TOXICITY OF 2', 5-DICHLORO-4'-NITROSALICYLANILIDE (BAYER 73) TO THREE GENERA OF LARVAL LAMPREYS

EFFECT OF pH ON THE TOXICITY OF TFM TO SEA LAMPREY LARVAE AND NONTARGET SPECIES DURING A STREAM TREATMENT

EFFECTS OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL ON DISSOLVED OXYGEN IN AQUATIC SYSTEMS

Great Lakes Fishery Commission

TECHNICAL REPORT 57

The Great Lakes Fishery Commission was established by the Convention on Great Lakes Fisheries between Canada and the United States, which was ratified on October 11, 1955. It was organized in April 1956 and assumed its duties as set forth in the Convention on July 1, 1956. The Commission has two major responsibilities: first, develop coordinated programs of research in the Great Lakes, and, on the basis of the findings, recommend measures which will permit the maximum sustained productivity of stocks of fish of common concern; second, formulate and implement a program to eradicate or minimize sea lamprey populations in the Great Lakes.

The Commission is also required to publish or authorize the publication of scientific or other information obtained in the performance of its duties. In fulfillment of this requirement the Commission publishes the Technical Report Series, intended for peer-reviewed scientific literature; Special Publications, designed primarily for dissemination of reports produced by working committees of the Commission; and other (non-serial) publications. Technical Reports are most suitable for either interdisciplinary review and synthesis papers of general interest to Great Lakes fisheries researchers, managers, and administrators or more narrowly focused material with special relevance to a single but important aspect of the Commission's program. Special Publications, being working documents, may evolve with the findings of and charges to a particular committee. Both publications follow the style of the *Canadian Journal of Fisheries and Aquatic Sciences.* Sponsorship of Technical Reports or Special Publications does not necessarily imply that the findings or conclusions contained therein are endorsed by the Commission.

October 1992

TOXICITY OF 2', 5-DICHLORO-4'-NITROSALICYLANILIDE (BAYER 73) TO THREE GENERA OF LARVAL LAMPREYS

Ronald J. Scholefield and James G. Seelye
U.S. Fish and Wildlife Service
Hammond Bay Biological Station, 11188 Ray Road
Millersburg, MI 49759

EFFECT OF pH ON THE TOXICITY OF TFM TO SEA LAMPREY LARVAE AND NONTARGET SPECIES DURING A STREAM TREATMENT

Terry D. Bills
U.S. Fish and Wildlife Service
National Fisheries Research Center--La Crosse
P. 0. Box 818
La Crosse, WI 54602

David A. Johnson
U.S. Fish and Wildlife Service
Marquette Biological Station
446 E. Crescent Street
Marquette, MI 49855

EFFECT OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL ON DISSOLVED OXYGEN IN AQUATIC SYSTEMS

Verdel K. Dawson
U.S. Fish and Wildlife Service
National Fisheries Research Center--La Crosse
P. 0. Box 818
La Crosse, WI 54602

David A. Johnson
U.S. Fish and Wildlife Service
Marquette Biological Station
446 E. Crescent St.
Marquette, MI 49855

John F. Sullivan
Wisconsin Department of Natural Resources
3550 Morman Coulee Road
La Crosse, WI 54601

TECHNICAL REPORT 57

Great Lakes Fishery Commission
2100 Commonwealth Blvd., Suite 209
Ann Arbor, MI 48105-1563

October 1992

TABLE OF CONTENTS

TOXICITY OF 2', 5-DICHLORO-4'-NITROSALICYLANILIDE (BAYER 73) TO THREE GENERA OF LARVAL LAMPREYS

Ronald J. Scholefield and James G. Seelye
U.S. Fish and Wildlife Service
Hammond Bay Biological Station
11188 Ray Road
Millersburg, MI 49759

ABSTRACT. Toxicity of 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) was determined for each of three genera of free-swimming larval lamprey *(Ichthyomyzon, Lampetra,* and *Petromyzon)* by conducting a series of static toxicity tests in 12°C Lake Huron water. Although the LC99.9 value of Bayer 73 for *Ichthyomyzon (70* μg/L) was significantly greater ($P<0.05$) than for *Lampetra* (49 μg/L) and *Petromyzon* (52 μg/L), there was no significant difference for the LC50 values among the three genera *(Ichthyomyzon 36* μg/L, *Lampetra* 33 μg/L, and *Petromyzon* 31 μg/L). Because of the small differences in the toxicity of Bayer 73 to these organisms, routine assessment or treatment of lentic areas for larval sea lampreys *(Petromyzon marinus)* will probably not cause any differential mortality of the other two genera of lampreys.

INTRODUCTION

The chemical 3-trifluoromethyl-4-nitrophenol (TFM) has been used to kill larval sea lampreys *(Petromyzon marinus)* in tributaries of the Great Lakes since 1958 (Applegate et al. 1961). A formulation of the ethanolamine salt of 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) was developed as a synergist to reduce the amount of the more expensive TFM needed during a stream treatment to control larval sea lampreys (Howell et al. 1964). Bayer 73 coated on sand granules (granular Bayer 73) was evaluated as a bottom-release toxicant and is regularly used for assessment of larval sea lamprey populations in lentic environments (Manion 1969). During the 1980s, granular Bayer 73 was used to kill sea lampreys in a large lentic area of Seneca Lake (Ho and Gloss 1987). Similar treatments with granular Bayer 73 are proposed for waters of Lake Champlain in New York where lampreys other than sea lampreys are likely to be exposed. Therefore, the toxicity of Bayer 73 to various genera of lampreys needs to be evaluated.

The toxicities of Bayer 73, TFM, and mixtures of Bayer 73 and TFM to larval sea lampreys and nontarget organisms have been studied extensively (Marking and Hogan 1967; Bills and Marking 1976; Gilderhus 1979; and Seelye et al. 1988). King and Gable (1985) determined that, among three species of lampreys, TFM toxicity was highest for sea lamprey, intermediate for northern brook lamprey *(Ichthyomyzon fossor),* and lowest for American brook lamprey

(Lampetra appendix). However, the toxicity of Bayer 73 to native lampreys of the Great Lakes has not been investigated. The National Research Council of Canada scientific panel on TFM and Bayer 73 recommended that the toxicity of Bayer 73 to native lampreys and sea lampreys be investigated (National Research Council of Canada 1985). The objective of this study was to determine the toxicity of Bayer 73 to three genera of larval lampreys found in the Great Lakes.

METHODS

Bayer 73 (70% active ingredient, wettable powder formulation) was obtained from Mobay Chemical Corporation, Kansas City, Missouri. A 1,000 mg/L Bayer 73 stock solution was prepared by dissolving 0.3571 g of 70% active Bayer 73 in dimethylfonnamide (DMF) and diluting to 250 mL with DMF. A 150 mg/L Bayer 73 working solution was prepared by diluting 15.0 mL of the 1,000 mg/L stock solution to 100 mL with DMF. Concentrations of Bayer 73 used in the tests ranged from 7-66 µg/L and were based on the percent active ingredient. Six concentrations of Bayer 73 were used in each test and the concentrations in each aquarium were verified with the method of Scholefield (1987). Each test was repeated four times on dates given in Table 1. Larval lampreys were collected from streams with electroshockers and returned to the laboratory to be sorted by genus or species. *Ichthyomyzon* spp. and American brook lampreys were collected from the upper Black River system of Cheboygan, Montmorency, and Presque Isle Counties, Michigan. Two species of *Ichthyomyzon,* the northern brook lamprey and the silver lamprey *(I. unicuspis),* are found in the Black River system (Morman 1979), but they cannot be differentiated in the larval stage. Larval *Petromyzon* were collected from the Chippewa River, Isabella County, Michigan. Larval lampreys were held in flowing Lake Huron water and maintained by the method of Hanson et al. (1974) for at least 90 days before the toxicity tests were conducted.

Table 1. 9-h LC50 and LC99.9 values (95% confidence interval in parentheses) for the toxicity of Bayer 73 to three genera of larval lampreys in 12°C Lake Huron water during June and July 1988.

Date	Ichthyomyzon		Lampetra		Petromyzon	
	LC50 (µg/L)	**LC99.9 (µg/L)**	**LC50 (µg/L)**	**LC99.9 (µg/L)**	**LC50 (µg/L)**	**LC99.9 (µg/L)**
June 8	30 (26-35)	50 (38-65)	33 (30-37)	50 (41-61)	35 (30-40)	56 (43-72)
June 16	38 (32-45)	85 (55-132)	31 (27-35)	56 (43-72)	32 (28-35)	52 (40-68)
June 21	37 (32-40)	73 (55-97)	31 (28-34)	48 (40-57)	26 (22-30)	57 (37-88)
July 6	40 (35-45)	73 (55-97)	36 (34-38)	42 (39-46)	30 (28-32)	41 (36-47)
Average	**36**	**70**	**33**	**49**	**31**	**52**
Standard error	2.1	7.3	1.2	5.8	1.9	3.7

Static toxicity tests were conducted as specified by the American Society for Testing and Materials (1985). The toxicity tests were conducted at 12^0C in 19-L glass aquariums that contained 15 L of aerated Lake Huron water with pH 8.2-8.4, total alkalinity ($CaCO_3$) 78-88 mg/L, and total hardness ($CaCO_3$) 98-105 mg/L.

