INVESTIGATIONS IN FISH CONTROL

60. **Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests**

61. **Toxicity of the Lampricide 3-Trifluoromethlyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests**

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United States Department of the Interior
Fish and Wildlife Service

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IN THIS COVER

FOREWORD

The lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), has been used extensively to control larvae of the sea lamprey (Petromyzon marinus) in the Great Lakes. Although the toxicity of TFM to lampreys is well documented, its effects on other organisms are unknown.

The use of any toxicant in the environment raises concern as to the safety of nontarget organisms. Since invertebrate and lower vertebrate populations provide the forage base for many sport and commercial fishes, data on how TFM affects these organisms are vital to any application for registration.

The three papers in this series represent a part of continuing research on the effects of TFM on aquatic organisms. The papers report the results of tests on 15 species of nontarget fish, the larvae of 3 species of frogs, and 16 species of invertebrates. Reports on the effects of TFM on algae, midges, mayflies, and selected other invertebrates were published as Nos.56, 57, 58, and 59 of Investigations in Fish Control; a complete review of the literature prior to 1972 related to the use of TFM as a lampricide was published in No. 44.

Fred P. Meyer, Director
Fish Control Laboratories

60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests

By Leif L. Marking and Lee E. Olson

CONTENTS

TOXICITY OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO NONTARGET FISH IN STATIC TESTS

By Leif L. Marking and Lee E. Olson [1]
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ABSTRACT

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is applied to tributary streams of the Great Lakes for controlling larvae of the sea lamprey (Petromyzon marinus). During treatments for lamprey control, cohabiting, nontarget fish are also exposed to TFM. Knowledge of the margin of safety for these fish is vitally important to the reduction of undesired effects of field applications. The lampricide is toxic to 15 species of coldwater and warmwater nontarget fish; the 96-h LC50's in static tests at 12 C range from 1.39 to 16.2μ 1/1 of field grade TFM (35%). The toxicity of TFM is influenced by temperature, water hardness, and pH. The most influential factor is pH. For certain species, more than 50 times as much chemical is needed to produce the same effect at pH 9.5 as at pH 6.5. In laboratory test water, TFM detoxifies slowly; solutions lose little or no activity over periods up to 8 wk. The margin of safety (LC01 for fish/LC99 for lamprey) for rainbow trout (Salmo gairdneri) in minimum lampricidal concentrations of TFM is influenced by pH and is greater in water of low pH (6.5) than in water of higher pH. Under laboratory conditions at pH 7.5 and 8.5, a 10% mortality of rainbow trout could be expected in lampricidal concentrations of field grade TFM.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is an effective toxicant against larval lampreys (Petromyzon marinus) living in tributary streams of the Great Lakes (Applegate et al. 1958). However, additional data on the toxicity of TFM to nontarget organisms are needed to satisfy regulatory requirements for toxicants (Lennon 1967). Previous laboratory and field information regarding the use of this lampricide was summarized by Schnick (1972).

The present study was designed to determine the toxicity of purified, field grade, and reduced TFM to fish in laboratory toxicity tests and to determine the influence of water hardness, pH, and temperature on the toxicity of TFM. The residual toxicity of TFM in water solutions was determined to evaluate the persistence of the toxicant under aerobic conditions. These data were used to derive the margin of safety for nontarget fish.

MATERIALS AND METHODS

The static test procedures used follow closely those of Lennon and Walker (1964) and Taras (1971). Ten fish were exposed to each concentration of TFM in glass jars containing 15 liters of oxygen-saturated test water. The test waters were prepared according to the schedule in Table 1 to produce desired water hardnesses. In separate studies, the pH of

Table 1.--Quantities of salts and characteristics of reconstituted waters

Water type	Salts added in mg/l				pH	mg/l as $CaCO_3$	
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl		Hardness	Alkalinity
Very soft	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft	48	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Hard	192	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Very hard	384	240.0	240.0	16.0	8.0-8.4	280-320	225-245

test waters was controlled with chemical buffers (Table 2). The solutions were adjusted to the appropriate pH before the test and readjusted with chemical buffers at 24-h intervals as necessary to maintain the selected pH ± 0.2 units. Test temperatures were regulated by immersing the test jars in constant-temperature water baths.

Table 2.--Buffer chemicals used to produce and maintain various pH's in soft, reconstituted water

pH	Milliliters of solutions for 15 liters of water		
	1N NaOH	1M KH_2PO_4	0.5M H_3BO_3
6.0	1.3	80.0	---
6.5	10.0	30.0	---
7.0	19.0	30.0	---
7.5[a]	---	---	---
8.0	19.0	20.0	---
8.5	12.0	11.5	---
9.0	8.8	---	30.0
9.5	11.0	---	20.0
10.0	16.0	---	18.0

[a]Unbuffered soft, reconstituted water.

Field grade TFM and analytical grade TFM (99% active ingredient) were obtained from Hoescht Chemical Company, Summerville, New Jersey.[2] Field grade TFM is formulated with DMF (N,N-dimethylformamide) and is approximately 35% active ingredient, but the purity varies slightly between batches. Purified TFM was prepared by Aldrich Chemical Company[2] (96% active ingredient). Dr. John Lech of the Department of Pharmacology of the Medical College of Wisconsin at Milwaukee also prepared purified TFM (94% active ingredient) and synthesized reduced TFM (RTFM) hydrochloride for these experiments. The purified materials were weighed on an electrobalance and dissolved in acetone; concentrations are expressed as mg/l. Field-grade TFM was measured volumetrically and dissolved in water; concentrations are expressed as μl/l.

Fish weighing 1 to 1.5 g each were obtained from Federal hatcheries and maintained according to the standard procedures of the Fish Control Laboratory (Hunn et al. 1968). The fish were acclimated to the desired water chemistries and temperature of each test. Mortalities were recorded at 1, 3, and 6 h on the first day of exposure and daily thereafter for the remainder of the 96-h test.

The methods of Litchfield and Wilcoxon (1949) were used in computation of the LC50's (concentrations producing 50% mortality) and

Table 3.--Toxicity of purified TFM[a] to fingerling fish in toxicity tests at 12 C

Species	LC50 and 95% confidence interval (mg/l) at				
	1 h	3 h	6 h	24 h	96 h
Coho salmon (Oncorhynchus kisutch)	6.80 6.24-7.41	5.60 4.69-6.69	5.60 4.69-6.69	4.30 3.78-4.89	2.70 2.26-3.22
Chinook salmon (Oncorhynchus tshaw- ytscha)	4.00 3.30-4.86	3.40 3.06-3.78	---	3.10 2.70-3.55	2.24 1.94-2.59
Rainbow trout (Salmo gairdneri)	4.40 4.02-4.82	3.08 2.87-3.31	2.92 2.65-3.22	2.91 2.57-3.31	1.97 1.78-2.18
Brown trout (Salmo trutta)	7.00 6.33-7.74	4.93 4.47-5.43	4.78 4.20-5.44	3.89 3.57-4.24	2.63 2.35-2.94
Lake trout (Salvelinus namaycush)	2.72 2.30-3.21	1.93 1.71-2.18	1.43 1.16-1.75	1.43 1.16-1.75	1.40 1.11-1.77
Northern pike (Esox lucius)	5.55 4.67-6.59	1.85 1.25-2.73	1.85 1.25-2.73	1.25 0.847-1.84	0.947 0.594-1.51
Carp (Cyprinus carpio)	---	---	2.10 1.85-2.37	1.74 1.43-2.11	1.25 1.00-1.56
Channel catfish (Ictalurus punctatus)	5.15 4.26-6.23	2.38 2.05-2.76	1.34 1.20-1.50	1.20 1.03-1.40	1.00 0.803-1.25
Bluegill (Lepomis macrochirus)	12.9 11.4-14.6	8.90 8.22-9.64	6.42 5.99-6.89	6.23 5.50-7.05	6.23 5.50-7.05
Smallmouth bass (Micropterus dolomieui)	11.1 10.2-12.1	7.96 7.10-8.93	6.42 5.66-7.28	6.42 5.66-7.28	6.30 5.63-7.04
Largemouth bass (Micropterus salmoides)	---	5.45 5.01-5.93	3.85 3.41-4.35	2.19 1.82-2.63	---
Yellow perch (Perca flavescens)	6.20 5.05-7.61	3.38 2.63-4.35	2.88 2.47-3.35	2.51 2.19-2.88	2.07 1.69-2.54
Walleye (Stizostedion vitreum)	7.10 4.96-10.1	3.00 2.47-3.65	2.05 1.84-2.28	1.88 1.61-2.19	1.88 1.63-2.16

[a]Purity (94%) for tests with coho salmon and (96%) for tests with others.

95% confidence intervals. Regressions were drawn and inspected for each set of data. All data fulfilled the Chi-square test requirement for acceptability.

Deactivation indices were derived for field grade TFM in water at four different pH's. Aged solutions of the toxicant were bioassayed to determine the biological activity remaining after selected time periods. The deactivation index was determined by dividing the LC50 of aged solutions by the LC50 of unaged solutions under corresponding test conditions (Marking 1972).

RESULTS

Purified TFM

Toxicity to selected species of fish

Purified TFM is toxic to coldwater and warmwater fish in soft water; the 96-h LC50's range from 0.947 to 6.30 mg/l of TFM (Table 3). Northern pike and channel catfish were most sensitive, and the 96-h LC50's were not significantly different from each other (P = 0.05). Smallmouth bass and bluegill are the most resistant; LC50's were significantly different from those with other species at all comparable exposure periods. Most of the other species were sensitive to 1 to 3 mg/l of TFM.

All species responded rapidly to the toxic effects of TFM (as shown by the 1-h LC50's), and the toxicity changed little with prolonged exposure (the 1-h LC50's which are only 2 to 5 times greater than 96-h LC50's). The LC50's for 24- and 96-h exposures were not significantly different for lake trout, northern pike, channel catfish, smallmouth bass, bluegill, yellow perch, and walleye.

Influences of temperature, water hardness, and pH

The toxicity of purified TFM to fish was altered considerably by water quality, and the alteration was fairly uniform for different species. Test results are presented for rainbow trout in Table 4 and for other species in Appendix Tables 1 to 7.

The lampricide was more toxic to rainbow trout in warm than in cold water. The 96-h LC50's were significantly different at temperatures of 7, 12, and 17 C. This influence was more consistent for coldwater species than for warmwater species. The 96-h LC50's for carp, for instance, were not significantly different at 12, 17, and 22 C.

Purified TFM was more toxic to rainbow trout in soft water (total hardness, 44 mg/l) than in hard or very hard water (total hardness, 170 and 300 mg/l, respectively). The respective 96-h LC50's at 12 C were 1.97, 5.47, and 9.45 mg/l. The hardness of test water influenced the toxicity to other species in a similar manner.

The toxicity of purified TFM to fish decreased substantially as the pH of test waters increased (Table 4 and Appendix Tables 1 to 7). The 96-h LC50's were significantly different for each pH increment for rainbow trout as well as for other species. The magnitude of change in toxicity can be compared by dividing the LC50 value at the lowest pH by that at the highest pH. The factors are as follows: coho salmon - 45, rainbow trout - 29, brown trout - 50, lake trout - 59, carp - 36, channel catfish - 23, bluegill - 21, and yellow perch - 26. The factor for rainbow trout is lower than that for other salmonids because the pH ranged only from 6.5 to 9.0, whereas for the other species the pH ranged from 6.5 to 9.5.

Field grade TFM

Toxicity to selected species of fish

Field grade TFM also was toxic to coldwater and warmwater fishes in soft water (Table 5). The 96-h LC50's for 15 species ranged from 1.39 to 16.2μ 1/1 of TFM (35.4%). Considering only the active ingredient in the formulation, the range was 0.39 to 4.58μ 1/1. Field grade TFM thus appeared to be more active than purified TFM; however, the difference was slight and may have been due to variations in sensitivity among the different groups of fish exposed. Among the families of fishes represented, centrarchids were the most resistant to TFM. Bluegill and green

Table 4.--Toxicity of purified TFM (96%) to fingerling rainbow trout at selected temperatures, water hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	6.20 5.61-6.85	4.00 3.73-4.29	3.00 2.78-3.24	2.89 2.64-3.16	2.44 2.16-2.75
12	Soft	7.5	4.40 4.02-4.82	3.08 2.87-3.31	2.92 2.65-3.22	2.91 2.57-3.30	1.97 1.78-2.18
17	Soft	7.5	3.10 2.93-3.28	2.62 2.33-2.94	2.62 2.33-2.94	2.05 1.70-2.47	1.58 1.35-1.85
12	Hard	7.8	---	9.00 8.41-9.63	8.43 7.67-9.26	8.00 7.43-8.61	5.45 4.74-6.27
12	Very hard	8.2	18.5 16.4-20.9	15.0 13.9-16.2	14.7 13.5-16.0	13.8 12.6-15.1	9.45 9.12-9.79
12	Soft	6.5	2.00 1.74-2.30	1.42 1.28-1.57	1.30 1.17-1.45	1.26 1.13-1.39	1.10 0.744-1.63
12	Soft	8.0	15.4 14.3-16.5	10.9 9.96-11.9	10.0 9.39-10.6	7.75 6.82-8.80	6.19 5.56-6.89
12	Soft	9.0	---	---	---	37.9 33.5-42.9	32.1 29.5-34.9

sunfish were the most resistant species to field grade TFM and smallmouth bass to purified TFM. Channel catfish were the most sensitive species to field grade TFM.

Influences of temperature, water hardness, and pH

The toxicity of field grade TFM to fish was influenced by temperature, water hardness, and pH in patterns similar to those observed with purified TFM. In general, TFM (35.7%) was most toxic to fish in warm, very soft, and low pH (6.5) water. Toxicity data for rainbow trout are in Table 6, and those for other species are in Appendix Tables 8-13. The greatest influence on the toxicity of TFM (35.7%) was from pH. Several 96-h LC50's were unavailable, but the 24-h exposure produced a good approximation of the 96-h results. The 24-h toxicity of TFM (35.7%) to rainbow trout

decreased by a factor of approximately 10 as pH increased from 6.5 to 8.5 and by a factor of nearly 100 as pH increased from 6.5 to 9.5 These factors were much greater than those for purified TFM at pH's of 6.5 to 9.0 (Table 4). Apparently the toxicity change accelerated above pH 9.0. Also, the data for most other species (Appendix Tables 8-13) showed a significant increase in toxicity from 24- to 96-h exposures at the high pH; the LC50's at pH 9.5 were 31 and 83 times greater than those at pH 6.5 for yellow perch and lake trout, respectively.

Reduced TFM

In waters of three different hardnesses, the reduced form of purified TFM was considerably less toxic to rainbow trout than the parent material. The 96-h LC50's ranged from 29.0 mg/l of RTFM in very soft to 48.0 mg/l in very hard water (Table 7); however, the

Table 5.--Toxicity of field grade TFM (35.4%)[a] to fingerling fish in toxicity tests at 12 C

Species	LC50 and 95% confidence interval (μl/l) at				
	1 h	3 h	6 h	24 h	96 h
Chinook salmon (Oncorhynchus tschawytscha)	11.5 9.66-13.7	8.66 7.86-9.54	7.62 6.48-8.96	5.98 5.09-7.03	4.20 3.52-5.02
Brown trout (Salmo trutta)	9.63 8.46-11.0	5.83 5.33-6.38	4.94 4.15-5.88	4.53 3.86-5.32	3.53 3.04-4.09
Rainbow trout (Salmo gairdneri)	5.83 5.36-6.34	4.83 4.33-5.39	4.46 4.05-4.91	3.83 3.31-4.43	3.83 3.31-4.43
Lake trout (Salvelinus namaycush)	14.5 12.5-16.9	4.94 3.81-6.38	4.52 3.50-5.82	3.84 3.11-4.72	2.94 2.64-3.28
Goldfish (Carassius auratus)	38.5 29.4-50.4	12.7 10.8-15.0	7.17 6.50-7.91	5.22 4.32-6.30	5.00 3.97-6.29
Carp (Cyprinus carpio)	8.27 7.00-9.77	4.51 3.24-6.29	3.35 2.37-4.74	3.35 2.37-4.74	3.35 2.37-4.74
Golden shiner (Notemigonus (crysoleucas)	18.8 18.3-19.3	11.4 9.98-13.0	10.0 9.01-11.1	8.20 7.10-9.48	7.62 6.29-9.23
Fathead minnow (Pimephales promelas)	17.5 15.9-19.3	10.5 8.14-13.5	5.54 4.65-6.60	4.79 4.19-5.47	4.79 4.19-5.47
White sucker (Catostomus commersoni)	10.0 8.24-12.1	6.50 5.16-8.19	6.26 4.94-7.93	4.50 3.22-6.28	3.95 2.69-5.81
Black bullhead (Ictalurus melas)	13.5 12.2-14.9	5.50 4.67-6.48	3.85 3.29-4.50	2.34 1.89-2.90	2.41 2.10-2.77
Channel catfish (Ictalurus punctatus)	11.9 10.2-13.9	4.64 4.09-5.25	3.86 3.30-4.51	2.40 2.08-2.77	1.39 1.12-1.73
Green sunfish (Lepomis cyanellus)	26.2 24.3-28.3	16.8 14.9-18.9	13.1 11.6-14.7	12.9 11.4-14.6	9.40 7.88-11.2
Bluegill (Lepomis macrochirus)	---	25.4 21.5-30.0	16.2 14.1-18.6	16.2 13.6-19.3	16.2 13.6-19.3
Largemouth bass (Micropterus salmoides)	---	---	10.0 5.28-18.9	6.04 4.07-8.97	6.04 4.07-8.97
Yellow perch (Perca flavescens)	11.4 10.0-12.9	7.00 6.35-7.71	5.85 5.27-6.49	5.80 4.98-6.76	4.35 3.45-5.48

[a]TFM (35.7%) for chinook salmon, brown trout, lake trout, and rainbow trout.

Table 6.--Toxicity of field grade TFM (35.7%) to fingerling rainbow trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	10.2 9.16-11.4	6.68 5.93-7.52	4.78 4.23-5.40	4.37 3.95-4.84	3.68 3.38-4.01
12	Soft	7.5	5.83 5.36-6.34	4.83 4.33-5.39	4.46 4.05-4.91	3.83 3.31-4.43	3.83 3.31-4.43
17	Soft	7.5	4.10 3.75-4.48	3.40 3.05-3.79	3.40 3.05-3.79	2.79 2.34-3.33	2.37 2.05-2.75
12	Very soft	6.6	3.77 3.32-4.28	3.27 2.85-3.75	---	---	---
12	Hard	7.8	50.3 43.5-58.2	26.0 23.0-29.4	19.0 16.7-21.6	14.1 12.8-15.5	8.38 7.41-9.48
12	Very hard	8.2	88.3 79.4-98.2	45.9 40.5-52.0	36.6 33.2-40.4	27.2 21.8-34.0	19.0 16.8-21.5
12	Soft	6.5	4.12 3.71-4.57	2.82 2.56-3.10	2.56 2.17-3.01	2.52 2.16-2.94	2.52 2.16-2.94
12	Soft	8.5	74.0 65.0-84.2	42.4 38.5-46.7	36.7 32.1-42.0	20.5 ---	---
12	Soft	9.5	>300	270 228-320	239 205-278	230 204-259	---

Table 7.--Toxicity of reduced TFM to fingerling rainbow trout in standard, reconstituted water at 12 C

Water hardness	LC50 and 95% confidence interval (mg/l) at		
	24 h	48 h	96 h
Very soft	30.0 24.6-36.6	30.0 24.6-36.6	29.0 26.2-32.1
Hard	64.0 51.7-79.2	60.0 49.2-73.2	49.0 42.7-56.3
Very hard	52.0 44.4-60.9	50.0 43.3-57.7	48.0 41.5-55.5

influence of water hardness was not as great as with the parent compound.

