INVESTIGATIONS IN FISH CONTROL

63. Laboratory Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM) as a Lampricide

64. Effects of 3-Trifluoromethyl-4-nitrophenol (TFM) on Developmental Stages of the Sea Lamprey

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66. Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in a Stream Ecosystem after Treatment for Control of Sea Lampreys

United States Department of the Interior
Fish and Wildlife Service

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IN THIS COVER

FOREWORD

Any program to develop data regarding the safe and efficacious use of a chemical must include investigations of its effects on nontarget organisms, on the target organism and its life stages, and should consider residue levels which might occur in natural ecosystems following application.

The lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), has been used extensively to control larvae of the sea lamprey, *Petromyzon marinus*, in the Great Lakes. Previous publications in *Investigations in Fish Control* (Nos. 56-62) have reported effects of TFM on algae, zooplankters, amphipods, isopods, crayfish, insects, and fishes under laboratory conditions. The following reports describe research on the effects of TFM on selected developmental stages of larval lampreys, and discuss residue levels that occur in fish tissue and in a stream ecosystem following treatment with TFM.

These papers represent part of a continuing series in *Investigations in Fish Control* which describes ecological effects of the use of TFM as a lampricide. The completed series of reports will be used to support petitions for the registration of TFM as an effective control for the sea lamprey.

Fred P. Meyer, Director
Fish Control Laboratories

INVESTIGATIONS IN FISH CONTROL

63. Laboratory Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM) as a Lampricide

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LABORATORY EFFICACY OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) AS A LAMPRICIDE

by

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ABSTRACT

The lampricidal activity of 3-trifluoromethyl-4-nitrophenol (TFM) was tested under controlled laboratory conditions to evaluate factors which may influence the efficacy of the chemical. TFM was tested at temperatures of 7, 12, 17, and 22 C, total water hardnesses of 12, 44, 170, and 300 mg/l as $CaCO_3$, and pH's of 6.5, 7.5, 8.5, and 9.0. TFM is an effective lampricide. It is more effective against larvae of the sea lamprey *(Petromyzon marinus)* than against embryos and prolarval stages and slightly more effective against larvae of sea lampreys than against those of the American brook lamprey *(Lampetra lamottei)*. Efficacy of the lampricide is affected very little by temperature, but is reduced in hard water, especially at high pH's. High pH decreases the activity of TFM and has the greatest influence on toxicity of any of the factors investigated. TFM is significantly more effective against exposed (free-swimming) larvae than against those in burrows.

INTRODUCTION

In 1964, 3-trifluoromethyl-4-nitrophenol (TFM) was registered by the Pesticide Registration Division of the U.S. Department of Agriculture for limited use in tributaries of the Great Lakes to control the sea lamprey *(Petromyzon marinus)*, against which the chemical had been shown to be selective (Applegate et al. 1958). In 1970, however, the registration was threatened with cancellation by the Environmental Protection Agency, and since then, extensions have been granted to provide time for preparation of information necessary to support continued registration.

A literature review by Schnick (1972) indicated that numerous tests of the lampricidal activity of TFM have been conducted in waters of various qualities and temperatures. However, because most of the early studies were conducted in natural waters from various sources and with different characteristics, the influence of individual factors was difficult to evaluate (Applegate et al. 1961; Applegate and King 1962; Kanayama 1963; Smith 1966; Zimmerman 1968; and Johnson 1970).

Recent studies have defined the influence of water chemistry on the toxicity of TFM to nontarget aquatic organisms (Marking and Olson 1975; Kawatski et al. 1975; and Chandler and Marking 1975). Additional information is needed, however, on the efficacy of TFM as a lampricide. The purpose of the present study was to determine: the toxicity of TFM to different species, sizes, and developmental stages of lampreys; the individual and combined influences of temperature, water hardness, and pH on the toxicity of TFM; and the toxicity to burrowed and exposed (free-swimming) lamprey larvae at the same stage of development.

MATERIALS AND METHODS

We used electrofishing gear to collect lamprey larvae from the Rifle River watershed in eastern Michigan. The lampreys were anesthetized in 100-mg/l solutions of MS-222 and sorted by species and size. The average lengths (and range in lengths) were 7 (4-9) cm for sea lamprey larvae and 7 (5-9) cm, and 16 (13-19) cm for two groups of American brook lamprey *(Lampetra lamottei)* larvae. The test organisms were placed in troughs containing a sifted sand substrate about 10 cm deep, and flowing well water at 12 C. The lampreys were maintained in the troughs for at least 2 wk prior to testing.

A three-dimensional testing model was used to evaluate the influences of temperature, hardness, and pH. Each of these factors was varied individually in the presence of selected combinations of the other two factors, so that the effect of each variable could be isolated and examined under a variety of controlled conditions.

The test water was deionized and reconstituted to four different hardnesses (Table 1), and the pH was adjusted with appropriate buffers (Table 2). The solutions were checked

Table 1. Ingredients and characteristics of reconstituted waters used in toxicity tests

Water type	Salts added (mg/l)				pH range	Total hardness (mg/l $CaCO_3$)	Total alkalinity (mg/l $CaCO_3$)
	$NaHCO_3$	$CaSO_4\ 2H_2O$	$MgSO_4$	KCl			
Very soft	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft[a]	48	30	30	2.0	7.2-7.6	40-48	30-35
Hard	192	120	120	8.0	7.6-8.0	160-180	110-120
Very hard	384	240	240	16	8.0-8.4	280-320	225-245

[a] Standard reconstituted water used in routine toxicity tests.

Table 2. Quantities of buffering chemicals used for adjusting the pH in reconstituted water of various hardnesses

pH	Buffer	Ml of solutions per 15 liters of water			
		Very soft	Soft	Hard	Very hard
6.5	1N NaOH	4	5	1	--
	1M KH_2PO_4	20	30	40	60
7.5	1N NaOH	14	14	13	12
	1M KH_2PO_4	20	20	20	20
8.5	1N NaOH	3.5	6.5	6	10
	0.5M H_3BO_3	20	40	30	30
9.0	1N NaOH	2	5.5	8	11
	0.5M H_3BO_3	20	20	20	20

daily with a pH meter and readjusted as necessary to maintain the desired pH. Water temperatures were controlled by placing the test vessels in water baths maintained at 7, 12, 17, or 22 C.

Field grade TFM (35.7%, obtained from American Hoechst Corporation), which is formulated with N,N-dimethylformamide (DMF), was measured volumetrically and dissolved in water. Since the entire formulation is applied in streams, concentrations are reported on the basis of total formulated lampricide and expressed as μl/l.

TFM was added to the test vessels about 20 h after the introduction of lampreys. We exposed 10 lamprey larvae to each concentration of the chemical in 15-liter glass jars using methods similar to those described for static toxicity tests (Lennon and Walker 1964). Dead fish were counted and removed at 1, 3, 6, and 12 h after initial exposure, and daily thereafter, during the 96-h tests. LC50's, LC99's, and 95% confidence intervals were computed according to the methods of Litchfield and Wilcoxon (1949). A P value of 0.05 was used to evaluate significance.

The toxicity of TFM to sea lampreys at selected stages of development as defined by Piavis (1961) was determined in soft, reconstituted water at 17 C. We obtained test specimens by collecting sexually mature sea lampreys from nest areas, spawning them artificially, and incubating the eggs in the laboratory. The immature specimens were exposed to TFM when they reached the desired stages of development. In additional tests we exposed sea lamprey prolarvae (Stage 17, burrowing) in water of selected hardnesses and pH's at 17 C.

Since stage 18 larvae in nature usually live in burrows in the substrate, the difference in toxicity of TFM to burrowed and free-swimming lampreys at this stage of development was determined. To eliminate the effect of adsorption of the chemical by the substrate, we conducted these tests in a flow-through test apparatus similar to that used by Marking et al. (1975), in which the test solution was introduced continuously. Thus, the concentration of TFM was essentially identical in tests containing the burrowed and free-swimming larvae.

RESULTS

Species and Sizes of Lampreys

The 96-h LC50's for TFM in soft, reconstituted water at 12 C for 7-cm sea lampreys and 7-cm American brook lampreys were 1.57 and 3.30 μl/l of formulated TFM, respectively—representing a significant difference in toxicity (Table 3). The corresponding LC50 for 16-cm American brook lampreys was 2.41 μl/l, which was not significantly different from the values for smaller sizes of the two species.

The toxicity of TFM to five stages of sea lampreys increased as the development of the

Table 3. Toxicity of TFM (35.7%) to two species and sizes of lamprey larvae in soft, reconstituted water at 12 C

Species	Average length (cm)	LC50 and 95% confidence interval (μl/l) at 24 h	48 h	96 h
Sea lamprey	7	1.57 1.10-2.06	1.57 1.10-2.06	1.57 1.10-2.06
American brook lamprey	7	4.45 2.92-6.77	3.30 2.45-4.44	3.30 2.45-4.44
American brook lamprey	16	2.52 1.95-3.25	2.41 2.02-2.87	2.41 2.02-2.87

lampreys advanced (Table 4). This trend was evident at all exposure periods, and is exemplified by a decrease in the 48- and 96-h LC50's from 8.65 to 1.57 μl/l of TFM as development progressed from Stage 14 (hatching) to Stage 18 (7-cm larvae).

Effect of Temperature

There was little difference in the toxicity of TFM to sea lamprey larvae at temperatures of 7, 12, 17, or 22 C (Table 5), regardless of the exposure period or the hardness or pH of the test water.

