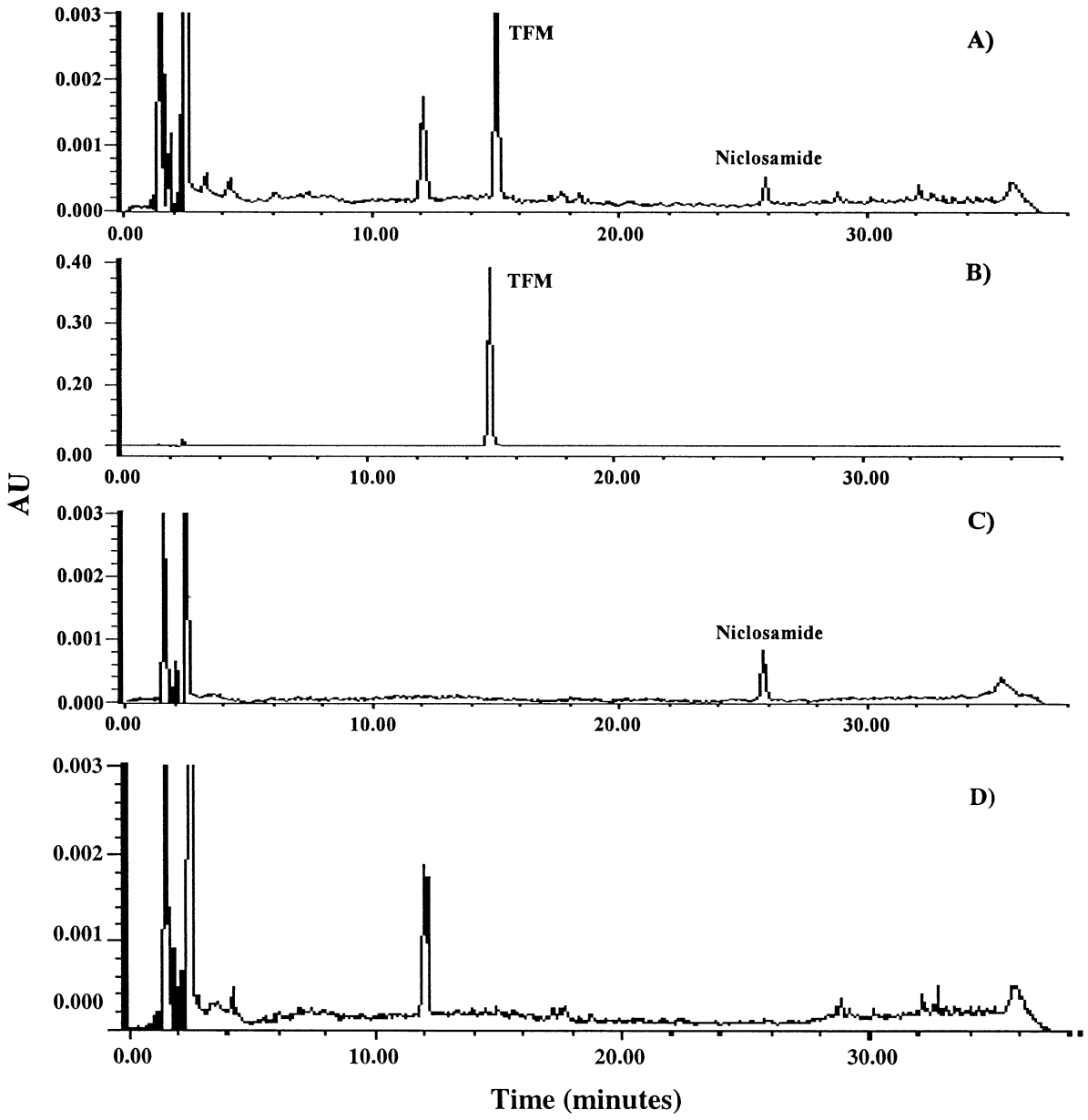

Figure 2. HPLC chromatograms of (A) acetone extract of fillet tissue taken from channel catfish exposed for 6 h to TFM and niclosamide in the Ford River, (B) TFM standard, (C) niclosamide standard, and (D) acetone extract of fillet tissue taken from 6-h control channel catfish.

controls. Fish acclimated in the river for ≥48 h before treatment. Fish designated for exposure were caged at the exposure site, located a sufficient distance downstream of the application site to ensure uniform mixing of the chemicals in the stream. Control fish were caged at the control site, upstream from the application site (**Figures 1** and **2**).

