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Great Lakes Fishery Commission

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ABSTRACT

At each of two water temperatures (7° and 17°C) and of three alkalinities (45, 90, and 180 mg/L as $CaCO_3$), the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to ammocetes was highest in the sea lamprey *(Petromyzon marinus),* intermediate in the northern brook lamprey *(Ichthyomyzon fossor),* and lowest in the American brook lamprey *(Lampetra appendix).* The exclusive use of sea lamprey ammocetes in pretreatment toxidity tests, therefore, results in selection of the lowest stream treatment concentration of TFM, which in turn reduces chemical costs and increases the margin of protection for nontarget organisms.

INTRODUCTION

The use of the selective lampricide 3-trifluoromethyl-4-nitrophenol (TFM) for treating streams to control sea lamprey *(Petromyzon marinus)* in the Great Lakes was described by Smith et al. (1974) and Smith and Tibbles (1980). Because the toxicity of TFM is strongly influenced by the chemical properties of the stream water into which it is introduced (Applegate et al. 1961), pretreatment toxicity tests are required in water from the stream to be treated (Howell and Marquette 1962). Data from these tests are used to estimate the minimum concentration of TFM required to kill 100% of the sea lamprey ammocetes and the maximum concentration that can be applied without causing significant harm to other fish.

Sea lamprey ammocetes, when readily available, were generally used in pretreatment toxicity tests during the early years of sea lamprey control; later, however, drastic reductions in the numbers of sea lamprey ammocetes after stream treatments led to the use of native species of ammocetes from untreated streams (Davis 1970). The four native species of lampreys in the Great Lakes drainage are the American brook lamprey *(Lampetra appendix),* northern brook lamprey *(Ichthyomyzon fossor),* chestnut lamprey *(Ichthyomyzon*

castaneus), and silver lamprey (Ichthyomyzon unicuspis). Laboratory tests in 1961 indicated that TFM was slightly more toxic to ammocetes of Ichthyomyzon sp. than to those of the sea lamprey or American brook lamprey (unpublished data, Hammond Bay Biological Station). The difference in toxicity was not considered significant, however, and it was believed that native and sea lamprey ammocetes could be used interchangeably in pretreatment tests. Subsequent observations of ammocete mortality during pretreatment tests indicated that the toxicity of TFM varied considerably when native ammocetes were used. The inconsistent results of tests in the field prompted Davis (1970) to conduct laboratory tests on the comparative toxicity of TFM to ammocetes of the three genera of Great Lakes lampreys. He found that TFM was considerably more toxic to ammocetes of the sea lamprey than to those of native lampreys. Ammocetes of the American brook lamprey were the most tolerant. Because these results differed significantly from those produced in 1961, we undertook a more comprehensive study to determine the acute toxicity of TFM to ammocetes of three species of Great Lakes lampreys at different water temperatures and alkalinities.

MATERIALS AND METHODS

Commercial grade TFM containing 35.7% active ingredient (free phenol equivalent) was obtained from American Hoechst Corporation and test concentrations were prepared on the basis of milligrams per liter of active ingredient.

Ammocetes were collected from streams with electroshockers. Those of American brook lamprey and Ichthyomyzon sp. were collected from the upper Black River, Cheboygan County, Michigan. Because species of the genus Ichthyomyzon cannot be identified in the ammocete stage, we may have tested more than one species of this genus. We consider this possibility unlikely, however, because only northern brook lampreys have been seen among adult Ichthyomyzon collected periodically over 25 years in the upper Black River. Sea lamprey ammocetes were collected from the Pigeon River, Cheboygan County, Michigan, and the Salmon-Trout River, Houghton County, Michigan. Ammocetes were held in flowing Lake Huron water in 189-L aquariums for at least 90 days before they were used in toxicity tests. During the acclimation period, aquariums containing ammocetes were supplied yeast as a food supplement, according to the method of Hanson et al. (1974).

Ten free-swimming ammocetes, 80-100 mm long, were used at each concentration in all tests. Tests were conducted with each of

the three species in three types of reconstituted water. The total hardness and total alkalinity, respectively, and pH of the three test waters were as follows: (1) 66 and 45, pH 7.8; (2) 130 and 90, pH 8.1; and (3) 246 and 180, pH 8.2. These water types were selected as being generally representative of natural waters in which ammocetes are found. Tests were conducted in 19-L aquaria containing 15 L of test solution. Temperature was maintained at either 7° or 17°C in water baths. Test procedures generally followed those established by the ASTM Committee E-35 on on Pesticides (1980). Test vessels were aerated to maintain dissolved oxygen near saturation. Vigorous aeration does not decrease the concentration of TFM (Applegate and King 1962). Mortality was determined after 9-h exposures to the lampricide. Morality that occurs between 5 and 12 h is used to determine the minimum lethal concentration (MLC) required to kill sea lampreys in a stream treatment (Howell and Marquette 1962; Smith et al. 1974). Cessation of branchial pulsations was the criterion used for determining death. Toxicity data were analyzed by the method of Litchfield and Wilcoxon (1949) to compute MLC 99.9 values and 95% confidence intervals.