Ten free-swimming larval lampreys (61-157 mm) were placed in each aquarium and mortalities were recorded hourly for 9 h. The 9-h exposure corresponds to the minimum time that lampricide is maintained at or above the minimum lethal concentration in a stream treatment (Kanayama 1963). The 9-h LC50 and LC99.9 values were calculated with the method of Litchfield and Wilcoxon (1949). Analysis of variance (ANOVA) was used to compare the LC values for the three genera. When a significant difference occurred, Duncan's multiple-range test ($P<0.05$) was used to establish which genus was significantly different.

RESULTS AND DISCUSSION

The average 9-h LC99.9 values were *70* µg/L for *Ichthyomyzon, 49* µg/L for *Lampetra,* and *52* µg/L for *Petromyzon* (Table 1). ANOVA indicated the 9-h LC99.9 values among the three genera were significantly different ($P = 0.031$). Duncan's multiple-range test indicated that the 9-h LC99.9 values were greater for *Ichthyomyzon* than for either *Lampetra* or *Petromyzon.* The LC99.9 values for *Lampetra* and *Petromyzon* were not significantly different.

We also compared the LC50 values for the three genera of lampreys (Table 1). The LC50 values are inherently more precise than the LC99.9 values when both are calculated from the same data by the method of Litchfield and Wilcoxon (1949). Average 9-h LC50 values were 36 µg/L for *Ichthyomyzon, 33* µg/L for *Lampetra,* and 31 µg/L for *Petromyzon.* ANOVA indicated no significant difference ($P = 0.18$) for the 9-h LC50 values (Table 1) among the three genera.

The variation in toxicity among the three genera was less pronounced for Bayer 73 than for TFM. Our Bayer 73 study indicated a difference of only 5 µg/L for the LC50 values among the three genera of lampreys. However, Davis (1970) reported that *L. lamottei* and P. *marinus* showed significant differences in their susceptibility to TFM. In 11-h TFM exposure tests, the LC50 values for larval *L. lamottei* and *P. marinus* were approximately 2,600 µg/L and 1,400 µg/L, respectively, a difference of approximately 86%. Dawson et al. (1977) also reported significant differences in the toxicity of TFM to *L. lamottei* and P. *marinus.* In 24-h TFM exposure tests, the LC50 values for *L. lamottei* were from 61%-183% greater than for *P. marinus.* King and Gabel (1985) noted that *L. appendix* and *Z. fossor* were more resistant to TFM than *P.* marinus and recommended that only *P. marinus* be used in pretreatment TFM toxicity tests. However, if pretreatment Bayer 73 toxicity tests are conducted, data indicate that larval *Lampetra* and *Petromyzon* could be used interchangeably.

Field observations indicate that most sea lampreys leaving their burrows and swimming to the water surface after exposure to granular Bayer 73 have received a lethal dosage of toxicant (Tibbles 1967). Granular Bayer 73 surveys and treatments apply the 5% active pesticide at 110 kg/ha and yield Bayer 73 concentrations that far exceed the LC99.9 values reported here. For example, Ho and Gloss (1987) reported concentrations of Bayer 73 in Seneca Lake that ranged from 148-573 µg/L at 0.1 m above the substrate. Therefore, the efficacy of Bayer 73 treatments at these concentrations would be identical for all three genera of lampreys.

REFERENCES

American Society for Testing and Materials. 1985. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. p. 282-306. In Annual book of ASTM standards. Vol. 11.04, Philadelphia, PA.

Applegate, V. C., J. H. Howell, J. W. Moffett, B. G. H. Johnson, and M. A. Smith. 1961. Use of 3-trifluormethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 1. 35 p.

Bills, T. D., and L. L. Marking. 1976. Toxicity of 3 trifluoromethyl-4-nitrophenol (TFM), 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) and a 98:2 mixture to fingerlings of seven fish species and to eggs and fry of coho salmon. US Fish Wildl. Serv. Invest. Fish. Control 69. 9 p.

Dawson, V. K., K. B. Cummings, and P. A. Gilderhus. 1977. Efficacy of 3-trifluoromethyl-4-nitrophenol (TFM), 2', 5-dichloro-4'-nitrosalicylanilide and a 98:2 mixture as lampricides in laboratory studies. US Fish Wildl. Serv. Invest. Fish. Control 77. 11 p.

Davis, W. A. 1970. Comparative susceptibility of three genera of larval lampreys to a lampricide. M.S. thesis, Univ. Guelph, Guelph, Ont. 52 p.

Gilderhus, P. A. 1979. Effects of granular 2', 5-dichloro4'-nitrosalicylanilide (Bayer 73) on benthic macroinvertebrates in a lake environment. Great Lakes Fish. Comm. Tech. Rep. 34: l-5.

Hanson, L. H., E. L. King, Jr., J. H. Howell, and A. J. Smith. 1974. A culture method for sea lamprey larvae. Prog. Fish-Cult. 36: 122-127.

Ho, K. T. Y., and S. P. Gloss. 1987. Distribution and persistence of the lampricide Bayer 73 following a control application. Can. J. Fish. Aquat. Sci. 44: 112-119.

Howell, J. H., E. L. King, Jr., A. J. Smith, and L. H. Hanson. 1964. Synergism of 5, 2'-dichloro-4'-nitrosalicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 8. 22 p.

Kanayama, R. K. 1963. The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluormethyl-4-nitrophenol required for treating lamprey streams. Great Lakes Fish. Comm. Tech. Rep. 7: l-10.

Ring, E. L. Jr., and J. A. Gable. 1985. Comparative toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol to ammocetes of three species of lampreys. Great Lakes Fish. Comm. Tech. Rep. 47: l-5.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. Pharmacol. Exp. Ther. 96(2): 99-113.

Manion, P. J. 1969. Evaluation of lamprey larvicides in the Big Garlic River and Saux Head Lake. Fish. Res. Board Can. 26: 3077-3082.

Marking, L. L., and J. W. Hogan. 1967. Toxicity of Bayer 73 to fish. US Fish Wildl. Serv. Invest. Fish. Control 19. 13 p.

Morman, R. H. 1979. Distribution and ecology of lampreys in the lower Peninsula of Michigan, 1957-75. Great Lakes Fish. Comm. Tech. Rep. 33: l-59.

National Research Council of Canada. 1985. TFM and Bayer 73: lampricides in the aquatic environment. Environ. Sec. Publ. No. NRCC 22488. 184 p.

Scholefield, R. J. 1987. A comparison of two methods for the simultaneous determination of TFM and Bayer 73 concentrations. Great Lakes Fish. Comm. Tech. Rep. 50: l-8.

Seelye, J. G., D. A. Johnson, J. G. Weise, and E. L. Ring, Jr. 1988. Guide for determining application rates of lampricides for control of sea lamprey ammocetes. Great Lakes Fish. Comm. Tech. Rep. 52. 23 p.

Tibbles, J. J. 1967. Appendix B: Lamprey control experiment in Canada. p. 41-46. *In* 1966 Annual Report of the Great Lakes Fishery Commission, Ann Arbor, MI.

EFFECT OF pH ON THE TOXICITY OF TFM TO SEA LAMPREY LARVAE AND NONTARGET SPECIES DURING A STREAM TREATMENT

Terry D. Bills
U.S. Fish and Wildlife Service
National Fisheries Research Center--La Crosse
P.O. Box 818
La Crosse, WI 54602

David A. Johnson
U.S. Fish and Wildlife Service
Marquette Biological Station
446 E. Crescent Street
Marquette, MI 49855

ABSTRACT. Treatment of tributaries to the Great Lakes with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) occasionally results in incomplete kills of sea lamprey larvae *(Petromyzon marinus)* or excessive mortality of nontarget fish. Laboratory studies indicate that changes in pH can significantly affect the toxicity of TFM. In continuous-flow toxicity tests conducted on the Millecoquins River, Michigan, TFM remained selective for sea lamprey at the ambient stream pH and at an increased pH (raised approximately 1 unit by the addition of sodium hydroxide). At all but one concentration, TFM killed all sea lampreys and none of the target fish. Selectivity decreased when the pH was lowered by approximately 1 unit (by the addition of hydrochloric acid). TFM at the lowest tested concentration (2.3 mg/L) killed 100% of the sea lampreys, 50% of the rainbow trout *(Oncorhynchus mykiss),* and 40% of the fathead minnows *(Pimephales promelas).* When the Millecoquins River was treated at a concentration of 4.2 mg/L of TFM, all the caged sea lampreys (but none of the nontarget fishes) were killed at the ambient stream pH (8.35). Treated stream water that was diverted through stainless steel tanks killed only 55% of the sea lampreys and none of the nontarget organisms when the pH was raised to 9.23. All of the sea lampreys and nontarget organisms were killed when the pH of the treated water was lowered to 7.25. These results indicate that diurnal changes in stream pH of approximately 1 pH unit can either cause TFM to become toxic to nontarget organisms or render the treatment ineffective for killing sea lampreys. Monitoring the pH of streams scheduled for treatment with TFM and postponing treatment of streams that exhibit a significant diurnal fluctuation in pH are recommended.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is used to kill larval sea lampreys *(Petromyzon marinus)* in tributaries of the Great Lakes (Applegate et al. 1958). Increases in stream pH, conductivity, and alkalinity reduce the toxicity of TFM to sea lamprey larvae (Applegate et al. 1961) and cause the effective treatment concentration of TFM to vary among streams and seasons of the year. Howell and Marquette (1962) reported that on-site bioassays with stream water were useful for predicting the concentration that was both lethal to larval sea lampreys and safe for nontarget fishes. Kanayama (1963) correlated the toxicity of TFM with the alkalinity and conductivity of stream water and projected a curve for estimating field-treatment rates. Alkalinity has since been used as the primary factor to predict the toxicity of TFM to aquatic organisms. Currently, stream-treatment rates are determined from previous applications, alkalinity measurements, or on-site toxicity tests (Smith et al. 1974).