Residual Toxicity

Field grade TFM (35.4%) was added to test waters and permitted to age for 1 wk before rainbow trout were introduced. A comparison of the toxicity of aged solutions and unaged reference solutions showed that TFM detoxified slowly, if at all, in water solutions (Table 8). The 96-h deactivation index (LC50 of aged solution/LC50 of unaged solution) was 0.91 for pH 6.5, 1.03 for pH 7.5, 1.09 for pH 8.0, and 1.14 for pH 9.0. Although detoxification tended to be slightly greater at high pH's, the toxicity of aged and unaged solutions was not significantly different at any pH's.

Additional deactivation studies were carried out for longer aging periods to determine the rate of detoxification at different pH's. The toxicity and deactivation indices of purified TFM (96%) were determined at four pH's after aging periods up to 8 wk at 12 C (Table 9). The indices were near 1.0 for pH's 6.5, 7.5, and 8.5 but were erratic and did not show significant detoxification of TFM with aging. TFM was much less toxic to rainbow trout at pH 9.5 than at lower pH's. Activity decreased with aging, but the decrease again was erratic. Although the index at 4 wk of aging (1.95) indicated a considerable decrease in activity, the activity remained constant in a series of solutions aged for 6 wk. Apparently, TFM was detoxified in some instances but not in others. Determination of the rate of detoxification

Table 8.--Toxicity of field grade TFM (35.4%) to fingerling rainbow trout in buffered solutions at 12 C freshly prepared (F) or aged 1 week (A)

pH	Type of solution	LC50 and 95% confidence interval (μl/l) at				
		1 h	3 h	6 h	24 h	96 h
6.5	(F)	3.45 3.10-3.84	3.00 2.68-3.35	2.85 2.59-3.13	2.85 2.59-3.13	2.46 2.17-2.79
	(A)	4.00 3.57-4.49	3.30 2.87-3.79	3.29 2.87-3.77	3.10 2.75-3.49	2.24 2.01-2.50
7.5	(F)	11.9 10.3-13.8	8.70 7.98-9.48	8.70 7.98-9.48	6.33 5.63-7.11	4.78 3.97-5.76
	(A)	19.1 16.7-21.9	11.6 9.96-13.5	11.1 9.39-13.1	8.58 7.34-10.0	4.90 3.61-6.66
8.0	(F)	42.0 36.0-49.0	35.0 30.6-40.0	32.0 28.4-36.1	26.0 23.6-28.7	11.0 10.1-12.0
	(A)	42.0 38.4-45.9	33.0 29.3-37.2	31.0 27.6-34.8	23.0 21.0-25.2	12.0 10.4-13.8
9.0	(F)	---	---	---	---	39.5 33.8-46.2
	(A)	238 187-303	142 121-167	137 118-159	117 99.2-138	45.0 38.5-52.5

Table 9.--Toxicity to rainbow trout of fresh and aged solutions of purified
TFM (96%) at four pH's in 12 C water. (Deactivation indices
shown in parentheses)

Aging period (weeks)	96-h LC50 and 95% confidence interval (mg/l) and (deactivation index)			
	pH 6.5	pH 7.5	pH 8.5	pH 9.5
0	0.962 0.850-1.09 (1.00)	2.08 1.73-2.50 (1.00)	5.39 4.84-6.00 (1.00)	40.5 35.8-45.8 (1.00)
1	---	2.32 1.98-2.71 (1.12)	5.81 5.24-6.45 (1.08)	71.2 64.9-78.2 (1.76)
2	---	2.17 1.83-2.57 (1.04)	5.63 5.09-6.22 (1.04)	66.3 59.2-74.2 (1.64)
3	---	---	4.40 --- (0.816)	---
4	---	1.78 --- (0.856)	---	79.0 69.4-89.9 (1.95)
6	1.20 0.973-1.48 (1.25)	2.05 1.70-2.47 (0.986)	5.81 5.23-6.46 (1.08)	51.8 46.8-57.3 (1.28)
8	0.842 --- (0.875)	2.05 1.71-2.46 (0.986)	---	---

from these erratic indices was not possible nor was it feasible to extend the aging periods. The data were sufficient, however, to indicate that TFM did not detoxify readily under laboratory conditions. Concentrations of TFM remaining in aged solutions were confirmed by spectrophotometric analysis (Olson and Marking 1973).

Comparison of Various Formulations

Some of the various formulations of TFM that have been prepared for laboratory use and for field applications were tested to determine their activity against rainbow trout (Table 10). The high-percentage formulations tested were comparable in activity but were slightly less active than field grade TFM (35.7%). The greater activity of the field grade TFM was expected because the formulating process overcomes some of the problems associated with solubility. The carrier used in the field grade formulation may increase dispersion, reduce particle size, or enhance ionic state of the TFM molecule since may carriers are used for such purposes.

Table 10.--Toxicity of TFM in various formulations to rainbow trout in soft water at 12 C

Percent active ingredient		Toxic unit	96-h LC50 and 95% confidence interval at	
			Total formulation	Active TFM
Analytical	(99+)	mg/l	1.39 1.17-1.66	1.39 1.17-1.66
Purified	(96)	mg/l	1.50 1.35-1.67	1.44 1.27-1.57
Purified	(94)	mg/l	1.55 1.36-1.76	1.46 1.28-1.65
Field	(35.7)	µl/l	3.38 2.91-3.92	1.21 1.03-1.40

DISCUSSION

Data on the toxicity of TFM to nontarget fishes helps assess the margin of safety for such fish. Because the toxicity of TFM is influenced significantly by water hardness and pH, the margin of safety can be determined accurately only if the tests for toxicity to target and nontarget organisms are done in comparable water media. Toxicity data from field applications usually cannot be compared with laboratory toxicity data because of the differences in water quality and the presence of other biota. Ideally, the margin of safety should be determined by testing field grade TFM against target and nontarget organisms in the water to be treated.

Efficacious concentrations of field grade TFM were determined in standardized laboratory tests by Dawson et al. (In press), who used test water identical to that used in our toxicity tests. For example, in soft water (44 mg/l total hardness; 12 C), the 24-h LC99 in µl/l of TFM (35.7%) against larval lamprey was 0.950 at pH 6.5, 3.25 at pH 7.5, and 12.0 at pH 8.5. In corresponding water quality, the 24-h LC01 in µl/l of TFM (35.7%) against rainbow trout was 1.60 at pH 6.5, 2.60 at pH 7.5, and 10.4 at pH 8.5. The margin of safety (LC01 for fish/LC99 for lamprey) at the respective pH's was 1.760, 0.800, and 0.866. Because values less than 1.0 indicate incom-

plete survival of the trout, some trout would be expected to die at minimum lampricidal concentrations at pH 7.5 or 8.5, but not at pH 6.5.

If the margin of safety is calculated on the basis of LC10's for fish and LC99's for lamprey, the value is near or higher than 1.0 at the three pH's. Therefore, a 10% mortality of rainbow trout could be expected at pH's 7.5 and 8.5 under these conditions. Trout in pH 6.5 water are safe (LC10/LC99 = 2.11).

CONCLUSIONS

1. Purified and field grade TFM are toxic to coldwater and warmwater fish in brief exposures (1, 3, and 6 h) as well as in 96-h exposures. The toxicity increased little during prolonged exposures.

2. TFM was generally more toxic to fish at higher temperatures, but the trend was not consistent for all warmwater species.

3. TFM was more toxic to fish in very soft water than in very hard water by a factor as great as 10.

4. TFM was considerably more toxic in acid than in alkaline water. The factor was more than 50 for pH's 6.5 to 9.5 for some species.

5. Reduced TFM was less toxic than TFM to fish, but the toxicity of RTFM was influenced less by water hardness.

6. TFM was very persistent in laboratory test waters; activity decreases were small or nil for periods up to 8 wk.

7. On the basis of active ingredient, field grade TFM appeared to be slightly more toxic than purified TFM.

8. The margin of safety for rainbow trout in minimum lampricidal concentrations of field grade TFM was influenced by pH and was greatest at pH 6.5.

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APPENDIX

Appendix Table 1.—Toxicity of purified TFM (94%) to fingerling coho salmon at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	9.60 8.74–10.5	6.10 5.37–6.93	5.60 4.69–6.69	4.80 4.20–5.49	4.30 3.73–4.95
12	Soft	7.5	6.80 6.24–7.41	5.60 4.69–6.69	5.60 4.69–6.69	4.30 3.78–4.89	2.70 2.26–3.22
17	Soft	7.5	3.61 3.35–3.89	3.47 3.11–3.87	3.47 3.11–3.87	2.68 2.39–2.99	2.00 1.64–2.43
12	Very soft	6.6	7.40 6.64–8.24	4.60 4.10–5.16	4.55 4.20–4.93	2.90 2.62–3.21	1.70 1.45–1.99
12	Hard	7.8	16.0 14.7–17.5	15.6 14.5–16.8	15.0 13.5–16.7	13.2 12.1–14.4	6.50 5.39–7.83
12	Very hard	8.2	33.0 30.6–35.6	33.0 30.6–35.6	32.0 29.8–34.3	25.4 23.8–27.1	17.0 15.3–18.9
12	Soft	6.5	2.30 2.05–2.58	1.72 1.54–1.92	1.69 1.52–1.88	1.67 1.45–1.92	1.18 1.02–1.36
12	Soft	8.5	18.5 16.2–21.1	16.9 15.2–18.7	16.3 14.2–18.7	11.9 10.2–13.8	8.25 7.18–9.49
12	Soft	9.5	---	---	---	---	51.3 39.4–66.8

Appendix Table 2.—Toxicity of purified TFM (96%) to fingerling brown trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	8.21 7.15–9.43	5.00 4.55–5.49	4.05 3.43–4.79	2.93 2.35–3.65	2.60 2.31–2.93
12	Soft	7.5	7.00 6.33–7.74	4.93 4.47–5.43	4.78 4.20–5.44	3.89 3.57–4.24	2.63 2.35–2.94
17	Soft	7.5	5.61 5.10–6.17	3.80 3.25–4.44	3.80 3.25–4.44	3.58 3.22–3.98	1.80 1.65–1.97
12	Very soft	6.6	3.60 3.17–4.09	2.48 2.18–2.82	2.30 2.08–2.54	2.04 1.87–2.22	1.20 1.02–1.41
12	Hard	7.8	10.8 9.87–11.8	8.41 7.65–9.25	8.00 7.35–8.70	8.00 7.35–8.70	5.80 ---
12	Very hard	8.2	16.3 15.0–17.7	11.9 10.4–13.6	11.9 10.4–13.6	11.9 10.4–13.6	9.80 9.25–10.4
12	Soft	6.5	2.37 2.05–2.74	1.33 1.19–1.48	1.30 1.15–1.47	1.12 1.02–1.23	0.990 0.881–1.11
12	Soft	8.5	26.8 21.3–33.7	17.0 15.3–18.9	14.8 12.8–17.2	12.8 11.5–14.2	8.28 7.31–9.37
12	Soft	9.5	>60.0	>60.0	>60.0	>60.0	50.0 47.0–53.2

Appendix Table 3.—Toxicity of purified TFM (96%) to fingerling lake trout at selected temperatures, water hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	6.02 4.89–7.41	2.23 1.83–2.71	1.89 1.61–2.22	1.74 1.55–1.95	1.62 1.41–1.86
12	Soft	7.5	2.72 2.30–3.21	1.93 1.71–2.18	1.43 1.16–1.75	1.43 1.16–1.75	1.40 1.11–1.77
12	Very soft	6.6	2.35 2.13–2.59	1.35 1.21–1.51	1.19 1.02–1.38	1.19 1.02–1.38	1.07 0.850–1.35
12	Hard	7.8	10.6 9.43–11.9	6.68 6.14–7.27	5.93 5.45–6.45	5.85 5.31–6.45	4.05 3.49–4.70
12	Very hard	8.2	18.0 16.5–19.7	12.2 11.6–12.9	11.1 10.4–11.8	10.7 9.88–11.6	8.42 7.64–9.28
12	Soft	6.5	1.81 1.61–2.04	0.842 0.750–0.945	0.704 0.593–0.836	0.704 0.593–0.836	0.690 0.569–0.837
12	Soft	8.5	---	9.42 8.04–11.0	8.52 7.73–9.39	7.75 7.06–8.51	4.58 3.48–6.03
12	Soft	9.5	52.0 39.5–68.4	50.0 38.8–64.5	50.0 38.8–64.5	50.0 38.8–64.5	40.8 34.5–48.3

Appendix Table 4.—Toxicity of purified TFM (96%) to fingerling carp at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	---	---	2.10 1.85–2.37	1.74 1.43–2.11	1.25 1.00–1.56
17	Soft	7.5	4.30 3.77–4.90	2.52 2.30–2.77	2.36 2.08–2.67	2.36 2.08–2.67	1.42 1.19–1.70
22	Soft	7.5	4.27 3.73–4.88	2.93 2.51–3.42	2.82 2.44–3.26	2.33 2.01–2.71	1.39 1.14–1.70
12	Very soft	6.6	3.46 3.22–3.72	1.87 1.65–2.12	1.46 1.32–1.62	1.28 1.06–1.54	1.03 0.793–1.34
12	Hard	7.8	13.8 12.2–15.6	7.75 7.20–8.34	6.00 5.35–6.73	5.37 4.69–6.14	3.63 3.08–4.28
12	Very hard	8.2	19.0 17.6–20.5	10.3 9.38–11.3	7.72 7.20–8.28	6.00 5.00–7.20	4.62 4.03–5.30
12	Soft	6.5	2.05 1.87–2.24	---	0.820 0.715–0.940	0.770 0.602–0.984	0.770 0.602–0.984
12	Soft	8.5	---	9.00 8.36–9.69	8.10 7.08–9.27	4.46 3.66–5.42	3.15 2.34–4.22
12	Soft	9.5	---	43.9 40.6–47.3	42.6 38.5–47.2	35.7 32.2–39.6	28.0 23.6–33.3

Appendix Table 5.—Toxicity of purified TFM (96%) to fingerling channel catfish at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	5.05 4.18-6.10	2.60 1.99-3.40	1.18 1.02-1.36	1.12 0.945-1.33	0.905 0.838-0.978
17	Soft	7.5	2.37 2.05-2.74	1.53 1.35-1.73	1.06 0.960-1.17	0.970 0.859-1.10	0.970 0.859-1.10
22	Soft	7.5	2.16 1.59-2.94	1.18 1.01-1.37	0.882 0.786-0.990	0.765 0.712-0.822	0.765 0.712-0.822
12	Very soft	6.6	>3.00	2.43 2.03-2.90	0.775 0.688-0.872	0.478 0.431-0.530	0.478 0.431-0.530
12	Hard	7.8	12.1 9.93-14.7	7.25 6.15-8.55	3.51 3.14-3.93	2.59 2.29-2.93	2.59 2.29-2.93
12	Very hard	8.2	25.5 22.0-29.6	8.90 7.95-9.96	7.40 6.45-8.50	5.39 4.82-6.02	5.39 4.82-6.02
12	Soft	6.5	>2.00	1.18 1.02-1.36	0.763 0.706-0.824	0.632 0.584-0.684	0.632 0.584-0.684
12	Soft	8.5	19.0 14.8-24.3	9.37 8.21-10.7	6.43 6.00-6.90	4.52 4.15-4.93	3.38 2.92-3.91
12	Soft	9.5	>60.0	71.0 52.7-95.6	50.0 43.7-57.2	---	14.4 13.1-15.8

Appendix Table 6.—Toxicity of purified TFM (96%) to fingerling bluegill at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	12.9 11.4–14.6	8.90 8.22–9.64	6.42 5.99–6.89	6.23 5.50–7.05	6.23 5.50–7.05
17	Soft	7.5	7.65 7.09–8.25	5.21 4.64–5.84	4.69 4.39–5.01	4.69 4.39–5.01	3.81 3.22–4.51
22	Soft	7.5	5.50 4.90–6.17	4.65 4.34–4.98	4.65 4.34–4.98	4.65 4.34–4.98	2.19 1.87–2.56
12	Very soft	6.6	7.18 6.54–7.88	—	3.33 2.96–3.75	3.00 2.77–3.25	2.92 2.61–3.27
12	Hard	7.8	22.9 20.4–25.7	—	11.9 10.9–13.0	11.6 10.5–12.8	11.6 10.5–12.8
12	Very hard	8.2	34.0 30.6–37.8	—	23.4 21.0–26.1	23.4 21.0–26.1	23.4 21.0–26.1
12	Soft	6.5	7.80 7.16–8.50	3.40 3.06–3.78	3.40 3.06–3.78	3.39 3.05–3.77	3.07 2.71–3.48
12	Soft	8.5	33.8 30.5–37.5	24.0 22.6–25.5	24.0 22.6–25.5	22.3 20.6–24.1	22.3 20.6–24.1
12	Soft	9.5	96.3 84.5–110	89.0 82.2–96.4	76.2 70.8–82.0	76.2 70.8–82.0	64.2 60.0–68.7

Appendix Table 7.--Toxicity of purified TFM (96%) to fingerling yellow perch at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	6.20 5.05-7.61	3.38 2.63-4.35	2.88 2.47-3.35	2.51 2.19-2.88	2.07 1.69-2.54
17	Soft	7.5	4.77 4.18-5.45	2.68 2.22-3.24	2.68 2.22-3.24	2.13 1.92-2.36	2.05 1.67-2.51
22	Soft	7.5	3.38 3.05-3.75	2.80 2.25-3.49	2.80 2.25-3.49	2.46 2.17-2.79	1.78 1.60-1.98
12	Very soft	6.6	---	---	---	1.68 1.24-2.28	1.22 0.932-1.60
12	Hard	7.8	---	12.0 10.5-13.7	9.00 7.55-10.7	7.00 5.91-8.30	6.28 5.24-7.53
12	Very hard	8.2	30.0 26.0-34.6	19.5 16.0-22.9	15.2 13.2-17.5	14.2 12.1-16.6	12.2 10.7-13.9
12	Soft	6.5	4.48 3.57-5.62	1.63 1.14-2.33	1.18 1.01-1.37	1.10 0.930-1.30	1.00 0.870-1.15
12	Soft	8.5	22.2 17.7-27.8	12.0 10.1-14.3	10.4 8.82-12.3	9.42 8.14-10.9	8.80 7.53-10.3
12	Soft	9.5	47.2 39.2-56.8	44.3 38.3-51.3	44.3 38.3-51.3	44.2 35.7-54.8	26.2 21.7-31.7