Test System. This study was conducted during a regularly scheduled treatment of the Ford River by U.S. Fish and Wildlife Service, Marquette Biological Station, Marquette, MI. The Ford River is located in the upper peninsula of Michigan (**Figure 1**). The exact location of the application site for this treatment was determined by service personnel before treatment and was based on criteria in the Sea Lamprey Control Standard Operating Procedure (*9*).

Test Exposure. Bayluscide was applied to the Ford River upstream from the bridge at Woodlawn at 0600 on June 19, 1996 (**Figure 1**). Zero hour for the exposure site was 1430 on the same day, when the first detectable levels of niclosamide were measured in the water (**Figure 1**). The entire reach of the Ford River was treated with TFM, but only the reach from Woodlawn to the mouth received the combination of lampricides.

Treatment of the Ford River was conducted in accordance with the Service Standard Operating Procedures for application of lampricides

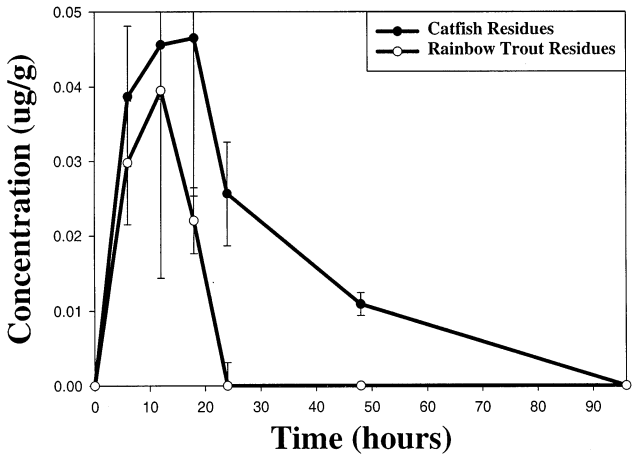

Figure 3. Mean concentration of niclosamide (μg/g) in acetone extracts from channel catfish and rainbow trout versus time (<0, 6, 12, 18, 24, 48, 96, and 192 h).