Several laboratory studies indicated that pH may also be a primary factor in the toxicity of TFM to nontarget fishes. Hunn and Allen (1974) observed higher residues of TFM in fish tissue at lower pH levels and postulated that an increase in pH would also increase the concentration of ionized TFM and decrease the transfer of TFM across the gill membranes. Marking and Olson (1975) showed in laboratory tests that the toxicity of TFM increased 50 times for salmonid species and 20 times for warmwater fishes as pH decreased from 9.5 to 6.5. Bills et al. (1988) demonstrated that the toxicity of TFM was affected more by pH than by alkalinity in laboratory tests with rainbow trout *(Oncorhynchus mykiss).* At a specific alkalinity, a decrease in pH of only 0.5 units significantly increased the toxicity. Despite the results of these laboratory studies, pH is generally not considered in the selection of TFM concentrations for stream treatments.

Weise (1984) reported that the pH levels of a stream may differ significantly at different locations or at a single location during a 24-h period. During a chemical treatment of South Sandy Creek, New York, pH values varied from 8.0 to 8.7 at the primary application point and from 7.9 to 9.1 at a point 10.4 km downstream. However, alkalinities at the primary application site did not change. During a treatment of the Little Salmon River, New York, pH values ranged from approximately 7.7 to 8.7 without a change in alkalinity. Although laboratory studies indicate that changes in stream pH of this magnitude would affect the toxicity of a TFM stream treatment, conditions in the natural environment might interact to decrease or enhance the toxicity of the chemical. Therefore, a field study was conducted to determine:

1) the minimum lethal concentration (MLC) of TFM for the sea lamprey (LC99) in a continuous-flow toxicity test in ambient river water,

2) the effect of an increase or decrease in pH of approximately 1 unit on the toxicity of TFM to sea lamprey and certain nontarget species during continuous-flow toxicity tests, and

3) the effect of raising and lowering pH by 1 unit on the toxicity of TFM as it passed a particular point in the stream during treatment.

MATERIALS AND METHODS

Tests were conducted on the Millecoquins River, Mackinac County, Michigan, in May 1988. Procedures closely followed those outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and American Society for Testing and Materials (1980). The continuous-flow toxicant delivery system of Garton (1980) was modified by:

1) an increase in dilution factors from 50% to 73%,

2) a reduction in aeration by elimination of the float valve on the water supply, installation of a large bore tube (7.5 cm) extending approximately 6 cm under the headbox water line, and the addition of four baffle plates in the headbox,

3) replacement of duplicate dilution tubes with single tubes,

4) substitution of plexiglass for double-strength glass in the dilution apparatus, and

5) an increase of the toxicant flow to 1 L/min/cell.

TFM (36% active ingredient) from the American Hoechst Chemical Company, Somerville, New Jersey, was used for all tests and the stream treatment. Stock solutions for high-performance liquid-chromatography (HPLC) standards and for on-site tests were prepared by diluting concentrated material with deionized water. TFM concentrations were measured hourly for the first 9 h and again at 12 h. Mean concentrations of TFM in mg/L ($\pm$ 1 SD) for the on-site tests were:

1) ambient river pH, 0 (control), 2.32 $\pm$ 0.22, 3.28 $\pm$ 0.25, 4.35 $\pm$ 0.21, 5.84 $\pm$ 0.36, 8.06 $\pm$ 0.42,

2) raised pH, 0, 2.21 $\pm$ 0.19, 3.03 $\pm$ 0.24, 4.15 $\pm$ 0.35, 5.70 $\pm$ 0.44, 7.71 $\pm$ 0.66, and

3) lowered pH, 0, 2.30 $\pm$ 0.18, 3.12 $\pm$ 0.22, 4.20 $\pm$ 0.31, 5.72 $\pm$ 0.52, and 7.75 $\pm$ 0.62.

Mean concentrations of TFM (mg/L ± 1 SD) used during the stream treatment were:

1) river, 4.24 ± 0.21,

2) raised pH, 4.21 ± 0.24, and

3) lowered pH, 4.23 ± 0.29.

Concentrations of TFM were quantified by HPLC with Dawson's (1982) analytical procedure as modified by Johnson (1982). Apparatus included the Waters model 440 detector, U6K injector, model 510 chromatography pump, Hewlett-Packard 3390A integrator, and Waters 3.9 mm x 150 mm Bondapacks C_{18} column. Samples of TFM-treated water were collected from each tank and from the river at approximately l-h intervals to ensure an accurate profile of exposure levels. Water samples were filtered through Whatman 2V filter discs (8 µm retention) prior to analysis.

Sodium hydroxide (0.5 N) or hydrochloric acid (0.5 N) was injected into the mixing box of the flow-through system with a Micromedic automatic pipette using a 1-mL pump. Approximately 200-300 µL/L of one solution were injected per minute to either raise or lower the pH of the river water by 1 pH unit. The pH of each tank and the river was monitored hourly with a Beckman pHI 21 meter.

Water hardness and total alkalinity ($CaCO_3$) were determined at approximately 6-h intervals. Water hardnesses were determined with a Hach test kit. Alkalinities were determined potentiometrically according to the procedure outlined by the American Public Health Association (1976). Temperature and dissolved oxygen were recorded continuously with a Yellow Springs Instrument Co. Model 56 monitor oxygen meter.

Test organisms included:

1) mayfly nymphs (Hexagenia spp.) from Polander Lake, Pool 6, of the upper Mississippi River,

2) sea lamprey ammocoetes (average length, 10.1 cm) from the Ford River, Michigan,

3) brook trout *(Salvelinus fontinalis)* (4.27 cm average length) from the Marquette (Michigan) State Fish Hatchery,

4) rainbow trout (7.39 cm average length) from the Thompson (Michigan) State Fish Hatchery, and

5) fathead minnows *(Pimephales promelas)* (3.19 cm average length) from the National Fisheries Research Center--La Crosse, La Crosse, Wisconsin.

Organisms were held in cages suspended in the Millecoquins River or in coolers filled with aerated river water (Tables 1 and 2).

Table 1. Water-quality characteristics (range or mean ± SD) of the Millecoquins River (R) and the adjusted test solutions (T) during continuous-flow toxicity tests with TFM.

	Ambient test type	Raised-pH test type	Lowered-pH test type
Temperature[a] (°C)	11.5-14.0	12.0-16.0	11.2-12.8
Dissolved oxygen[a] (mg/L)	10.0-11.0	9.8-10.8	--
Hardness (mg/L as $CaCO_3$)			
R	101.7 (± 0.5)	102.0 (± 2.6)	102.7 (± 1.1)
T	--	102.3 (± 2.7)	102.5 (± 0.6)
Alkalinity (mg/L as $CaCO_3$)			
R	94.3 (± 2.2)	98.3 (± 0.8)	94.7 (± 4.2)
T	--	111.7 (± 1.7)	85.1 (± 4.6)
PH			
R	8.36 (± 0.15)	8.30 (± 0.08)	8.14 (± 0.12)
T	--	9.23 (± 0.05)	7.16 (± 0.08)

[a] Continuously monitored. Values represent ranges during exposures.

Table 2. Water-quality characteristics (range or mean ± SD) of the river and of pH-altered test solutions during treatment of the Millecoquins River with the lampricide TFM.

	Source		
	River	Raised pH	Lowered pH
Temperature [a] (“C)	12.0-14.5	13.0-15.0	13.0-15-o
Dissolved oxygen [a] (mg/L)	9.0-11.0	10.0-11.0	10.0-11.0
Hardness (mg/L as $CaCO_3$)	104.7 (± 1.1)	104.0 (± 0.0)	105.7 (± 1.5)
Alkalinity (mg/L as $CaCO_3$)	94.0 (± 2.0)	105.3 (± 2.3)	82.7 (± 3.1)
PH	8.35 (± 0.15)	9.28 (± 0.06)	7.25 (± 0.08)

[a] Continuously monitored. Values represent ranges during exposures.

Nalgene tanks (53 L) containing approximately 6 cm of stream sediment were used for on-site toxicity tests of ambient pH, raised pH, and lowered pH. Stainless steel tanks containing 6 cm of stream sediment were used for adjusted pH exposures during stream treatment. Cages placed in the stream served as controls. Observations on survival and mortality were taken hourly. Lethal concentrations that would produce 25% or 99% mortality were calculated by the method of Litchfield and Wilcoxon (1949).

RESULTS

Effect of Adjusting pH on Water Characteristics

Total hardness ($CaCO_3$) Millecoquins River water ranged from 102-105 mg/L during the two-week test period (Tables 1 and 2). The addition of sodium hydroxide or hydrochloric acid did not alter water hardness. Water temperatures and dissolved oxygen levels remained relatively constant. Temperatures increased from a minimum of 12^0C in the early morning to a maximum of 16^0C in late afternoon. Dissolved oxygen remained near saturation at approximately 10 mg/L.

The average pH of the river water was 8.3 during exposures. The pH cycled daily and rose approximately 0.3 pH units by late afternoon. The lowest pH recorded in the river was 7.99 and the highest was 8.54. The mean pH for the two raised-pH tests were 9.28 and 9.23 during the treatment and continuous-flow toxicity tests, respectively, with standard deviations of 0.06 or fewer units. For the reduced-pH tests, the means were 7.16 and 7.25, respectively, with a standard deviation of 0.08 or fewer units.