Appendix Table 8.—Toxicity of field grade TFM (35.7%) to fingerling chinook salmon at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	15.9 / 13.0–19.4	8.47 / 7.70–9.31	6.78 / 6.12–7.51	6.22 / 5.51–7.03	4.27 / 3.89–4.69
12	Soft	7.5	11.5 / 9.66–13.7	8.66 / 7.86–9.54	7.62 / 6.48–8.96	5.98 / 5.09–7.03	4.20 / 3.52–5.02
17	Soft	7.5	8.83 / 7.91–9.85	7.58 / 6.66–8.63	6.85 / 6.16–7.62	5.38 / 4.83–5.99	3.54 / 3.19–3.92
12	Very soft	6.6	6.80 / 6.10–7.59	4.58 / 4.08–5.15	4.45 / 4.00–4.95	3.68 / 3.20–4.23	1.94 / 1.62–2.33
12	Hard	7.8	38.5 / 33.9–43.7	28.2 / 25.7–31.0	22.3 / 20.0–24.9	16.0 / 15.2–18.7	10.0 / 8.98–11.1
12	Very hard	8.2	95.0 / 80.3–112	60.5 / 53.5–68.5	58.0 / 51.2–65.8	41.5 / 36.0–47.8	19.0 / 16.2–22.2
12	Soft	6.5	4.70 / 3.97–5.56	3.58 / 3.19–4.02	3.49 / 3.13–3.90	3.12 / 2.77–3.52	2.45 / 2.16–2.77
12	Soft	8.5	73.5 / 67.4–80.1	39.5 / 35.2–44.4	36.0 / 31.1–41.6	33.4 / 28.9–38.6	18.0 / 15.4–21.0
12	Soft	9.5	>300	>300	362 / 284–462	254 / 215–300	171 / 154–190

Appendix Table 9.—Toxicity of field grade TFM (35.7%) to fingerling brown trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (μl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	15.8 13.6–18.4	9.96 8.85–11.2	5.58 5.13–6.07	5.18 4.60–5.83	3.98 3.47–4.56
12	Soft	7.5	9.63 8.46–11.0	5.83 5.33–6.38	4.94 4.15–5.88	4.53 3.86–5.32	3.53 3.04–4.09
17	Soft	7.5	6.53 5.68–7.51	4.72 4.17–5.34	4.72 4.17–5.34	3.82 3.22–4.53	2.14 1.71–2.68
12	Very soft	6.6	7.00 5.98–8.20	3.58 3.15–4.06	3.42 3.07–3.81	3.00 2.48–3.63	2.32 2.08–2.59
12	Hard	7.8	28.8 25.0–33.2	13.8 11.4–16.7	12.8 10.9–15.0	11.2 10.2–12.3	7.22 6.19–8.42
12	Very hard	8.2	59.8 55.2–64.8	39.8 34.7–45.6	35.7 32.5–39.3	22.6 18.7–27.3	18.3 15.1–22.1
12	Soft	6.5	4.10 3.68–4.57	2.46 2.17–2.79	---	2.22 1.99–2.48	2.11 1.91–2.33
12	Soft	8.5	80.0 68.9–92.9	38.8 34.0–44.3	---	28.9 23.9–35.0	25.5 21.5–30.2
12	Soft	9.5	>150	>150	>150	>150	120 113–127

Appendix Table 10.—Toxicity of field grade TFM (35.7%) to fingerling lake trout at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence intervals (μl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	28.2 23.6–33.7	14.0 11.1–17.7	9.63 8.51–10.9	7.28 6.34–8.36	6.18 5.25–7.28
12	Soft	7.5	18.2 14.9–22.2	9.20 8.18–10.4	8.00 7.27–8.80	7.78 7.16–8.46	7.18 6.64–7.77
17	Soft	7.5	>10.0	7.03 5.58–8.86	7.03 5.58–8.86	7.03 5.58–8.86	6.78 5.58–8.23
12	Very soft	6.6	>15.0	10.9 9.98–11.9	7.21 6.57–7.91	4.45 4.06–4.88	3.36 3.01–3.76
12	Hard	7.8	60.3 50.0–73.3	29.2 26.7–31.9	27.3 24.6–30.3	24.7 21.4–28.5	17.2 15.8–18.8
12	Very hard	8.2	92.3 80.5–106	54.2 50.9–57.7	54.0 49.9–58.4	48.3 45.5–51.2	36.2 33.2–39.5
12	Soft	6.5	4.58 4.22–4.97	2.60 2.30–2.94	2.45 2.16–2.77	2.45 2.16–2.77	2.45 2.16–2.77
12	Soft	8.5	>90.0	>90.0	117 89.6–153	76.0 65.7–87.9	45.6 36.4–57.1
12	Soft	9.5	> 300	> 300	> 300	300 267–338	203 180–229

Appendix Table 11.—Toxicity of field grade TFM (35.7%) to fingerling carp at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	13.1 11.6–14.8	6.62[a] 6.10–7.19	6.28 5.55–7.11	5.60 4.65–6.74	5.23 4.37–6.26
17	Soft	7.5	12.2 10.8–13.8	9.60[a] 8.40–11.0	9.58 8.37–11.0	8.20 7.11–9.45	5.42 4.71–6.23
22	Soft	7.5	15.0 14.0–16.1	10.7[a] 9.06–12.6	10.7 9.06–12.6	6.03 4.96–7.33	5.09 4.32–5.99
12	Very soft	6.6	5.99 5.41–6.64	3.58 3.27–3.92	2.68 2.41–2.98	2.52 2.14–2.96	2.52 2.14–2.96
12	Hard	7.8	43.2 34.1–54.7	24.3 20.4–29.0	20.6 18.6–22.9	17.7 16.0–19.6	13.8 12.7–15.0
12	Very hard	8.2	78.5 68.6–89.8	47.2 46.5–47.9	36.0 32.2–40.3	26.9 22.1–32.8	22.3 18.9–26.3
12	Soft	6.5	5.62 5.14–6.15	2.90 2.60–3.24	2.24 1.86–2.69	2.12 1.91–2.35	1.98 1.66–2.36
12	Soft	8.5	84.2 75.0–94.5	38.4 32.2–45.8	29.1 26.2–32.3	16.7 13.9–20.0	14.5 12.9–16.3
12	Soft	9.5	> 300	> 300	279 223–350	169 145–197	96.0 75.2–123

[a] 4-h observation.

Appendix Table 12.—Toxicity of field grade TFM (35.7%) to fingerling bluegill at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (µl/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	44.4 / 41.2-47.9	19.8 / 17.2-22.7	15.6 / 13.6-17.9	13.7 / 11.7-16.1	13.0 / 11.5-14.7
17	Soft	7.5	34.0 / 30.7-37.7	17.9 / 16.0-20.0	16.0 / 14.0-18.3	14.6 / 13.4-15.9	14.6 / 13.4-15.9
22	Soft	7.5	20.5 / 18.4-22.8	16.9 / 15.2-18.7	---	15.5 / 13.6-17.6	15.0 / 13.4-16.8
12	Very soft	6.6	30.0 / 26.8-33.6	11.7 / 10.2-13.4	8.58 / 7.73-9.52	7.63 / 7.08-8.23	7.35 / 6.74-8.01
12	Hard	7.8	>120	60.8 / 53.1-69.7	51.0 / 46.9-55.5	44.6 / 40.1-50.0	36.8 / 33.9-40.0
12	Very hard	8.2	>200	120 / 111-130	92.0 / 84.5-100	71.8 / 66.4-77.7	67.0 / 62.2-72.2
12	Soft	6.5	16.9 / 15.2-18.8	10.5 / 9.46-11.5	6.23 / 5.51-7.04	5.62 / 5.09-6.20	5.62 / 5.09-6.20
12	Soft	8.5	>200	153 / 142-165	98.0 / 86.0-112	66.5 / 60.8-72.8	65.4 / 58.5-73.1
12	Soft	9.5	>400	>400	478 / 400-572	368 / 320-423	368 / 320-423

Appendix Table 13.—Toxicity of field grade TFM (35.7%) to fingerling yellow perch at selected temperatures, hardnesses, and pH's

Temp. (°C)	Water hardness	pH	LC50 and 95% confidence interval (μl/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	14.3 11.3-18.1	5.98 5.05-7.09	5.85 4.85-7.05	4.12 3.74-4.54	3.60 2.97-4.37
17	Soft	7.5	9.35 8.31-10.5	5.30 4.76-5.90	5.30 4.76-5.90	4.74 4.18-5.37	4.18 3.63-4.81
22	Soft	7.5	8.30 6.89-10.0	6.00 5.36-6.72	6.00 5.36-6.72	4.05 3.35-4.90	3.76 3.19-4.43
12	Very soft	6.6	6.30 5.60-7.08	2.88 2.26-3.68	2.60 2.22-3.05	2.28 1.92-2.70	2.28 1.92-2.70
12	Hard	7.8	45.0 39.1-51.9	17.2 15.0-19.8	16.9 14.5-19.7	13.4 12.0-15.0	10.3 9.32-11.4
12	Very hard	8.2	93.0 81.4-106	47.6 42.8-53.0	33.3 30.4-36.4	30.4 27.3-33.8	27.0 24.8-29.4
12	Soft	6.5	2.68 2.40-3.00	1.70 1.45-1.99	1.25 0.962-1.62	1.06 0.859-1.31	1.00 0.827-1.21
12	Soft	8.5	42.4 38.3-46.9	20.0 17.3-23.2	13.8 10.3-18.4	9.30 8.19-10.6	5.55 4.44-6.93
12	Soft	9.5	>200	165 147-186	109 92.5-128	82.5 71.8-94.9	31.2 26.8-36.3

61. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests

By Leif L. Marking, Terry D. Bills, and Jack H. Chandler

CONTENTS

TOXICITY OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO NONTARGET FISH IN FLOW-THROUGH TESTS

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ABSTRACT

Field grade 3-trifluoromethyl-4-nitrophenol (TFM) was tested for acute and chronic toxicity to 11 species of nontarget fish in 4- and 30-day exposures, respectively. The species used were coho salmon (Oncorhynchus kisutch), rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), brook trout (Salvelinus fontinalis), lake trout (Salvelinus namaycush), goldfish (Carassius auratus), golden shiner (Notemigonus crysoleucas), channel catfish (Ictalurus punctatus), bluegill (Lepomis macrochirus), red-ear sunfish (Lepomis microlophus), and yellow perch (Perca flavescens). The 96-h LC50's for the lampricide in flow-through tests ranged from 8.79 to 32.1 μl/l in hard water and from 2.15 to 17.5 μl/l in soft water. The toxicity of the TFM formulation to two species of salmonids did not change significantly (P = 0.05) between 1- and 30-day exposures. The results of simultaneous static and flow-through acute toxicity tests with channel catfish were not significantly different in two experiments.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is effective for killing larval lampreys (Petromyzon marinus) living in tributary streams of the Great Lakes without decimating the endemic fish populations (Applegate et al. 1958). The effects of TFM on fish have been observed during numerous stream applications and in the laboratory (Marking and Olson 1975, Dawson et al. (In press), and Schnick 1972). The registration of TFM as a lampricide has been supported primarily by laboratory data developed in static test systems. However, flow-through toxicity tests simulate the use pattern of TFM more closely than static toxicity tests.

This study was designed to determine acute and chronic toxicities of field grade TFM to nontarget fish in flow-through toxicity tests. In addition, the acute toxicity of TFM was compared in static and flow-through systems.

MATERIALS AND METHODS

Field grade TFM, obtained from American Hoechst Chemical Company, Somerville, New Jersey,[1] was used for these experiments. Because the percentage of active ingredient varies from one batch to another, purity is specified.

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

The liquid formulations were measured volumetrically and diluted in water to prepare stock solutions, and toxicity values were calculated and reported on a formulation volume to volume (μl/l) basis. The concentration of TFM in each test aquarium was determined daily by colorimetric analysis (Olson and Marking 1973), and the toxicity was calculated on the basis of the mean values for the concentrations.

The flow-through toxicity tests were conducted in an apparatus similar to that described by Mount and Brungs (1967) but with modifications according to McAllister et al. (1972). The apparatus was designed to deliver 1 liter of test solution each cycle. Each glass aquarium contained 45 liters of test medium. The rate of flow was sufficient to replace the entire volume of test medium at least four times each day. The flow-through units were designed to deliver seven successively lower concentrations of the toxicant; each concentration was approximately 25% less than the preceding one. The control for each test contained dilution water but no toxicant. The temperature of test solutions was maintained with a water bath.

Two types of water were used in the flow-through tests. Reconstituted water, prepared according to Marking (1969), was used for some 96-h tests and for comparing the toxicity of TFM in static and flow-through tests. Charcoal filtered municipal well water was used for other 96-h tests and for 30-day exposures. The reconstituted water was soft (total hardness of 44 mg/l and pH of 7.5), whereas the well water was hard (total hardness of 300 mg/l and pH of 7.7). Procedures for the static tests followed those of Lennon and Walker (1964).

National Fish Hatcheries furnished the fish for these experiments. Fish used in 96-h tests were not fed during acclimation before each test nor during exposure (Hunn et al. 1968). Fish for the 30-day tests were fed dry commercial pellets during acclimation and exposure. The fish ranged in size from 1.1 to 19.9 g; for tests in which the weight is not specified, the fish weighed 2 to 5 g. Observations on survival and mortality were recorded daily, and dead fish were removed during each observation.

The toxicity of TFM was calculated according to the statistical procedures of Litchfield and Wilcoxon (1949). Toxicity was defined by LC50's (concentrations calculated to produce 50% mortality) and 95% confidence intervals. Chi-square tests were applied to each set of data to test for goodness of fit.

RESULTS

Four species of fish were exposed to field grade TFM (39.45%) in flow-through toxicity tests using soft, reconstituted water at 17 ± 1 C (Table 1). Channel catfish are the most sensitive and red-ear sunfish the most resistant; the 96-h LC50's were 2.15 and 17.5 μl/l of TFM, respectively. The toxicity of the lampricide did not change significantly (P= 0.05) after 24 h for goldfish, golden shiner, and red-ear sunfish.

Seven species were exposed to field grade TFM (35.7%) in charcoal filtered municipal well water at 12 C (Table 2). The 96-h LC50's ranged from 8.79 to 32.1 μl/l of the formulation. Larger fish of a given species were more resistant than smaller ones to the toxicant. For instance, the 96-h LC50 for TFM was 10.5 μl/l against 1.3-g coho salmon and 29.0 μl/l against 7.4-g coho salmon and was 8.79 μl/l against 1.3-g rainbow trout and 13.8 μl/l against 19.7-g rainbow trout. Lake trout (17.0 g) were more resistant than rainbow trout of similar size (96-h LC50 = 16.9 μl/l).

Toxicosis was apparent in very short exposures (1 to 6 h) to TFM, and the toxicity did

Table 1. Toxicity of TFM (39.45%) to fingerling fish in flow-through tests with soft, reconstituted water at 17 ± 1 C

Species	LC50 and 95% confidence interval (μl/l) at			
	3 h	6 h	24 h	96 h
Goldfish (Carassius auratus)	---	8.10 5.65-11.6	4.85 3.26-7.01	4.25 2.86-6.31
Golden shiner (Notemigonus crysoleucas)	---	13.2 9.96-17.5	10.6 8.56-13.1	8.50 5.79-12.5
Channel catfish (Ictalurus punctatus)	7.00 5.22-9.38	4.80 3.82-6.03	4.05 3.11-5.27	2.15 1.52-3.03
Red-ear sunfish (Lepomis microlophus)	30.0 25.5-35.3	---	17.5 14.2-21.6	17.5 14.2-21.6

not change significantly for many exposure time increments (Table 2). In fact, toxicity did not change significantly between 6- and 96-h exposures for coho salmon (7.4 g), rainbow trout, brown trout, brook trout, lake trout, and bluegill. Considering only small sizes of fish, brook trout were more resistant than other salmonids, bluegills, or yellow perch.

Three species of fish were exposed to field grade TFM (35.7%) for 30 days in charcoal filtered municipal well water at 12 C (Table 3). The 30-day LC50's against coho salmon, brook trout, and lake trout ranged from 10.5 to 19.6 μl/l of the formulation. As in shorter exposures, brook trout were most resistant even though they were smaller than lake trout. Also, toxicity did not change significantly between

10 and 30 days with coho salmon and between 1 and 30 days with lake trout.

Channel catfish were exposed to TFM in simultaneous static and flow-through tests to compare the toxicity and to assess the need for establishing the toxicity of TFM in flow-through facilities. Three separate tests showed that TFM (39.45%) was uniformly toxic in the two types of tests (Table 4). All three tests showed that TFM was more toxic in static than in flow-through facilities; however, the difference was significant only in the second trial. Therefore, additional tests in the flow-through facility with water of different temperature, hardness, and pH are perhaps unnecessary because those characteristics have been examined intensively in previous work.

Table 2. Toxicity of TFM (35.7%) to selected fish in 4-day flow-through tests using charcoal filtered municipal well water at 12 C

Species	Average weight (g)	LC50 and 95% confidence interval (μl/l) at				
		1 h	3 h	6 h	24 h	96 h
Coho salmon (Oncorhynchus kisutch)	1.3	14.4 13.2-15.7	14.2 13.2-15.7	14.2 13.2-15.7	12.6 11.3-14.1	10.5 9.21-12.0
Coho salmon	7.4	34.9 28.8-42.3	29.9 25.5-35.0	29.8 25.1-35.3	29.8 25.1-35.3	29.0 25.2-33.4
Rainbow trout (Salmo gairdneri)	1.3	22.0 13.1-36.8	19.7 12.6-30.8	13.2 7.70-22.6	11.4 8.70-14.9	8.79 7.28-10.6
Rainbow trout	19.7	14.8 13.2-16.6	13.8 12.8-14.9	13.8 12.8-14.9	13.8 12.8-14.9	13.8 12.8-14.9
Brown trout (Salmo trutta)	2.7	12.9 12.1-13.7	10.8 10.0-11.7	10.8 10.0-11.7	10.8 10.0-11.7	10.8 10.0-11.7
Brook trout (Salvelinus fontinalis)	2.2	35.4 30.6-41.0	32.1 28.1-36.7	32.1 28.1-36.7	32.1 28.1-36.7	32.1 28.1-36.7
Lake trout (Salvelinus namaycush)	17.0	22.3 18.7-26.6	16.9 15.3-18.7	16.9 15.3-18.7	16.9 15.3-18.7	16.9 15.3-18.7
Bluegill (Lepomis macrochirus)	1.1	---	22.2 19.3-25.2	16.1 14.9-17.4	16.0 14.8-17.3	15.9 14.9-17.0
Yellow perch (Perca flavescens)	2.6	20.6 15.7-27.1	17.6 13.9-22.3	15.1 11.8-19.3	13.2 11.2-15.6	9.44 8.06-11.0

Table 3. Toxicity of TFM (35.7%) to fish in 30-day flow-through tests using charcoal filtered municipal well water at 12 C

Species	Average weight (g)	LC50 and 95% confidence interval (μl/l) at			
		1 day	10 days	20 days	30 days
Coho salmon	1.3	12.6	10.5	10.5	10.5
		11.3-14.1	9.30-11.7	9.30-11.7	9.30-11.7
Brook trout	2.2	32.1	32.1	22.5	19.6
		28.1-36.7	28.8-35.8	19.0-26.6	15.4-24.9
Lake trout	17.0	16.9	16.9	16.9	16.9
		15.3-18.7	15.3-18.7	15.3-18.7	15.3-18.7

Table 4. Toxicity of TFM (39.45%) to channel catfish in static and flow-through tests with soft, reconstituted water at 17 $\pm$ 1 C

Type of assay	LC50 and 95% confidence interval (μl/l) at				
	1 h	3 h	6 h	24 h	96 h
Static	14.2	6.00	4.25	3.55	2.85
	13.3-15.2	5.39-6.68	3.89-4.65	3.16-3.99	2.45-3.32
Flow-through	---	---	---	3.50	3.28
				3.17-3.87	2.99-3.60
Static	12.5	6.25	3.75	3.15	2.75
	11.6-13.5	5.53-7.06	3.07-4.58	2.72-3.64	2.29-3.30
Flow-through	---	---	4.85	4.30	3.42
			4.57-5.14	3.88-4.77	3.05-3.84
Static	11.8	6.60	4.60	3.48	1.80
	10.4-13.3	5.76-7.56	4.01-5.28	2.94-4.12	1.26-2.58
Flow-through	---	7.00	4.80	4.05	2.15
		5.22-9.38	3.82-6.03	3.11-5.27	1.52-3.03

DISCUSSION

The use of the flow-through technique has been recommended over the static technique for certain kinds of toxicity determinations. The flow-through technique is more complicated and expensive than the static technique; however, and the use of water with different characteristics is not as practical in flow-through tests as in static tests. Because the toxicity of field grade TFM is similar in both techniques, the static procedure probably is sufficient to estimate the acute toxicity of TFM to fish. However, the flow-through technique must be used for determining chronic toxicity.