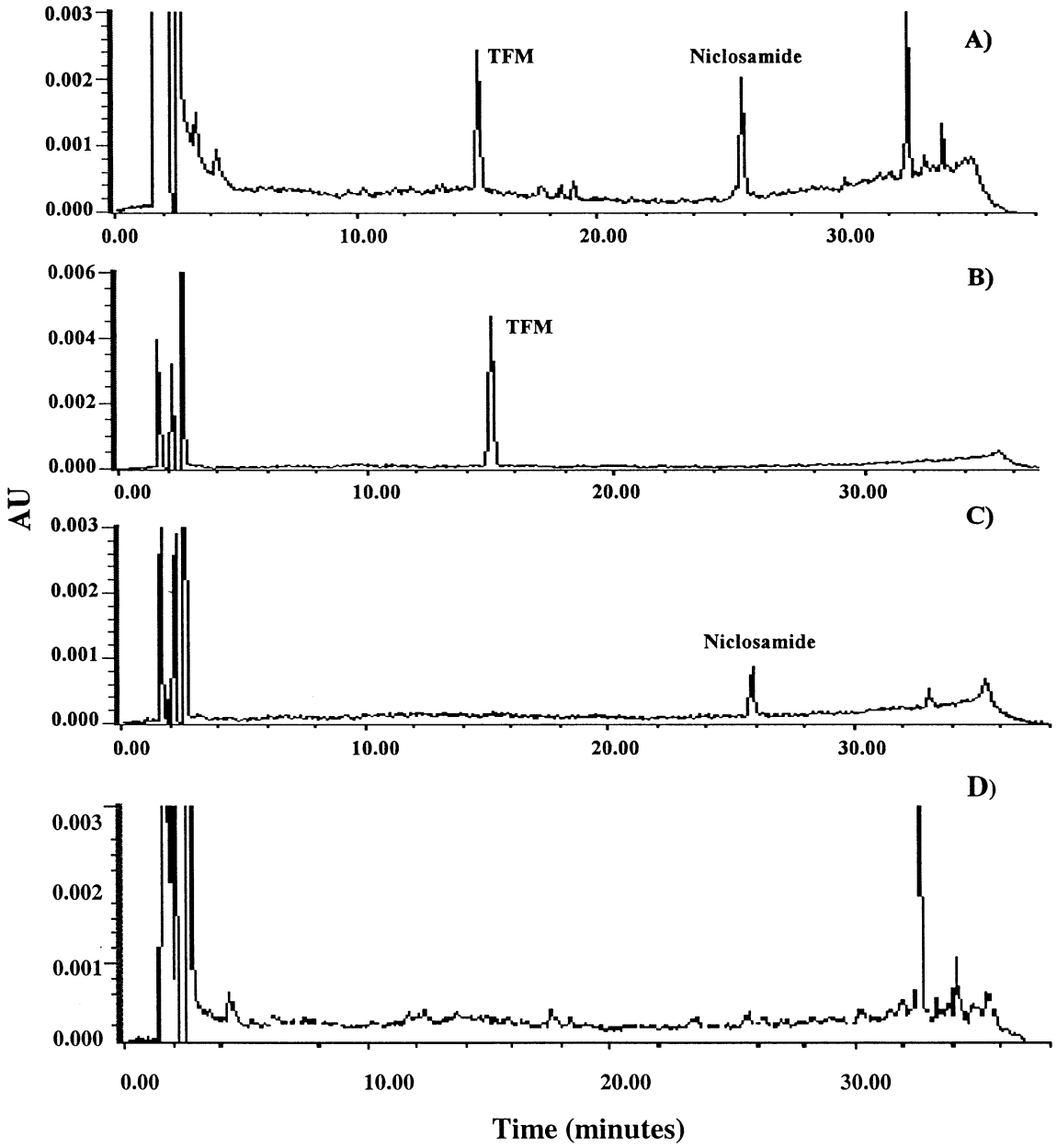

Figure 4. HPLC chromatograms of (A) acetone extract of fillet tissue taken from rainbow trout exposed for 6 h to TFM and niclosamide in the Ford River, (B) TFM standard, (C) niclosamide standard, and (D) acetone extract of fillet tissue taken from 6-h control rainbow trout.

(*9*). Service personnel were responsible for determining the appropriate concentrations of lampricides for the treatment, conducting the application of the lampricides, monitoring the lampricide concentrations in the water, and making adjustments to the concentrations when needed.

Total alkalinity and total hardness of water from the exposure site were analyzed using standardized procedures (*1*). Temperature, pH, and dissolved oxygen were monitored with a Yellow Springs Instruments water quality monitoring system.

Sample Collection. Water samples were collected in duplicate just below the surface of the water in the middle of the stream at each sampling time at the exposure site. Each sample consisted of approximately 300 mL collected into a 500-mL amber glass bottle with a screw-cap lid containing a Teflon liner.

Samples of five rainbow trout and five channel catfish were collected from the exposure site 1 day before the scheduled treatment (pretreatment) and at 6, 12, 18, 24, 48, 96, and 192 h after the lampricides reached the site. Samples of three control fish of each species were collected at the control site 6, 12, 18, 24, 48, 96, and 192 h after the lampricides reached the exposure site. The fish were rinsed with fresh water and euthanized, and the weight and length of each fish were recorded. Only the edible fillets (skin on for rainbow trout and skinless for channel catfish) were collected for analysis. Fillets from each fish were rinsed with fresh water, weighed, wrapped in aluminum foil, and placed in sealable freezer bags for storage. Fillet samples were frozen until analysis.