Table 4. Toxicity of TFM (35.7%) to various developmental stages of sea lamprey larvae in soft, reconstituted water at 17 C

Stage[a]	LC50 and 95% confidence interval (μl/l) at		
	24 h	48 h	96 h
14 (hatching)	10.0 8.24-12.1	8.65 7.83-9.55	8.65 7.83-9.55
15 (pigmentation)	6.20 4.67-8.23	6.20 4.67-8.23	6.15 4.65-8.14
16 (gill cleft)	6.20 5.37-7.16	5.60 5.07-6.18	5.60 5.07-6.18
17 (burrowing)	3.88 3.14-4.84	3.88 3.14-4.84	3.88 3.14-4.84
18 (7-cm larvae)	1.57 1.10-2.06	1.57 1.10-2.06	1.57 1.10-2.06

[a] According to Piavis (1961).

Effect of Water Hardness

The toxicity of formulated TFM to sea lamprey larvae declined as water hardness increased, especially at the higher pH's. For example, the 12-h LC99's at 12 C and 12 and 300 mg/l of total hardness, were 0.950 and 1.58 μl/l of TFM, respectively, at pH 6.5 and 8.50 and 32.5 μl/l at pH 8.5 (Table 5).

Similar effects of water hardness on the toxicity of TFM to 7-cm sea lamprey larvae also were evident for mortalities reported as LC50's and at all exposure periods (See Appendix). The influence of water hardness on the toxicity of TFM was similar in tests against sea lamprey prolarvae at Stage 17 (Table 6).

Effect of pH

The pH of the test water had more influence on the toxicity of TFM to sea lampreys than either temperature or hardness. In soft water at 12 C the 12-h LC99's for 7-cm larvae at pH 6.5, 7.5, 8.5, and 9.0 were 1.17, 4.10, 12.0, and 33.0 μl/l of TFM, respectively (Table 5). The influence of pH on the activity of TFM was observed at all temperatures and water hardnesses tested, but was especially evident in the harder waters. This general toxicity pattern also applied to Stage 17 prolarvae (Table 6).

Effect of Substrate

Sea lampreys burrowed in sand are less vulnerable to TFM than are lampreys confined without a substrate (Table 7). A concentration of 5.2 μl/l killed 100% of the free-swimming lampreys in 4 h, but did not kill all of the burrowed lampreys until after 48 h. At 24 h, the LC50 for burrowed lampreys (4.75 μl/l) was more than four times that for free-swimming lampreys (1.17 μl/l). A concentration of 1.7 μl/l produced 40% mortality among the free-swimming lampreys in 12 h and 100% mortality in 48 h, but this concentration failed to kill any of the burrowed lampreys in 96 h.

Table 5. Toxicity of TFM (35.7%) to 7-cm sea lamprey larvae after 12 h of exposure in waters of selected temperatures, pH's, and hardnesses

pH	Water hardness (mg/l $CaCO_3$)	12-h LC99 and 95% confidence interval (μl/l) at temperatures (°C) of			
		7	12	17	22
6.5	12	0.860 0.682-1.08	0.950 0.785-1.15	1.00 0.800-1.25	1.30 0.985-1.72
	44	1.24 0.790-1.95	1.17 0.886-1.54	1.25 1.00-1.56	1.25 0.874-1.79
	170	1.00 0.699-1.43	1.02 0.843-1.23	1.10 0.880-1.38	2.00 1.52-2.64
	300	1.60 1.28-2.00	1.58 1.01-2.48	1.62 1.13-2.32	1.63 1.30-2.04
7.5	12	2.32 1.92-2.81	2.90 2.03-4.15	2.73 2.07-3.60	3.50 2.80-4.38
	44	2.61 1.66-4.10	4.10 2.87-5.86	4.15 3.32-5.19	3.80 3.14-4.60
	170	5.10 3.86-6.73	6.80 4.76-9.72	5.18 4.28-6.27	6.50 4.92-8.58
	300	10.0 6.99-14.3	6.61 5.28-8.26	6.40 5.29-7.74	7.03 5.81-8.51
8.5	12	11.0 8.33-14.5	8.50 5.41-13.3	10.0 7.58-13.2	12.5 10.0-15.6
	44	29.5 24.4-35.7	12.0 8.39-17.2	21.1 16.9-26.4	22.1 16.7-29.2
	170	27.6 22.8-33.4	18.2 14.6-22.8	33.0 23.1-47.2	23.7 16.6-33.9
	300	35.0 28.0-43.8	32.5 24.6-42.9	27.5 17.5-43.2	29.6 23.7-37.0
9.0	12	--- ---	8.40 6.36-11.1	--- ---	--- ---
	44	--- ---	33.0 23.1-47.2	--- ---	--- ---
	170	51.5 39.0-68.0	41.0 28.7-58.6	58.0 47.9-70.2	66.2 54.7-80.1

Table 6. Toxicity of TFM (35.7%) to sea lamprey prolarvae (Stage 17) in waters of selected hardness and pH at 17 C

pH	Water hardness (mg/l $CaCO_3$)	LC50 and 95% confidence interval (μl/l) at					
		3 h	6 h	12 h	24 h	48 h	96 h
6.5	12	1.40 1.13-1.73	1.40 1.13-1.73	0.710 0.531-0.949	0.610 0.492-0.757	0.610 0.492-0.757	0.610 0.492-0.757
	44	1.40 1.11-1.76	1.40 1.11-1.76	1.21 0.943-1.55	1.21 0.943-1.55	1.21 0.943-1.55	1.21 0.943-1.55
	170	1.74 1.49-2.04	1.74 1.49-2.04	1.74 1.49-2.04	1.74 1.49-2.04	1.23 0.955-1.58	1.23 0.955-1.58
	300	1.41 1.14-1.74	1.41 1.14-1.74	1.41 1.14-1.74	1.41 1.14-1.74	1.41 1.14-1.74	1.41 1.14-1.74
7.5	12	6.40 5.12-7.99	6.35 5.06-7.97	3.64 2.93-4.52	3.49 2.94-4.14	3.49 2.94-4.14	3.49 2.94-4.14
	44	6.75 5.64-8.08	4.40 3.69-5.25	3.90 3.34-4.56	3.88 3.14-4.84	3.88 3.14-4.84	3.88 3.14-4.84
	170	5.65 4.57-6.99	5.65 4.57-6.99	5.65 4.57-6.99	5.65 4.57-6.99	5.65 4.57-6.99	5.02 4.11-6.13
	300	7.99 6.55-9.75	7.99 6.55-9.75	6.25 5.48-7.12	5.20 4.31-6.27	5.20 4.31-6.27	5.20 4.31-6.27
8.5	12	34.5 29.6-40.3	22.0 19.6-24.7	15.0 13.0-17.3	10.1 7.84-13.0	10.0 8.00-12.5	9.50 7.55-12.0
	44	>40.0[a]	32.0 26.4-38.8	32.0 26.4-38.8	27.5 25.6-29.5	17.3 15.5-19.3	17.2 14.4-20.6
	170	74.0 57.4-95.5	40.5 33.8-48.5	40.0 33.0-48.6	35.1 29.8-41.3	35.1 29.8-41.3	35.1 29.8-41.3
	300	60.0 54.1-66.5	46.0 40.8-51.9	31.5 31.1-31.9	24.0 20.7-27.9	24.0 20.7-27.9	24.0 20.7-27.9
9.0	12	62.0 55.2-69.7	61.9 54.7-70.1	46.0 40.8-51.9	29.3 26.4-32.6	29.3 26.4-32.6	24.5 21.1-28.4
	44	62.1 51.3-75.2	54.6 46.0-64.9	46.0 42.5-49.8	26.0 18.7-36.1	26.0 18.7-36.1	22.0 16.9-28.7
	170	>100[a]	71.5 61.4-83.3	62.0 54.4-70.7	55.5 46.5-66.3	45.0 39.5-51.2	43.5 35.8-52.9
	300	83.0 71.8-96.0	81.6 64.4-103	37.1 29.6-46.4	35.5 28.9-43.7	30.5 23.7-39.2	26.0 18.7-36.1

[a] No mortality at highest concentration tested.

DISCUSSION

Piavis (1962) recognized that the sensitivity of sea lamprey to TFM increased in the more advanced stages of embryonic development, and suggested that control of the sea lamprey would be most effective if conducted at a time when the lampreys reached the more sensitive larval stage. Information on the toxicity of TFM to sea lampreys of various developmental stages presented in this paper supports his findings.

Table 7. Toxicity of TFM (35.7%) to burrowed and free-swimming sea lamprey larvae in a flow-through diluter containing carbon-filtered city water at 12 C

Exposure time (hours)	LC50 and 95% confidence interval (μl/l) for	
	Free-swimming lampreys	Burrowed lampreys
3	6.50 5.19-8.13	>7.00
4	4.61 4.25-5.00	>7.00
6	4.06 3.73-4.43	>7.00
7	3.17 2.64-3.81	6.85 5.42-8.66
12	1.71 1.55-1.88	5.20 --- ---[a]
24	1.17 1.02-1.35	4.75 3.80-5.93
48	<1.07	2.99 2.57-3.48
96	<1.07	2.50 2.22-2.81

[a] Data insufficient to compute confidence intervals.