RESULTS AND DISCUSSION

The toxicity of TFM was highest for sea lampreys, intermediate for northern brook lampreys, and lowest for American brook lampreys (Table 1). Our results thus support those of Davis (1970), who determined the same order of sensitivity among sea lampreys and native lampreys, and Dawson et al. (1975), who reported that sea lampreys were significantly more sensitive than American brook lampreys to TFM. The toxicity of TFM to ammocetes of the three species decreased as water alkalinity increased. These results corresponded to those obtained for sea lampreys in earlier TFM tests conducted in natural waters (Applegate et al. 1961; Kanayama 1963).

Temperature had little effect on the toxicity of TFM to ammocetes at 7° and 17°C (Table 1). The toxicity of TFM increased slightly for the native lampreys at the higher temperature and decreased slightly for sea lampreys, but the differences were not considered significant. The order of sensitivity among the three species exposed to TFM remained the same at both temperatures. Our results supported those of previous studies, which showed that temperature had little effect on the toxicity of TFM to lampreys (Applegate et al. 1961; Dawson et al. 1975).

Our data showed that the use of native lampreys in pretreatment toxicity tests would result in concentrations of TFM higher than needed to kill sea lampreys in stream treatments.

Compared with the MLC 99.9 values for sea lampreys, those for northern brook lampreys were 6 to 43% higher (mean 25%) and those for American brook lampreys were 6 to 79% higher (mean 44%). In sea lamprey control, the exclusive use of sea lamprey ammocetes in pretreatment toxicity tests results in lower treatment concentrations of TFM, lower treatment costs, and greater protection for nontarget organisms.

Table 1. Minimum lethal concentration (mg/L)[1] of TFM required to kill three species of lampreys at different water temperatures and alkalinities (95% confidence intervals in parentheses).

Temperatures and total alkalinity (mg/L $CaCO_3$)	Species		
	Sea lamprey	Northern brook lamprey	American brook lamprey
7°C			
45	1.4 (1.3 - 1.5)	2.0 (1.6 - 2.3)	2.5 (2.0 - 3.1)
90	3.0 (2.8 - 3.3)	4.0 (3.4 - 4.7)	5.0 (4.3 - 5.8)
180	6.4 (5.1 - 8.1)	8.4 (6.2 - 11.3)	9.8 (8.5 - 11.3)
17°C			
45	1.6 (1.4 - 1.8)	1.7 (1.3 - 2.2)	1.7 (1.4 - 2.0)
90	3.0 (2.7 - 3.3)	3.8 (2.6 - 5.4)	4.0 (3.4 - 4.7)
180	7.5 (6.6 - 8.4)	8.2 (6.4 - 10.5)	9.2 (7.5 - 11.3)

1 Minimum concentration of TFM required to kill 99.9% of test specimens during 9 h of exposure.

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SOLID BARS OF 3-TRIFLUOROMETHYL-4-NITROPHENOL: A SIMPLIFIED METHOD OF APPLYING LAMPRICIDE TO SMALL STREAMS

by

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ABSTRACT

A newly developed solid-bar formulation of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) consists of the active ingredient incorporated in a matrix of surfactant-like materials. The bars dissolve at a nearly constant rate over a period of 8 to 10 h. The rate of dissolution is affected only slightly by water temperature but somewhat more by water velocity. Field trials in three streams resulted in apparent elimination of larval sea lampreys (Petromyzon marinus). The bars offer a more efficient method than the presently used liquid formulation and metering pumps for treating small streams with TFM.

INTRODUCTION

Sea lampreys *(Petromyzon marinus)* spawn in tributary streams of the Great Lakes and the offspring live their larval life burrowed in stream bottom sediments. Sea lampreys have been controlled since 1958 by treating infested streams with the chemical 3-trifluoromethyl-4-nitrophenol (TFM). The usual procedures has been to use mechanical pumps to meter a liquid formulation of TFM into the streams for 8 to 12 h (Smith et al. 1974). Each pump requires the continuous presence of an operator, thus placing heavy demands on available manpower. In some large stream systems, the number of tributaries requiring treatment exceeds the number of personnel available to install and monitor pumps. In response to the need for an easier method for treating small streams, a solid bar formulation of TFM was developed that dissolves at a nearly constant rate over time. In this paper, I describe the development of the bar and field trials that were conducted to assess the dissolution rates and efficacy of the new formulation.