Alkalinity ($CaCO_3$) of the river water ranged from 94-98 mg/L during the exposures. The addition of sodium hydroxide or hydrochloric acid changed the alkalinity by approximately 12 mg/L. During stream treatment, the mean alkalinity was:

1) 94 mg/L for the river,

2) 105.3 mg/L for the raised-pH test, and

3) 82.7 mg/L for the lowered-pH test.

Preliminary Tests

The ambient on-site toxicity test showed the selectivity of TFM for sea lampreys over nontarget organisms (Table 3). All ammocoetes were killed at the lowest concentration (2.32 mg/L) in 12 h with minimal mortality among the nontarget species. The projected minimum contact time needed for treatment of the river was 9 h and the calculated 9-h LC99 for ammocoetes was 3.5 mg/L. This concentration compared satisfactorily with the prediction chart value of 3.2 mg/L based on an alkalinity of 95 mg/L (Seelye et al. 1988).

Table 3. Mortality (%) among selected species after 12 h of exposure to TFM in continuous-flow toxicity tests in Millecoquins River water at ambient, raised, and lowered pH.

Test type and pH	Species [a]	TFM concentration (mg/L)					
Ambient		Control	2.32	3.28	4.35	5.84	8.06
8.36 (± 0.15)[b]	Sea lamprey	0	100	100	100	100	100
	Brook trout	0	0	0	0	0	10
	Rainbow trout	0	0	0	0	0	0
	Fathead minnow	0	0	0	0	0	40
Raised pH		Control	2.21	3.03	4.15	5.70	7.75
9.23 (± 0.05)	Sea lamprey	0	0	30	80	90	100
	Brook trout	0	0	0	0	0	0
	Rainbow trout	0	0	0	0	0	0
	Fathead minnow	0	0	0	0	0	0
Lowered pH		Control	2.30	3.12	4.20	5.72	7.75
7.16 (± 0.08)	Sea lamprey	0	100	100	100	100	100
	Brook trout	0	0	60	100	100	100
	Rainbow trout	0	50	100	100	100	100
	Fathead minnow	0	40	100	100	loo	100

[a] Ten organisms of each species were used in all tests except that only five rainbow trout were used in the raised-pH test.

[b] Standard deviation.

The raised-pH test with TFM was also selective for sea lamprey. All ammocoetes were killed at the highest concentration (7.75 mg/L) in 12 h with no mortality among nontarget organisms (Table 3). The calculated 9-h LC99 for ammocoetes was 6.75 mg/L. However, this concentration was nearly double the predicted MLC of 3.7 mg/L, based on an alkalinity of 110 mg/L. In this case, treatment concentrations established on prediction chart values (Seelye et al. 1988) would have led to an unsuccessful treatment.

The lowered-pH test also showed TFM was slightly selective for sea lamprey; however, nontarget organisms were adversely affected (Table 3). The lowest concentration (2.3 mg/L) killed 100% of the sea lampreys, 50% of the rainbow trout, and 40% of the fathead minnows. No mortality occurred among

the brook trout at this concentration. The calculated 9-h LC99 for ammocoetes was less than 2.3 mg/L, the lowest concentration tested. The 9-h LC25 was 2.06 mg/L for rainbow trout, 2.79 mg/L for brook trout, and 2.12 mg/L for fathead minnows. Selectivity was reduced to the point that TFM concentrations necessary to kill sea lamprey larvae (even at the MLC) would have caused significant mortality among nontarget organisms.

Stream Treatment

Characteristics of the lo-mile treated section of the Millecoquins River during treatment were:

1) conductivity of 204 micromhos/centimeter at 25°C,

2) total alkalinity of 96 mg/L,

3) pH of 8,

4) flow rate of 130 cubic feet per second,

5) hours chemical applied of 12,

6) minimum lethal time of 9 h, and

7) target concentration of TFM of 4.2 mg/L based on the predictive charts and results of the on-site toxicity test.

Analytical (HPLC) determinations of water samples showed that the target concentration of 4.2 mg/L was achieved and maintained for the desired time period. No unusual increases or decreases in TFM concentrations occurred during the treatment.

All caged sea lamprey ammocoetes in the river (control) died during the stream treatment (Table 4). No mortality occurred among any of the vertebrate nontarget organisms (brook trout, rainbow trout, or fathead minnows), but all the mayfly nymphs were killed. In contrast, in the raised-pH exposures, only 55% of the sea lampreys were killed in 12 h and no nontarget organisms (vertebrate or invertebrate) were killed. In the lowered-pH test, all the sea lamprey ammocoetes and nontarget organisms were killed in 12 h.

Table 4. Mortality (%) among selected species exposed to 4.2 mg TFM for 12 h during a lampricide treatment of the Millecoquins River at three different pH factors.

Test type and pH	Species [a]	Time in hours 1	3	6	9	12
River (control) pH 8.35 (± 0.15)[b]	Sea lamprey	0	70	95	100	100
	Brook trout	0	0	0	0	0
	Rainbow trout	0	0	0	0	0
	Fathead minnow	0	0	0	0	0
	Mayfly nymphs	--[c]	--	--	--	100
Raised pH 9.23 (± 0.05)	Sea lamprey	0	0	0	35	55
	Brook trout	0	0	0	0	0
	Rainbow trout	0	0	0	0	0
	Fathead minnow	0	0	0	0	0
	Mayfly nymphs	--[c]	--	--	--	0
Lowered pH 7.25 (± 0.08)	Sea lamprey	0	15	8.5	100	100
	Brook trout	0	95	100	100	100
	Rainbow trout	0	100	100	100	100
	Fathead minnow	0	50	100	100	100
	Mayfly nymphs	--[c]	--	--	--	100

[a] Twenty organisms of each species were used in each exposure except for mayfly nymphs in which 10 were used.

[b] Standard deviation.

[C] For mayfly nymphs, mortality was only assessed at the end of exposure.

An interesting observation was the time-to-death for the sea lamprey. It took 9 h of exposure to kill the ammocoetes in both the control and lowered-pH tests. However, all nontarget organisms that were unaffected in the control were killed in the lowered-pH test within 6 h. Most nontarget organisms were dead within 3 h of exposure.

DISCUSSION

In laboratory studies, pH has been shown to be the primary factor affecting the toxicity of TFM to fish and sea lampreys (Dawson et al. 1975; Marking and Olson 1975). Decreases in pH increased the toxicity of TFM in laboratory studies even when the alkalinity of the solution remained the same (Bills et al. 1988). Conversely, increases in pH reduce the toxicity of TFM and might result in incomplete kills of sea lamprey larvae.

Schleen (1979) reported an incomplete kill of sea lamprey larvae in Mayhew Creek, Ontario, where TFM treatment concentrations were based only on alkalinity measurements, but pH values ranged from 9.24 to 9.89. However, retreatment of the stream at a similar TFM concentration, but at a pH from 7.7 to 7.8, resulted in a successful kill of the residual sea lamprey larvae.

Although pH and alkalinity correlate well with each other, pH is more variable, primarily because of the effects of photosynthesis and respiration (Hynes 1970). Frey (1963) noted that it is common for the pH of a stream to vary by more than 1 unit during the course of a day. Our data on the Millecoquins River showed a pH fluctuation of 0.3 pH units on any given day and 0.5 pH units over several days during a two-week period. Although treatment concentrations derived from the prediction charts (Seelye et al. 1988) based on alkalinity are usually accurate, data from on-site tests showed that a decrease in pH can significantly increase the toxicity of TFM to nontarget organisms. Conversely, an increase in pH reduced the toxicity of TFM for sea lamprey larvae, but the toxicity for nontarget organisms was reduced even more. For example, during the stream treatment (4.2 mg/L of TFM with a 12-h exposure), pH tests at 7.25 (low) and 8.35 (ambient) killed all mayfly nymphs and all sea lamprey larvae. However, the test at a pH of 9.28 (high) killed 55% of the ammocoetes and no mayfly nymphs.

During stream treatment, a reduction of pH from 8.35 to 7.25 significantly increased the mortality among most nontarget organisms. Most nontarget organisms were killed within 3 h; however, a 9-h exposure was still required at both pH levels to kill 100% of the sea lampreys.

In most cases, TFM treatment concentrations can be determined from stream alkalinity measurements and on-site toxicity tests. However, pH should be monitored before a treatment to determine if the stream exhibits a significant diurnal change in pH. Changes in pH of 1 unit can (depending on the initial pH of the stream) cause TFM to become toxic to nontarget organisms or render the treatment ineffective for killing sea lampreys. It is recommended that treatments be postponed until a later time if diurnal fluctuations in pH are significant or that treatment concentrations of TFM be adjusted to compensate for pH fluctuations.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1976. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

American Society for Testing and Materials Committee E-35 on Pesticides. 1980. Standard practice for conducting toxicity tests with fishes, macroinvertebrates, and amphibians, E729-80. p. l-25. In Annual book of ASTM standards, part 4b. End use and consumer products. ASTM, Philadelphia, PA.

Applegate, V. C., J. H. Howell, and M. A. Smith. 1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science (Wash., DC) 127(3294): 336-338.

Applegate, V. C., J. H. Howell, J. W. Moffett, B. G. H. Johnson, and M. A. Smith. 1961. Use of 3-trifluormethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 1. 35 p.

Bills, T. D., L. L. Marking, G. E. Howe, and J. J. Rach. 1988. Relation of pH to toxicity of lampricide TFM in the laboratory. Great Lakes Fish. Comm. Tech. Rep. 53: 9-16.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. National Environmental Research Center, U.S. Environ. Protection Agency. EPA-660/3-75-009. 61 p.