Large fish of a species were more resistant than smaller ones. The increase in resistance with size is perhaps related to the greater ability of larger fish to metabolize TFM. Lech and Costrini (1972) demonstrated the formation of TFM glucuronide (reduced TFM) in vitro and suspected that the same metabolite was formed in vivo in rainbow trout. Other studies showed that TFM and reduced TFM are excreted in the urine of rats (Lech 1971). Thus TFM is apparently readily metabolized and excreted. Mature fish probably have more effective enzyme systems than do juveniles for metabolizing TFM and adjusting to a continuous exposure to the toxicant.

The lampricide kills fish in short exposures (1 to 6 h) at concentrations equal to or nearly equal to those required in long exposures (4 to 30 days). In fact, the toxicity did not change after 3 h with salmon (7.4 g), rainbow trout (19.7 g), brown trout, brook trout, and lake trout in 4-day tests (Table 2). The same trend occurred in the 30-day trials in which the LC50's were identical for lake trout after 1 and 30 days of exposure (Table 3). The change in toxicity of TFM to brook trout in 1- and 30-day exposures was significant. However, considering the magnitude of change for brook trout and tests with other species, fish generally succumb immediately or survive chronic exposure by employing enzymatic defenses.

CONCLUSIONS

1. Field grade TFM was toxic to nontarget coldwater and warmwater fish in brief ex-

posures (1 to 6 h) as well as in 96-h exposures in flow-through tests.

2. In hard water, the 96-h LC50's ranged from 8.79 to 32.1 μl/l of TFM formulation and in soft water from 2.15 to 17.5 μl/l of TFM formulation.

3. Field grade TFM was chronically toxic to nontarget fish; however, the toxicity changed little between 1- and 30-day exposures.

4. Field grade TFM was more toxic to small than to large sizes of fish of the same species in 96-h exposures.

5. The toxicity of TFM to fish was greater in static tests than in flow-through tests, but the difference was not significant in two of three experiments.

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62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae

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CONTENTS

TOXICITY OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO SELECTED AQUATIC INVERTEBRATES AND FROG LARVAE

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ABSTRACT

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) was tested against various groups of nontarget aquatic organisms. Invertebrates exposed were flatworms (Catenula sp.), annelids (Tubifex tubifex), daphnids (Daphnia magna), seed shrimps (Cypridopsis sp.), glass shrimp (Palaemonetes kadiakensis), mayfly nymphs (Callibaetis sp.), backswimmers (Notonecta sp.), mosquito larvae (Culex sp. and Anopheles sp.), bivalve mollusks (Corbicula sp., Sphaerium sp., Elliptio sp., and Plectomerus sp.), and snails (Physa sp., Helisoma sp., and Pleurocera sp.). Vertebrates exposed to TFM were larvae of gray tree frog (Hyla versicolor), leopard frog (Rana pipiens), and bullfrog (Rana catesbeiana). Larvae of tree frogs were the most sensitive organism to TFM (96-h LC50 = 1.98 mg/l), and backswimmers were the least sensitive (96-h LC50 = 555 mg/l). Soft-bodied invertebrates were less sensitive than snails and bivalve mollusks to TFM. The invertebrates tested were not as susceptible as larval lampreys (Petromyzon marinus) in similar standardized tests.

INTRODUCTION

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been effective for controlling sea lamprey (Petromyzon marinus) in the Great Lakes. The lampricide is applied to streams in which the larvae live and is more toxic to larval lampreys than to other fishes (Applegate et al. 1958). Schnick (1972) reviewed the literature on the lampricide and summarized the data available that support existing registration of this pesticide. Recently completed studies have defined the toxicity of TFM to selected nontarget organisms (Marking and Olson 1975; Marking et al. 1974; Maki et al. 1974; Fremling 1974; Kawatski et al. 1974; Sanders and Walsh 1974)

and the efficacy against larval lampreys (Dawson et al. In press).

The present study was designed to determine the toxicity of TFM to selected aquatic invertebrates and larvae of frogs.

MATERIALS AND METHODS

Field grade TFM (39.45% active ingredient) was measured gravimetrically and diluted with deionized water to prepare stock solutions for static and flow-through toxicity tests. Concentrations were calculated on the basis of the formulation used in the field rather than on active ingredient.

Static tests were conducted in small jars containing 3 liters of test water or in large jars containing 15 liters of water. The jars were immersed in a water bath to the level of the test fluids; the water bath was equipped with a commercial chilling device (Frigid Units, Inc.[1]). At least 10 concentrations and 1 water control were employed in each test.

Flow-through tests were performed in a modified version of the Mount and Brungs (1967) apparatus, but a different chemical metering device (Chandler et al. 1974) was substituted for the conventional form. Five concentrations and a control were used in each of the flow-through systems. Each of the 5 test chambers held 45 liters of diluted toxicant in which each successive concentration was approximately 50% less than the previous one. The colorimetric method of Olson and Marking (1973) was used periodically to determine actual concentrations of toxicant in each of the aquaria. A rate of flow was maintained which ensured a minimum of three complete replacements of test solutions per day in each of the chambers. Test media were cooled with water bath equipment similar to that used in static tests. Tests were conducted at 16 to 17 C.

Most of the tests were conducted in spring water to which lime was added (hereafter called limed water) to bring the total hardness (as $CaCO_3$) to approximately 20 mg/l. The pH of the test waters varied from 6.8 to 7.0. Reconstituted waters routinely used for toxicity tests involving fish (Marking 1969) were used only in tests with clams, because it appeared in initial tests that the soft-bodied invertebrates might have been adversely affected by the test media.

Most test organisms were collected in ponds and streams. A few were reared outdoors in partly shaded, vinyl pools or in the laboratory. All forms collected in the field were retained for a minimum of 7 days in waters identical with those used in the tests. Only vigorous individuals of uniform sizes were used in tests. Small or delicate organisms were placed in cylindrical cages fabricated from Nitex screen. The cages were suspended in the test chamber of the flow-through appa-

ratus to facilitate observation and to prevent loss or damage to organisms by turbulent water.

Mortality determinations were made on an appropriate hourly or daily basis, and dead organisms and detritus were removed after each examination. Mortalities were based on immobility or lack of response of test organisms to various mechanical stimuli. Snails were assumed to be dead when they failed to retract the "foot" into the shell, and bivalves when they were unable to close their shells.

The statistical procedures of Litchfield and Wilcoxon (1949) were used to calculate the concentration of toxicant necessary to produce 50% mortality (LC50's) and to obtain 95% confidence intervals.

RESULTS AND DISCUSSION

Eight groups of invertebrates (hereafter referred to as "soft-bodied" invertebrates) were exposed to TFM in static or flow-through tests. Of the eight groups, tubificids (Tubifex tubifex) were the most sensitive and flatworms ranked next (the 96-h LC50's were 2.50 and 11.6 mg/l, respectively, Table 1). The sensitivity of these organisms may be greater in laboratory tests than in their natural environment. Tubificids normally live in bottom substrates, whereas those used in our test were exposed to TFM in water solutions with no substrate. Flatworms (Catenula sp.) were exposed to TFM in hard water (160 mg/l total hardness) and other organisms were exposed in soft water (20 mg/l total hardness). Because TFM is less toxic to invertebrates in hard or high pH water (Fremling 1974; Kawatski et al. 1974), the 96-h LC50 for TFM against flatworms in soft water would probably be less than 11.6 mg/l as shown in Table 1.

Organisms of intermediate sensitivity (96-h LC50's, 21.3 to 89.0 mg/l) were daphnids, seed shrimp, mayfly nymphs, and mosquito larvae. The toxicity of TFM to mayfly nymphs (Callibaetis sp. - a form that lives in streams) exposed in static and in flow-through tests did not differ significantly in either case (Table 1). The least sensitive species were glass shrimp (96-h LC50 = 125 mg/l) and backswimmers (96-h LC50 = 555 mg/l).

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

Table 1. Toxicity of TFM (39.45%) to selected aquatic invertebrates in static tests at 17 C with limed water (20 mg/l total hardness)

Species	LC50 and 95% confidence interval (mg/l) at				
	1 h	3 h	6 h	24 h	96 h
Flatworm[a] Catenula sp.	---	44.5 30.2–66.0	35.0 27.2–45.0	25.0 18.3–34.2	11.6 9.66–14.0
Annelid Tubifex tubifex	17.4 13.8–21.9	16.3 11.7–22.6	12.5 7.20–21.6	8.70 7.25–10.4	2.50 1.23–5.09
Daphnid Daphnia magna	186 164–212	130 104–163	122 102–146	65.0 47.4–88.0	29.0 21.0–41.1
Seed shrimp Cypridopsis sp.	117 80.1–171	60.0 40.0–91.1	60.0 40.0–91.1	52.0 36.2–75.0	37.0 19.0–73.2
Glass shrimp Palaemonetes kadiakensis	---	660 555–785	550 436–694	215 161–286	125 90.6–173
Mayfly Callibaetis sp.	372 214–441	182 157–211	166 135–204	100 60.4–166	21.3 13.8–32.7
Callibaetis sp.[b]	---	---	---	---	22.4 15.4–32.5
Backswimmer Notonecta sp.	14,250 9,901–20,510	7,900 5,901–10,576	2,000 1,248–3,206	795 718–881	555 472–653
Mosquito larvae Anopheles sp.	---	---	---	---	80.0 55.5–115
Culex sp.	---	---	---	---	89.0 66.5–119

a Exposed in hard water (160 mg/l total hardness).
b Flow-through toxicity tests.

Exposures for 96 h indicated greater toxicity than exposures for shorter periods. Invertebrates reacted differently than fish in that respect; changes in the toxicity of TFM to fish were small or nil in 3- to 96-h exposures (Marking and Olson 1975). Since TFM is applied over shorter periods (8 to 12 h) to control lamprey larvae, the values at 24 h of exposure are perhaps more important than 96-h values for estimating the sensitivity of these organisms during lampricidal treatments.

Snails and bivalves which were exposed to TFM in soft water at 17 ± 1 C, generally were more sensitive than soft-bodied invertebrates; 96-h LC50's ranged from 2 to 9 mg/l of TFM for all the species except fingernail clams (Table 2). Fingernail clams were more resistant than other mollusks, and 96-h LC50's were 16.3 and 15.3 mg/l in static and flow-through tests. TFM appeared to be more toxic to snails in static tests than in flow-through tests.

Table 2. Toxicity of TFM (39.45%) to snails, bivalves, and frog larvae in soft water (44 mg/l total hardness) at 17 ± 1 C (based on ability of organisms to respond to tactile stimulus)

Test organism	96-h LC50 and 95% confidence interval (mg/l)	
	In static tests	In flow-through tests
Snails		
Physa sp.	3.05 2.35-3.95	4.60 3.03-6.97
Helisoma sp.	3.75 3.03-4.64	4.10 2.89-5.82
Pleurocera sp.	3.90 2.96-5.14	8.65 5.51-13.6
Bivalves		
Asiatic clam Corbicula sp.	2.30 1.54-3.43	4.10 2.77-6.06
Mussels Elliptio sp.	---	3.65 2.66-5.00
Plectomerus	---	8.10 6.77-9.69
Fingernail clam Sphaerium sp.	16.3 10.6-25.0	15.3 7.42-31.3
Amphibians		
Gray tree frog larvae Hyla versicolor	1.98 1.77-2.22	---
Leopard frog larvae Rana pipiens	2.76 2.45-3.11	---
Bullfrog larvae Rana catesbeiana	---	3.55 2.62-4.82

Frog larvae also were more sensitive than soft-bodied invertebrates to TFM. In static tests larvae of the gray tree frog were the most sensitive (96-h LC50 = 1.98 mg/l), and larvae of the bullfrog were most resistant (96-h LC50 of 3.55 mg/l). Bullfrog larvae were exposed to TFM in flow-through tests and the other frog larvae in static tests. There is little difference in sensitivity among the three species.

Dawson et al. (197_) tested TFM (35.7%) for its effectiveness against larval lampreys (Petromyzon marinus) in standardized laboratory tests. In soft water (pH = 7.5) at 17 C, the 96-h LC50 was 1.60 mg/l and the 12-h LC99 was 2.90 mg/l. Thus larval lampreys were much more susceptible than the soft-bodied invertebrates.

Although the 96-h LC50 values for TFM against snails, bivalves, and frog larvae indicated sensitivity for some species at larvicidal concentrations, these organisms would be less sensitive in 12-h exposures used to treat streams for larval lampreys. Few, if any, of these organisms should be affected by stream treatments with the lampricide.

CONCLUSIONS

1. The lampricide (39.45%) was toxic to aquatic invertebrates in standardized laboratory tests, but the invertebrates were not as susceptible as larval sea lampreys under similar test conditions.

2. Most of the soft-bodied invertebrates were less sensitive than snails and bivalve mollusks to TFM.

3. Larvae of gray tree frogs were the most sensitive to TFM (96-h LC50 = 1.98 mg/l), and backswimmers were the most resistant (96-h LC50 = 555 mg/l).

4. The toxicity of TFM to invertebrates increased in longer exposures (up to 96 h), whereas the reported toxicity of TFM to fish changes little after 3-h exposures.

5. TFM appeared to be more toxic to snails in static than in flow-through tests.

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United States Department of the Interior

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INVESTIGATIONS IN FISH CONTROL

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

By Verdel K. Dawson, Kenneth B. Cumming, and Philip A. Gilderhus

CONTENTS

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	8.65	8.65
	8.24–12.1	7.83–9.55	7.83–9.55
15 (pigmentation)	6.20	6.20	6.15
	4.67–8.23	4.67–8.23	4.65–8.14
16 (gill cleft)	6.20	5.60	5.60
	5.37–7.16	5.07–6.18	5.07–6.18
17 (burrowing)	3.88	3.88	3.88
	3.14–4.84	3.14–4.84	3.14–4.84
18 (7-cm larvae)	1.57	1.57	1.57
	1.10–2.06	1.10–2.06	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 ($\mu l/l$) 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 CaCO₃)	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 CaCO₃)	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	> 5.00ᵃ	> 5.00ᵃ	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	> 30.0ᵃ	> 30.0ᵃ	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	> 1.00ᵃ	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	> 2.50ᵃ	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	> 2.50ᵃ	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	> 8.00ᵃ	> 8.00ᵃ	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

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.

Stage 7—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.

CONTENTS

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

by

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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 $(\mu g/g)^a$ | |
Hardness (as mg/l CaCO₃)	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

CONTENTS

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 m³/sec (20-25 cfs) in its middle reaches and about 1.43 m³/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 concentra-

tion 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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As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interests of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

INVESTIGATIONS IN FISH CONTROL

67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals

United States Department of the Interior

Fish and Wildlife Service

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67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals

By Leif L. Marking and Verdel K. Dawson

CONTENTS

METHOD FOR ASSESSMENT OF TOXICITY OR EFFICACY OF MIXTURES OF CHEMICALS

by

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ABSTRACT

The individual toxic contributions of poisons were summed, and the additive toxicity was defined by a linear index for two chemicals in combination. This index expresses the toxicity quantitatively: zero indicates additive toxicity, negative values indicate less than additive toxicity, and positive values indicate greater than additive toxicity. We selected examples from the literature and conducted tests in the laboratory to assess the additive toxicity of selected chemical mixtures to fish. The values ranged from -1.37 for zinc and cyanide to 7.20 for malathion and Delnav $^{(R)}$. The method quantifies additive toxicity or efficacy, and assists in evaluating the advantages as well as environmental hazards resulting from chemical mixtures.

INTRODUCTION

The effect of mixtures of two or more chemicals is commonly referred to as additive, synergistic, or antagonistic depending on the relation of the toxicity of the mixtures to that of the individual components. Because these terms are abiguous and nonquantitative (Fingl and Woodbury 1965), a better system of terminology and quantification is needed.

British researchers have investigated methods of predicting the toxic effects of chemical mixtures in water by adding up "toxic units" of individual toxic materials (Lloyd 1961; Herbert and Shurben 1964; Herbert and Vandyke 1964; Brown et al. 1968; Brown et al. 1969; and Brown and Dalton 1970). Brown (1968) stated his reservations about the use of this technique and cautioned that actual bioassays of polluted water are to be preferred. Sprague and Ramsey (1965) used the "toxic unit" method to predict the toxicity of copper and zinc mixtures to Atlantic salmon *(Salmo salar).*

Other techniques for evaluating the toxicity of mixtures of chemicals to mammals have been advanced (Keplinger and Deichmann 1967; Smyth et al. 1969); most of which follow the mathematical model for additive joint toxicity that yields the harmonic mean of the LD50's for the components (Finney 1952). This model tests the hypothesis that the toxicity of chemical mixtures is simply additive. Smyth et al. (1969) normalized the values obtained from Finney's equation with a frequency distribution curve and adjusted the values to indicate additive toxicity with zero. Smyth et al. (1970) derived values in terms of adjusted ratios for mixtures of industrial organic chemicals fed to rats.

The objective of this paper is to adapt current methods and terminology to quantitatively describe additive toxicity of chemicals in water and to assign significance to the additive toxicity index.