The reduced toxicity of TFM at the higher pH's presumably results from an increased ionization of the molecule (pKa = 6.07; Applegate et al. 1961). The un-ionized form of certain molecules is lipid-soluble, and therefore more easily transported across the gills of fish (Sills and Allen 1971).

The reduced toxicity of TFM in hard water, especially at the higher pH's, may result from the formation of a complex between the ionized form of the TFM molecule and divalent cations. As the hardness of the water increases, the availability of more cations for complexing shifts the ionization equilibrium and results in a decrease in the concentration of the more active, un-ionized form of TFM.

Although temperature changes have been blamed for incomplete kills during stream treatments (U.S. Bureau of Commercial Fisheries 1958; Smith and King 1970), laboratory studies have indicated that temperature has little effect on the toxicity of TFM (U.S. Bureau of Commercial Fisheries 1960; Applegate et al. 1961). However, Applegate et al. (1961) reported that the rate of death slowed as the temperature decreased and that the selectivity against lampreys increased as the temperature dropped near freezing.

We found that free-swimming lampreys were more vulnerable to TFM than burrowed lampreys. Presumably the free-swimming lampreys are more excited and their rate of metabolism and uptake is greater than that of the burrowed lampreys. Also, the burrowed lampreys may be somewhat protected from exposure to TFM in the water. Since the field-use concentration of TFM for each stream has been determined by on-site bioassays of TFM against free-swimming lampreys, these tests could indicate treatment concentrations which are insufficient to produce complete elimination of the burrowed lamprey unless the lethal concentrations are maintained over an extended period. These results do not support those of Applegate et al. (1958) who reported that concentrations of TFM lethal to all larval lampreys were essentially the same in jar tests and in treatments of a simulated stream.

CONCLUSIONS

1. TFM is effective as a lampricide.
2. Sea lampreys are more sensitive to TFM than American brook lampreys, but the difference is not enough to permit selective removal of sea lampreys.
3. Early developmental stages of sea lampreys are more resistant to TFM than the larval stage.
4. Temperature has very little influence on the toxicity of TFM.
5. The toxicity of TFM is significantly reduced in water of high pH.
6. The lampricide is less toxic in hard than in soft water, especially at high pH's.
7. Burrowed sea lamprey larvae are significantly less vulnerable to TFM than are free-swimming sea lamprey larvae.

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Appendix. Toxicity of TFM (35.7%) to free-swimming 7-cm sea lamprey larvae in waters of selected temperatures, pH's, and hardnesses

Temp. (°C)	pH	Total hardness (mg/l $CaCO_3$)	LC50 and 95% confidence interval (μl/l) at					
			3 h	6 h	12 h	24 h	48 h	96 h
7	6.5	12	1.79 1.31-2.44	1.20 1.05-1.38	0.545 0.462-0.643	0.500 0.319-0.783	0.385 0.294-0.504	0.330 0.237-0.459
		44	2.00 1.74-2.29	0.915 0.773-1.08	0.655 0.518-0.829	0.225 0.137-0.370	0.225 0.137-0.370	0.225 0.137-0.370
		170	2.60 1.71-3.96	1.38 1.05-1.82	0.560 0.467-0.671	0.452 0.335-0.611	0.275 0.181-0.418	0.275 0.181-0.418
		300	1.70 1.27-2.28	1.42 1.15-1.76	0.770 0.587-1.01	0.390 0.291-0.522	0.332 0.237-0.466	0.277 0.209-0.367
7	7.5	12	>4.00[a]	5.60 3.44-9.12	1.53 1.15-2.04	1.32 1.00-1.73	0.720 0.509-1.02	0.600 0.418-0.861
		44	4.25 3.43-5.26	3.17 2.64-3.81	1.72 1.48-2.00	1.72 1.48-2.00	1.30 1.03-1.64	1.18 0.906-1.54
		170	7.60 5.21-11.1	7.60 5.21-11.1	3.12 2.61-3.73	2.00 1.64-2.43	1.41 1.14-1.74	1.41 1.14-1.74
		300	6.40 5.55-7.39	6.30 5.44-7.30	3.70 2.83-4.83	2.38 1.87-3.02	1.75 1.25-2.46	1.75 1.25-2.46
7	8.5	12	20.7 14.6-29.4	12.0 10.4-13.8	7.80 6.67-9.12	2.70 2.03-3.58	2.16 1.27-3.66	1.95 1.24-3.06
		44	>15.0[a]	>15.0[a]	15.0 11.6-19.4	10.4 8.93-12.1	7.80 5.80-10.5	4.55 3.43-6.03
		170	29.5 24.5-35.6	29.5 24.5-35.6	13.1 10.8-15.9	8.60 7.37-10.0	7.00 5.66-8.66	6.10 4.75-7.84
		300	35.0 27.6-44.4	28.5 23.0-35.3	28.0 22.6-34.7	10.2 8.57-12.0	8.60 7.46-9.91	6.90 5.47-8.70
7	9.0	170	54.0 40.4-72.2	54.0 40.4-72.2	26.1 22.0-31.0	17.2 14.8-20.0	11.5 8.82-15.0	11.0 8.34-14.5
12	6.5	12	1.10 0.847-1.43	0.740 0.616-0.889	0.450 0.343-0.590	0.420 0.339-0.520	0.420 0.339-0.520	0.381 0.290-0.500
		44	>0.600[a]	>0.600[a]	0.640 0.476-0.860	0.420 0.339-0.520	0.420 0.339-0.520	0.420 0.339-0.520
		170	1.22 0.996-1.49	1.22 0.996-1.49	0.542 0.460-0.639	0.490 0.389-0.618	0.490 0.389-0.618	0.490 0.389-0.618
		300	1.25 1.02-1.53	1.10 0.929-1.30	0.780 0.646-0.941	0.550 0.465-0.651	0.550 0.465-0.651	0.550 0.465-0.651
12	7.5	12	3.90 2.66-5.71	1.85 1.26-2.72	1.62 1.30-2.02	1.40 1.13-1.73	1.40 1.13-1.73	1.31 0.997-1.72
		44	2.50 1.67-3.74	2.50 1.67-3.74	2.50 1.67-3.74	1.57 1.20-2.05	1.57 1.20-2.05	1.57 1.20-2.05

Appendix—*(Cont'd)*

Temp. (°C)	pH	Total hardness (mg/l $CaCO_3$)	LC50 and 95% confidence interval (μl/l) at					
			3 h	6 h	12 h	24 h	48 h	96 h
		170	4.62 3.66-5.83	4.62 3.66-5.83	2.90 2.10-4.01	2.45 2.04-2.94	2.04 1.61-2.58	2.04 1.61-2.58
		300	6.40 5.49-7.46	3.85 3.29-4.50	3.80 3.09-4.68	2.83 2.11-3.79	2.83 2.11-3.79	2.83 2.11-3.79
12	8.5	12	8.40 6.86-10.3	7.70 6.38-9.29	5.40 4.58-6.37	3.90 3.00-5.08	3.28 2.40-4.47	3.28 2.40-4.47
		44	>5.00[a]	>5.00[a]	4.90 3.51-6.84	4.90 3.51-6.84	4.90 3.51-6.84	4.35 3.24-5.84
		170	22.9 20.4-25.7	--- ---	13.6 12.3-15.1	9.40 7.81-11.3	8.60 7.45-9.93	8.60 7.45-9.93
		300	34.5 27.2-43.8	23.1 19.9-26.8	14.2 11.9-16.9	11.6 9.85-13.7	10.0 8.19-12.2	10.0 8.19-12.2
12	9.0	12	42.0 31.4-56.2	38.5 29.9-49.6	33.0 23.5-46.3	20.0 16.4-24.3	17.1 14.4-20.3	15.0 11.6-19.4
		44	>30.0[a]	>30.0[a]	24.5 21.3-28.2	20.0 17.8-22.4	17.8 15.7-20.2	17.2 14.6-20.3
		170	36.5 30.6-43.5	--- ---	22.2 17.7-27.8	20.8 17.0-25.5	18.0 15.3-21.2	18.0 15.3-21.2
17	6.5	12	1.59 0.985-2.57	0.820 0.632-1.06	0.600 0.495-0.727	0.455 0.337-0.614	0.455 0.337-0.614	0.455 0.337-0.614
		44	>1.00[a]	1.38 0.800-2.38	0.560 0.453-0.693	0.560 0.453-0.693	0.560 0.453-0.693	0.560 0.453-0.693
		170	1.65 1.24-2.20	0.850 0.687-1.05	0.520 0.434-0.623	0.519 0.431-0.625	0.519 0.431-0.625	0.519 0.431-0.625
		300	1.71 1.27-2.29	1.22 0.993-1.50	0.840 0.659-1.07	0.640 0.460-0.890	0.620 0.450-0.855	0.600 0.439-0.820
17	7.5	12	>2.50[a]	2.05 0.984-4.27	1.78 1.51-2.09	1.23 1.02-1.48	1.23 1.02-1.48	1.23 1.02-1.48
		44	>2.50[a]	2.50 1.75-3.57	1.91 1.56-2.34	1.60 1.27-2.02	1.60 1.27-2.02	1.60 1.27-2.02
		170	10.0 6.35-15.7	4.25 3.43-5.26	3.20 2.68-3.82	2.80 2.26-3.47	2.80 2.26-3.47	2.80 2.26-3.47
		300	12.7 8.42-19.2	6.30 5.46-7.27	6.20 5.39-7.13	2.35 1.86-2.97	2.30 1.82-2.90	2.30 1.82-2.90
17	8.5	12	8.90 7.16-11.0	8.81 7.07-11.0	7.60 5.81-9.95	4.60 3.38-6.26	1.75 1.25-2.46	1.75 1.25-2.46
		44	>8.00[a]	>8.00[a]	11.3 6.40-20.0	10.0 5.85-17.1	6.80 4.53-10.2	4.25 3.43-5.26