Dawson, V. K. 1982. A rapid high-performance liquid-chromotographic method for simultaneously determining concentrations of TFM and BAYER 73 in water during lampricide treatments. Can. J. Fish. Aquat. Sci. 39: 778-782.

Dawson, V. K., K. B. Cummings, and P. A. Gilderhus. 1975. Laboratory efficacy of 3-trifluoromethyl-4-nitrophenol (TFM) as a lampricide. US Fish Wildl. Serv. Invest. Fish. Control 63. 13 p.

Frey, D. G. 1963. Wisconsin: the Birge-Juday era. p. 3-54. *In* D. G. Frey [ed.] Limnology in North America. Univ. Wisc. Press, Madison, WI.

Garton, R. R. 1980. A simple continuous-flow toxicant delivery system. Water Res. 14(3): 227-230.

Howell, J. H., and W. M. Marquette. 1962. Use of mobile bioassay equipment in the chemical control of sea lamprey. US Fish Wildl. Serv. Spec. Sci. Rep. Fish. 418. 9 p.

Hunn, J. B., and J. L. Allen. 1974. Movement of drugs across the gills of fishes. Ann. Rev. Pharmacol. 14: 47-55.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press. The Chaucer Press Ltd. Bungay, Suffolk, Great Britain. 555 p.

Johnson, D. A. 1982. Composition of sediment in a drum of TFM exposed to subfreezing temperatures. US Fish Wildl. Serv. Sea Lamprey Control Internal Rep. 82-6. 3 p.

Kanayama, R. K. 1963. The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluormethyl-4-nitrophenol required for treating lamprey streams. Great Lakes Fish. Comm. Tech. Rep. 7. 10 p.

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

Marking, L. L., and L. E. Olson. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to non-target fish in static tests. US Fish Wildl. Serv. Invest. Fish. Control 60 27 p.

Schleen, L. P. 1979. Effects of high pH's on a lampricide (TFM) treatment of Mayhew Creek, 1979. Sea Lamprey Control Tech. Rep. 3, Sea Lamprey Control Centre, Sault Ste. Marie, Ontario. 7 p.

Seelye, J. G., D. A. Johnson, J. G. Weise, and E. L. King, Jr. 1988. Guide for determining application rates of lampricides for control of sea lamprey ammocetes. Great Lakes Fish. Comm. Tech. Rep. 52. 23 p.

Smith, B. R., J. J. Tibbles, and B. G. H. Johnson. 1974. Control of the sea lamprey *(Petromyzon marinus)* in Lake Superior, 1953-70. Great Lakes Fish. Comm. Tech. Rep. 26. 60 p.

Weise, J. G. 1984. Summary of chemical use to manage sea lamprey *(Petromyzon marinus)* populations in Canada and New York State, 1958-84. Sea Lamprey Control Tech. Rep. 4, Sea Lamprey Control Centre, Sault Ste. Marie, Ontario. 84 p.

EFFECTS OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL ON DISSOLVED OXYGEN IN AQUATIC SYSTEMS

Verdel K. Dawson
U.S. Fish and Wildlife Service
National Fisheries Research Center--La Crosse
P. O. Box 818
La Crosse, WI 54602

David A. Johnson
U.S. Fish and Wildlife Service
Marquette Biological Station
446 E. Crescent St.
Marquette, MI 49855

John F. Sullivan
Wisconsin Department of Natural Resources
3550 Morman Coulee Road
La Crosse, WI 54601

ABSTRACT. The effects of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) on dissolved oxygen and other water-quality characteristics were evaluated in a series of test chambers under selected combinations of water, sediment, TFM, and exposure to sunlight. Concentrations of TFM, dissolved oxygen, ammonia, and total alkalinity, plus pH and sunlight exposure, were monitored throughout the 48-h tests. Concentrations of TFM gradually decreased over time, especially in the presence of sediment and sunlight. The lampricide did not directly cause a reduction in dissolved oxygen concentration, but appeared to inhibit photosynthetic production of oxygen during daylight. Dissolved oxygen concentrations were significantly reduced by the presence of TFM in chambers exposed to sunlight. Concentrations of total ammonia were significantly higher in chambers with sediment than in those without sediment. In chambers that contained river water and were exposed to sunlight, ammonia concentrations were low because of either oxidation by the elevated dissolved oxygen concentrations or the assimilation of nutrients by algae. The observed changes in dissolved oxygen and ammonia because of the presence of TFM were subtle, but statistically significant.

INTRODUCTION

Applegate et al. (1958) reported that 3-trifluoromethyl-4-nitrophenol (TFM) was selectively toxic to the sea lampreys *(Petromyzon marinus).* In 1964,

the compound was registered by the Pesticide Registration Division of the United States Department of Agriculture for limited use to control sea lamprey larvae in tributaries of the Great Lakes. Most treatments with TFM have been effective against sea lampreys without causing significant mortalities of nontarget fishes. A TFM treatment in May 1987 of Kelly Brook, a tributary of the Oconto River, Wisconsin, resulted in an unexpected fish kill. Dissolved oxygen (DO) concentrations were approximately 10 mg/L and total ammonia concentrations were at acceptable levels (0.2-0.3 mg/L) before the treatment. However, the DO dropped to 2.5 mg/L, and total ammonia concentrations increased to 1.5 mg/L during the treatment. According to water-quality criteria established by the United States Environmental Protection Agency (USEPA) (United States Environmental Protection Agency 1977) for freshwater aquatic life, DO concentrations should be above 5 mg/L and concentrations of unionized ammonia should be below 0.02 mg/L. Although 1.5 mg/L of total ammonia (0.04-0.1 mg/L of un-ionized ammonia) is not likely to be toxic to fish at the pH of Kelly Brook (8.2-8.4), this concentration could stress fish (United States Environmental Protection Agency 1977). Another explanation for the fish kill is that TFM might have had an adverse effect on community metabolism. Maki and Johnson (1976) reported that TFM depressed the production of DO by algal communities and increased the respiration rate. Huang and Gloyna (1968) showed that halogenated and nitrated phenols decreased chlorophyll content and suppressed DO production in *Chlorella* pyrenoidosa; however, they did not include TFM in their tests. Also, Carey and Fox (1981) indicated that DO is consumed during the photodecomposition of TFM.

We do not know if the changes in DO and ammonia concentrations observed in Kelly Brook were caused directly by the treatment or by some other factor. The purpose of the study was to evaluate whether treatment with TFM causes water-quality changes in aquatic systems. Particular emphasis was given to factors that may contribute to the depression of DO.

MATERIALS AND METHODS

Field-grade TFM (36.1% active ingredient) was obtained from Hoechst Chemical Company, Frankfurt, Germany. Dilution water used in the study was either:

1) very-hard reconstituted water (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975; American Society for Testing and Materials 1980) with total alkalinity ($CaCO_3$) 225-245 mg/L and pH 8.0-8.4, or

2) natural surface water collected from the Root River, Minnesota (total alkalinity 140-250 mg/L and pH 8.0-8.5).

These two sources were selected because they were representative of the water quality observed in Kelly Brook during the 1987 treatment (total alkalinity 225-230 mg/L and pH 8.2-8.4).

Three series of four 50-L stainless steel tanks were placed outdoors in baths of flowing water to maintain a constant temperature. One series contained reconstituted water and the other two contained Root River water (one series was covered to prevent exposure to sunlight). In each series, one tank served as a control and contained only 30 L of water. The second tank contained 15 mg/L of TFM in 30 L of water. The third contained 5 cm of sediment from the Root River, no TFM, and 30 L of water. The fourth tank contained 5 cm of sediment, 30 L of water, and TFM at 15 mg/L. Enough aeration was provided to all tanks to bring the initial DO to near saturation. Aeration was discontinued just before TFM was added at 10:30 a.m. on a sunny day in early July.

Each solution was monitored for DO and temperature with a YSI membrane electrode for two days on the following schedule: 0, 1, 2, 3, 4, 5, 6, 8, 12, 18, 20, 22, 24, 26, 28, 30, 32, 36, 44, 46, and 48 h. In addition, water samples were taken at 0, 2, 4, 6, 12, 24, and 48 h and analyzed for TFM residues with a Waters [1] High-Performance Liquid Chromatograph (Dawson 1982) and for pH, total ammonia, and total alkalinity by standard procedures (United States Environmental Protection Agency 1979; American Public Health Association 1985). Water samples were collected just after the addition of TFM and incubated at 20°C for five days to determine the biochemical oxygen demand (BOD) of each solution (American Public Health Association 1985).

Li-Cor Quantum Sensors that are responsive to photosynthetically active radiation (400-700 nm) were used to monitor the intensity of incidental sunlight at the surface and at a depth of approximately 4 cm in the Root River water control tank. An electronic data logger collected data (at lo-minute intervals with lo-second averaging) from the light sensors, a thermistor, and a DO meter.

All data collection and analyses conformed with good laboratory practices, standard quality assurance, and quality-control procedures. All data were stored in computer files and statistically analyzed for means, standard deviations, standard error of the mean, minimum and maximum values, quantiles, normal probability plots, analysis of variance, and paired t-tests, using the Statistical Analysis System software package (Statistical Analysis System 1987). Statistical significance was established at $P \leq 0.05$.

[1] Reference to trade names does not imply U.S. Government endorsement of commercial products.

RESULTS AND DISCUSSION

Initial concentrations of TFM in the test chambers were within 15% of the target concentration of 15 mg/L (Table 1). Residues declined in all chambers during the 48-h test. The reduction was greatest (19%) in chambers that contained sediment and were exposed to sunlight. Average reductions were 15% in chambers exposed to sunlight but contained no sediment, and only 2% in shaded chambers.