MATERIALS AND METHODS

DEVELOPMENT OF THE BAR FORMULATION

Development of the TFM bar started with three basic materials, an 80% TFM concentrate and two non-ionic surfactants, called Pluronic ® polyols[1] used as the matrix. The TFM concentrate was prepared by mixing a liquid formulation of the sodium salt of TFM (37% active ingredient) with 20% HCl at a 2:1 ratio in a separatory funnel, thereby converting the TFM, from sodium salt to free phenol which settled to the bottom. The bottom layer, consisting of TFM, dimethyl formamide, and water, was drained into pans, and concentrated by evaporation in a stream of dry air in a fume hood until the resultant material contained 80% or more of active TFM. The two specific Pluronics® (labeled F-68 and F-38), which were purchased from BASF Wyandotte Corporation have different melting points and solubilities. Testing of two matrix materials in different ratios made it possible to produce a final product with the needed solubility and gelling characteristics that included the largest amount of active ingredient that could be included in a hard, solid bar. The mixture contained Pluronics®, F-38 and F-68, and 80% TFM concentrate in a 10:8:7 ratio. The toxicity of the Pluronics was assessed in static tests according to the methods of the Committee on Methods for Acute Toxicity Tests with Aquatic Organisms (1975). Each of the Pluronics alone and the two combined in a 10:8 ratio proved to be nontoxic to rainbow trout *(Salmo gairdneri)* and fathead minnows *(Pimephales promelas)* at 1,000 mg/L.

The procedure used to produce the bar was to melt the Pluronics® at 65°C in a glass or metal container, adding the TFM concentrate, mixing gently until a homogeneous mixture was obtained, cooling to 30°C, pouring into rectangular molds, and letting the mixture solidify at room temperature for 8 h or more.

A flat, rectangular shape allowed for retention of nearly constant surface area as the bar dissolved, thereby, permitting application of TFM at a reasonably constant rate over a given period of time. Thickness governed the length of time a bar took to dissolve in water; a bar 2.5 cm thick lasted for the 8 to 10 h needed for treatment of lamprey streams.

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

The final formulation was a bar that contained 23% active ingredient and 77% inert material by weight, measured 22.9 x 15.2 cm, and weighed 0.92 kg. Under slowly flowing (0.03 m/s) conditions in the laboratory, each bar treated 0.007 m^3/s (7.1 L/s) of water at a concentration of 1 mg/L for 8 h at 18°C. At 12°C, a bar lasted about 10 h and yielded a slightly lower concentration (0.8 mg/L). Thus, under average conditions in tributary streams, the use of four bars for each 28.3 L/s (1 cfs) would provide a concentration of 0.8-1.0 mg/L over an 8 to 10 h period.

FIELD METHODS

Field trials were conducted in three small streams in the Upper Peninsula of Michigan-Five Mile and Johnson creeks in summer 1981, and Section 13 Tributary in summer 1982. About 1.6 km of each stream was treated. The streams were chosen because they harbored significant populations of larval sea lampreys and were typical (Table 1) of the streams in which the bar formulation was expected to be especially useful.

Table 1. Characteristics of streams used for field trials of a solid bar formulation of TFM.

Stream	Tributary to	Date of treatment	Discharge[a]		Water		
			Cubic meters	Liters	Temperature (C)	Alkalinity (mg/L)	pH
Five Mile Creek	Lake Superior	August 1981	0.07	67.9	14-17	12	6.4
Johnson Creek	Lake Michigan	August 1981	0.008	8.5	10-15	105	8.1
Section 13 Tributary	Sturgeon River	June 1982	0.03	22.6	12-15	80	7.7

[a]At application point.

Each trial was preceded by on-site toxicity tests in water from the stream, conducted according to methods of Howell and Marquette (1962). The toxicity tests defined the minimum lethal concentrations for lamprey ammocetes and the maximum safe concentrations for salmonid fishes. In each field trial, the concentration selected for application was 66 to 100% higher than the indicated minimum, to compensate for dilution and dispersion as the chemical moved downstream. The volume of water flow in each stream was determined within the day preceding each application. Velocities were measured with Price-type mechanical current

meters. During chemical applications, water samples were collected just downstream from the application points and near the mouth of each stream. TFM concentrations were measured with a Klett-Summerson colorimenter. In the streams, each bar was placed on a small rack made of 0.64 cm mesh hardware cloth. The rack held the bottom of the bar about 2.5 cm above the bottom of the stream to permit water movement across all surfaces of the bar.

STREAM TREATMENTS

FIVE MILE CREEK

Five Mile Creek contained very soft acidic water and due to the low pH, the minimum effective and maximum allowable concentrations of TFM were unusually low (0.75 and 2.0 mg/L). The selected application of 1.25 mg/L for 8 h was intended to maintain 1.0 mg/L for 4 h, even at the mouth of the stream. The 12 bars needed to treat the flow with 1.25 mg/L were placed in the stream where the velocity was relatively fast (0.37 m/s) to ensure rapid mixing of the TFM in the stream water.