MATERIALS AND METHODS

For toxicity tests, hatchery-reared rainbow trout *(Salmo gairdneri)* and bluegill *(Lepomis macrochirus)* were maintained as described by Hunn et al. (1968) and acclimated to test waters. The toxicities of individual chemicals to fish were determined according to standard laboratory procedures (Lennon and Walker 1964; Marking 1969a). The toxicities of chemicals in mixtures were determined similarly except that two chemicals were added in a fixed ratio to the test vessels. Toxicity was defined by the LC50's (concentrations producing 50% mortality) and their 95% confidence intervals (Litchfield and Wilcoxon 1949).

The additive indices of mixtures of chemicals were derived as follows. The effective contributions of each chemical (A and B) in a mixture are represented by the formula: $\dfrac{A_m}{A_i} + \dfrac{B_m}{B_i} = S$, where A and B are chemicals, i and m are the toxicities (LC50's) of the individual chemicals and the mixtures, respectively, and S is the sum of the biological activity. If the sum of toxicity of the chemicals is simply additive, S = 1.0; sums that are less than 1.0 indicate greater than additive toxicity, and sums greater than 1.0 indicate less than additive toxicity (Fig. 1). This sum alone could function as a quantitative indication of additive toxicity, except that values greater than 1.0 are not linear with values less than 1.0.
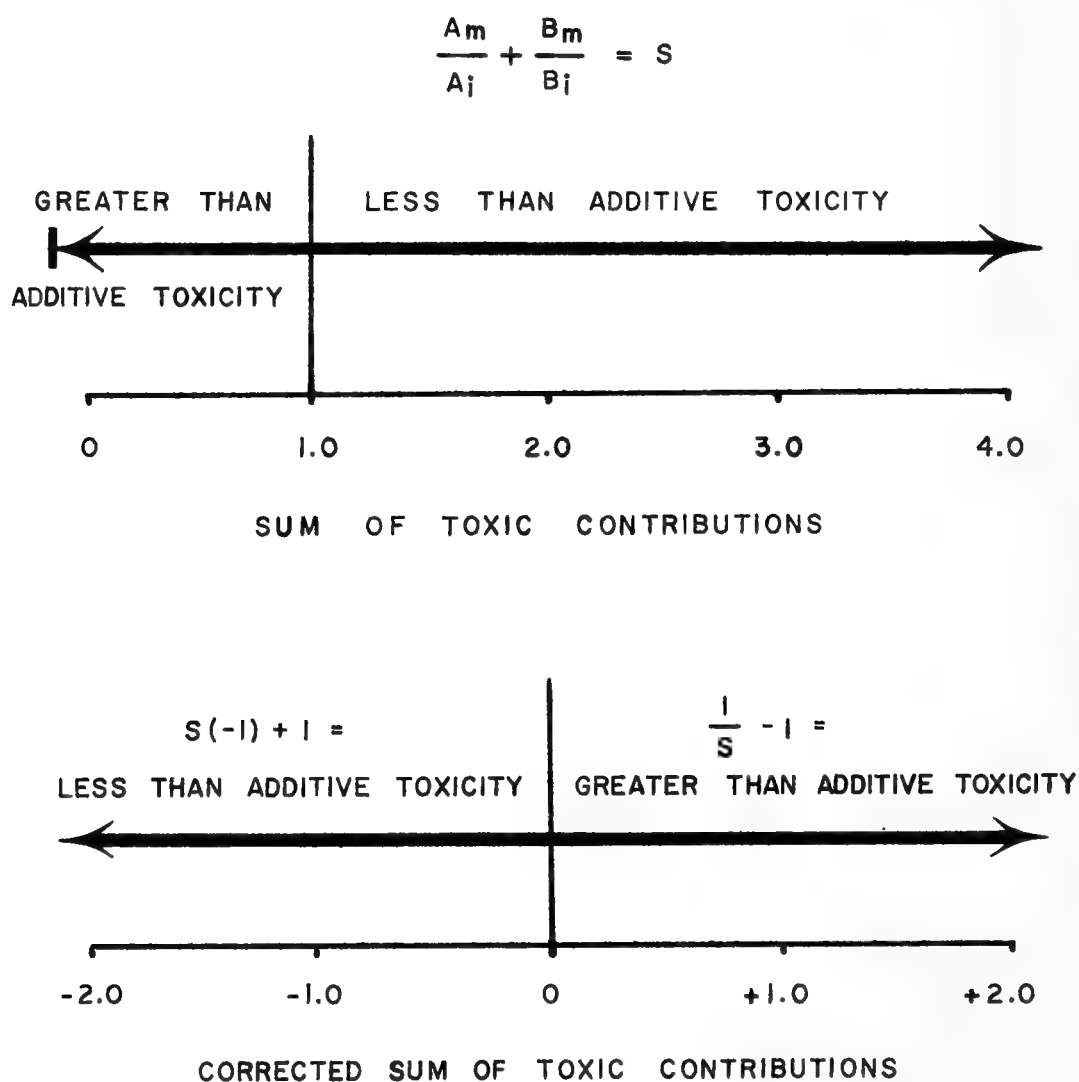
Figure 1.—Sums (S) of toxic contributions for a chemical mixture, which are nonlinear for less than additive and greater than additive toxicity (upper illustration) were corrected for linearity and direction of plus and minus values (lower illustration).

A system in which the index represents simple additive, greater than additive, and less than additive effects by zero, positive, and negative values, respectively, would be desirable. Such a system can be developed by establishing linearity and by assigning a reference point of zero for simple additive toxicity (Fig. 1). We established linearity by using the reciprocal of the values of S which were less than 1.0, and a zero reference point was achieved by subtracting 1.0 (the expected sum for simple additive toxicity) from the reciprocal $[(\frac{1}{S})-1]$. Thus greater than additive toxicity is represented by index values greater than zero. Index values representing less than additive toxicity were obtained by multiplying the values of S which were greater than 1.0 by –1 to make them negative, and a zero reference point was achieved by adding 1.0 to this negative value $[S(-1)+1]$. Thus, less than additive toxicity is represented by negative index values. The sum of effective contributions (S) is modified by one of two procedures: either the additive index $= \frac{1}{S} - 1$ for $S \leq 1.0$ (greater than additive toxicity) or $S(-1) + 1$ for $S \geq 1.0$ (less than additive toxicity). A sum (S) of 1 yields an index value of zero by either modifying procedure and represents simple additive toxicity. A summary of the procedure follows.

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S, \text{ the sum of biological effects}$$

Additive index $= \frac{1}{S} - 1.0$ for $S \leq 1.0$ and

Additive index $= S(-1) + 1.0$ for $S \geq 1.0$

We assessed the significance of additive indices close to zero in our data by substituting values from the 95% confidence intervals into the formula to determine whether the range for additive indices overlapped zero (simple additive toxicity). The range was derived by selecting values of the 95% confidence interval yielding the greatest deviation from the additive index. The lower limits of the individual toxicants (A_i and B_i) and the upper limits of the mixtures (A_m and B_m) were substituted for LC50's to determine the lower limit of the index. Correspondingly, the upper limits of the individual toxicants (A_i and B_i) and the lower limits of the mixtures (A_m and B_m) were substituted into the formula to determine the upper limit of the index.

Applications and Discussion

To test the method on existing information, we selected toxicity data from published papers in which the author had provided the toxicity for the individual components and for the mixtures. In some instances observed mortality was reported and used in place of statistically derived LC50's. The significance of additive indices derived from data in the literature was not assigned because 95% confidence intervals were usually not reported.

Doudoroff (1952) studied the toxic activity of mixtures of zinc and copper, two suspected metal ion contaminants in some effluents. He showed extraordinary toxic activity against fathead minnows (Pimephales promelas) in 8-h tests in which survival was recorded rather than median lethal concentrations (LC50's). The additive index is

$$\frac{1.0}{8.0} + \frac{0.025}{0.20} = 0.250; \ (\frac{1}{0.250}) - 1 = 3,$$

a value which definitely supports Doudoroff's conclusions (Table 1). Lloyd (1961) and Sprague and Ramsey (1965) found less or no potentiation in zinc and copper mixtures, but they defined the toxicity with LC50's for longer exposures; however, they did report that lethal mixtures of those metal ions act 2 to 3 times as fast as the metals singly.

Cairns and Scheier (1968) reported "slight antagonistic interaction" of zinc and cyanide to fathead minnows, in contrast to the opposite activity of zinc and copper. They attributed the effect to complexation of these ions. The additive index (–1.37) as computed in the present study indicates considerable antagonism. Chen and Selleck (1969) also considered the zinc-cyanide combination to be very antagonistic, but they did not quantify the toxicity.

Howland (1969) reported additive effects for mixtures of two fish toxicants, antimycin and rotenone, whereas our index is –0.39. Although our value suggests slightly less than additive toxicity, the significance of the value should be defined before the results are interpreted.

Antimycin could be applied to water with a fluorescent tracer, rhodamine B. The tracer

Table 1. Toxicity or efficacy of chemicals applied individually and in combination against fishes and the calculated additive index

Chemical mixtures	Toxic unit	96–h LC50 or EC50 of chemical		Additive index	Reference
		Individually	In combination		
Zinc[a]	mg/l	8.0	1.0		
and				3.00	Doudoroff 1952
Copper[a]	mg/l	0.2	0.025		
Zinc	mg/l	4.2	3.90		
and				-1.37	Cairns and Scheier 1968
Cyanide	mg/l	0.18	0.26		
Antimycin	μg/l	0.032	0.027		
and				-0.39	Howland 1969
Rotenone	μg/l	57.0	31.0		
Antimycin	μg/l	0.048	0.047		
and				0.00	Marking 1969b
Rhodamine B	mg/l	217	5.0		
MS–222	mg/l	80	30		
and				0.29	Berger 1969
QdSO₄	mg/l	25	10		
Malachite green	mg/l	0.2	0.05		
and				0.83	Leteux and Meyer 1972
Formalin[b]	mg/l	50	15		

[a] An 8–h time response, based on survival rather than LC50.

[b] Concentrations effective against parasites.

interacts little, if any, with the toxicant (additive index = 0.00), and the suitability of this tracer was supported (Marking 1969b).

The additive index method can be used for characteristics other than toxicity. For instance, the efficacy of MS–222 (tricaine methanesulfonate) and QdSO₄ (quinaldine sulfate), two fish anesthetics, shows the rapid and sustaining anesthetic qualities, respectively, and an improved safety factor when the two chemicals are mixed rather than applied separately. The index of 0.29 suggests greater than additive efficacy and agrees with Berger's (1969) interpretation that the interaction was synergic. Schoettger and Steucke (1970) discussed advantages of the anesthetic mixture, including a cost reduction of 60 to 80%. Mixtures of some fish therapeutants are more effective than individual disease treatment chemicals. For

example, the additive index for the efficacy of malachite green and formalin is 0.8 (Leteux and Meyer 1972).

Several pairs of toxic chemicals were chosen for determining additive toxicity in our laboratory (Table 2). When the fish toxicants antimycin and TFM (lampricide) were tested individually and in combination against bluegills, the additive index was 0.343 and the range computed from the 95% confidence interval was -0.189 to 1.17. Since the range overlaps zero, the toxicity for these two chemicals in this particular test was merely additive.

The index for mixtures of antimycin and Dibrom[(R)] against rainbow trout was -0.574 (range, -1.12 to -0.173). Since the range did not overlap zero, the toxicity of the mixture was less

Table 2. Toxicity of toxicants applied individually and in combination to fish in 96–h, standardized tests at 12°C

Species, toxicants, and toxic unit	LC50 and 95% confidence interval		Additive index
	Individually	In combination	
Bluegill			
Antimycin (μg/l)	0.0710	0.0390	
	0.0574–0.0879	0.0296–0.0506	
and			0.340
			–0.189 to 1.17
TFM (mg/l)	4.96	0.970	
	4.10–5.99	0.744–1.26	
Rainbow trout			
Antimycin (μg/l)	0.0312	0.0300	
	0.0266–0.0366	0.0272–0.0331	
and			–0.574
			–1.12 to 0.173
Dibrom (mg/l)	0.0490	0.0300	
	0.0279–0.0633	0.0272–0.0331	
Antimycin (μg/l)	0.0412	3.79	
	0.0371–0.0457	2.78–5.16	
and			–91.8
			--- ---
$KMnO_4$ (mg/l)	1.22	1.0	
	1.08–1.38	--- ---	
Malathion (μg/l)	70.0	3.44	
	59.2–82.7	2.92–4.06	
and			7.20
			5.09 to 10.0
Delnav (μg/l)	47.2	3.44	
	42.4–52.6	2.92–4.06	

than additive. Berger (1971) reported that mixtures of those toxicants are synergistically toxic to black bullhead *(Ictalurus melas)*, largemouth bass *(Micropterus salmoides)*, and yellow perch *(Perca flavescens)*. These data suggest that additive toxicity may vary for different species of fish, ratios of toxicants, or test conditions. For example, Hoff and Westman (1965) reported that a 3:2 ratio of Dibrom[R] and malathion showed promising selectivity toward bluegills and pumpkinseeds *(Lepomis gibbosus)* in the presence of largemouth bass. For these reasons, the additive index for toxicants should be determined for nontarget as well as target species.

Antimycin is readily detoxified by oxidizing agents such as potassium permanganate (Walker 1967). Both chemicals were toxic to fish, but the counteraction through oxidation greatly decreased the toxicity of the mixture. Potassium permanganate was added at 1.0 mg/l to each concentration of antimycin because that concentration of permanganate has been effective in fish management applications. As expected, the combination produced extreme antagonistic activity against rainbow trout; the additive index was –91.8 in standardized tests at pH 7.5 (Table 2). This procedure could be used to assess the effectiveness of permanganate for detoxifying antimycin under other conditions of

different pH or temperature.

Mixtures of malathion and Delnav[R], two organic phosphates reported to be synergistic against insects, are extremely toxic to fish. The additive index is 7.20 for rainbow trout. That value is the highest for any toxicant mixture evaluated by our method and further emphasizes the potency of certain pesticide mixtures.

The 95% confidence interval influences the significance of the additive index, and significance becomes more difficult to show as the range widens because of the greater likelihood that the range will overlap zero. The 95% confidence interval is influenced by the number of concentrations and the number of test organisms per concentration. Therefore, well planned toxicity tests which result in narrow confidence intervals are the most useful in the assignment of effects of chemical mixtures.

The study of mixtures of toxic chemicals in water and the resultant benefits or hazards is fairly new, and only a few methods have been investigated. Conceivably, the additive toxicity of more than two chemicals could be evaluated by simply adding the contributions of additional chemicals according to the following formula,

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} + \frac{C_m}{C_i} + - - - = S.$$

This additive toxicity index method could be useful for assessing the economics of mixtures of chemicals, for determining toxicity advantages against target organisms, for determining hazards or disadvantages against nontarget organisms, for assessing the additive toxicity of different ratios of chemicals in a mixture, and for assessing physical influences of the environment on additive toxicity. Advantages of this method over existing methods include linearity for all index values and a procedure for determining the significance of indices.

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8

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68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations

United States Department of the Interior

Fish and Wildlife Service

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INVESTIGATIONS IN FISH CONTROL

68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations

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INVESTIGATIONS IN FISH CONTROL

68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations

By Ralph M. Burress

CONTENTS

DEVELOPMENT AND EVALUATION OF ON-SITE TOXICITY TEST PROCEDURES FOR FISHERY INVESTIGATIONS

by

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ABSTRACT

A simple, inexpensive procedure was developed for conducting on-site tests (bioassays) of the toxicity of various concentrations of antimycin on target and nontarget fishes in waters to be treated. After preliminary laboratory experiments with seven types of containers showed that large polyethylene bags were best for use in field toxicity tests, year-round field experiments were performed in ponds. Measurements of water quality in large bags containing 284 liters of water were closely similar to water quality measurements in ponds throughout the test periods. The maximum safe loading level for 96–h tests at temperatures above 18.3 C was about 852 g of fish per bag, but this amount could be increased in colder water or in shorter tests. Final modifications and evaluations of the method were made on the basis of treatments of ponds with antimycin in accordance with data derived from on-site tests. The procedure described is adaptable for tests of many other chemical compounds commonly used by fishery workers.

INTRODUCTION

With the development of more potent and more selective fish toxicants, fishery managers have an increasing need to determine the concentration of toxicant which is most effective for a specific purpose in a body of water. The efficacy of chemicals used by fishery workers is affected by water quality, physical and biological conditions in the aquatic habitat, and the relative susceptibility of different kinds and sizes of target organisms. The lack of specific information on which to base application rates has often resulted in inadvertent underdosing, deliberate overdosing, and repeated dosing, all of which were detrimental to the success of the treatments or to the habitats treated.

The on-site toxicity test (bioassay) procedure is one of the more promising methods for determining both the concentration and the length of exposure required to produce the desired level of control of target organisms. Despite the pronounced need for information of this kind, the on-site test method apparently has not been widely used; relatively few investigators who have applied toxicants to manipulate fish populations in streams have reported using fish and water from the streams to be treated to conduct preliminary on-site tests. The facilities employed have varied from a simple arrangement of fish hatchery troughs beside a stream (Lennon and Parker 1959) to a

fully equipped mobile laboratory (Howell and Marquette 1962). On-site tests conducted in lakes before the application of fish toxicants have been performed in large wastebaskets (Berger et al. 1967), in 91-liter plastic garbage cans (Pfeiffer 1968), in open-ended, 208-liter barrels that were pushed into the lake bottom a few inches (Vaughn et al. 1974), and in 76-liter capacity plastic bags that were included in kits provided by Ayerst Laboratory Inc., for on-site tests of antimycin (G. C. Radonski, personal communication).

The utility and economy of using small plastic bags as bioassay vessels are well documented. Davis and Hardcastle (1959) used 25-liter bags enclosed in cardboard cartons to conduct herbicide toxicity tests in the laboratory; Falk (1972) conducted acute toxicity bioassays of pollutants by placing 20-liter plastic bags inside larger nylon mesh bags supported by rigid aluminum frames situated in shallow lake waters. In both these studies, the authors placed 10 fish in each bag and supplied continuous aeration to maintain adequate concentrations of dissolved oxygen for 48 or 96 h.

Although dissolved oxygen diffuses through polyethylene film (Fremling and Evans 1963), the rate of diffusion is so slow that, if small plastic bags are used as bioassay containers, either accessory aeration must be supplied or the loading levels (grams of fish per liter of water) must be light and tests must be of short duration. In the present study, the objective was to use more and larger fish in 96-h tests of antimycin without employing either aeration devices or bulky supports for rigid vessels. Consequently, the study centered on determining the feasibility of employing plastic bags large enough to contain 284 liters of water and on developing simple experimental procedures for their use. During the period July 1968 to June 1970, numerous laboratory and field experiments were conducted at the Southeastern Fish Control Laboratory, Warm Springs, Georgia and in four nearby ponds. Bioassay procedures developed were tested in field trials in Illinois and Arkansas in 1972.