Appendix—*(Cont'd)*

Temp. (°C)	pH	Total hardness (mg/l $CaCO_3$)	LC50 and 95% confidence interval (μl/l) at 3 h	6 h	12 h	24 h	48 h	96 h
		170	26.2 22.1-31.1	26.2 22.1-31.1	18.9 16.4-21.8	14.4 13.0-15.9	14.4 13.0-15.9	14.4 13.0-15.9
		300	3.15 25.7-38.6	15.0 13.4-16.8	11.7 10.1-13.5	8.60 7.45-9.93	8.60 7.45-9.93	8.60 7.45-9.93
17	9.0	170	52.0 42.7-63.3	52.0 42.7-63.3	33.0 26.7-40.8	28.0 22.6-34.7	28.0 22.6-34.7	28.0 22.6-34.7
22	6.5	12	0.900 0.719-1.13	0.580 0.474-0.710	0.460 0.308-0.687	0.450 0.364-0.557	0.450 0.364-0.557	0.450 0.364-0.557
		44	>0.800[a]	0.800 0.543-1.18	0.560 0.453-0.693	0.560 0.453-0.693	0.560 0.453-0.693	0.560 0.453-0.693
		170	1.40 1.05-1.87	0.960 0.785-1.17	0.860 0.690-1.07	0.860 0.690-1.07	0.760 0.628-0.919	0.760 0.628-0.919
		300	1.40 0.935-2.10	1.40 0.935-2.10	0.860 0.639-1.16	0.860 0.639-1.16	0.860 0.639-1.16	0.860 0.639-1.16
22	7.5	12	>2.50[a]	2.00 1.64-2.43	1.90 1.62-2.24	1.25 1.09-1.43	1.25 1.09-1.43	1.25 1.09-1.43
		44	>2.50[a]	1.87 1.54-2.27	1.87 1.54-2.27	1.87 1.54-2.27	1.84 1.52-2.22	1.84 1.52-2.22
		170	>6.00[a]	4.20 3.39-5.20	4.20 3.39-5.20	3.65 3.06-4.35	3.65 3.06-4.35	3.46 2.97-4.04
		300	5.41 4.59-6.38	3.85 3.29-4.50	3.85 3.29-4.50	3.35 2.62-4.28	2.51 1.95-3.23	2.51 1.95-3.23
22	8.5	12	16.0 10.0-25.6	9.50 7.52-12.0	4.60 3.08-6.87	4.20 3.14-5.62	4.20 3.14-5.62	4.20 3.14-5.62
		44	>10.0	12.2 8.24-18.1	9.40 6.82-13.0	8.20 6.71-10.0	8.20 6.71-10.0	8.20 6.71-10.0
		170	37.5 29.1-48.4	17.0 15.3-18.8	16.2 14.1-18.6	15.2 13.5-17.2	15.2 13.5-17.2	15.2 13.5-17.2
		300	26.0 19.7-34.3	15.6 13.8-17.7	12.2 10.8-13.8	10.0 8.27-12.1	9.00 7.19-11.3	8.70 7.47-10.1
22	9.0	170	125.0 74.2-2.11	46.6 36.7-59.2	40.0 33.0-48.5	37.5 31.2-45.0	37.5 31.2-45.0	37.5 31.2-45.0

[a] No mortality at highest concentration tested.

INVESTIGATIONS IN FISH CONTROL

64. Effects of 3-Trifluoromethyl-4-nitrophenol (TFM) on Developmental Stages of the Sea Lamprey

By George W. Piavis and John H. Howell

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CONTENTS

EFFECTS OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) ON DEVELOPMENTAL STAGES OF THE SEA LAMPREY[1]

by

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ABSTRACT

Developing sea lampreys *(Petromyzon marinus)* in stages 1 (zygote) through 17 (burrowing prolarva) were exposed for 24 h to a 10-mg/l (active ingredient) solution of 3-trifluoromethyl-4-nitrophenol (TFM) at 18 C. Embryonic development, incidence of abnormalities, and mortality in the experimentals were compared with those in unexposed controls. Although exposed embryos in the first eight developmental stages exhibited no immediate effects, the number of viable stage-18 larvae produced was drastically reduced, incidence of abnormalities increased markedly, hatching was sometimes delayed, and hemoglobin production was retarded or lacking. The laboratory findings suggest that the treatment of streams with TFM at the customary rates probably does not effect a complete kill of sea lampreys in all developmental stages.

INTRODUCTION

The selective lampricide 3-trifluoromethyl-4-nitrophenol (TFM), used in tributaries of the Great Lakes to control the sea lamprey *(Petromyzon marinus)*, is toxic to lampreys from stage-18 larvae (Piavis 1962) to spawning adults (Applegate et al. 1961). Its toxicity to earlier developmental stages of the sea lamprey, however, has not been determined. Piavis (1962), who subjected several developmental stages to six selective lamprey larvicides—TFM, four other nitrophenols, and a thiocarbamate—showed that some lampreys survived exposure to a 10-mg/l concentration at stages 10 (neural plate and groove) or 13-14 (prehatching and hatching), but not at certain other stages: 8 (blastula), 9 (gastrula), and during transition from stage 17 (burrowing prolarva) to stage 18 (larva). He concluded that chemical treatment of streams with larvicides would be most effective if carried out at least 40 days after all spawning ceased. Forty days is the longest approximate time required for lamprey development to progress from fertilization to stage 18 (Piavis 1961).

The spawning season of the sea lamprey in the upper Great Lakes usually lasts about 2 mo, June and July, and development of the last embryos extends through August. Stream treatments during these months are facilitated, however, by favorable physical and biological factors such as low stream flow, suitable water temperature, and acceptable biological activity of TFM. Consequently it was highly desirable to determine whether TFM is toxic to sea lamprey embryos at all stages of development.

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MATERIALS AND METHODS

Gametes were obtained from spawning pairs of sea lampreys collected in the Ocqueoc River, Presque Isle County, Michigan. Two batches of eggs were fertilized by the procedures described by Piavis (1961); one male and one female were used for each batch. Fertilized eggs were apportioned among 18-cm glass bowls that were immersed in water in 10-liter battery jars. The bath water in each jar consisted of 6 to 7 liters of filtered Lake Huron water, tempered to 18 C in constant-temperature troughs. Each bowl was aerated by a stone air breaker.

The developmental stages and their distinguishing features, and (in parentheses) the time between the first and last appearance of the stage in samples reared at 18 C (from Piavis 1961) are as follows: stage 0, ovulated but unfertilized ovum; 1, zygote (0-2 h); 2, 2 cells (2-8 h); 3, 4 cells (8-11 h); 4, 8 cells (10-15 h); 5, 16 cells (13-15 h); 6, 32 cells (16-19 h); 7, 64 cells (19-24 h); 8, full blastula (24-64 h); 9, gastrula (64-104 h); 10, neural plate and groove (4-5 days); 11, neural rod (5-6 days); 12, head distinguishable (6-8 days); 13, prehatching (8-12 days); 14, hatching (10-13 days); 15, pigmentation (13-16 days); 16, gill clefts (15-17 days); 17, burrowing larva (17-33 days); and 18, larva (33-40 days).

As animals in each stage of development became successively available, they were removed from one of the two batches, counted, and exposed for 24 h to a 10-mg/l (active ingredient) solution of TFM at 18 C. Exposures were made in a glass bowl and battery jar combination like that previously described. The remaining embryos served as controls. After exposure, the embryos were washed and transferred to fresh water. Further development, abnormalities, and mortalities of the exposed embryos were compared with those of the controls on the basis of periodic sample counts. Abnormal embryos which were so grossly deformed that no precise stage could be assigned to them were counted as dead in the final samples. Terminal samples were taken at stage 17 for all lampreys that reached this stage; the living lampreys (normal and abnormal) were then held long enough to allow them to advance to stage 18. All terminal-sample percentages are cumulative from the egg through the indicated stage.

EFFECTS OF EXPOSURE TO TFM ON EMBRYONIC DEVELOPMENT

Embryonic development and mortality were roughly similar in the two batches of eggs used as controls. Of the 6,686 fertilized eggs obtained from one pair of lampreys, 2,747 were exposed to TFM at stages 1-7 and 17; survival to normal stage 18 among the remainder (controls) was 68% (Table 1). Of the 4,616 fertilized eggs obtained from the second pair, 2,107 were exposed to TFM at stages 8-16, survival to normal stage 18 among the remainder was 75%.

The effects of exposure of embryos and prolarvae (stages 15-17) to 10-mg/l TFM (a.i.) for 24 h varied considerably with the stage of development at the time of exposure. Embryos in stages 1-7 advanced to successive stages while they were being exposed to the 24-h treatment. More advanced stages (8-17) did not, primarily because the duration of the stage normally would be expected to exceed the 24-h exposure period.

The principal results, listed according to stage at the time of exposure, are summarized below and in Table 1.

Stage 1—Normal development through stage 12; abnormalities developed during stage 13; mortality increased progressively to 100% during stage 17.