The concentration of TFM just below the application point was 2.2 mg/L for the first hour, indicating that the fast current was dissolving the bars more rapidly than expected. After the first hour, three bars were removed to lower the concentration and thus, maintain a safety margin for fish. The treatment resulted in concentrations of TFM that exceeded 1 mg/L for about 4 h at the application point and the mouth, effective treatment rates at both points (Fig. 3.).

JOHNSON CREEK

Johnson Creek was a hardwater, alkaline stream which required higher concentrations of TFM than those used in Five Mile Creek. The minimum effective and maximum allowable concentrations were 3 and 8 mg/L, respectively. The selected application rate of 5 mg/L for 10 h was intended to maintain 4 mg/L for 7 h throughout the stream. Six bars were placed in the stream where the current velocity was 0.09 m/s. The concentration/time relations observed in the treatment were very close to those predicted; the bars dissolved in about 10 h and a concentration of 4 mg/L or more was maintained for 8 h at the mouth of the stream (Fig. 1).

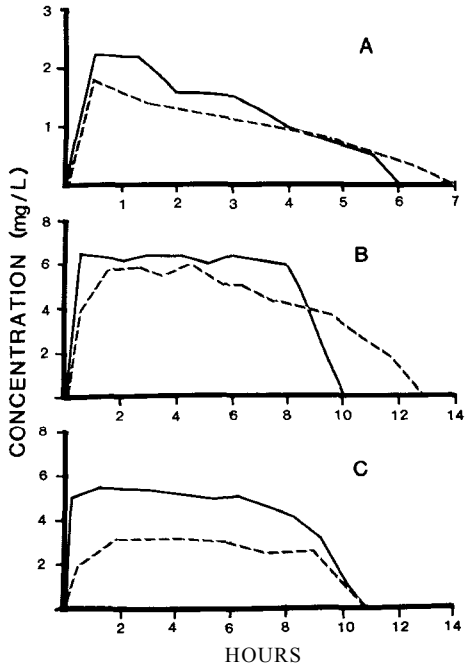

Fig. 1. Concentraton of TFM in water at the application point (solid line) and mouth (broken line) during the treatment of Michigan streams with TFM bars: A-Five Mile Creek, Alger County; B-Johnson Creek, Schoolcraft County; and C-Section 13 Tributary, Delta County.

SECTION 13 TRIBUTARY

The third trial was done in Section 13 Tributary, which flows into the Sturgeon River in Delta County, Michigan. The water was moderately hard and the working range was determined to be 2.6 to 6.3 mg/L of TFM. The selected treatment of 4.5 mg/L for 8 h at the application point was expected to maintain at least 3.6 mg/L for 6 h at the mouth of the stream. An effective concentration was maintained at the application point, but not at the mouth of the stream. Two factors were considered responsible for the failure to maintain the antipated concentration at the mouth. First, the bars dissolved faster than expected (6 h instead of the anticipated 8 h), even in a current of 0.12 m/s. Second, an orginally unperceived increase in volume of flow between the application point and the

mouth (later measured to be 20%) and rain during the treatment caused dilution of the concentration.

The stream was retreated on the following day with a slightly higher concentration and the bars were placed where the current velocity was slower (0.06 m/s). The second treatment was calculated to maintain 5 mg/L for 8 h at the application point. The resultant concentraton curve was very close to that predicted (Fig. 1) and the bars lasted for 9 h. Analysis showed a concentration of 4 mg/L for 8 h at the application point and 2.6 mg/L for 8 h at the mouth--effective treatment at both points according to the pretreatment toxicity tests.

POST TREATMENT SURVEYS

LAMPREYS

Dead larval lampreys were observed in each of the streams during the treatments and electrofishing surveys after the treatments revealed no live sea lampreys. One live lamprey was found where Five Mile Creek emptied into Lake Superior.

FISH

In Five Mile Creek, the high concentration during the first hour of treatment caused minor mortalities of fish. About 40 dead young-f-the-year salmonids including coho salmon *(Oncorhynchus* kisutch), rainbow trout, and brook trout *(Salvelinus fontinalis)* were found. The fish kill was considered to be minor, based on the abundance of these fish in the stream. No dead fish were seen during surveys of the other two streams, even though brook trout and minnows were known to be present in Johnson Creek.

DISCUSSION

When placed at properly selected locations in a stream, the bar formulation of TFM performed essentially as intended. It dissolved at a relatively even rate over time and higher volumes of flow could be accommodated by simply placing more bars in the stream. The TFM concentration in the stream remained nearly constant for 5 to 6 h, then declined slowly as the bars became thinner.