Sample Analysis. The niclosamide in the water samples were identified and quantified with reference standards by HPLC. For the HPLC analyses, a YMC ODS-AQ column (250 mm × 4.6 mm, 5 μm, 120 A) and a binary mobile phase consisting of 25:75 acetonitrile:58 mM sodium acetate buffer (pH 3.8) gradient with a flow rate of 1.0 mL/min were used. The gradient elution series was 25:75, 35:65, 50:50, 60:40, 80:20, and 100:0 at 0, 5, 10, 20, 25, and 30 min. The total sample run time was 38 min. A four-point standard curve was used to quantify the niclosamide concentration, and interval standards were included approximately every tenth injection for quality control.

The method detection limit for analysis of residues of niclosamide in fish fillet tissue was determined using the seven replicate method (*7*). For each sampling time (<0, 6, 12, 18, 24, 48, 96, and 192 h), three control fish and five exposed fish were sampled for each test species. Approximately 5 g of homogenized fillet tissue was sampled from the controls. Approximately 5 g of homogenized fillet tissue was

sampled from four of the exposed fish, and triplicate samples were taken from the remaining exposed fish. The homogenized tissue samples were extracted with acetone, and the residues were purified by solid-phase partitioning according to the method of Schreier et al. (*14*).

HPLC analyses of the extracts were conducted on a Phenomenex Prodigy ODS[3] reverse-phase column, 150 mm × 4.6 mm, 5 μm, 100 Å, with a YMC ODS AQ guard column. Samples were analyzed under gradient conditions using a mobile phase of acetonitrile:58 mM acetate buffer (pH 3.8). The gradient elution series was 30:70, 35:65, 50:50, 60:40, 80:20, and 100:0 at 0, 5, 10, 20, 25, and 30 min. The total sample run time was 38 min. The analyses were run at 40 °C, and the flow rate was 1.0 mL/min. A three-point standard curve was used to quantify the niclosamide concentration, and interval standards were included approximately every tenth injection for quality control. For channel catfish fillet extracts, the injection volume was 50 μL, and the detector wavelength was 335 nm. The percent recovery from fortified channel catfish tissue samples was 113 ± 10.0%, and the method detection limit was 0.0063 μg/g. The injection volume for rainbow trout fillet extracts was 100 μL, and the detector wavelength was 360 nm. The absorption peak for niclosamide is 335 nm, but extracts of rainbow trout tissue contained an interference at that wavelength. The interference was avoided at 360 nm, and niclosamide retained a strong absorbance at that wavelength. The percent recovery from fortified rainbow trout tissue samples was 77.2 ± 5.9%, and the method detection limit was 0.0107 μg/g. Statistical analyses were limited to descriptive statistics that included simple expressions of mean and standard deviation or *t* test comparisons (*13*).

Storage Stability. Fillets of rainbow trout and channel catfish were sampled 2 d before treatment to determine storage stability. Three channel catfish fillets and three rainbow trout fillets were spiked with niclosamide at a concentration of 0.5 μg/g of fillet tissue. Spiked samples were stored under the same conditions as the samples taken from exposed and control fish. The spiked fillet samples were homogenized (*4*), and one subsample of each homogenate was processed and analyzed for niclosamide concentration before and after test samples were analyzed. Recoveries of niclosamide from channel catfish fillet tissue were 88.3 ± 12.5% before the study samples were processed (5 months after fortification) and 76.0 ± 21.2% after all study samples were analyzed (7.5 months after fortification). Recoveries from rainbow trout tissues were 78.4 ± 5.1% and 68.2 ± 2.4% before and after the study samples were processed, respectively. Although recoveries tended to be lower among the fortified samples processed at the end of the analysis period than at the beginning, the differences were not statistically significant ($p > 0.05$).