Stage 2—Normal development through stage 9; abnormalities and mortalities were significant during stage 10, and increased progressively to 100% during stage 17.

Stage 3—Normal development through stage 12; abnormalities and mortalities became significant during stage 13; mortality reached 81% during stage 17. Of the 369 embryos in the lot, 70 (19%) yielded normal stage-18 larvae.

Stage 4—Normal development through stage 12; abnormalities and mortalities significantly increased during stage 13; mortalities increased progressively to 100% during stage 17.

Table 1. Effect of TFM on developing sea lampreys: percentage of normal stage–18 larvae produced after lampreys were exposed at different developmental stages to 10–ppm TFM for 24 h at 18.4 C

Stage at exposure	Number exposed	Most advanced stage attained	Percentage of surviving stage–18 larvae
1	354	17	0
2	275	17	0
3	369	18	19.0
4	199	17	0
5	286	18	1.4
6	603	17	0
7	611	18	5.7
8	275	17	0
9	246	16	0
10	252	17	0
11	212	16	0
12	314	16	0
13	200	17	0
14	203	15	0
15	245	15	0
16	160	16	0
17	50	17	0
Total	4,854	—	2.2
Control 1[a]	3,939	18	68.1
Control 2[a]	2,509	18	74.9

[a] Lampreys exposed at developmental stages 1–7 and 17 were from the same parents as those from Control 1; the animals exposed at stages 8–16 were from the same parents as those from Control 2.

Stage 5—Normal development through stage 9; abnormalities appeared during stage 11; mortalities reached 48% during stage 13 and 99% during stage 17. Of the 286 embryos in the lot, 4 (1%) advanced to normal stage-18 larvae.

Stage 6—Normal development through stage 12; mortality reached 68% during stage 13; abnormalities increased to 75% of the survivors at stage 15; mortalities increased progressively to 100% during stage 17.

Stage7—Normal development through stage 9; mortality was 27% at stage 10 and 94% at stage 17. Of the 611 embryos in this lot, 35 (6%) survived to become normal stage-18 larvae.

Stage 8—Normal development through stage 13; mortality during exposure, 18%; abnormalities in 45% of the specimens through stage 16; mortality reached 100% during stage 17.

Stage 9—Mortality during exposure, 18%; abnormalities appeared at stage 10; mortality increased progressively to 100% during stage 17.

Stage 10—No mortality during exposure; abnormalities increased to 49% at stage 15; mortality reached 21% at stage 15 and increased to 100% at stage 17.

Stage 11—Mortality during exposure, 19%; abnormalities increased to 87% and mortality reached 60% at stage 16; mortality reached 100% during stage 16.

Stage 12—Mortality was 21% during exposure, and reached 100% during stage 16.

Stage 13—Mortality 19% during exposure; 100% abnormalities after exposure; mortality reached 100% during stage 17.

Stage 14—Mortality 89% in stage 14, after exposure; mortality reached 100% during stage 15.

Stages 15, 16, and 17—Total mortality during exposure.

Lamprey embryos exposed to TFM during the early stages of development (1-8) exhibited no immediate effects; usually the effects were delayed until stage 13. The embryos either attained stage 8 during the exposure period or, if exposed at stage 8, remained at that stage; the time sequence of development of the embryos exposed at these stages was thus identical with that for the controls and within the normal range reported by Piavis (1961). Embryos exposed to TFM during stages 8-14 remained in the stage of exposure during exposure; all prolarvae in stages 15-17 died during exposure (Table 1). Thus, except for a few normal larvae that developed after exposure within 24 h after fertilization, at stages 3, 5, and 7, all embryos and prolarvae exposed to TFM became grossly abnormal or died.

Exposure of lampreys, at any stage of development, to 10-mg/l TFM (a.i.) for 24 h greatly increased the incidence of abnormalities. There seemed to be no marked distinction between stage 1 through 8 and stages 9 through 17; abnormalities in lots exposed at each stage far exceeded the rate in the controls. The number of abnormal larvae that reached stage 18 after exposure at different stages were as follows: exposure at stage 3, 95; stage 5, 41; stage 7, 15; stage 8, 6; and stage 10, 10. Abnormal stage-18 larvae in the controls numbered 271 in Control No. 1 and 61 in Control No. 2.

Although many of the embryos exposed during cellular stages (1-7) continued development and eventually hatched, they failed to do so at the normal stage (14). Development within the egg, however, did not cease. Elongation of the embryo continued, resulting in curling and coiling within the chorionic space, until the embryo completely filled it. Pigment spots, heart beat, velum movement, and open gill slits (all characteristics of stage-16 embryos) were often present in extreme cases of "delayed stage 13's." Although most of these embryos eventually hatched, they were grossly deformed, usually into the shape of the letters C, J, P, or O (Fig. 1). Such deformed animals did not straighten after hatching, never swam normally, and were unable to burrow.

Normal sea lamprey embryos initiate synthesis of hemoglobin during stage 15, as indicated by the reddish cast of the blood or the reddish color of the red blood cells under high magnification. In the present study, however, hemoglobin was not detected at this stage, in any of the exposed embryos—confirming similar findings by Piavis (1962) after he exposed sea lamprey embryos to several halogenated nitrophenols. Apparently the lack of hemoglobin inhibited further development in most embryos. The nearly normal velum movements and heart beats of the exposed embryos that attained stage 16 decreased greatly with passage of time within that stage. Movement diminished to the extent that prodding with a teasing needle produced only localized body twitches. The gallbladder became light green and was greatly distended. Although a few embryos eventually developed eyespots (a criterion for stage 17), they were unable to burrow. Even the few embryos that reached stage 18 in normal condition (after exposure at stages 3, 5, and 7) appeared to have lower than normal hemoglobin levels, had abnormally large gallbladders, and moved sluggishly. These symptoms disappeared, however, as development continued in stage 18.

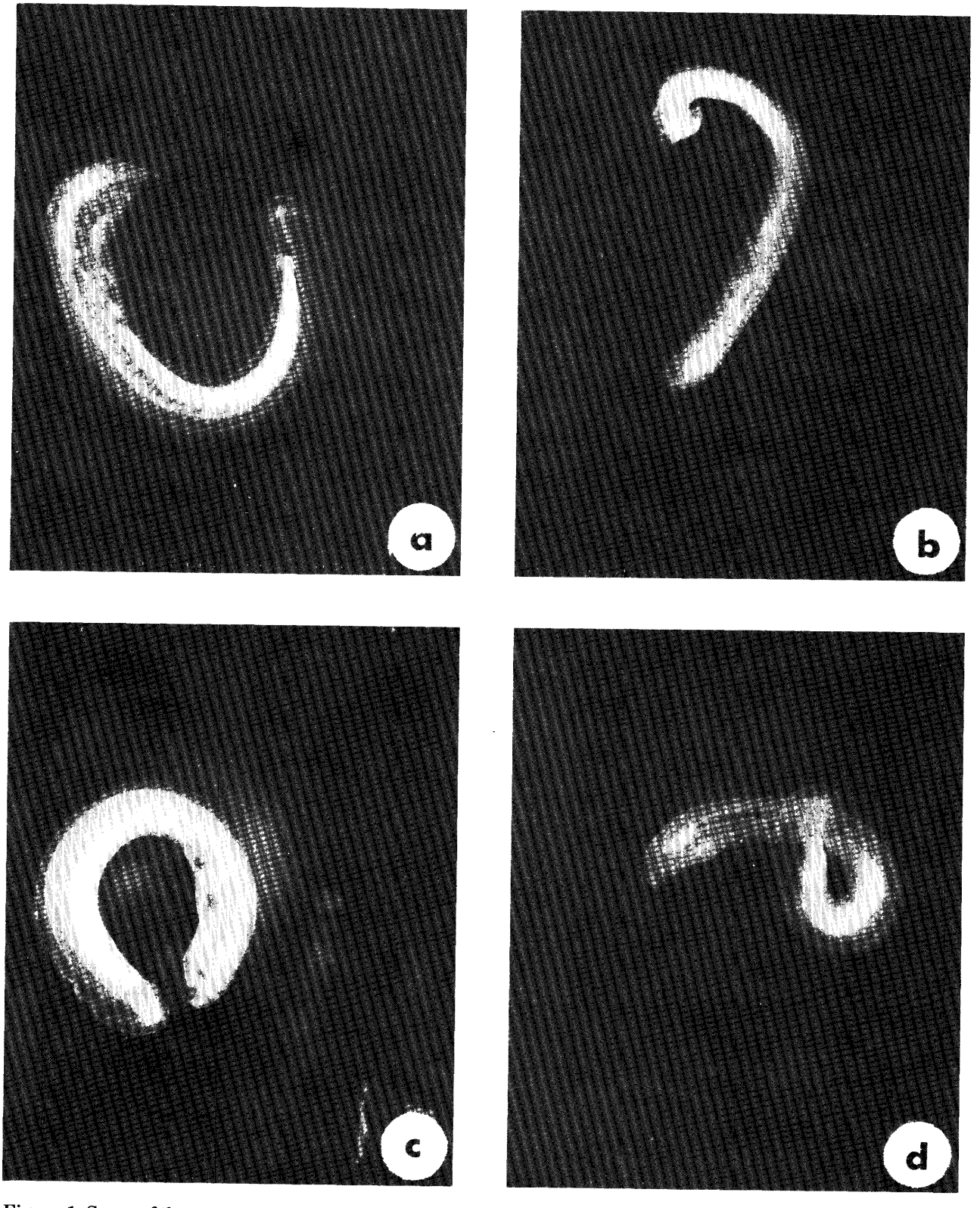

Figure 1. Some of the more prominent changes in body contour effected by exposure of developmental stages of the sea lamprey to 10 mg/l TFM. (a) "C" shaped prolarva, (b) "J" shaped prolarva, (c) "O" shaped prolarva, (d) "P" shaped prolarva.