Current velocity at point of placement in the stream significantly affected the rate of dissolution. If the velocity was too high, the bars dissolved at a faster rate than expected and delivered a shortened but elevated concentration peak in the stream. This effect was amplified if the water warmed during the day. In the

instances where the bars dissolved more rapidly than expected, the resulting higher concentrations tended to compensate for the shorter exposure times. It appears that for best results, the bars should be placed in an area in a stream where the velocity is 0.08 m/s or less. Through further experience in field use of the new formulation, it should be possible to predict the dissolution rates at higher velocities. The option of higher concentrations and decreased exposure times could then be selected from the data provided by on-site toxicity tests. Another option in fast-flowing water is to use stacked bars to provide greater thickness and a longer treatment.

The TFM bars appear to be highly effective in eliminating larval sea lampreys from small streams. Once intial analyses are done to ensure that proper concentrations are being applied, little monitoring should be necessary during treatments. The bars should improve efficiency by reducing manpower requirements during treatments. The new formulation has been granted a registration by the U.S. Environmental Protection Agency, affirming that all safety and efficacy requirements have been met.

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TOXICITY OF THE LAMPRICIDES 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) AND 2',5-DICHLORO-4'-NITROSALICYLANILIDE BAYER 73) TO EGGS AND NYMPHS OF THE MAYFLY (HEXAGENIA SP.)

by

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ABSTRACT

Eggs and nymphs of mayflies *(Hexagenia* sp.) were exposed to the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dichloro-4'-nitrosalcylanilide (Bayer 73) and to a mixture of 98% TFM and 2% Bayer 73 (TFM-2B) to determine the sensitivity of various life stages to these compounds. Some eggs and newly hatched nymphs survived concentrations of TFM up to 10 mg/L; all nymphs of the other groups tested (7, 16, 23, and 27 mm long) died at concentrations of 5 mg/L or more. Eggs and nymphs were unaffected by Bayer 73 concentrations up to 0.4 mg/L, the highest concentrations tested. The toxicity pattern observed for the mixutre was similar to that for TFM alone. Stream treatments simulated by using exposure periods of 6, 12, 18, and 24 h and concentrations of TFM, or TFM plus Bayer 73 of 2.5, 5.0, and 10.0 mg/L led to significant mortalities of mayfly nymphs at nearly all periods of concentrations. No significantly mortality resulted from exposure to Bayer 73 for any time period or at any concentration tested. Burrowed nymphs were twice as resistant to TFM as free-swimming nymphs; no difference in resistence to Bayer 73 was apparent.

INTRODUCTION

The lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73) have been used extensively in tributaries of the Great Lakes to control the sea lamprey *(Petromyzon, marinus).* The selective toxicity of TFM to sea lamprey ammocetes was first reported by Applegate et al. (1958). In 1964 it was registered for use as a lampricide in a sea lamprey control program. Howell et al. (1964) showed that, although Bayer 73 was extremely toxic to sea lamprey ammocetes, it also was very toxic to certain nontarget organisms. These investigators further determined that the addition of small amounts (up to 2%) of Bayer

73 to TFM did not affect the selectivity of TFM and significantly reduced the quantity of TFM required in stream treatments. Mixtures of the two chemicals have been used for lamprey control in the United States and Canada since the mid-1960s (Hamilton 1974).

The toxicity of TFM and Bayer 73 to target and nontarget organisms has been studied extensively (Smith 1967; Chandler and Marking 1975; Fremling 1975; Kawatski et al. 1975; Maki et al. 1975; Marking and Olson 1975; Marking et al. 1975; Sanders and Walsh 1975; Dawson et al. 1975, 1977; Bills and Marking 1976; Rye and King 1976; Dwyer et al. 1978; Gilderhus 1979; Hudson 1979). Various studies have demonstrated the toxicity of the lampricides to many nontarget fishes and to some of the invertebrates present in streams being treated.

Fishermen and bait dealers have recently reported possible reductions in the number of burrowing mayflies (Hexagenia sp.) in some of the streams that have been treated with lampricides. This concern about possible adverse effects on mayflies prompted the U.S. Fish and Wildlife Service to investigate the situation. The objectives of the present study were to determine (1) toxicity of the individual lampricides and the normally used mixture to eggs and nymphs of various life stages, (2) toxicity of lampricides for various exposure periods, (3) if TFM or Bayer 73 affect burrowed and free-swimming nymphs differently, and (4) to compare the test results with toxicity data for lampreys and mayflies reported by other researchers.

MATERIALS AND METHODS

CHEMICALS AND PROCEDURES

Field grade TFM (35.7% active ingredient) was obtained from American Hoechst Corporation, Somerville, New Jersey. Bayer 73 (70% active ingredient, wettable powder form) was obtained from Mobay Chemical Corporation, Kansas City, Missouri. The TFM:Bayer 73 (TFM-2B) mixture, prepared by us in the laboratory contained 98% TFM and 2% Bayer 73. Concentrations of each chemical used in tests were based on the active ingredient.