Table 1. Concentrations (mg/L) of TFM up to 48 h after treatment in Root River or reconstituted water, with or without bottom sediments, and covered or exposed to sunlight.

	Root River water				Reconstituted water	
	Exposed		Covered		Exposed	
Time (hours)	No sediment	With sediment	No sediment	With sediment	No sediment	With sediment
0	14.9	14.4	14.9	13.8	14.7	12.9
2	14.7	14.4	14.7	15.3	14.5	12.5
4	14.4	14.1	14.5	14.3	14.4	12.4
6	14.5	14.2	14.8	14.4	14.5	12.5
12	12.9	12.5	14.6	14.3	12.3	10.7
24	13.1	12.7	14.6	12.7	13.1	11.0
48	13.0	11.8	14.7	13.5	12.3	10.4

Water temperatures in test chambers averaged 15^0C but fluctuated diurnally, in spite of the flowing-water baths. Temperatures dropped to approximately 13^0C at night and increased to 18^0C during the day. There were no significant differences in temperatures among the test chambers, including those exposed to direct sunlight.

The DO concentrations ranged from 3.9 to more than 20 mg/L during the 48-h test (Fig. 1). Chambers that contained Root River water exposed to sunlight fluctuated diurnally and were generally supersaturated with DO (Fig. la). In general, concentrations of DO were significantly lower in TFM solutions exposed

to sunlight than in the respective control solutions. This reduction could have resulted from an interruption of photosynthesis by TFM, a halogenated, nitrated phenol (Huang and Gloyna 1968), or from oxygen consumption during photodecomposition of TFM (Carey and Fox 1981). The DO gradually decreased in chambers that were shaded from sunlight, especially in those that contained sediment (Fig. lb). Among the shaded chambers, the presence of TFM did not result in decreased concentrations of DO. In fact, among shaded chambers containing sediment, those with TFM present actually contained higher DO concentrations. This indicates that the observed decreases in DO were not the direct result of the presence of TFM but were rather caused by an interruption of photosynthesis. In chambers exposed to sunlight, the diurnal DO cycles reached significantly higher peaks when TFM was not present (Figs. la and 1c). In the chamber containing Root River water with sediment but no TFM, supersaturation of DO resulted in concentrations higher than 20 mg/L, the upper limit of the DO-meter calibration (Fig. la).

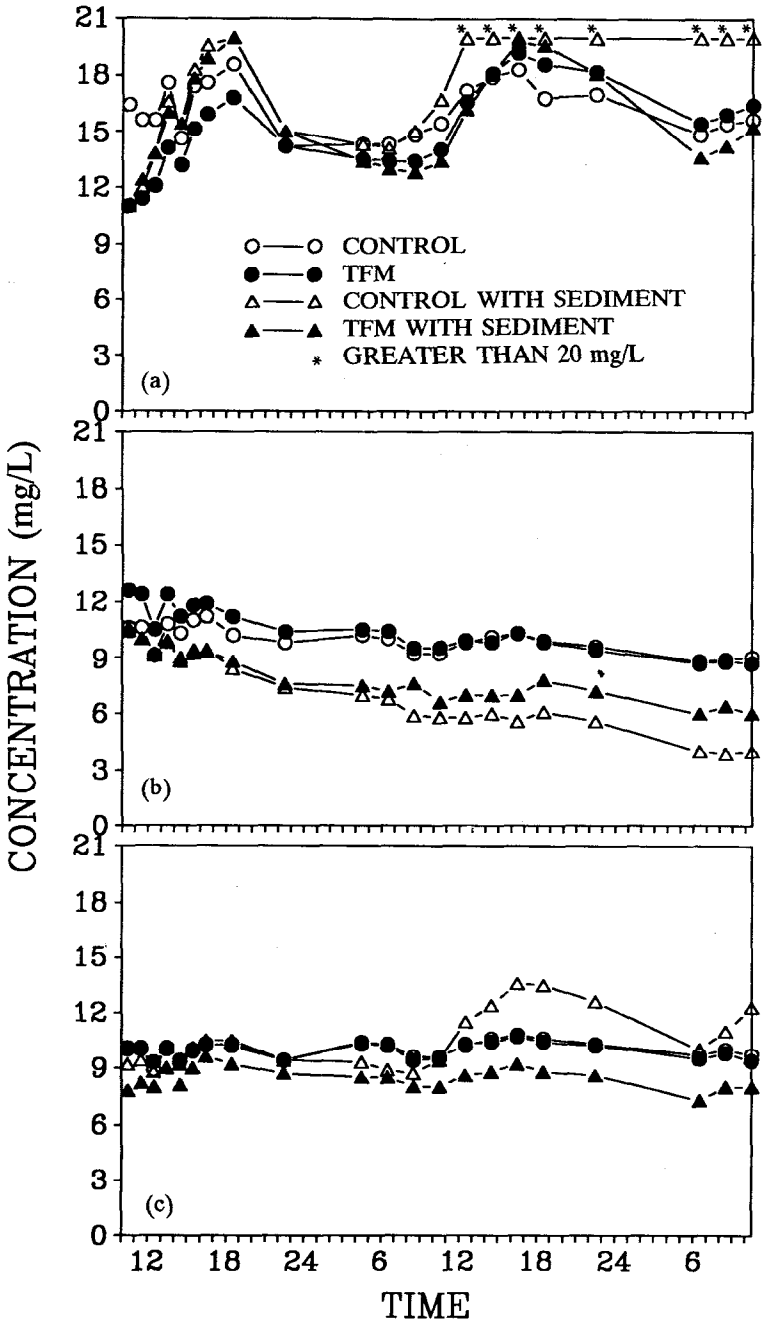

Fig 1. Effects of TFM and sediment on the concentrations of dissolved oxygen in Root River water (a) exposed to sunlight, Root River water (b) shaded from sunlight, and reconstituted water (c) exposed to sunlight.

Concentrations of DO in chambers containing less-fertile reconstituted water and no sediment were relatively stable and unaffected by the presence of TFM. A diurnal cycle of DO concentration developed during the second day in chambers that contained reconstituted water, sediment, and no TFM--probably as a result of algal seeding from the sediment (Fig. 1c).

Among the chambers that were shaded from sunlight, DO was significantly decreased in those containing sediment. However, the presence of sediment did not reduce the concentration of DO in the chambers that were exposed to sunlight. Oxygen generated through photosynthesis in test chambers exposed to sunlight apparently made up for the amount consumed by BOD in the sediment.

Concentrations of total ammonia were significantly higher in chambers that contained sediment than in those without sediment (Table 2). In Root River water exposed to sunlight, ammonia concentrations were low, possibly because of oxidation by the elevated DO or assimilation by algae. In Root River water shaded from sunlight, ammonia concentrations increased over time and reached approximately 1.3 mg/L in the chamber containing sediment and TFM (Table 2). Reconstituted water typically has little ammonia but concentrations greater than 0.5 mg/L were observed in chambers that contained sediment. Ammonia concentrations declined in the reconstituted-water chamber that contained sediment but no TFM (Table 2). The DO was elevated in this chamber.

Table 2. Concentrations (mg/L) of total ammonia in Root River or reconstituted water, with or without bottom sediments, and covered or exposed to sunlight up to 48 h after treatment with 15 mg/L of TFM.

ROOT RIVER WATER								
	Exposed				Covered			
	No sediment		With sediment		No sediment		With sediment	
Time (hours)	Control	TFM	Control	TFM	Control	TFM	Control	TFM
0	<0.050	<0.050	0.302	0.153	<0.509	<0.050	0.209	0.452
2	<0.050	<0.050	0.256	0.102	<0.050	<0.050	0.168	0.481
4	0.050	0.053	0.203	0.079	<0.050	0.052	0.197	0.461
6	0.063	0.085	0.166	0.070	<0.050	0.055	0.241	0.491
12	<0.050	<0.050	0.076	<0.050	< 0.050	0.051	0.215	0.511
24	<0.050	<0.050	<0.050	<0.050	<0.050	0.064	0.246	OS42
48	<0.050	<0.050	<0.050	<0.050	<0.050	0.157	0.770	1.294

RECONSTITUTED WATER				
	Exposed			
	No sediment		With sediment	
Time (hours)	Control	TFM	Control	TFM
0	<0.050	0.065	0.756	0.553
2	0.058	0.067	0.782	0.553
4	0.055	0.063	0.732	0.491
6	0.056	0.073	0.732	0.531
12	0.081	0.097	0.704	0.501
24	0.078	0.080	0.561	0.463
48	0.060	0.117	0.585	0.791

Values of pH were generally lower in water samples that contained TFM than in control samples, but the differences were not significant. The range of pH among all samples during the 48-h test was 8.14-8.94. Fluctuations in pH were limited by the buffering capacity (total alkalinity ($CaCO_3$) ranging from 140-230 mg/L) of the water. The presence of sediment in Root River water resulted in slightly increased alkalinity values. Alkalinity gradually decreased in all chambers during the test.

Data on sunlight exposure collected by the data logger (Fig. 2a) showed that there was substantial sunshine but occasional cloud cover on both days. The DO profile recorded by the data logger in the control chamber (Fig. 2b) was similar to those collected manually (Fig. la).