IMPLICATIONS OF THE EXPERIMENT

Although production of viable stage-17 prolarvae was very markedly reduced, from 71% in the controls to only 4% after exposure of stages 1-7 to 10-mg/l TFM and nil after exposure of stages 8-17, this experiment indicates that treatments of streams with TFM during the sea lamprey spawning season probably do not kill all sea lamprey embryos. Some embryos less than 24 h old at the time of treatment would be expected to survive (at water temperatures near 18 C), and the percentage of survivors would be expected to increase as concentration and time of exposure decreased from 10 mg/l and 24 h.

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INVESTIGATIONS IN FISH CONTROL

65. Accumulation and Loss of Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in Fish Muscle Tissue: Laboratory Studies

By Joe B. Sills and John L. Allen

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ACCUMULATION AND LOSS OF RESIDUES OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) IN FISH MUSCLE TISSUE: LABORATORY STUDIES

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ABSTRACT

Residues of 3-trifluoromethyl-4-nitrophenol (TFM) in muscle tissue of eight species of fish, after they were exposed under controlled conditions, were determined by gas chromatography. The mean concentration of TFM residue in samples from various species immediately after a 12-h exposure to 1 to 4 mg/l of TFM ranged from 0.02 to 5.09 μg/g depending on pH, temperature, hardness of the test solutions, and TFM concentration. Residues decreased rapidly after the fish were withdrawn from the test media, and were near the limit of detection (0.01 μg/g) within 24 h.

INTRODUCTION

Control of the parasitic sea lamprey *(Petromyzon marinus)* in the Great Lakes is dependent on the application of selective toxicants to streams inhabited by the larvae. The toxicant that has been most widely used is 3-trifluoromethyl-4-nitrophenol (TFM). To retain the registered use of TFM, the U.S. Environmental Protection Agency requires that its safety for use in natural waters be evaluated. The concentration of TFM residues in fish exposed to TFM and the persistence of the residues is part of the safety information required to retain its registration. In the present study we measured the concentration and persistence of TFM residue in fish muscle after exposure of eight species of fish to measured quantities of TFM.

MATERIALS AND METHODS

Eight species of fish representative of Great Lakes populations were exposed to selected concentrations of field grade TFM (39.4% sodium salt with 35.7% free phenol) for 12 h in 100-liter polyethylene tanks with aeration. The 12-h exposure period (usually to a TFM concentration of 1 mg/l) was used to simulate the time that fish are commonly exposed during stream treatments. After each test the fish were placed in a flow of fresh water of the same temperature for recovery. Concentrations were calculated on the free phenol basis. Water hardness, pH, and temperature were varied in some tests to evaluate their effects on TFM uptake and residue elimination. The hardness and pH of the water used during the exposure periods were controlled as described by Marking and Dawson (1973). Fish weighing 100 g or more were used in all tests to ensure enough muscle tissue for analysis. Samples consisting of five fish each were selected for analysis usually at intervals of 4 h for 24 h after transfer to fresh water. Fish were filleted and frozen immediately after selection.

Frozen fish samples were prepared for analysis by the method of Benville and Tindle (1970) and extracted by the column technique of Hesselberg and Johnson (1972). The fish extracts were analyzed by the gas chromatographic method of Allen and Sills (1974). The method is capable of detecting 0.01 ng of TFM; samples containing less than 0.01 μg/g of TFM residue are reported as 0.00.

Rainbow trout *(Salmo gairdneri)*, brown trout *(S. trutta)*, lake trout *(Salvelinus namaycush)*, carp *(Cyprinus carpio)*, and white bass *(Morone chrysops)* were treated and sampled at the Fish Control Laboratory, La Crosse, Wis. Frozen fillets were sent to the Southeastern Fish Control Laboratory, Warm Springs, Ga. for analysis. The channel catfish *(Ictalurus punctatus)*, bluegill *(Lepomis macrochirus)*, and largemouth bass *(Micropterus salmoides)* were treated, sampled, and analyzed at the Southeastern Fish Control Laboratory. We tested rainbow trout, representing the coldwater species, and channel catfish, representing the warmwater species, more extensively than the other species to determine effects of temperature, hardness, concentration of TFM, and pH on residue accumulation and elimination.

Accumulation and Loss of TFM Residues in Eight Species of Fish

Rainbow Trout

Rainbow trout ranging in weight from 100 to 600 g were exposed to 1-mg/l solutions of TFM in waters adjusted to four different hardnesses and three different temperatures (Table 1). Temperature changes per se had relatively little effect on the accumulation and elimination of TFM residue. The mean residue levels immediately after the 12-h exposure ranged from 0.30 to 1.01 μg/g, and showed no consistent change with different temperatures. Residues were near the lowest detectable limit of 0.01 μg/g 24 h after withdrawal in all three tests.

Increases or decreases in hardness, which were accompanied by corresponding changes in pH, produced noticeable differences in the accumulation and elimination of TFM residue when temperature was held constant. The mean concentration of TFM residue at withdrawal was lowest in fish from the hardest water (0.11 μg/g) and highest in the softest water (2.77 μg/g). The rates of accumulation and elimination were both greatest among fish in the softest water tested; and the slightly higher residue level remaining after a 24-h withdrawal probably resulted from the much higher initial accumulation. The residues after 24 h in the other tests were 0.00 or 0.01 μg/g.

Channel Catfish

Channel catfish weighing from 400 to 1,200 g were exposed to 1-mg/l solutions of TFM adjusted to three different temperatures and four different water hardnesses (Table 2), and to solutions containing 4 mg/l of TFM at three temperatures in water having a hardness of 160 to 180 mg/l (Table 3). As in rainbow trout, accumulation and elimination of TFM residue were not closely related to temperature. Fish exposed to 4-mg/l concentrations of TFM accumulated more residue than those exposed to 1-mg/l concentrations. The elimination of residue, however, followed the pattern described for rainbow trout, and the TFM concentration

Table 1. Residues of TFM in rainbow trout exposed to 1-mg/l concentrations of TFM for 12 h

Test solutions			Withdrawal interval (hours)	TFM residue (μg/g)[a]	
Hardness (as mg/l $CaCO_3$)	pH	Temp. (°C)		Mean	Range
10-13	6.4-6.8	12	Control	0.00	0.00-0.01
			0	2.77	2.40-3.10
			4	1.42	1.30-1.55
			12	0.11	0.08-0.12
			24	0.04	0.02-0.05
40-48	7.2-7.6	7	Control	0.00	0.00-0.00
			0	1.01	0.74-1.31
			12	0.19	0.02-0.53
			24	0.01	0.01-0.02
40-48	7.2-7.6	12	Control	0.00	0.00-0.00
			0	0.30	0.01-0.79
			4	0.02	0.01-0.03
			8	0.02	0.00-0.06
			12	0.01	0.01-0.01
40-48	7.2-7.6	17	Control	0.00	0.00-0.00
			0	0.74	0.60-1.07
			8	0.03	0.01-0.06
			24	0.01	0.01-0.01
160-180	7.6-8.0	12	Control	0.00	0.00-0.00
			0	0.15	0.04-0.27
			4	0.03	0.02-0.05
			8	0.05	0.02-0.09
			12	0.01	0.01-0.02
			24	0.01	0.01-0.02
280-320	8.0-8.4	12	Control	0.00	0.00-0.00
			0	0.11	0.05-0.23
			4	0.04	0.01-0.10
			8	0.00	0.00-0.01
			12	0.00	0.00-0.00
			24	0.00	0.00-0.00

[a] Each mean and range represents five analyses.

Table 2. Residues of TFM in channel catfish exposed to 1-mg/l concentrations of TFM for 12 h

Test solutions			Withdrawal interval (hours)	TFM residue (μg/g)[a]	
Hardness (as mg/l $CaCO_3$)	pH	Temp. (°C)		Mean	Range
20-22	7.1-7.6	12	Control	0.00	0.00-0.00
			0	1.75	1.10-2.20
			4	0.67	0.45-1.04
			8	0.32	0.08-0.70
			12	0.07	0.03-0.10
			24	0.02	0.01-0.04
20-22	7.1-7.6	18.5	Control	0.00	0.00-0.00
			0	1.67	1.10-2.40
			4	0.18	0.10-0.26
			8	0.06	0.02-0.10
			12	0.02	0.00-0.05
			24	0.02	0.00-0.06
20-22	7.1-7.6	27	Control	0.00	0.00-0.00
			0	1.41	1.00-2.00
			4	0.03	0.03-0.04
			8	0.01	0.01-0.01
			12	0.01	0.01-0.02
			24	0.01	0.00-0.01
40-48	7.2-7.6	18.5	Control	0.00	0.00-0.00
			0	0.77	0.64-0.93
			4	0.10	0.07-0.12
			8	0.02	0.01-0.03
			12	0.01	0.01-0.01
			24	0.00	0.00-0.00
160-180	7.6-8.0	18.5	Control	0.00	0.00-0.00
			0	0.33	0.16-0.60
			4	0.02	0.01-0.03
			8	0.00	0.00-0.01
			12	0.00	0.00-0.00
			24	0.00	0.00-0.00
280-320	8.0-8.4	18.5	Control	0.00	0.00-0.00
			0	0.13	0.01-0.30
			4	0.01	0.00-0.02
			8	0.00	0.00-0.01
			12	0.00	0.00-0.00
			24	0.00	0.00-0.00

[a] Each mean and range represents five analyses.