RESULTS AND DISCUSSION

Analysis of Water. Niclosamide was not detected in water samples from the control site. The concentration of niclosamide in water samples at the exposure site was below the limit of detection (<0.49 μg/L) during all sampling periods except at 6 h. The mean ± SD concentration of niclosamide in water samples taken from that site at 6 h was 17.6 ± 0.04 μg/L; the peak concentration was 20 μg/L. The target concentration for niclosamide for this stream treatment was 20 μg/L.

The stream water temperature was 18.3 ± 1.5 °C. Total alkalinity (as $CaCO_3$) and total hardness (as $CaCO_3$) of water from the exposure site were 137 ± 3.8 and 144 ± 0.71 mg/L, respectively. The mean pH and dissolved oxygen of the water were 8.25 ± 0.14 and 9.1 mg/L, respectively.

Analysis of Fillet Tissue. HPLC analysis of the acetone extracts of channel catfish fillets produced three peaks at retention times of 12, 15, and 25.9 min (**Figure 2A**). The first peak was also found in the extracts of control channel catfish tissue (**Figure 2D**) and therefore did not result from exposure to niclosamide. Peaks 2 and 3 were at the same retention times and had the same spectra as TFM and niclosamide standards (**Figure 2B**,**C**). Maximum niclosamide residues in channel catfish tissue (0.0465 ± 0.0212 μg/g) were found during the 18-h sampling (**Figure 3**). Niclosamide residues were below the limit of detection (<0.0063 μg/g) in the 96- and 192-h channel catfish samples. Results of analyses of channel catfish fillet tissue at times 0−192 h are given in **Figure 4**.

HPLC analysis of the acetone extracts of rainbow trout fillet tissues produced two peaks at retention times of 15 (TFM) and 25.9 min (niclosamide) (**Figure 4**). Maximum niclosamide residues in rainbow trout tissue (0.0395 ± 0.0251 μg/g) were found during the 12-h sampling (**Figure 4**). Niclosamide residues were below the limit of detection (<0.0107 μg/g) in the 24-, 48-, 96-, and 192-h rainbow trout samples. Results of analyses of rainbow trout fillets at times 0−192 h are given in **Figure 3**. In general, concentrations of niclosamide were greater and tended to persist longer in channel catfish than in rainbow trout. The glucuronide conjugate and sulfate ester metabolites of niclosamide observed in previous laboratory exposures to greater concentrations of niclosamide (*5*) were, in this study, not detected in any samples.

Residues decreased rapidly after the lampricides had passed the exposure site and were below the limit of detection in rainbow trout and channel catfish fillets within 24 and 96 h after the block of lampricide reached the exposure site. Niclosamide was the only detectible residue resulting from exposures to Bayluscide.

Use of Bayluscide as a lampricide to treat streams tributary to the Great Lakes is regulated in the United States by the EPA and in Canada by Health Canada. Both agencies require that registrations of chemicals be maintained and updated on a continuing basis to ensure their safety to humans and the environment. Residue data from this study are used by these regulatory agencies to evaluate persistence of residues and safety of the use of Bayluscide as a lampricide. Because residues of niclosamide in aquatic organisms are rapidly eliminated once a stream treatment is complete, there is little concern that niclosamide will bioaccumulate in the food chain.

ABBREVIATIONS USED

TFM, 3-trifluoromethyl-4-nitrophenol; HPLC, high-performance liquid chromatography; MLC, minimum lethal concentration; UMESC, Upper Midwest Environmental Sciences Center; FWS, U.S. Fish and Wildlife Service.

ACKNOWLEDGMENT

We are grateful to Mark L. Hanson, Christine T. Myers, Margie M. Savoldelli, Lisa Coplan, and Dennis M. Wasley for their assistance with collection and analysis of study samples.

LITERATURE CITED

(1) American Public Health Association (APHA). *Standard methods for the examination of water and wastewater*, 14th ed.; American Water Works Association, and Water Pollution Control Federation: Washington, DC, 1976.