MATERIALS AND METHODS

Laboratory experiments included comparisons of the utility, efficacy, cost, and safety of seven types of potential on-site test vessels: polyethylene wastebaskets, fiber glass containers, metal lard cans, aluminum pails, stainless steel pails, large glass jars, and polyethylene bags. No single container was superior to the others in every respect, but polyethylene bags appeared to be the most useful, primarily because they are readily portable and are available in large sizes from numerous firms that manufacture plastic film or plastic containers. Consequently, large polyethylene bags (0.96 m × 1.65 m, made of material 0.076 mm [3 mils] thick) were used in conducting 19 on-site toxicity tests with antimycin in four ponds during the period April 1969 to June 1970. The ponds varied in type from a clear, infertile pond to a highly eutrophic pond polluted with dairy wastes. Loading rates ranged from 0.11 to 2.76 g/l, but were less than 1 g/l in 13 of the tests. Certain procedures were modified in the exploratory stage of the study, but the methods outlined here were followed consistently thereafter.

Experimental Fish

In most experiments, antimycin was tested against bluegills (*Lepomis macrochirus*); in others, a few subadult largemouth bass (*Micropterus salmoides*) or channel catfish (*Ictalurus punctatus*) were included. Most of the fish were seined from the ponds in which the tests were conducted. These fish, usually captured a day or two before the tests, were held in uncrowded live-cages in the ponds while they recovered from the stress of collection, and only vigorous specimens were selected for testing. The few fish from the laboratory that were used in field tests also were held at least 24 h in live-cages for acclimation to pond conditions. If the supply of fish was adequate, 10 fish were placed in each vessel except when the use of large fish made it necessary to reduce the number to avoid exceeding the loading capacity.

Preparation of Stock Solutions

Stock solutions were prepared by using a 1-ml glass pipette graduated in hundredths to

measure 1.42 ml of Ayerst's undiluted 20% stock solution of antimycin [Fintrol-Concentrate $^{(R)}$] into a 1-liter volumetric flask and adding acetone to bring the volume up to 1 liter. The addition of 1 ml of this stock solution to 284 liters of water yielded a $1-\mu g/l$ (1-ppb) concentration of antimycin (active ingredient), a procedure which simplified operations and reduced the likelihood of error. The small amounts of toxicant needed for determining the concentrations required for selective kills were also measured with a 1-ml glass pipette.

On-site Toxicity Test Arrangement

In selecting the site, the crew avoided areas where rocks, roots, or other objects might puncture the bags. Where no protective enclosure for the bags was needed, two steel fence posts were driven into the bottom of the pond about 7 m apart in waist-deep water, a rope was stretched between the posts, and the bags were suspended from the rope with stout twine (Fig. 1). When necessary, bags were protected from sources of mechanical damage (e.g., turtles, boats, and flotsam) by making a simple exclosure of netting material supported by four steel fence posts. The ropes used to support the bags were tied diagonally across the exclosure, providing support for a cover made of netting and eliminating bird depredations that sometimes occurred when treated fish surfaced in distress.

Filling Bags

In the laboratory, 284 liters of water were pumped into a bag and the water level was marked. The bag was then emptied, and about 50 more bags were marked at the same level for use in the field. Two men standing in waist-deep water were able to fill and handle bags without difficulty. Water of this depth permits crewmen in chest waders to handle full bags without dragging them over the pond bottom. The preferred method for filling bags involved the use of a large plastic waste can from which the bottom had been removed. The can, when fully inserted into the mouth of the bag, held the bag open and gave good support as the bag was filled. When water reached the desired level, the can was removed, the bag neck was twisted shut and secured with a stout rubber band, and the bag was suspended from the rope with twine. After all bags were filled, each was reopened; the toxicant was added and mixed into the water thoroughly with a dip net; fish were added; the bag was resealed, returned to its place, and numbered. In each experiment, two controls (untreated) were used; one contained fish and the other only water.

Observations

In clear ponds dead fish could be seen at the bottom of the bags and removed easily with a dip net when observations were made. If the water was not clear enough to permit direct observation, all fish were dipped out into a container. Live fish then were returned to the bags; dead fish were discarded. Use of a rectangular dip net expedited removal of dead fish from the corners of bags.

Monitoring water quality

Most tests were conducted for 96 h. Measurements of pH and dissolved oxygen in the pond, in the control bag containing fish, and in the control bag without fish, generally were made in early morning and late afternoon. A two-man crew could set up and conduct a test without undue difficulty if work schedules were efficiently arranged.

EFFICACY OF POLYETHYLENE BAGS FOR USE IN ON-SITE TOXICITY TESTS

The results of the brief initial experiments with large polyethylene bags were encouraging. Bags of 0.076-mm wall thickness proved to be sufficiently strong. Furthermore, struggling fish in their death throes invariably retracted their fins when they came in contact with the bag wall and thus did not puncture the bags. If seams leaked or the bag was punctured, the medium was not diluted because the water flow always was outward.

Results of routine static tests conducted to compare the toxicity of antimycin to fingerling bluegills in glass jars and in plastic bags were closely comparable; this indicates that no

appreciable loss of toxicant resulted from absorption, adsorption, or reaction with the plastic. Before pond tests were begun, other tests were performed in outdoor plastic pools containing phytoplankton. Water temperatures in the bags were virtually identical with those at corresponding depths outside the bags, and the pH of water in the bags corresponded more closely with that in the surrounding water than did the pH in other types of containers. Concentrations of dissolved oxygen remained higher in clear bags than in opaque bags, presumably because photosynthesis continued at a higher rate in clear bags.

The influence of 2-, 3-, 4-, and 5-g/l loading rates on pH and dissolved oxygen concentrations were determined in a summer field test. When surface temperatures were high (28.0–31.9 C), the 3-g/l loading with bluegills of intermediate size was the highest that could be used for a 96-h test. A 4-g/l load could have been used for as long as 48 h, but a 5-g/l load was too great for even a 24-h test.

Comparing pH values and dissolved oxygen concentrations in bags and various types of ponds during different seasons was a primary concern during the development and evaluation of toxicity test procedures. Water quality data collected during the 19 field tests indicated that the highest and lowest pH values measured in the control bags containing fish were neither consistently higher nor lower than those measured at the pond surface (Table 1). Thus, test results were not biased by differences in pH

attributable to the bags. Comparisons of dissolved oxygen concentrations showed that average readings in the control bags containing fish tended to be slightly lower than those in the pond, whereas the readings in the control bags without fish generally were somewhat higher. However, no test results were invalidated because of oxygen depletion in the bags. If bags made from different grades of raw materials have different physical and chemical characteristics, oxygen may diffuse less readily through some bags than others. Preliminary tests with each new lot of bags would help determine safe loading rates.

After the 19 field trials were completed and basic test methods were established, on-site toxicity tests were conducted in one pond in Illinois and four in Arkansas. The antimycin treatments were successful, and were reported by Cumming and Gilderhus (in press) and by Cumming, Burress, and Gilderhus (1975). Properly secured bags withstood heavy rains, winds, and even strong currents induced by outboard motor operation. There is good reason to believe that such tests also could be conducted in streams with a slow or moderate current, if bags were protected from floating objects. Because of the essentially neutral buoyancy of the bags, it is possible to anchor them and conduct tests below the surface. G. C. Radonski (personal communication) stated that he has conducted toxicity tests in plastic bags under ice and in the hypolimnion at depths as great as 9.1 m.

RECOMMENDATIONS FOR CONDUCTING ON-SITE TESTS

The methods outlined above provide the basic guidelines for conducting an on-site toxicity test. The first day's preparations include securing the experimental fish; setting up the posts, ropes, and protective netting; and determining pH and other water quality factors. On the day of the test, the bags are filled, the toxicant is added and thoroughly mixed, and the fish are added in rapid sequence at the time of day when the full-scale treatment will be applied. This ensures that water quality conditions during the test and the treatment will be as similar as possible.

Selecting Test Concentrations

The range of toxicant concentrations to be tested depends largely on the size and relative susceptibility of the target fishes, the amount of population reduction desired, and the water quality characteristics (particularly temperature and pH). Not less than five test concentrations should be used, and a sixth bag, with fish, should be used as a control. The following concentrations of antimycin (μg/l) are suggested when total kills of susceptible species are desired: 2.5, 4.0, 5.5, 7.0, and 8.5. If the

target fishes include resistant species such as goldfish (*Carassius auratus*), gar (*Lepisosteus* spp.), or bowfin (*Amia calva*), concentrations of 12, 15, 18, 21, and 24 µg/l are more appropriate. Less antimycin is required when the temperature is above 15.6 C or the pH is below 8.5.

Selective reduction of target species by complete treatment of the aquatic system requires much lower concentrations of antimycin. When the temperature exceeds 15.6 C and there are marked diurnal fluctuations in pH, the following test concentrations of antimycin (µg/l) are suggested: 0.20, 0.35, 0.50, 0.75, and 1.0. If pH levels are so high that the 1.0-µg/l concentration is not adequate, the range of concentrations should be shifted upward or the treatment postponed until water conditions become more favorable. The following concentrations (µg/l) are more appropriate for use at temperatures lower than 15.6 C: 0.4, 0.8, 1.2, 1.6, and 2.0. The duration of toxicity tests should be extended by 1 to 3 days when water temperatures are low.

Test Animals

In tests where the biologist must determine the ability of fish to withstand exposure to a toxicant in order to ensure selection of a safe concentration for treatment (e.g., removal of scalefish from a catfish pond), use of fish from the pond to be treated is mandatory. In other situations as well, test animals from the waters to be treated should be used if they can be collected in good condition and in adequate numbers. If this is not possible, target and nontarget fishes of appropriate sizes must be brought in from other sources and allowed to acclimate for 24 h in live-cages before the test. Sizes and numbers needed depend on the loading levels that can be used under conditions existing at the time of the test.

Loading Rates

The following information provides broad guidelines regarding loading rates. At temperatures of 25 to 30 C, up to a 3-g/l load (852 g per bag) of fish can be used for 96-h tests. For each 5-degree reduction in temperature below 25 C, it may be possible to increase the loading by 1 g/l (284 g per bag). For 24-h tests, these amounts can be increased, depending upon the circumstances. If excessive loading rates are used, results of the test are likely to be too biased to be

usable. Temperature, dissolved oxygen, and pH should be measured each morning and afternoon throughout the test period.

Duration of Toxicity Tests

Test results are apparent much sooner at high than at low temperatures, and the concentration of antimycin required for a complete kill in warm weather can safely be selected on the basis of a 24-h test. In cold weather such tests should be conducted for at least 48 h to avoid the use of unnecessarily high concentrations of toxicant.

Selection of concentrations to effect partial kills can be based on 24-h tests only if temperature and pH are high enough to ensure complete detoxification of the antimycin within that time. When temperatures are low and the pH is neutral or acid, tests for partial kills should be carried out for 96 h or until the full effects of the various concentrations are discernible.

Selecting Treatment Concentrations

The results of on-site toxicity tests can be used in selecting treatment concentrations if two conditions are met: (1) mortality patterns must be consistent, i.e., mortalities must increase as toxicant concentrations increase, and (2) mortality of the control fish must not exceed 10%. Inconsistent patterns of mortality often are a result of using poor quality test animals. If test results are good, the lowest concentration at which all target fish are killed should be used when the entire volume of water is treated to effect a selective kill. The concentration of toxicant used in each bag can be calculated precisely because the volume of water is known. Success in applying test results to actual treatments is directly proportional to the accuracy with which the volume of water to be treated is determined.

When a complete kill is intended and large specimens of the target species are used in the toxicity test, the lowest completely effective concentration tested should be increased by at least 1.0 µg/l to ensure effectiveness of the treatment. If large specimens are not used, a safety factor of at least 1.5 to 3.0 µg/l should be added to the lowest completely effective bioassay concentration, depending on the susceptibility of the target species.

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Table 1.—Temperature, pH, and dissolved oxygen ranges in ponds where on-site bioassay experiments were conducted.

Date	Test period (h)	Loading level (g/l)	Surface temperature range (°C)	pH range			Dissolved oxygen range (mg/l)		
					Plastic bags			Plastic bags	
				Pond	Fish	No fish	Pond	Fish	No fish
4/69	99	0.75	17.8–23.4	6.7– 8.2	6.8– 8.0	7.2– 8.1	6.6–10.0	6.0– 9.5	7.5– 9.5
5/69	70	0.93	21.7–30.6	6.7– 8.2	6.3– 6.8	6.7– 7.3	5.4– 9.6	0.5– 6.5	4.9– 8.2
5/69	70	0.68	18.9–27.2	6.8– 8.4	6.9– 8.5	7.2– 8.7	6.3–10.5	5.7– 9.2	5.9–10.3
5/69	107	0.45	22.2–30.0	6.7– 8.9	6.6– 7.6	6.6– 8.9	6.1–13.0	3.3–10.4	5.8–14.3
5/69	83	0.55	23.4–30.6	6.6– 9.3	7.2– 9.4	7.8– 9.4	5.4–13.5	8.2–14.1	7.2–15.4
6/69	72	0.31	29.5–35.0	7.3– 8.9	7.2– 9.0	7.8– 9.1	6.8–10.7	7.5–10.3	7.7–10.8
7/69	72	a	26.1–31.7	7.6–10.3	7.9–10.4	7.9–10.3	3.0–14.0	6.2–24.0	8.6–19.9
8/69	72	1.41	26.7–31.1	6.6– 8.3	6.6– 8.5	6.6– 8.3	6.1– 9.0	5.2– 8.7	6.5– 9.0
11/69	96	0.46	13.7–16.2	5.9– 6.4	5.1– 6.6	6.1– 6.5	8.8– 9.8	9.2–10.0	9.6–10.0
11/69	96	1.23	13.7–16.2	5.9– 6.4	5.5– 6.1	6.1– 6.5	8.8– 9.8	5.0–10.6	9.6–10.0
11/69	120	0.62	11.2–14.5	5.8– 6.3	6.3– 6.8	6.3– 6.8	7.4–10.0	7.8– 9.0	8.6–10.4
11/69	120	1.24	11.2–14.5	5.8– 6.3	6.0– 6.2	6.3– 6.8	7.4–10.0	4.7– 6.3	8.6–10.4
11/69	72	0.99	8.9–12.2	7.0– 7.3	6.6– 7.0	7.2– 7.4	10.2–13.3	10.4–11.2	10.8–12.8
12/69	72	0.89	6.8– 8.9	7.3– 7.6	7.3– 7.4	7.3– 7.4	10.0–14.0	12.6–13.2	13.5–13.6
1/70	81	2.76	8.9–15.6	7.3– 8.8	6.8– 8.6	—	11.4–14.5	8.6–13.8	—
3/70	96	0.85	12.2–15.6	5.3– 6.4	5.9– 6.1	6.0– 6.1	5.0– 8.2	—	—
4/70	96	0.11	17.8–21.1	6.8– 7.5	6.8– 7.5	6.8– 7.2	6.6–10.4	8.6–10.4	8.4–10.5
6/70	96	0.49	23.4–26.1	7.5– 8.9	7.4– 8.7	7.7– 8.8	8.6–12.2	8.0–12.4	9.4–12.2
6/70	96	0.55	25.0–31.7	7.0– 9.3	7.0– 8.6	7.0– 8.3	6.2–11.2	8.5– 9.8	8.1–11.2

a Turtles damaged bag, allowing fish to escape.

8

Figure 1. Sample apparatus for suspending large plastic bags used to conduct on-site toxicity tests.

(Reports 53 through 55 are in one cover.)

53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish, by Verdel K. Dawson and Leif L. Marking. 1973. 11 pp.

54. The Efficacy of Quinaldine Sulfate:MS-222 Mixtures for the Anesthetization of Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.

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(Reports 56 through 59 are in one cover.)

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(Reports 63 through 66 are in one cover.)

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67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals, by L. L. Marking and V. K. Dawson. 1975. 7 pp.

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INVESTIGATIONS IN FISH CONTROL

69. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM),
    2',5-dichloro-4'-nitrosalicylanilide (Bayer 73),
    and a 98:2 Mixture to Fingerlings of Seven Fish
    Species and to Eggs and Fry of Coho Salmon

70. The Freshwater Mussel (*Anodonta* sp.) as an
    Indicator of Environmental Levels of
    3-trifluoromethyl-4-nitrophenol (TFM)

United States Department of the Interior

Fish and Wildlife Service

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INVESTIGATIONS IN FISH CONTROL

69. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture to Fingerlings of Seven Fish Species and to Eggs and Fry of Coho Salmon

By Terry D. Bills and Leif L. Marking

70. The Freshwater Mussel (*Anodonta* sp.) as an Indicator of Environmental Levels of 3-trifluoromethyl-4-nitrophenol (TFM)

By Alan W. Maki and Howard E. Johnson

TOXICITY OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM), 2′,5-DICHLORO-4′-NITROSALICYLANILIDE (BAYER 73), AND A 98:2 MIXTURE TO FINGERLINGS OF SEVEN FISH SPECIES AND TO EGGS AND FRY OF COHO SALMON

by

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ABSTRACT

We determined the toxicity of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73) and a 98:2 mixture of these compounds against fingerlings of seven species of fish—brown trout (*Salmo trutta*), rainbow trout (*Salmo gairdneri*), lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), bluegill (*Lepomis macrochirus*), and yellow perch (*Perca flavescens*)—and to eggs and fry of coho salmon (*Oncorhynchus kisutch*). Channel catfish were the most sensitive to TFM and brown trout to Bayer 73. Bluegills were the most resistant to both TFM and Bayer 73. The toxicity of TFM and Bayer 73 individually was influenced far more by pH than was the mixture in standard laboratory tests with rainbow trout. Toxicity of the mixture was additive or less than additive to all species and life stages tested. The mixture was slightly more toxic to larval lampreys (*Petromyzon marinus*) than to other fish in comparable laboratory toxicity tests. The margin of safety was narrow, however, when the 24-h toxicity for brown trout or rainbow trout was compared with the 24-h LC99 for sea lamprey larvae.

INTRODUCTION

Before 1964, 3-trifluoromethyl-4-nitrophenol (TFM) was the only compound used for the control of larval sea lampreys (*Petromyzon marinus*) in tributaries of the Great Lakes (Applegate et al. 1961). Howell (1964) pointed out the ineffectiveness of TFM in water with certain physicochemical characteristics. This ineffectiveness prompted the U.S. Fish and Wildlife Service to search for compounds that could replace TFM or increase the biocidal activity of the compound.

Howell et al. (1964) reported that the molluscicide 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73, also known as Bayluscide®) was extremely toxic to larval sea lampreys. However, the compound also was highly toxic to other fishes (Marking and Hogan 1967). Howell et al. (1964) determined that by mixing from 0.5 to 4% Bayer 73 with TFM, the efficacy was increased without loss of the selective toxicity displayed by TFM. Since use of the TFM:Bayer 73 (98:2) mixture began in 1964, few, if any, data on its safety or toxicity to nontarget organisms have been published. Lennon (1967) pointed out the need for such data to meet regulatory agency requirements for continued use. The history, chemistry, and uses of TFM, Bayer 73, and the mixture were summarized by Schnick (1972), and Hamilton (1974a, 1974b).

The objectives of the present study were to determine (1) the toxicity of TFM, Bayer 73, and a 98:2 mixture of the compounds against several species of fish in soft water; (2) the effects of water temperature, hardness, and pH on the toxicity of these lampricides to rainbow trout (*Salmo gairdneri*); (3) the toxicity of these lampricides to eggs and fry of coho salmon (*Oncorhynchus kisutch*); and (4) the margin of safety for each compound and the mixture for nontarget fishes.