Table 3. Residues of TFM in channel catfish exposed to 4-mg/l concentrations of TFM for 12 h

Test solutions			Withdrawal interval (hours)	TFM residue (μg/g)[a]	
Hardness (mg/l as $CaCO_3$)	pH	Temp. (°C)		Mean	Range
160-180	7.6-8.0	12	Control	0.00	0.00-0.00
			0	5.09	2.07-8.60
			4	2.28	0.80-4.60
			8	0.33	0.19-0.62
			12	0.66	0.20-1.28
			24	0.04	0.01-0.09
160-180	7.6-8.0	17	Control	0.00	0.00-0.00
			0	2.09	1.90-2.53
			4	0.64	0.36-0.92
			8	0.39	0.08-1.12
			24	0.03	0.02-0.04
160-180	7.6-8.0	27	Control	0.00	0.00-0.00
			0	2.59	2.07-3.17
			4	0.10	0.07-0.12
			8	0.03	0.02-0.05
			12	0.01	0.01-0.02
			24	0.01	0.00-0.04

[a] Each mean and range represents five analyses.

was only slightly above the background concentration after 24 h. The elimination of TFM was slightly accelerated at the highest temperature. In fish exposed to a 1-mg/l concentration of TFM, the mean residue at withdrawal ranged from 0.13 to 1.75 μg/g immediately after withdrawal and from 0.00 to 0.02 μg/g after 24 h of withdrawal. In fish exposed to the higher concentration, these values ranged from 2.09 to 5.09 μg/g at withdrawal and from 0.01 to 0.04 μg/g after 24 h of withdrawal.

The accumulation of TFM again was closely related to the hardness of the test medium. As in rainbow trout, channel catfish accumulated the highest level of TFM residue in the softest test solution. However, the elimination was essentially complete after 24 h in all tests.

In tests of the effect of pH on the accumulation of TFM in channel catfish the temperature, hardness, and concentration of TFM were held

Table 4. Residues of TFM in channel catfish muscle tissue immediately after exposure to a 1-mg/l concentration of TFM for 12 h at 18.5 C, a water hardness of 45 mg/l as $CaCO_3$, and various pH's

pH	μg/g of TFM in muscle	
	Mean[a]	95% confidence interval
6	3.21	0.00-6.51
7	1.53	1.22-1.84
8	0.33	0.29-0.37
9	0.03	0.03-0.05

[a] Each mean and range represents five analyses.

constant in solutions that were buffered to pH's 6, 7, 8, and 9 (Table 4). The analysis of fish immediately after withdrawal clearly showed that the uptake of TFM decreased as pH increased. The mean concentration of TFM was 3.21 μg/g at pH 6 and 0.03 μg/g at pH 9. The decrease was 10-fold from pH 6 to pH 8 and 100-fold from pH 6 to pH 9. The pH variation between the unbuffered test solutions was the most probable cause of TFM residue variation rather than increases in hardness per se.

Other Species of Fish

Persistence of TFM in muscle tissue of other fish (Table 5) was similar to that in rainbow trout and channel catfish. TFM residue was essentially eliminated from fish muscle after 24 h in fresh water even in brown trout and carp, which retained low concentrations slightly longer than the other species.

Table 5. Residues of TFM in muscle tissue of six species of fish exposed to a 1-mg/l concentration of TFM for 12 h

Test solutions			Withdrawal interval (hours)	TFM residue (μg/g)[a]	
Hardness (mg/l as $CaCO_3$)	pH	Temp. (°C)		Mean	Range
Brown trout					
40-48	7.2-7.6	12	Control	0.00	0.00-0.00
			0	0.77	0.54-0.92
			4	0.35	0.15-0.63
			8	0.25	0.07-0.35
			12	0.13	0.04-0.22
			24	0.10	0.06-0.14
40-48	7.2-7.6	7	Control	0.00	0.00-0.00
			0	0.35	0.24-0.63
			4	0.04	0.02-0.06
			8	0.04	0.01-0.12
			24	0.03	0.02-0.06
Lake trout					
40-48	7.2-7.6	12	Control	0.00	0.00-0.00
			0	0.11	0.07-0.14
			4	0.04	0.02-0.04
			8	0.02	0.01-0.03
			12	0.02	0.01-0.02
			24	0.00	0.00-0.01
Carp					
40-48	7.2-7.6	12	Control	0.00	0.00-0.00
			0	1.69	1.40-1.90
			4	0.78	0.45-1.00
			8	0.34	0.27-0.53
			12	0.24	0.13-0.33
			24	0.06	0.02-0.07
White bass					
40-48	7.2-7.6	12	Control	0.00	0.00-0.00
			0	0.02	0.02-0.02
			4	0.02	0.01-0.04
			8	0.01	0.00-0.01
			12	0.00	0.00-0.00
			24	0.00	0.00-0.00

Table 5—Continued

Test solutions			Withdrawal interval (hours)	TFM residue (μg/g)[a]	
Hardness (mg/l as $CaCO_3$)	pH	Temp. (°C)		Mean	Range
Bluegill					
20-22	6.5-6.9	18.5	Control	0.00	0.00-0.00
			0	0.21	0.18-0.26
			4	0.07	0.03-0.10
			8	0.04	0.03-0.06
			12	0.04	0.01-0.13
			24	0.01	0.01-0.02
Largemouth bass					
20-22	6.5-6.9	18.5	Control	0.00	0.00-0.00
			0	0.32	0.16-0.57
			4	0.01	0.01-0.01
			8	0.00	0.00-0.01
			12	0.00	0.00-0.01
			24	0.00	0.00-0.00

[a] Each mean and range respresents five analyses.

DISCUSSION

Residues of TFM do accumulate in the muscle of fish, and the amount varies with species and exposure conditions. Though there was wide variation in residue concentrations immediately after exposure, the concentration decreased rapidly after the fish had been placed in fresh water. After 24 h of withdrawal in fresh water the TFM residues were almost completely eliminated (less than 0.01 to 0.04 μg/g). The factors which have the greatest influence on uptake of TFM are concentration and pH of the medium. High pH has the effect of lowering the concentration of available TFM which lowers uptake. An increase in pH from 6 to 9 caused a 100-fold reduction in TFM uptake in channel catfish. Water hardness seems to influence uptake, but may in fact be a result of pH changes because elevated pH accompanies increased hardness. Temperature affects fish activity and metabolism, and thus probably exerts some influence on residue uptake and elimination.

CONCLUSIONS

1. The concentration of TFM residues in the muscle of fish exposed to 1.0 to 4.0 mg/l of TFM for 12 h ranged from 0.02 to 5.09 μg/g; however the chemical disappeared almost completely within 24 h after withdrawal.
2. The accumulation of TFM residue in fish was more dependent upon pH than on water hardness or temperature.
3. The rate of elimination of TFM residue increased slightly as the temperature was increased.

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INVESTIGATIONS IN FISH CONTROL

66. Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in a Stream Ecosystem after Treatment for Control of Sea Lampreys

By Philip A. Gilderhus, Joe B. Sills, and John L. Allen

United States Department of the Interior
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RESIDUES OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) IN A STREAM ECOSYSTEM AFTER TREATMENT FOR CONTROL OF SEA LAMPREYS

by

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ABSTRACT

Samples of water, bottom soil, plants, invertebrates, and fish for residue analysis were collected from two stations on the East Au Gres River in Michigan before, during, and after treatment of the stream with 3-trifluoromethyl-4-nitrophenol (TFM) for control of sea lampreys *(Petromyzon marinus)*. The residues were highest in samples collected as the last portion of full-strength TFM flowed past each station, and were much higher in water and organisms than in the bottom soil. Fish retained higher residues than other organisms 24 h after treatment (up to 6 μg/g); however, the residues decreased to less than 0.08 μg/g at 96 h after treatment. Residues in soil were among the lowest found in all samples collected during the study.

INTRODUCTION

The first experimental stream treatments with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) for the control of sea lampreys *(Petromyzon marinus)* in the Great Lakes were conducted in the spring and summer of 1958 (Applegate et al. 1961), and operational treatments began in the fall (Great Lakes Fishery Commission 1958). Since then, most of the tributaries to the Great Lakes which harbor sea lamprey larvae have been treated with TFM and some streams have been treated several times.

The concentration and exposure time required for each stream are determined in on-site bioassays conducted just before treatment (Howell and Marquette 1962). Treatment crews apply TFM to a stream at various points along the section to be treated to ensure the maintenance of lamprey-killing concentrations and exposure times.

Concern about the possible accumulation of TFM in the environment led to early studies to assess the residues. Billy et al. (1965) determined concentrations in water from streams during and after treatment, but were unable to recover TFM from fish exposed to field-use concentrations in stream treatments or in raceway tests. They were, however, able to detect residues in fish exposed to exceptionally high concentrations. Kempe (1973) documented the decay rate of TFM in static water-soil systems under laboratory conditions.

Recent environmental protection legislation has prescribed stricter requirements for registration of pest-control chemicals and periodic review of existing registrations. During review of the TFM registration in 1970, the determination was made that the existing data on TFM residues were inadequate to satisfy the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act.