(2) Applegate, V.; Howell, J.; Moffett, J.; Johnson, B.; Smith, M. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide. Technical Report No. 1; Great Lakes Fishery Commission: Ann Arbor, MI, 1961; pp 1−35.

(3) Associate Committee on Scientific Criteria for Environmental Quality. TFM and Bayer 73: lampricides in the aquatic environment. NRCC No. 22488; National Research Council Canada: Ottawa, Canada, 1985.

(4) Benville, P.; Tindle R. Dry ice homogenization procedure for fish samples in pesticide residue analysis. *J. Agric. Food Chem.* **1970**, *18*, 948−949.

(5) Dawson, V.; Schreier, T.; Boogaard, M.; Gingerich, W. Uptake, metabolism, and elimination of niclosamide by fish. In *Xenobiotics in Fish*; Smith, D., Gingerich, W., Beconi-Barker, M., Eds.; Plenum: New York, 1999; pp 167−176.
(6) Eddy, S.; Underhill J. *How to know the freshwater fishes*, 3rd ed.; Wm. C. Brown Co. Publishers: Dubuque, IA, 1978; 215 pp.
(7) Definition and procedure for the determination of the method detection limit. *Code of Federal Regulations*, Part 136; Appendix B; U.S. GPO: Washington, DC, 1984; *Fed. Regist.* **1984**, *49* (No. 209), 198−199.
(8) Hunn, J.; Allen, J. Movement of drugs across the gills of fishes. *Annu. Rev. Pharmacol*. **1974**, *14*, 47−55.
(9) Klar, G.; Johnson, D.; Morse, T. Standard operating procedures for the application of lampricides in the U.S. Fish and Wildlife Service sea lamprey (*Petromyzon marinus*) control program. Study Reference Number SCL 92-001.2. U.S. Fish and Wildlife Service, Marquette Biological Station: Marquette, MI, 1993.
(10) Lech, J. Isolation and identification of 3-trifluoromethly-4-nitrophenol glucuronide from bile of rainbow trout exposed to 3-trifluoromethyl-4-nitrophenol. *Toxicol. Appl. Pharmacol*. **1973**, *24*, 114−124.
(11) Lech, J.; Statham C. Role of glucuronide formation in the selective toxicity of 3-trifluoro methyl-4-nitrophenol (TFM) for the sea lamprey: comparative aspects of TFM uptake and conjugation in sea lamprey and rainbow trout. *Toxicol. Appl. Pharmacol.* **1975**, *31*, 150−158.
(12) Marking, L.; Olson, L. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to nontarget fish in static tests. *U.S. Fish Wild. Serv. Invest. Fish Control* **1975**, 60.
(13) SAS. *SAS/STAT guide for personal computers*, Version 6; SAS Institute, Inc.: Cary, NC, 1987.
(14) Schreier, T.; Dawson, V.; Choi, Y.; Spanjers, N.; Boogaard, M. Determination of niclosamide residues in rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) fillet tissue by high-performance liquid chromatography. *J. Agric. Food Chem*. **2000**, *48*, 2212−2215.
(15) Smith, M.; Applegate, V.; Johnson, B. Colorimetric determination of halogenated nitrophenols added to streams as sea lamprey larvicides. *Anal. Chem.* **1960**, *32*, 1670−1675.
(16) United States Environmental Protection Agency. Pesticide Assessment Guidelines, Subdivision O: Residue Chemistry. PB83-153981; U.S. Environmental Protection Agency, Washington, DC, 1982; 25 pp.
(17) Le Maire, E. Experiments to determine the effect of pH on the biological activity of two chemicals toxic to ammocoetes. Biological Report Series No. 690; Fisheries Research Board of Canada: Ottawa, 1961; 3 pp.

Received for review April 16, 2002. Revised manuscript received August 17, 2002. Accepted August 24, 2002. This study was performed with financial assistance from the U.S. State Department and the Great Lakes Fishery Commission.

JF020443H