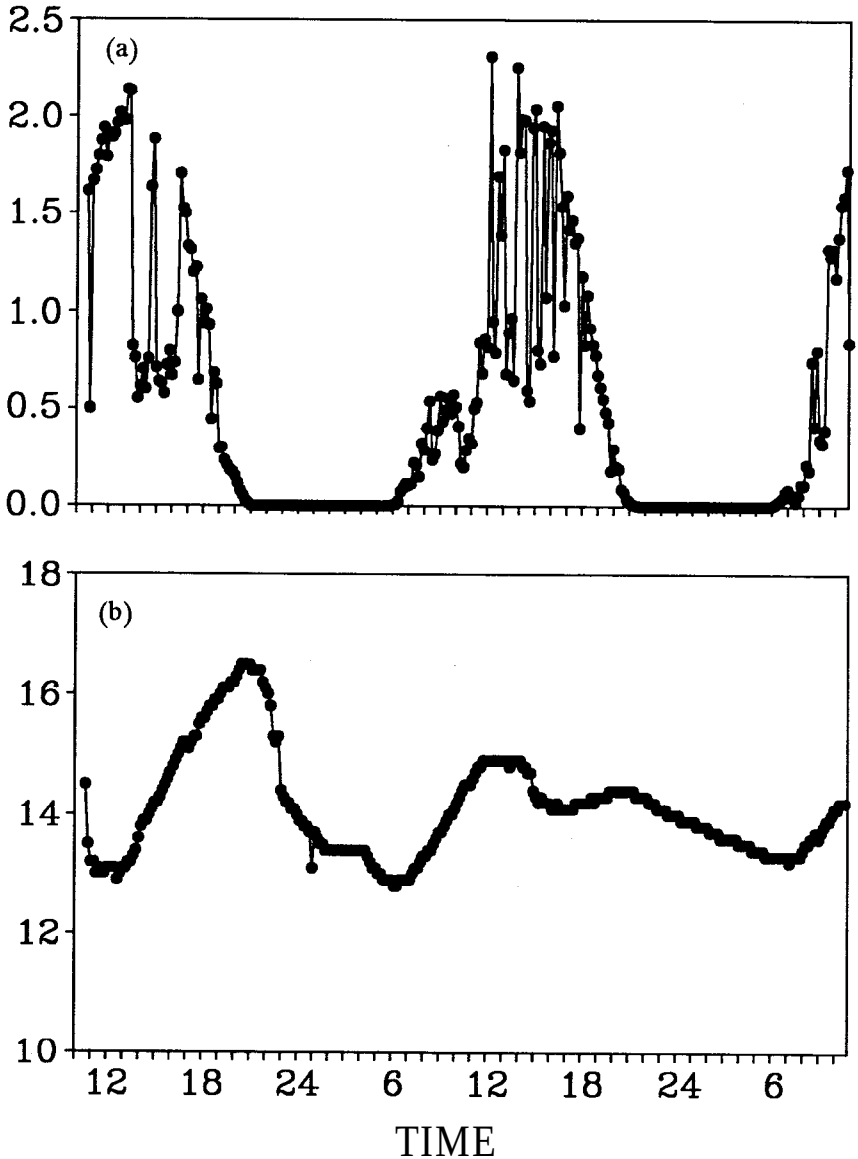

Fig. 2. Sunlight intensity (a) and diurnal concentrations of dissolved oxygen (b) in the Root River water control during the 48-h test.

Significantly higher BOD levels were obtained in Root River water than in reconstituted water. BOD was not significantly affected by the presence of sediment or TFM in either type of water (Table 3).

Table 3. BOD of Root River or reconstituted water, with and without bottom sediments, after treatment with 15 mg/L of TFM.

Water source and bottom sediments	Chemical	5-day BOD (mg/L)
Root River		
No sediment	control	6.7
No sediment	TFM	6.8
With sediment	control	5.2
With sediment	TFM	7.0
Reconstituted		
No sediment	control	2.4
No sediment	TFM	1.6
With sediment	control	2.6
With sediment	TFM	2.4

Seelye and Scholefield (1990) concluded that low DO did not in itself increase the toxicity of TFM to sea lampreys or rainbow trout (Oncorhynchus mykiss). However, interference with photosynthesis caused by TFM could have an indirect effect on nontarget fish in several ways. It could result in reduced DO during treatments of certain productive streams that lacked riffles for aeration. Lowering the DO could result in a buildup of ammonia. Ammonia is directly toxic to fish and can stress them at sublethal levels, making them more susceptible to other challenges (United States Environmental Protection Agency 1977). In water of lowered oxygen content, the toxicity is further increased because of the reduction in the concentration of excreted carbon dioxide at the gill surface (Lloyd 1961). Photosynthetic removal of carbon dioxide increases the pH of poorly buffered systems (Cole 1975). If photosynthesis is interrupted, the pH may decline because of the carbon dioxide produced by community metabolism. A decline in pH might jeopardize nontarget fish by shifting TFM to its more toxic un-ionized form (Dawson et al. 1975). The individual effects of these factors

might not be significant. In combination, however, they could result in mortalities of nontarget fish during lampricide treatments.

CONCLUSIONS

Concentrations of TFM in test chambers gradually decreased over time, especially in the presence of sunlight and sediment. The presence of TFM did not directly cause a reduction in DO; however, it might have inhibited photosynthetic production of DO during daylight hours. Concentrations of total ammonia were significantly higher in chambers with sediment than in those without sediment. Ammonia released from sediment did not build up in chambers that exhibited photosynthetic production of DO. The high buffering capacity (high alkalinity) of water from both sources resulted in relatively stable pHs. The BOD of Root River water was significantly higher than that of reconstituted water, but was not significantly affected by either the presence of sediment or TFM. The effects of TFM on the concentrations of DO and ammonia in aquatic systems were subtle, but they could have an impact on the success of lampricide treatments.

ACKNOWLEDGMENTS

We thank Ruth Davis, Linda Gardner, Tod Noltemeyer, and Julie Schreck of the National Fisheries Research Center--La Crosse, La Crosse, Wisconsin, for technical assistance.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1985. Standard methods for the examination of water and wastewater. 16th ed., Washington, DC. 1268 p.

American Society for Testing and Materials Committee E-35 on Pesticides. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians E729-80. p. l-25. In Annual Book of ASTM Standards, Part 4b. End use and consumer products. ASTM, Philadelphia, PA.

Applegate, V. C., J. H. Howell, and M. A. Smith. 1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science (Wash., DC) 127(3294): 336-338.

Carey, J. H., and M. E. Fox. 1981. Photodegradation of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). 1. Pathway of the direct photolysis in solution. J. Great Lakes Res. 7(3): 234-241.

Cole, G. A. 1975. Textbook of limnology. C. V. Mosby Co. St. Louis, MO. 283 p.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. National Environmental Research Center, U.S., Environ. Protection Agency EPA-66013-75-009. 61 p.

Dawson, V. K. 1982. A rapid high-performance liquid-chromatographic method for simultaneously determining the concentrations of TFM and Bayer 73 in water during lampricide treatments. Can. J. Fish. Aquat. Sci. 39: 778-782.

Dawson, V. K., K. B. Cummings, and P. A. Gilderhus. 1975. Laboratory efficacy of 3-trifluoromethyl-4-nitrophenol (TFM) as a lampricide. US Fish Wildl. Serv. Invest. Fish. Control 63. 13 p.

Huang, J., and E. F. Gloyna. 1968. Effect of organic compounds on photosynthetic oxygenation--I. Chlorophyll destruction and suppression of photosynthetic oxygen production. Water Res. 2: 347-366.

Lloyd, R. 1961. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdnerii* Richardson). J. Exp. Biol. 38: 447-455.

Maki, A. W., and H. E. Johnson. 1976. Evaluation of a toxicant on the metabolism of model stream communities. J. Fish. Res. Board Can. 33: 2740-2746.

Seelye, J. G., and R. J. Scholefield. 1990. Effects of changes in dissolved oxygen on the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to sea lamprey and rainbow trout. Great Lakes Fish. Comm. Tech. Rep. 56: 6-13.

Statistical Analysis System (SAS). 1987. SAS/STAT guide for personal computers. Version 6. SAS Institute, Inc., Gary, NC.

United States Environmental Protection Agency. 1977. Quality criteria for water. U.S. Government Printing Office, Washington, DC. 256 p.

1979. Methods for chemical analysis of water and wastes. Office of Technology, Environmental Monitoring and Support Laboratory, Cincinnati, OH. EPA-600/4-79-020. 460 p.

Technical Report Series

1 Use of 3-trifluormethyl-4-nitrophenol as a selective sea lamprey larvicide. May 1961. V. C. Applegate, J. H. Howell, J. W. Moffett, B. G. H. Johnson, and M. A. Smith. 36 p.

2 Fishery statistical districts of the Great Lakes. September 1961. S. H. Smith, H. J. Buettner, and R. Hile. 24 p.

3 Commercial fish production in the Great Lakes 1867-1977. September 1979. N. S. Baldwin, R. W. Saalfeld, M. A. Ross, and H. J. Buettner. 192 p. (Supersedes 1962 edition and 1970 supplement.)

4 Estimation of the brook and sea lamprey ammocoete populations of three streams. September 1962 B. R. Smith and A. L. McLain. p. l-18.

A photoelectric amplifier as a dye detector. September 1962 W. J. Ebel. p. 19-28.

5 Collection and analysis of commercial fishery statistics in the Great Lakes. December 1962. R. Hile. 34 p.

6 Limnological survey of Lake Erie 1959 and 1960. November 1963. A. M. Beeton. 34 p.

7 The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluormethyl-4-nitrophenol required for treating lamprey streams. November 1963. R. K. Kanayama. 10 p.