The present study was undertaken in response to the need for further information on the persistence of TFM residues in representative components of a stream environment. Fish, invertebrates, water, plants, and bottom soil were sampled at selected intervals during and after an operational stream treatment for eradication of sea lamprey larvae, and analyzed for residues of TFM.

MATERIALS AND METHODS

The East Au Gres River in Iosco and Arenac counties, Michigan, was chosen for sampling because it is a fertile and productive stream inhabited by a diversity of organisms. The stream is about 40 km (25 mi) long, and has flow volumes of 0.57 to 0.71 m^3/sec (20–25 cfs) in its middle reaches and about 1.43 m^3/sec (50 cfs) near the mouth. The stream has a moderate gradient and extensive riffle areas.

The stream treatment of 25–26 July 1972 was designed to maintain 11 mg/l of TFM for about 12 h at any given point in the stream. Treatment was started in the headwaters and the concentration was boosted at selected locations downstream to compensate for dilution that resulted from increases in volume of flow between application points. The lampricide was applied at each site for several hours by means of battery-powered fuel pumps.

Two sampling stations were chosen on the basis of the abundance and diversity of organisms available, the different types of habitat represented, and their location in the stream system. Station 1 was in the middle reaches of the stream, where a peak concentration of 11 mg/l of TFM was maintained for 12 h. Passage time for the chemical at this station, including the buildup to full strength and decline was about 20 h. The samples were taken in a 350-m long area consisting of riffles and runs where the stream bottom was mostly rubble and gravel. Station 2 was about 800 m from the mouth of the river and about 10 km (6 mi) downstream from the last TFM application point. The chemical passed this point over a period of 20–25 h, and the peak concentration of 10–11 mg/l lasted about 7 h. The samples were taken in a 100-m long area where the bottom consisted mostly of clay overlain by large rubble and boulders, with small pockets of sand.

We collected samples at each station at four intervals: before treatment; near the end of the peak period of concentration of the chemical (hereafter called treatment samples); and at 24 and 96 h after treatment. We assumed that the treatment samples would contain the maximum residue because they were collected after maximum exposure to the chemical and immediately before exposure to untreated water. Samples included water, bottom soil (sand with some

Table 1. Organisms collected in the East Au Gres River, Michigan for analysis of residues of TFM

Common Name	Classification
Plants	
Algae	*Cladophora* sp.
Water weed	*Elodea* sp.
Invertebrates	
Oligochaetes (Aquatic earthworms)	Annelida, Oligochaeta
Crayfish	Crustacea, Decapoda
Stonefly	Insecta, Plecoptera, *Pteronarcys* sp.
Mayfly	Insecta, Ephemeroptera, *Ameletus* sp.
Dragonfly	Insecta, Odonata, Anisoptera
Dobsonfly	Insecta, Megaloptera, Corydalidae
Cranefly	Insecta, Diptera, Tipulidae
Snipefly	Insecta, Diptera, Rhagionidae
Snails	Mollusca, Gastropoda, *Physa* sp.
Fish	
Rainbow trout	*Salmo gairdneri*
Cyprinids	*Rhinichthys cataractae* and *Notropis* sp.
Sculpin	*Cottus* sp.

detritus), oligochaetes (aquatic earthworms), crayfish, aquatic insect larvae, snails, algae, aquatic vascular plants, and fish. Two species of cyprinids were pooled as a sample representative of fish which feed at a low trophic level. Organisms sampled (Table 1) were those for which we could get an adequate weight of material for analysis in a reasonable time (2-3 h). Due to habitat and sampling variations, some species were found at only one of the stations, and some were not found in all samples from the same station.

Water samples were collected in plastic bottles at the surface of the stream. Bottom soil samples were taken manually from the top 2.5 cm of the substrate. Fish, crayfish, and mayflies were collected by electrofishing. In the collection of other aquatic insects and oligochaetes, one man disturbed the bottom material with a shovel immediately upstream from a screen held by another. Snails and aquatic vegetation were picked by hand. All samples were frozen shortly after collection, in a styrofoam box containing dry ice. They remained frozen during transportation to the laboratory for analysis.

Samples were analyzed for TFM residues by the method of Allen and Sills (1974), which has a detection limit of 0.01 μg/g. Organisms were analyzed on the basis of wet weight, whole-body residues. The extraction procedure was modified to accommodate the small sample weights of the insects, which were homogenized in the hexane-ethyl ether (3+1) extracting solvent. Each soil sample was blended in three, 100-ml quantities of solvent. Water samples were acidified and extracted with three portions of hexane-ethyl ether (3+1). The extracts were cleaned up, concentrated, and analyzed by the gas chromatographic method of Allen and Sills (1974).

RESULTS AND DISCUSSION

No pretreatment samples at either station (Tables 2 and 3) showed TFM with the exception of oligochaetes, which yielded a small chromatographic peak (Table 2) with nearly the same retention time as TFM. Treatment samples contained the highest concentrations of TFM. Posttreatment samples showed a rapid decrease in TFM residues with time.

Highest residues were accumulated by oligochaetes and snails, the treatment samples of which contained 21.4 and 15.3 μg/g of TFM, respectively. No oligochaetes were collected in the 24- and 96-h samples. They were not abundant even before treatment at station 1 because the largely gravel and rubble bottom there was poor oligochaete habitat. There is, however, previous evidence of a significant reduction of oligochaete populations after treatment of streams with TFM (Torblaa 1968). Snails were the only organisms which retained more than 0.1 μg/g of TFM 96 h after treatment; they retained 0.37 μg/g, a 98% reduction from residue levels observed in treatment samples.

Fish ranked next in terms of residues present immediately after maximum exposure to TFM. Residues in fish ranged from 4.3 μg/g in rainbow trout to 11.4 μg/g in cyprinids, both at station 2. Fish also retained the highest residues at 24 h after treatment. The reduction in residues after 24 h ranged from 45 to 87% with the exception of rainbow trout at station 2, in which the reduction was only 4%. By 96 h after treatment, residues had declined 99% in all fish samples.

Residues in aquatic insects at the end of treatment ranged from 0.8 μg/g in cranefly larvae to 5.73 μg/g in mayfly nymphs. Concentrations of TFM in insects declined by 85 to 92% after 24 h and by 93 to 99% after 96 h in fresh water.

Aquatic plants accumulated residues of TFM in about the same range as insects. Although the number of samples was limited, the data indicate that algae *(Cladophora* sp.*)* absorbed TFM more rapidly than higher plants *(Elodea* sp.*)*. The concentrations of TFM in plants declined by 89 to 97% after 24 h and 98 to 99% after 96 h in fresh water.

Concentrations of TFM at the end of treatment were lower in soil samples than in other samples, indicating that TFM does not have an affinity for soil particles. The maximum concen-

tration of TFM in a soil sample was only 0.8 μg/g. By 96 h after treatment, no TFM was detectable in soil samples.

TFM apparently does not persist in the food chain. Sills and Allen (1975) showed that residues of TFM in fish muscle decreased to near the detection limit by 24 h after exposure to 1 to 4 mg/l of TFM in controlled laboratory studies. Mechanisms of elimination from fish have been outlined by Lech and Costrini (1972) and Hunn and Allen (in press). The present study showed the loss of residues from whole fish under field conditions also to be rapid, but somewhat slower than under the laboratory conditions of Sills and Allen (1975). The difference was likely due to the higher concentrations to which fish were exposed in the field. Residues of TFM were more readily eliminated from aquatic insects than from fish. No evidence of biomagnification was noted and residues in all major components of the stream ecosystem were reduced by 93 to 99% in 96 h.

Table 2. TFM residues in samples from the east branch of the East Au Gres River (Sample station 1[a]) at State Road

Sample	TFM residues (μg/g)			
	Before treatment	During treatment	24 h post-treatment	96 h post-treatment
Water	0.00	9.45	0.00	0.00
Soil	0.00	0.44	0.08	0.00
Plants (vascular)	0.00	2.90	0.32	0.08
Oligochaetes	0.02	21.4	NS	NS
Crayfish	NS	NS	0.33	0.01
Stonefly	0.00	2.89	0.25	0.04
Dragonfly	NS	0.85	0.07	0.04
Dobsonfly	NS	NS	NS	0.04
Cranefly	0.00	0.80	0.06	0.06
Snipefly	0.00	1.13	0.18	0.04
Rainbow trout	0.00	9.80	1.33	0.01
Sculpin	0.00	10.9	6.00	0.04

[a] Values less than 0.01 μg/g are reported as 0.00; NS indicates no sample was collected.

Table 3. TFM residues in samples from lower East Au Gres River (Sample station 2[a])

Sample	TFM residues (μg/g)			
	Before treatment	During treatment	24 h post-treatment	96 h post-treatment
Water	0.00	6.10	0.00	0.00
Soil	0.00	0.80	0.14	0.00
Algae	0.00	6.75	0.22	0.03
Crayfish	0.00	1.14	0.12	0.03
Stonefly	0.00	4.08	0.38	0.06
Mayfly	0.00	5.73	0.17	0.02
Cranefly	0.00	NS	NS	0.01
Snails	0.00	15.3	0.58	0.37
Rainbow trout	0.00	4.30	4.15	0.08
Cyprinids	0.00	11.4	5.00	0.07

[a] Values less than 0.01 μg/g are reported as 0.00; NS indicates no sample was collected.

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