Test procedures closely followed those set by the U.S. Environmental Protection Agency Committee on Methods for Acute Toxicity Tests with Aquatic Organisms (1975). Water used for all tests was soft reconstituted well water, prepared by adding 48 mg/L of $NaHCO_3$, 30 mg/L each of $CaSO_4 \cdot 2\ H_2O$ and $MgSO_4$, and 2.0 mg/L of KCl. Chemical characteristics of the test water were: pH of 7.2-7.6, total hardness of 40-44 mg/L as $CaCO_3$, and total alkalinity of 30-35 mg/L as $CaCO_3$. The methods of Litchfield and

Wilcoxon (1949) were used to calculate LC50s and 95% confidence intervals.

CULTURE AND 24-H STATIC TEST PROCEDURES

Fertilized eggs were collected during a July emergence on Pool 7 of the Upper Mississippi River. Nymphs used in tests on the effects of lampricides on various size groups were produced by the methods described by Fremling (1967). Eggs and newly hatched nymphs (about 1 to 2 mm long), and nymphs 7, 16, 23, and 27 mm long were used in toxicity tests. Test vessels contained 50 eggs, or 50 newly hatched or 10 larger nymphs; two replications were used for each concentration tested. Embryos and newly hatched nymphs were exposed in 250-mL glass beakers containing 200 mL of water; nymphs 7, 16, 23 mm long were exposed in 3.78-L glass jars containing 2 L of water, and nymphs 27 mm long were exposed in 18.9-L glass jars containing 15 L of water. Water temperatures were maintained at 17°C by partial immersion of the test vessels in water baths.

Special precautions were taken to ensure the viability of eggs before exposure. The eggs were removed from holding tanks with a pipette, placed in glass vessels held at 20°C, and observed daily until the viability of the embryos could be determined by using special lighting similar to that described by Marking et al. (1983). The lighting system produced an effect similar to dark-field microscopy, making the embryo inside the egg case readily visible. Viable eggs were then counted into test vessels and exposed to test chemicals. Observations were made immediately after 24 h of exposure. Embryos were considered alive if they could be observed moving inside the egg, or if they had hatched.

SIMULATED STREAM TREATMENT EXPOSURE PROCEDURES

Nymphs used for determining the effects of short-term exposures were collected with a Ponar dredge from a backwater area of Pool 7 of the Upper Mississippi River and held in tanks for 1 wk to allow them to recover from handling stress. Groups of 40 nymphs each (average length 25 mm) were exposed in 18.9-L glass jars containing 15 L of water. Concentrations of 2.5, 5.0, and 10.0 mg/L of TFM, Bayer 73, and TFM-2B were tested. Nymphs were collected, held, and tested at 12°C. The 12°C versus 17°C test temperature was selected because nymphs that are 25 mm or longer are near emergence, and we observed previously **that attempts to** raise the temperature to 17°C for the 1 wk acclimation period resulted in some nymphs emerging during the pretest or test

period. In order to prevent emergence we exposed the nymphs at 12°C. Temperatures were maintained at 12°C by immersion of the jars in water baths. After 6, 12, 18, and 24 h, we transferred groups of 10 nymphs to fresh water and held them for 24 h before determining mortality. These concentrations and time periods were chosen to simulate exposures during stream treatments for sea lamprey control.

BURROWED VS. FREE-SWIMMING NYMPHS

Nymphs used in tests to determine the effects of lampricides on burrowed vs. non-burrowed organisms were also collected with a Ponar dredge from a backwater area of Pool 7 (River mile 704.0) of the Upper Mississippi River and held in tanks for 1 wk to allow recovery from handling stress. Groups of 10 nymphs each (average length 23 mm) were exposed in 3.78-L glass jars containing either 2 L of water or 2 L of water plus bottom sediment 5 cm deep. The sediment was allowed to settle for 24 h before the organisms were introduced. Aeration was provided during the test to ensure that dissolved oxygen concentration would not be affected by biochemical oxygen demand of the sediment; aeration was also provided for vessels without sediment. After 96 h of exposure, we assessed survival by washing the sediment throught a fine-mesh screen and counting the number of live nymphs.