METHODS AND MATERIALS

The chemicals used in the toxicity tests were field grade TFM (35.7% active ingredient) supplied by American Hoechst Chemical Co., Somerville, New Jersey, and Bayer 73 (70% wettable powder) supplied by Chemagro Corporation, Kansas City, Missouri. Stock solutions of both were prepared in water (the liquid formulation of TFM measured volumetrically and diluted with water). All concentrations were based on active ingredients, and the mixture used was 98% TFM:2% Bayer 73. To prepare test solutions of the desired concentrations, we pipetted portions of stock solutions into the test vessels and stirred the resulting media to ensure homogeneity.

Fish and coho salmon eggs were obtained from National Fish Hatcheries and maintained according to procedures outlined by Hunn et al. (1968) for fish and by Bills (1974) for fish eggs. In addition to eggs and fry of coho salmon, fish tested were fingerlings (0.5–1.5 g) of brown trout (*Salmo trutta*), rainbow trout, lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), bluegill (*Lepomis macrochirus*), and yellow perch (*Perca flavescens*). Fish and coho salmon were acclimated to test conditions for 24 h preceding chemical additions. Procedures outlined by Lennon and Walker (1964) were followed for the toxicity tests with fingerlings, and procedures presented by Bills (1974) were followed for tests with eggs. Toxicity tests with coho salmon eggs and fry were conducted in 2.5-liter glass vessels; all other toxicity tests were conducted in 15-liter glass vessels.

Test waters of different hardnesses were prepared by adding inorganic salts to deionized water (Marking 1969). In toxicity tests in which the effects of pH were assessed, chemical buffers were added to soft water (Marking and Dawson 1973). The pH's were checked daily and adjusted to within ± 0.2 pH units. Temperature in the test vessels was maintained by immersing the units in a water bath.

Data were analyzed to determine LC50's and 95% confidence intervals according to methods described by Litchfield and Wilcoxon (1949). Additive indices and their ranges were calculated from the LC50's and 95% confidence intervals according to methods described by Marking and Dawson (1975). Additive indices quantitate the combined activity of the mixture, and the confidence intervals define significance. Positive index values indicate greater than additive toxicity, and negative values indicate less than additive toxicity. Indices for which confidence intervals overlap zero are considered to indicate that the toxicity is neither greater nor less than additive.

RESULTS

Channel catfish and brown trout were most sensitive to TFM, followed by yellow perch, lake trout, rainbow trout, brook trout, and bluegill (Table 1). The 96-h LC50's in soft water at 12 C ranged from 0.750 mg/l for channel catfish to 4.89 mg/l for bluegill. The comparative toxicity (96-h LC50) of Bayer 73 to the selected species ranged from 0.0282 mg/l for brown trout to 0.152 mg/l for bluegill. On the basis of the additive index concept, the toxicity of the mixture was strictly additive, indicating that from a toxicological standpoint there is no advantage in applying the compounds as a mixture.

In tests to determine the effects of water temperature, hardness, and pH on toxicity of the lampricides to rainbow trout, water temperatures influenced toxicity least (Table 2). TFM and Bayer 73 individually were slightly more toxic to rainbow trout in soft water at 17 C than at lower temperatures, but the mixture was not affected by temperature.

The effect of water hardness on the toxicity of the lampricides to rainbow trout was determined in tests with waters of differing hardnesses: very soft (10 mg/l as $CaCO_3$), soft (44 mg/l), hard (160 mg/l), and very hard

(300 mg/l). The toxicity of TFM was about 1.5 times greater in very soft water than in soft, hard, or very hard water (Table 2). In contrast, the toxicity of Bayer 73 was not affected by water hardness. The toxicity of the mixture was strictly additive. However, separate evaluation of the components of the mixture showed that the 96-h LC50's for the Bayer 73 component were not significantly different from the 96-h LC50's for Bayer 73 singly, but that the amount of TFM required to produce the same effect was reduced twofold to threefold. This difference indicates that it could be economically advantageous to apply the mixture if the response of sea lampreys is similar to that of rainbow trout and if the selectivity of TFM toward the sea lamprey could be maintained.

Of the water quality characteristics examined, pH had the most distinguishable effect (Table 2). In producing toxicosis to rainbow trout in soft water, TFM was 4% as effective at pH 9.5 (96-h LC50, 25.2 mg/l) as at pH 6.5 (0.949 mg/l). The toxicity of Bayer 73 showed a similar trend; in producing toxicosis in soft

water, this chemical was 14.3% as effective at pH 9.5 (96-h LC50, 0.185 mg/l) as at pH 6.5 (0.0261 mg/l). As in the other tests, the toxicity of the mixture was additive or less than additive. The individual evaluation of the components showed that the 96-h LC50's for Bayer 73 were similar to those of the compound individually, whereas the LC50's for TFM were reduced by more than 50% in soft water of pH 9.5. This relation suggests that most of the toxicosis produced by the mixture is attributable to the Bayer 73 component in water at pH 9.5.

Among life stages of coho salmon tested, green eggs were most sensitive to TFM, followed by fry, sac fry, and eyed eggs; the 96-h LC50's ranged from 0.639 mg/l for green eggs to 3.49 mg/l for eyed eggs (Table 3). The toxicity of Bayer 73 to eggs and fry was dissimilar to that of TFM; sac fry and fry were most sensitive, and green eggs and eyed eggs were more resistant. The 96-h LC50's ranged from 0.066 mg/l for sac fry to 0.509 mg/l for eyed eggs. The additive index shows that the toxicity of the mixture is additive.

Table 1.—Toxicity and additive indices of TFM:Bayer 73 (98:2), based on active ingredient, to seven species of fish in soft water at 12 C.

| Species and toxicant | 96-h LC50 and 95% confidence interval (mg/l) | | Additive index |
	Individually	Combination	
Brown trout			
TFM	0.940 0.791–1.17	0.980 0.810–1.19	
			–0.752 –1.61 to –0.147
Bayer 73	0.0282 0.0219–0.0363	0.0200 0.0165–0.0242	
Rainbow trout			
TFM	1.81 1.53–2.14	1.16 0.998–1.35	
			–0.326 –0.808 to +0.0295
Bayer 73	0.0346 0.0297–0.0404	0.0237 0.0204–0.0275	

Table 1.—Toxicity and additive indices of TFM:Bayer 73 (98:2), based on active ingredient, to seven species of fish in soft water at 12 C (Con't).

Species and toxicant	96-h LC50 and 95% confidence interval (mg/l)		Additive index
	Individually	Combination	
Lake trout			
TFM	1.78 1.51–2.10	1.39 1.12–1.72	
			−0.360 −0.950 to +0.0571
Bayer 73	0.0490 0.0433–0.0555	0.0284 0.0229–0.0351	
Brook trout			
TFM	1.83 1.35–2.48	1.16 0.786–1.71	
			−0.138 −1.23 to +0.723
Bayer 73	0.0470 0.0364–0.0607	0.0237 0.0160–0.0349	
Channel catfish			
TFM	0.750 0.621–0.906	0.615 0.542–0.697	
			−0.158 −0.599 to +0.190
Bayer 73	0.0370 0.0298–0.0459	0.0125 0.0111–0.0142	
Bluegill			
TFM	4.89 4.27–5.60	3.18 2.57–3.94	
			−0.0687 −0.506 to +0.320
Bayer 73	0.152 0.135–0.172	0.0636 0.0514–0.0788	
Yellow perch			
TFM	1.71 1.47–1.98	0.900 0.723–1.12	
			+0.228 −0.165 to +0.757
Bayer 73	0.0639 0.0568–0.0726	0.0184 0.0148–0.0229	

Table 2.—Toxicity and additive indices of TFM:Bayer 73 (98:2) based on active ingredients to rainbow trout in laboratory tests at selected temperatures, water hardnesses, and pH's.

Temp. (C)	Water hardness	pH	Toxicant	96-h LC50 and 95% confidence interval (mg/l)		Additive index
				Individually	Combination	
7	Soft	7.5	TFM	2.13 1.73–2.62	1.42 1.15–1.76	−0.134 −0.648 to +0.275
			Bayer 73	0.0620 0.0568–0.0677	0.0290 0.0234–0.0358	
12	Soft	7.5	TFM	1.81 1.53–2.14	1.16 0.998–1.35	−0.326 −0.808 to +0.0295
			Bayer 73	0.0346 0.0297–0.0404	0.0237 0.0204–0.0275	
17	Soft	7.5	TFM	1.74 1.33–2.27	1.41 1.14–1.74	−0.466 −1.21 to +0.0219
			Bayer 73	0.0439 0.0396–0.0487	0.0288 0.0232–0.0366	
12	Very soft	8.1	TFM	9.00 8.13–9.96	3.89 3.18–4.76	−0.425 −1.08 to +0.0216
			Bayer 73	0.0800 0.0650–0.0984	0.0794 0.0649–0.0970	
12	Soft	8.1	TFM	14.1 11.4–17.4	3.62 3.16–4.14	−0.234 −0.665 to +0.0901
			Bayer 73	0.0755 0.0649–0.0878	0.0738 0.0646–0.0845	
12	Hard	8.1	TFM	14.1 11.4–17.4	4.85 4.03–5.83	−0.333 −0.968 to +0.104
			Bayer 73	0.100 0.0817–0.122	0.0989 0.0823–0.119	

Table 2.—Toxicity and additive indices of TFM:Bayer 73 (98:2) based on active ingredients to rainbow trout in laboratory tests at selected temperatures, water hardnesses, and pH's (Con't).

Temp. (C)	Water hardness	pH	Toxicant	96-h LC50 and 95% confidence interval (mg/l)		Additive index
				Individually	Combination	
12	Very hard	8.1	TFM	17.3 14.0–21.4	4.65 3.90–5.54	−0.366 −0.890 to +0.0139
			Bayer 73	0.0865 0.0756–0.0990	0.0949 0.0796–0.113	
12	Soft	6.5	TFM	0.949 0.840–1.07	0.750 0.669–0.841	−0.377 −0.871 to −0.0194
			Bayer 73	0.0261 0.0197–0.0345	0.0153 0.0136–0.0172	
12	Soft	8.5	TFM	5.40 4.58–6.37	3.70 3.09–4.43	−0.756 −1.60 to −0.181
			Bayer 73	0.0705 0.0552–0.0901	0.0755 0.0630–0.0898	
12	Soft	9.5	TFM	25.2 20.4–31.1	9.30 8.20–10.5	−0.396 −1.13 to +0.0947
			Bayer 73	0.185 0.133–0.257	0.190 0.167–0.215	

Table 3.—Toxicity and additive indices of TFM:Bayer 73 (98:2), based on active ingredient, to eggs and fry of coho salmon in soft water at 12 C.

Stage of development and toxicant	96-h LC50 and 95% confidence interval (mg/l)		Additive index
	Individually	Combination	
Green eggs			
TFM	0.639 0.461–0.886	0.860 0.609–1.21	
			−0.407 −1.74 to +0.390
Bayer 73	0.286 0.212–0.387	0.0175 0.0124–0.0247	
Eyed eggs			
TFM	3.68 3.17–4.27	2.59 1.97–3.41	
			+0.238 −0.264 to +0.928
Bayer 73	0.509 0.369–0.703	0.0528 0.0402–0.0696	
Sac fry			
TFM	3.49 3.14–3.88	1.41 1.13–1.75	
			+0.198 −0.159 to +0.669
Bayer 73	0.0655 0.0582–0.0737	0.0282 0.0227–0.0350	
Swim-up fry			
TFM	2.39 2.12–2.69	1.89 1.57–2.28	
			−0.359 −0.874 to +0.0125
Bayer 73	0.0679 0.0582–0.0792	0.0386 0.0320–0.0465	

DISCUSSION

Howell et al. (1964) first recognized that the effectiveness of TFM was reduced in hard, alkaline waters. This phenomenon was later quantitated by Marking and Olson (1975) and Dawson et al. (1975). Marking and Olson reported a 59-fold decrease in the toxicity of TFM to lake trout as pH increased from 6.5 to 9.5, and Dawson et al. (in press) reported an 8-fold decrease in the toxicity of TFM to sea lamprey ammocetes as the pH increased from 6.5 to 8.5. As the pH shifts to the alkaline range, a concomitant reduction in the availability of the free phenol form of TFM decreases the amount available to produce toxicosis. Hunn and Allen (1974) pointed out that the decrease in toxicity at higher pH's is probably caused by the reduction in concentration of the lipid-soluble free phenol form. Our data for TFM individually show the same results—about a 25-fold decrease in the toxicity of TFM to rainbow trout between pH's 6.5 and 9.5.

Howell et al. (1964) killed all sea lamprey ammocetes with a mixture of TFM and Bayer 73 at concentrations which were nontoxic when the compounds were applied singly. They interpreted this as synergistic activity. Dawson et al. (in press), using the additive index concept, determined that the toxicity of the mixture to ammocetes was additive or less than additive—not synergistic. Data from our study with fish also show less than additive or strictly additive toxicity. Howell et al. (1964) pointed out the economic advantage of applying the mixture; i.e., the reduction in the amount of TFM required to produce toxicosis without loss of selectivity. The reduction is especially significant in the treatment of alkaline waters, when use of the mixture reduces the amount of TFM needed by as much as 50%. However, 24-h TFM toxicity data for rainbow trout and brown trout from the present study and comparable laboratory data for lamprey larvae (Dawson et al. in press) show that the margin of safety to nontarget fish is reduced when the mixture is applied. A 38% mortality of rainbow trout and 10% mortality of brown trout can be expected during a treatment that kills 99% of the ammocetes.

CONCLUSIONS

1. Of the species tested, channel catfish proved to be the most sensitive to TFM; the 96-h LC50 was 0.750 mg/l.

2. Brown trout were the most sensitive salmonid tested, and bluegills were the most resistant to both TFM and Bayer 73.

3. The toxicity of TFM or Bayer 73 was influenced little by water hardness; both were slightly more toxic at relatively high temperatures, and both were significantly more toxic at low than at high pH's; as pH increased from 6.5 to 9.5, toxicity of TFM decreased by a factor of 25 and Bayer 73 by a factor of 7.

4. Among early life stages of coho salmon, green eggs were most sensitive to TFM, followed by fry, sac fry, and eyed eggs. Bayer 73 was most toxic to sac fry, followed by fry, green eggs, and eyed eggs.

5. The mixture of TFM:Bayer 73 was additive or less than additive (not synergistic) in toxicity to fish under various test conditions.

6. Toxicity of the mixture was influenced less than that of the individual toxicants by temperature, water hardness, and pH.

7. The mixture was more toxic to larval sea lampreys than to nontarget fish in comparable laboratory toxicity tests. However, the margin of safety based on the 24-h toxicity data with brown trout or rainbow trout and the 24-h LC99 with sea lamprey larvae was narrow.

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THE FRESHWATER MUSSEL (*ANODONTA* SP.)
AS AN INDICATOR OF ENVIRONMENTAL LEVELS OF
3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM)[1]

by

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ABSTRACT

After freshwater mussels (*Anodonta* sp.) were exposed to 8.68-mg/l solutions of 3-trifluoromethyl-4-nitrophenol (TFM; ^{14}C-TFM and analytical grade TFM) in a model stream for 24 h, uptake and elimination rates of TFM residues for three body components were determined by radioassay. The average residue concentrations (μgTFM/g wet wt) after the 24-h exposure were 44.4 in the foot, 37.7 in the gill, and 38.5 in the viscera. The average calculated half-time for residue elimination from the three components was 20.2 h. The rate of uptake and ultimate residue concentration was widely variable, presumably because the feeding and locomotor activity of individual mussels varied greatly during the exposure period.

INTRODUCTION

The use of benthic invertebrates as indicators of water quality has long been a useful procedure in pollution investigations (American Public Health Association 1971). Among bivalve mollusks used for monitoring insecticides, oysters have been used in the marine environment (Bugg et al. 1967; Casper 1967) and several species of mussels (Unionidae) in fresh waters (Bedford et al. 1968; Miller et al. 1966). The filter feeding habit of freshwater mussels results in the accumulation of many elements in their tissues from water, against concentration gradients (Gaglione and Ravera 1964). The sedentary nature of the mussel makes it an ideal candidate for pesticide monitoring because the animal cannot escape a toxicant by drifting or swimming away.

We report here the results of experiments designed to evaluate the freshwater mussel (*Anodonta* sp.) as an indicator of residues of the larval lampricide 3-trifluoromethyl-4-nitrophenol (TFM) after a simulated treatment for lamprey control in a model stream system.

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METHODS AND MATERIALS

Mussels of the genus *Anodonta*, 8 to 10 cm long, were collected from the Muskegon River at Evart, Michigan. Inasmuch as species identification could not be confirmed without sacrificing the animals, all individuals were simultaneously collected from the same pool and identified to the genus *Anodonta* from external characters. The mussels were maintained in hatchery channels at the State Fish Hatchery in Paris, Michigan, at water temperatures of 10 to 12 C.

The rate of uptake and elimination of ^{14}C-TFM by the mussels was tested in a model stream system inside the hatchery in fall 1973. The stream consisted of a concrete trough 4.0 m long and 0.6 m wide which drained into a second trough of the same dimensions. Water was supplied to the model stream at 100 l/min from Cheney Creek, a small natural stream adjacent to the hatchery. In the upper trough, which was designated as a pool, the water depth was maintained at 25 cm. Gravel and rubble were transferred from a nearby stream to the lower trough, which was designated as a riffle section. Drifting organisms and organic matter from Cheney Creek settled in the troughs over a period of about 1 yr before the tests were conducted. Overhead fluorescent lamps (Coolwhite) provided light intensity of 9688 ± 538 lx at the stream surface. Photoperiod was controlled to conform to natural daylength, with seasonal adjustments.

One month before the experimental treatment, 50 mussels were placed in the experimental pool of the model stream for acclimation. On 13 November 1973, water flow to the troughs was stopped and the drains were plugged. Water was then recirculated by placing a large diaphragm pump in the downstream end of the stream, with the discharge at the head of the channel. The pump capacity was slightly more than 100 l/min, which caused the introduction of some air into the pump and facilitated re-aeration of the stream water. The stream volume was 500 to 525 liters. The entire model stream was treated with an isotope dilution of about 1.0 mCi of ^{14}C-TFM and 4.500 g of analytical grade TFM, all in 10 ml of acetone. This dilution gave approximately 2.6×10^6 counts/min/l at time zero and an actual concentration of 8.68 mg/l labeled and unlabeled TFM in the stream water. Water temperature was 11 C, pH 7.8, and hardness 211 mg/l as $CaCO_3$.

After the 24-h exposure period, the discharge from the pump was directed into a 980-liter metal container and a flow of fresh water was immediately reestablished in the model stream. The ^{14}C-TFM was then recovered by acidifying the water to pH 4.0 and passing it through a column of non-ionic polymeric adsorbent (Lech 1971).