8 Synergism of 5, 2'-dichloro-4'-nitro-salicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. May 1964. J. H. Howell, E. L. King, Jr., A. J. Smith, and L. H. Hanson. 22 p.

9 Detection and measurement of organic lampricide residues. 1965. S. L. Daniels, L. L. Kempe, T. J. Billy, and A. M. Beeton. 18 p.

10 Experimental control of sea lampreys with electricity on the south shore of Luke Superior, 1953-60. 1965. A. L. McLain, B. R. Smith, and H. H. Moore. 48 p.

11 The relation between molecular structure and biological activity among mononitrophenols containing halogens. December 1966. V. C. Applegate, B. G. H. Johnson, and M. A. Smith. p. l-20.

Substituted nitrosalicylanilides: A new class of selectively toxic sea lamprey larvicides. December 1966. R. J. Starkey and J. H. Howell. p. 21-30.

12 Physical limnology of Saginaw Bay, Lake Huron. September 1967. A. M. Beeton, S. H. Smith, and F. F. Hooper. 62 p.

13 Population characteristics and physical condition of alewives, *Alma pseudoharengus,* in a massive dieoff in Lake Michigan, 1967. December 1968. E. H. Brown, Jr. 22 p.

14 Limnological survey of Lake Ontario, 1964 (five papers). April 1969. H. F. Allen, J. F. Reinwand, R. E. Ogawa, J. K. Hiltunen, and L. Wells. 60 p.

15 The ecology and management of the walleye in western Lake Erie. May 1969. H. A. Regier, V. C. Applegate, and R. A. Ryder, in collaboration with J. V. Manx, R. G. Ferguson, H. D. Van Meter, and D. R. Wolfert. 104 p.

16 Biology of larval sea lampreys (Petromyzon marinus) of the 1960 year class, isolated in the Big Garlic River, Michigan, 1960-1965. October 1971. P. J. Manion and A. L. McLain. 36 p.

17 New parasite records for Lake Erie fish. April 1972 A. 0. Dechtiar. 20 p.

18 Microbial degradation of the lamprey larvicide 3-trifluoromethyl-4-nitrophenol in sediment-water systems. January 1973. L. L. Kempe. 20 p.

19 Lake Superior--A case history of the lake and its fisheries. January 1973. A. H. Lawrie and J. F. Rahrer. 74 p.

20 Lake Michigan--Man's effects on native fish stocks and other biota. January 1973. L. Wells and A. L. McLain. 58 p.

21 Lake Huron--The ecology of the fish community and man's effects on it. January 1973. A. H. Berst and G. R. Spangler. 42 p.

22 Effects of exploitation, environmental changes, and new species on the fish habitats and resources of Lake Erie. April 1973. W. L. Hartman. 44 p.

23 A review of the changes in the fish species composition of Lake Ontario. January 1973. W. J. Christie. 66 p.

24 Lake Opeongo--The ecology of the fish community and of man's effects on it. March 1973. N. V. Martin and F. E. J. Fry. 34 p.

25 Some impacts of man on Kootenay Lake and its salmonoids. April 1973. T. G. Northcote. 48 p.

26 Control of the sea lamprey (Petromyzon marinus) in Lake Superior, 1953-70. March 1974. B. R. Smith, J. J. Tibbles, and B. G. H. Johnson. 60 p.

27 Movement and recapture of parasitic-phase sea lampreys (Petromyzon marinus) tagged in the St. Marys River and Lakes Huron and Michigan, 1963-67. July 1974. H. H. Moore, F. H. Dahl, and A. K. Lamsa. 20 p.

28 Changes in the lake trout population of southern Lake Superior in relation to the fishery, the sea lamprey, and stocking, 1950-70. July 1975. R. L. Pycha and G. R. King. 34 p.

29 Chemosterilization of the sea lamprey (Petromyzon marinus). July 1978. L. H. Hanson and P. J. Manion. 16 p.

30 Biology of larval and metamorphosing sea lampreys *(Petromyzon marinus)* of the 1960 year class in the Big Garlic River, Michigan, Part II, 1966-72. October 1978. P. J. Manion and B. R. Smith. 36 p.

31 Walleye stocks in the Great Lakes, 1800-1975; fluons and possible causes. February 1979. J. C. Schneider and J. H. Leach. 54 p.

32 Modeling the western Lake Erie walleye population: a feasibility study. April 1979. B. J. Shuter, J. F. Koonce and

34 Effects of granular 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) on benthic macroinvertebrates in a lake environment. May 1979. P. A. Gilderhus. p. l-5.
Efficacy of antimcin for control of larval sea lampreys (Petromyzon marinus) in lentic habitats. May 1979. P.A. Gilderhus. p. 6-18.

35 Variations in growth, age of transformation, and sex ratio of sea lampreys reestablished in chemically treated tributaries of the upper Great Lakes. May 1979. H. A. Purvis. 36 p.

36 Annotated list of the fishes of the Lake Ontario watershed. June 1979. E. J. Crossman and H. D. Van Meter. 28 p.

37 Rehabilitating Great Lakes ecosystems. December 1979. Edited by G. R. Francis, J. J. Magnuson, H. A. Regier, and D. R. Talheim. 100 p.

38 Green Bay in the future--a rehabilitative prospectus. September 1982. Edited by H. J. Harris, D. R. Talhelm, J. J. Magnuson, and A. M. Forbes. 60 p.

39 Minimum size limits for yellow perch *(Perca flavescens)* in western Lake Erie. March 1980. W. L. Hartman, S. J. Nepszy, and R. L. Scholl. 32 p.

40 Strategies for rehabilitation of lake trout in the Great Lakes: proceedings of a conference on lake trout research, August 1983, August 1984. Edited by R. L. Eshenroder, T. P. Poe, and C. H. Olver. 64 p.

41 Overfishing or pollution? Case history of a controversy on the Great Lakes. January 1985. F. N. Egerton. 28 p.

42 Movement and capture of sea lampreys (Petromyzon marinus) marked in northern Lake Huron, 1981-82 February 1985. J. W. Heinrich, W. C. Anderson, and S. D. Oja. p. l-14.
Response of spawning-phase sea lampreys (Petromyzon marinus) to a lighted trap. February 1985. H. A. Purvis, C. L. Chudy, E. L. Ring, Jr., and V. K. Dawson. p. 15-28.

43 A prospectus for the management of the Long Point ecosystem. March 1985. G. R. Francis, A. P. L. Grima, H. A. Regier, and T. H. Whillans. 112 p.

44 Population dynamics and interagency management of the bloater *(Coregonus hoyi)* in Lake Michigan, 1967-1982. March 1985. E. H. Brown, Jr., R. W. Rybicki, and R. J. Poff. 36 p.

45 Review of fish species introduced into the Great Lakes, 1819-1974. April 1985. L. Emery. 32 p.

46 Impact of sea lamprey parasitism on the blood features and hemopoietic tissues of rainbow trout. June 1985. R. E. Kinnunen and H. E. Johnson. 20 p.

47 Comparative toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol to ammocetes of three species of lampreys. August 1985. E. L. Ring, Jr. and J. A. Gabel. p. l-5.
Solid bars of 3-trifluoromethyl-4-nitrophenol: a simplified method of applying lampricide to small streams. August 1985. P. A. Gilderhus. p. 6-12.
Toxicity of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2', 5-dichloro-4'-nitrosalicyianilide (Bayer 73) to eggs and nymphs of the mayfly (Hexagenia sp.). August 1985. T. D. Bills, L. L. Marking, and J. J. Rach. p. 13-24.

48 Pathology of sea lamprey inflicted wounds on rainbow trout. December 1986. R. E. Kinnunen and H. E. Johnson. 32 p.

49 Using the lake trout as an indicator of ecosystem health--application of the dichotomous key. February 1987. T. R. Marshall, R. A. Ryder, C. J. Edwards, and G. R. Spangler. 38 p.

so A comparison of two methods for the simultaneous determination of TFM and Bayer 73 concentrations. November 1987. Ronald J. Scholefield. p. 1-8.
Elimination of **^{14}C-Bisazir** residues in adult sea lamprey (Petromyzon marinus). November 1987. J. L. Allen and V. K. Dawson. p. 9-20.

51 Parasites of fishes in the Canadian waters of the Great Lakes. April 1988. Edited by S. J. Nepszy. 106 p.

52 Guide for determining application rates of lampricides for control of sea lamprey ammocetes. May 1988. J. G. Seelye, D. A. Johnson, J. G. Weise, and E. L. Ring, Jr. 24 p.

53 Sterilizing effect of Cesium-137 irradiation on male sea lampreys released in the Big Garlic River, Michigan. October 1988. P. J. Manion, L. H. Hanson, and M. F. Fodale. p. 1-7.
Relation of pH to toxicity of lampricide TFM in the laboratory. October 1988. T. D. Bills, L. L. Marking, G. E. Howe, and J. J. Rach. p. 9-20.

54 Economics of Great Lakes Fisheries: A 1985 assessment. November 1988. D. R. Talhelm. 54 p.

55 Effects of the lampricide 3-trifluoromethyl-4-nitrophenol on macroinvertebrate populations in a small stream. May 1990. H. J. Lieffers. 28 p.

56 Resistance to 3-trifluoromethyl-4-nitrophenol (TFM) in sea lamprey. July 1990. R. J. Scholefield and J. G. Seelye. p. 1-5.
Effects of changes in dissolved oxygen on the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to sea lamprey and rainbow trout. July 1990. J. G. Seelye and R. J. Scholefield. p. 6-16.