RESULTS OF DISCUSSION

SENSITIVITY AT VARIOUS LIFE STAGES

TFM was highly toxic to mayfly nymphs in 24-h exposures at all the nominal concentrations tested. The egg stage was the most resistant, and sensitivity increased with time after hatching-i.e. the nymphs 16, 23, and 27 mm long were 2 to 3 times more sensitive than those 7 mm long (Fig. 1). Similarly, Olson and Marking (1973) and Niblett and McKeown (1977) determined that eggs of rainbow trout *(Salmo gairdineri)* were more resistant to TFM than the newly hatched sac fry; however, the fry became slightly more resistant to the lampricide as they developed. About 50% of the nymphs 23 mm long died after exposure to a concentration of 1.5 mg/L TFM. This percentage agrees closely with the results of Fremling (1975) who determined that the 24-h LC50 for nymphs 20-22 mm long was 1.69 mg/L TFM, and with those of Smith (1967), who found that TFM produced partial mortalities to burrowing mayflies at concentrations as low as 2 mg/L. Rye and King (1976) found TFM-2B to be more toxic than Bayer 73 alone to burrowing mayflies, and determined

that the 24-h LC50 for the mixture was about 4.0 mg/L. These studies indicated that mayfly populations could be adversely affected by conventionally used stream treatment concentrations of TFM, although the exposure time in stream treatments is usually much less than 24 h.

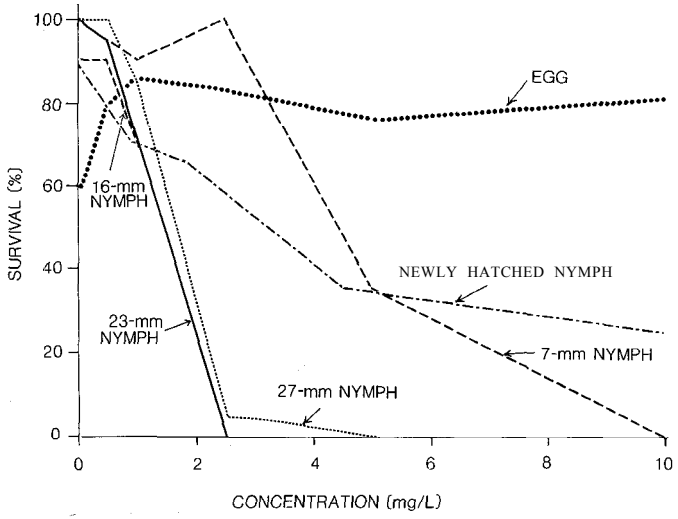

Fig. 1. Survival of selected life stages of mayflies (Hexagenia sp.) exposed to TFM for 24 h in soft water at 17°C.

Bayer 73 had little effect on any of the life stages exposed for 24 h. Survival of eggs and nymphs was similar to that of controls at concentrations up to 0.4 mg/L the highest concentration tested in life stage exposures (Fig. 2). This concentration of Bayer 73 would be used in a field treatment that required about 20 mg/L of TFM (a concentration this high is rarely used for sea lamprey control). The maximum concentration of TFM used for treating any Lake Superior stream was about 13 mg/L (Smith et al. 1974); the corresponding Bayer 73 concentration, if the TFM:Bayer 73 mixture was used, would be about 0.2-0.3 mg/L. Tributaries of Lakes Huron and Michigan require slightly higher TFM concentrations because water hardnesses and alkalinities are higher; however, even in these drainages, TFM concentrations as high as 20 mg/L are rarely used.

The toxicity of TFM-2B was similar to that of TFM alone (Fig. 3). Egg stages were most resistant, followed by nymphs that were newly hatched or 7 mm long. The nymphs 16, 23, 27 mm long were about twice as sensitive as the 7 mm long nymphs.

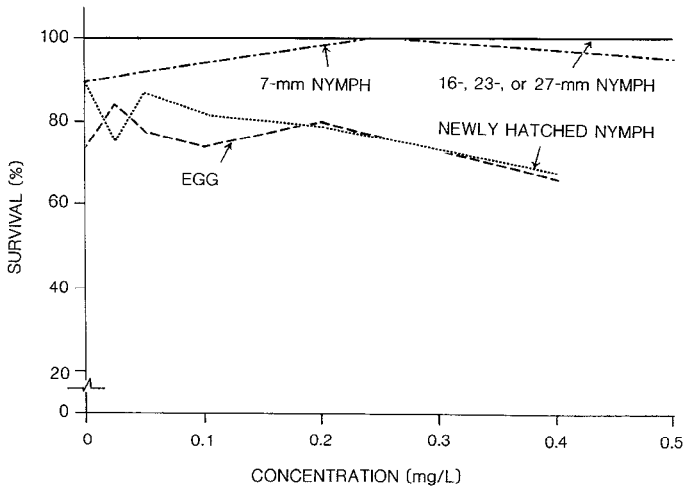

Fig. 2. Survival of selected life stages of mayflies (*Hexagenia* sp.) exposed to Bayer 73 for 24 h in soft water at 17°C.

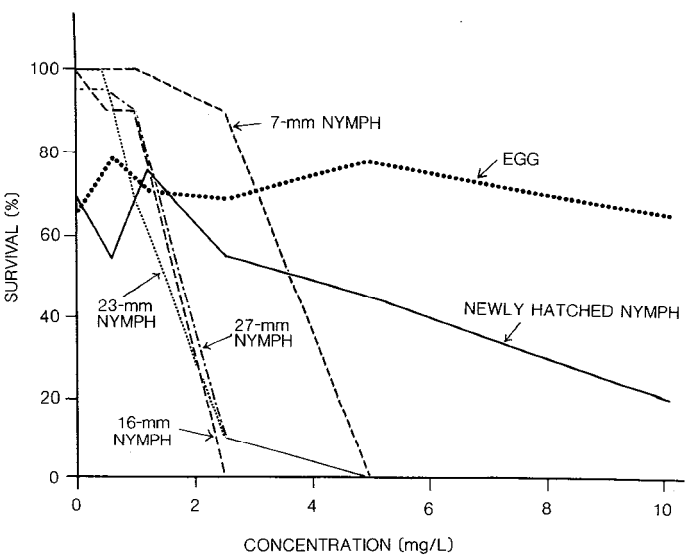

Fig. 3. Survival of selected life stages of mayflies (*Hexagenia* sp.) exposed to TFM:2B for 24 h in soft water at 17°C.

LETHAL EXPOSURES FOR NYMPHS

Groups of 40 nymphs averaging 25 mm long were exposed to concentrations as high as 10 mg/L of TFM, Bayer 73, or TFM-2B for periods of 6, 9, 12, or 24 h in soft water at 12°C. After the desired exposure period they were transferred to fresh water and examined after 24 h. Exposures to TFM caused some mortality at all treatment regimens (Table 1). All nymphs were killed at concentrations of 5 mg/L during 9 h of exposure. The mixture of the two lampricides produced a mortality pattern similar to that for TFM along. No significant mortality was observed in any of the tests with Bayer 73 alone. Our data supported those of other researchers who observed that Bayer 73 alone is much less toxic than TFM or TFM-2B to mayflies (Rye and King 1976).

Table 1. Toxicity of TFM, Bayer 73, and a TFM-2B mixture: precentages of mayfly nymphs 25 mm long killed in soft water at 12°C after various periods of exposure.

Chemical and concentration (mg/L)	Exposure period (h)			
	6	9	12	24
TFM				
0	0	0	0	0
2.5	10	20	60	100
5	30	100	100	100
10	100	100	100	100
Bayer 73				
0	0	0	0	0
2.5	0	10	0	10
5	20	0	0	10
10	10	0	10	0
TFM-2B				
0	0	0	0	0
2.5	0	0	60	100
5	60	100	100	100
10	100	100	100	100

EFFECTS OF BURROWING LAMPRICIDE TOXICITY

Burrowed nymphs exposed to TFM were twice as resistant as freeswimming ones (Table 2). Burrowed nymphs perhaps do not respire as rapidly as the free-swimming nymphs and therefore may not absorb the chemical as rapidly. At the higher concentrations of TFM, some nymphs left their burrows, apparently due to irritation caused by the chemical. No statistical difference (P = 0.5) was found in the 96 h LC50 between burrowed and free-swimming nymphs exposed to Bayer 73 and no burrowed nymphs left their tunnels after exposure to Bayer 73.

Table 2. Toxicity (96-h LC50 and, in parentheses, 95 confidence interval) of TFM and Bayer 73 to burrowed and free-swimming mayfly nymphs in soft water at 12°C.

Chemical	Condition of nymphs	
	Free-swimming	Burrowed
TFM	0.710 (0.472-1.07)	1.69 (1.27-2.25)
Bayer 73	2.82 (1.38-5.75)	2.05 (1.00-4.18)

COMPARATIVE TOXICITY AND POTENTIAL IMPACTS

Dawson et al. (1975), who determined LC-99s for sea lamprey ammocetes under various water hardnesses, pHs, and temperatures, found that, under test conditions similar to ours, 99% of the ammocetes were killed at concentrations of 4.1 mg/L of TFM after 12 h of exposure. We also found that high percentages of mayfly nymphs would be killed by concentrations of TFM or TFM-2B lethal to sea lamprey larvae, if the nymphs were free-swimming-i.e. had been driven from their burrows by chemical irritation. These findings may be biased toward increased mortality because the tests involved placing the nymphs in an unnatural situation. Burrowed nymphs were about twice as resistant as free-swimming forms. Two other factors also negate the probability of long-term effects on mayfly populations. First, sea lamprey ammocetes occur in only

about 7.5% of the streams entering the Great Lakes and only small portions of these streams are treated because physical barriers prevent upstream migration of spawning sea lampreys; and second, exposure is infrequent, because most streams are usually treated only once every 3-5 years.

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