Two mussels were removed at intervals of 1, 2.5, 10, 14, and 24 h, thoroughly rinsed in clean water, placed in labeled bags, and immediately frozen. Additional samples were removed at intervals up to 30 days after exposure for determination of elimination rates. The samples were kept frozen for about 1 wk before analysis. The mussells were dissected from their shells and separated into three components: foot, gills, and viscera. Four replicate analyses were conducted for each component. Each sample was dried at 50 C for 48 h, and its weight adjusted to 100 to 150 mg. Samples were combusted to $^{14}CO_2$ and water in a semi-automated Nuclear Chicago combustion apparatus. The $^{14}CO_2$ was taken up in 10 ml of monoethanolamine-methyl cellosolve, 1:2 (V/V). A 2-ml portion was then radioassayed in 15 ml of a mixture of toluene-methyl cellosolve, 2:1 (V/V), and fluor with a dual channel Nuclear Chicago Unilux I (Model 6850) liquid scintillation spectrometer. At each sampling interval 2-ml aliquots of the water samples were radioassayed in 15 ml of a toluene-trition X-100 fluor, 2:1 (V/V).

We established efficiency curves for the instrument by using a series of internally quenched standards, and converted all sample counts to actual disintegrations per minute and μ Ci values, using the channel ratio and efficiency curve. The isotope dilution factor was the basis for calculation of actual residue concentrations on a wet and dry weight basis for all samples.

RESULTS AND DISCUSSION

All individual mussels concentrated residues of TFM by about 3 to 4 times over the ambient water concentration during the 24-h exposure (Table 1). However, significant variations existed between individuals collected within each sampling period. The amount of locomotor activity and length of time the shell is open with foot extended apparently has a direct bearing on the bioconcentration of TFM residues by the soft internal portions of the mussel. During the exposure, mussels were observed in all stages of locomotion, ranging from foot extended to a completely nonmotile state with a closed shell. These individual variations in behavior probably explain the wide variation in uptake observed among the individual mussels.

The data were further characterized by the use of a simple linear regression of μg TFM/g tissue on a dry weight basis against exposure time in hours. The equation was of the general form:

$$Y = a + b\,(X)$$

where Y = concentration of total TFM residue expressed as μg/g dry weight, a = the Y-intercept of regression, b = rate of loss or slope of the regression, and X = exposure time in hours.

The regression intercept, regression coefficient, sample standard deviation of the regression coefficient S_b, and confidence intervals of the slope were calculated according to Steele and Torrie (1960). The calculated equations for foot, gill, and visceral fractions demonstrated the relatively rapid uptake rates after initial exposure; the slopes were 10.5, 7.8, and 9.0, respectively (Table 2).

The mussels eliminated most TFM residues within 24 h after exposure but detectable residues were present in most samples taken as long as 4 wk after exposure (Table 3). Neither the TFM residue concentrations nor the elimination rates differed significantly among the foot, gill, and viscera fraction. The same wide variation

Table 1.—Average concentration (± one standard deviation in parentheses) of TFM residues (μg TFM/g tissue) in the foot, gills, and viscera of mussels (*Anodonta* sp.) after exposure to 8.68 mg/l solutions of TFM for the indicated periods. Each value is the mean of four samples from two mussels.

Body component and type of weight (μg/g)	Exposure time (hours)				
	1	2.5	10	14	14
Foot					
Dry	18.1 (19.2)	79.3 (13.2)	66.4 (17.9)	209.2 (110.6)	268.8 (103.2)
Wet	3.2 (3.4)	11.8 (1.6)	12.4 (6.0)	36.6 (27.7)	44.4 (37.7)
Gill					
Dry	34.9 (36.1)	93.9 (37.8)	98.2 (41.5)	168.9 (48.1)	232.3 (97.5)
Wet	6.4 (6.8)	9.3 (1.1)	13.0 (7.0)	32.5 (11.7)	37.7 (9.3)
Viscera					
Dry	18.4 (20.3)	96.6 (32.0)	78.1 (23.5)	185.4 (43.8)	248.4 (106.2)
Wet	1.9 (3.1)	15.8 (3.5)	11.2 (4.9)	26.7 (8.1)	38.5 (11.3)

Table 2.—Regression equations describing relation between concentrations of TFM (Y) and time (X) for the foot, gill, and viscera portions of mussels (*Anodonta* sp.) with 95% confidence intervals of the slope.

Body component and stage of experiment	Regression equation	95% Confidence intervals ($\pm$)
Foot		
Uptake	$Y = 20.6 + 10.5\ (X)$	7.9
Elimination	$Y = 30.9 - 11.8\ (\log X)$	0.05
Gill		
Uptake	$Y = 45.3 + 7.8\ (X)$	4.4
Elimination	$Y = 40.2 - 15.4\ (\log X)$	0.06
Viscera		
Uptake	$Y = 37.6 + 9.0\ (X)$	6.9
Elimination	$Y = 32.6 - 12.5\ (\log X)$	0.05

among individuals observed in the uptake rates was apparent during the elimination period.

The rate of TFM elimination was described by a regression of actual concentrations of TFM determined from radioassay of each body component against the log time in hours. The data are described by the following general equation:

$$Y = a + (-b)\ (\log X)$$

where Y = concentration of total TFM residue in the organism expressed as $\mu g/g$ dry weight, a = the Y-intercept of regression or initial concentration in tissue at the initiation of the elimination period, b = rate of loss or slope of the regression, and $\log X$ = log of time in hours.

The calculated data for elimination of TFM from each of the mussel body components indicate that the half-lives of TFM residue concentrations were 20.4, 20.2, and 20.1 h for foot, gill, and visceral components, respectively (Table 2). More rapid elimination rates were determined from mussels collected after an actual TFM treatment of the Ocqueoc River. The mussels sampled had eliminated 96% of their body lampricide residues within 24 h and more than 99% within 96 h (J. L. Allen and J. B. Sills, unpublished data). These more rapid elimination rates may be due to the much higher flow rate and water volume of the Ocqueoc River, which diluted the residue more rapidly than it was diluted in our model stream.

Table 3.—Average concentration (± one standard deviation in parentheses) of TFM residues (μg TFM/g tissue) in the foot, gills, and viscera of mussels (*Anodonta* sp.) at indicated times after a 24-h exposure to 8.68 mg/l TFM.

Withdrawal period (h)	Foot		Gill		Viscera	
	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight
7	1.4 (0.5)	7.1 (1.7)	1.9 (0.5)	12.0 (2.9)	1.5 (0.1)	8.2 (1.0)
9	8.5 (1.0)	39.4 (3.7)	7.7 (1.1)	55.0 (6.4)	6.6 (1.2)	39.0 (9.2)
12	0.8 (0.5)	3.4 (2.3)	0.8 (0.2)	5.4 (1.0)	0.8 (0.2)	5.6 (0.8)
20	6.5 (7.1)	39.4 (43.3)	5.4 (5.9)	36.7 (39.4)	5.7 (6.0)	40.7 (42.8)
34	0.8 (0.3)	4.3 (1.7)	1.2 (0.4)	11.6 (2.8)	1.0 (0.5)	6.1 (3.6)
57	0.4 (0.3)	2.1 (1.6)	0.2 (0.3)	1.5 (2.7)	0.1 (0.2)	1.0 (1.2)
300	0.2 (0.1)	1.1 (0.8)	0.2 (0.1)	1.3 (0.4)	0.1 (0.1)	0.4 (0.5)
325	0.1 (0.1)	0.5 (0.9)	0.1 (0.1)	0.8 (0.7)	0.2 (0.1)	1.3 (0.4)
710	0.0 (- -)	0.2 (0.3)	0.1 (0.6)	0.7 (0.8)	0.0 (- -)	0.0 (- -)

CONCLUSIONS

The mussel *Anodonta* sp. can concentrate TFM to a level of 3 to 4 times the ambient water concentration during a 24-h exposure. TFM is rapidly eliminated after exposure ceases; more than half the residue is lost within 24 h. Total residue concentrations vary widely among individual mussels, probably because of individual variations in activity during the period of exposure. Although *Anodonta* sp. may be a useful indicator of recent TFM contamination, the variable rate of uptake among individual organisms limits its value as a quantitative method for monitoring TFM concentrations.

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INVESTIGATIONS IN FISH CONTROL

71. Field Tests of Isobornyl Thiocyanoacetate (Thanite) for Live Collection of Fishes

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Contents

FIELD TESTS OF ISOBORNYL THIOCYANOACETATE (THANITE) FOR LIVE COLLECTION OF FISHES

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Abstract

Eight ponds containing a total of 28 species of fish were treated with isobornyl thiocyanoacetate (Thanite) to test its efficacy for the live collection of fish. Twenty-six species were collected alive after 1- to 4-μl/l applications of Thanite. Most scalefishes except carp *(Cyprinus carpio)* were relatively easy to collect, and catfishes (Ictaluridae) were generally the most resistant to effects of the chemical. With the exception of northern pike *(Esox lucius)*, most fish recovered quickly after being placed in fresh water. Most fish collected within 1.5 h after treatment survived, but survival rates decreased with time of exposure. The concentrations effective for collection of live fish did not routinely eliminate all fish; small numbers of at least eight species of fish survived treatments of 1.5 μl/l or more. The high percentages of fish (of most species) collected alive demonstrated that Thanite is effective for the intended purpose.

Introduction

The development of a safe, efficacious, and relatively inexpensive chemical that would permit the live collection of desirable fishes from a body of water would be of distinct benefit to fishery management programs. The availability of a chemical that facilitated live collection would make it possible to harvest predator fish at comparatively low cost. Sport fishing could benefit in yet another way: desirable fishes that are otherwise lost when ponds and lakes are renovated or when flood waters dry up could be salvaged for stocking in public fishing areas.

Various investigators have tested many compounds to determine their advantages and limitations as collecting aids: cresol (Embody 1940; Wilkins 1955; Howland 1969); sodium sulfite (Westman and Hunter 1956); sodium sulfite catalyzed with cobalt chloride (Vanderhorst and Lewis 1969); sodium cyanide (Lewis and Tarrant 1960; Tatum 1969); fresh walnut hull extracts (Westfall et al. 1961); LSD-25 (Loeb 1962); derivatives of d-lysergic acid (Loeb et al. 1965); rotenone (Tate et al. 1965); rotenone followed by immediate immersion of treated fish in a solution of methylene blue (Bouck and Ball 1965); Aqualin (Louder and McCoy 1965); eight anesthetics, including Anileridine, Tribromoethanol, Ethinamaie, and RO 40403 (Blanchard 1966); and isobornyl thiocyanoacetate, or Thanite (Lewis 1968; Buckner and Perkins 1975; Burress and Bass 1975). Factors that must be considered in evaluating the potential usefulness of such compounds include efficacy, cost, availability, effects on target and nontarget organisms, rate of degradation, and hazards to users.

Thanite (82% isobornyl thiocyanoacetate and 18% other active terpenes) is an insecticide with low mammalian toxicity (Hercules Powder Company 1962). It has been widely used for some 30 years to control common household pests and external human parasites. Lewis (1968), who was the first to use Thanite for the live collection of fishes, reported excellent results in collecting largemouth bass *(Micropterus salmoides)* from two ponds in southern Illinois. In 1968, Buckner and Perkins (1975) began

using Thanite in the management of ponds in southwestern Georgia. They collected and moved 2,000 to 4,000 largemouth bass annually and made live collections of at least 14 other species of fish. Burress and Bass (1975) collected largemouth bass and 12 other species alive from two ponds in Florida and reported that Thanite was relatively safe and inexpensive.

The field trials reported here were conducted primarily to document the efficacy of Thanite as an aid in the live collection of fish and to facilitate the process of registering the compound with the U.S. Environmental Protection Agency (EPA) for that use. These efficacy tests were conducted in four states, in eight ponds with different physical, chemical, and biological characteristics (Table 1).

Materials and Methods

The general methods and procedures described by Burress and Bass (1975) were used in mixing and applying Thanite solutions; collecting, holding, and measuring (total length) live fish; collecting and counting fish killed by the Thanite treatment; and later treating the waters with a fish toxicant. The formulation used in six of the eight ponds consisted of Thanite, kerosene, and the emulsifier Atlox 1045A mixed in a ratio of 70:20:10 parts by volume. The other formulations used consisted of an 80:20 mixture of Thanite and Atlox 1045A in Ebert Pond, and an 80:20 mixture of Thanite and Atlox 3408F in West Sunken Camp Lake. Additional information on minor variations in methodology, descriptions of the ponds, and environmental or other factors that influenced test results is included with the results to facilitate discernment of possible cause and effect relationships. The species of fish present in the various ponds are listed in Table 2. During the treatment and collection periods, we also tried to observe the effects of Thanite on macroscopic nontarget organisms.

Results

Fish that were exposed to effective concentrations of Thanite tended to surface in distress and swim about in a disoriented manner. About 20% of them reacted by jumping or briefly skittering across the pond surface. As sedation deepened, many fish moved toward shore. Some sought cover while others floated listlessly at the surface or settled to the bottom. In general, small fish were affected first and died sooner than large ones. Occasionally, a large fish in noticeably poor physical condition surfaced before healthy young fish were sedated. Low temperatures slowed responses and delayed the onset of mortality, enabling pickup crews to cover larger areas more effectively. Fish that were collected in early stages of sedation recovered quickly after being placed in fresh water, but recovery times and mortality rates increased as exposures lengthened.

Additional observations in each of the eight ponds are detailed in the following sections.

McGraw Pond, Dundee, Illinois

This pond, on property of the Max McGraw Wildlife Foundation, was the only pond not of conventional construction. It was C-shaped, contained three islands, and had been dug with bulldozers. The bottom was covered with deposits of soft muck overlying a substrate of coarse gravel that contained a mixture of rocks 15 to 20 cm in diameter. Parrot feather (*Myriophyllum* sp.) was distributed along most of the shoreline to a depth of 1.2 m. Before treatment, the water level was lowered about 35 cm to prevent overflow of the treated water. At this lowered level, the pond had a surface area of 2.83 ha. We treated a 0.61-ha section at the deeper end, which was blocked off with a net (1.3-cm mesh, stretched measure).

We made three applications in a period of 2 h to observe the response of six species of fish (Table 2) to increasing concentrations of Thanite. The cumulative concentrations produced by successive applications were 0.8, 1.2, and 1.6 µl/l. Differences between the methods followed in this test and those described by Burress and Bass (1975) included the use of a pump to apply Thanite to the pond surface; a search of the pond bottom by divers for both living and dead fish on the second day after treatment; retention of captured fish in live cages in an adjacent pond for 24 h to evaluate their survival; and the use of antimycin to eradicate the fish remaining in the pond. Four boats operated by two-man crews were used to collect fish as they surfaced during the 3.5 h after the first application. The average area covered by each crew was 0.15 ha.

The first application of Thanite (0.8 µl/l) was too light to be effective, but the second (0.4 µl/l applied below the surface in deep water) quickly facilitated collection of numerous sunfish and intermediate-sized bass. A third application (0.4 µl/l, at the surface) was needed, however, before adult sunfish and large bass were affected. Many sunfish were captured along the block net, and numerous small white crappies were gilled in it. Wind-induced currents carried Thanite beyond the net, and distressed crappies were seen 6 m beyond the treated area. No fish were killed in the untreated area, however, suggesting that Thanite can safely be used to collect fish from selected areas of larger bodies of water.

Table 1.—*Physical and chemical characteristics of ponds treated with Thanite.*

Pond, and month and year of treatment	Surface area (ha)	Depth (m)		Volume (m³)	Temperature (°C)		Secchi disk transparency (cm)	pH at surface	Total alkalinity (μg/l)	Conductance (mhos)
		Average	Maximum		Surface	Bottom				
McGraw Pond (10/72)	0.61	1.52	2.13	9,251	16.0	13.9	53.3	8.2	287	1,683
Barnes Pond (1/74)	0.47	1.07	1.82	5,033	13.9	10.0	10.2	7.0	25	82
Watkins Pond (1/74)	0.32	0.98	1.82	3,121	15.0	13.0	10.2	8.0	—[a]	130
Scott Pond A (2/74)	0.30	0.67	1.59	2,072	13.5	13.0	15.2	9.3	—[a]	20
Scott Pond B (2/74)	1.30	1.24	2.58	16,302	13.5	12.7	30.4	9.3	—[a]	20
Reeves Pond (2/74)	0.49	1.58	3.04	7,907	8.5	8.5	3.8	6.9	11	3
Ebert Pond (10/74)	0.81	1.25	2.75	10,135	15.0	12.0	67.0	7.5	174	496
West Sunken Camp Lake (6/75)	1.21	2.52	4.87	30,837	20.0	16.5	256.5	6.4	7	—[a]

[a] Not measured.

4

Table 2.—*Species of fish present in ponds treated with Thanite, 1972-75[a].*

Species	McGraw Pond	Barnes Pond	Watkins Pond	Scott Pond A	Scott Pond B	Reeves Pond	Ebert Pond	West Sunken[b] Camp Lake
Centrarchidae								
Largemouth bass *(Micropterus salmoides)*	X	X	X	X	X	X	S	S
Bluegill *(Lepomis macrochirus)*	X	X	X	X	X	X	X	S
Redear sunfish *(Lepomis microlophus)*		X	X			X		
Pumpkinseed *(Lepomis gibbosus)*								S
Green sunfish *(Lepomis cyanellus)*	X		X					
Rock bass *(Ambloplites rupestris)*								S
White crappie *(Pomoxis annularis)*	X							
Black crappie *(Pomoxis nigromaculatus)*								S
Warmouth *(Lepomis gulosus)*		X						
Dollar sunfish *(Lepomis marginatus)*		X						
Ictaluridae								
Channel catfish *(Ictalurus punctatus)*		X	X			X	S	
Brown bullhead *(Ictalurus nebulosus)*		X	S			X		
Yellow bullhead *(Ictalurus natalis)*		X						
Black bullhead *(Ictalurus melas)*							X	
Cyprinidae								
Golden shiner *(Notemigonus crysoleucas)*		X		X	X			X
Carp *(Cyprinus carpio)*	X							
Percidae								
Walleye *(Stizostedion vitreum vitreum)*								S
Yellow perch *(Perca flavescens)*	X							X
Esocidae								
Northern pike *(Esox lucius)*								S

Table 2.—*Species of fish present in ponds treated with Thanite, 1972-75[a]. (Continued)*

Species	McGraw Pond	Barnes Pond	Watkins Pond	Scott Pond A	Scott Pond B	Reeves Pond	Ebert Pond	West Sunken[b] Camp Lake
Chain pickerel (*Esox niger*)		X						
Redfin pickerel (*Esox americanus americanus*)		X						
Catostomidae								
White sucker (*Catostomus commersoni*)								X
Redhorse (*Moxostoma* sp.)								S
Lake chubsucker (*Erimyzon sucetta*)		X						
Clupeidae								
Gizzard shad (*Dorosoma cepedianum*)					X			
Poecilliidae								
Mosquitofish (*Gambusia affinis*)		X						
Salmonidae								
Chinook salmon (*Oncorhynchus tshawytscha*)							S	

[a] S= stocked within 2 to 6 weeks before treatment; X= long-term residents of the waters treated.

[b] Two bullheads of undetermined species and one longnose sucker *(Catostomus catostomus)* stocked in West Sunken Lake are not included in the test or tables. The bullheads were found dead after treatment; the longnose sucker was not recovered.

The primary objective of the pickup crews was to collect every largemouth bass that could be captured; they collected 89% of the total population (Table 3). Forty-four of 50 largemouth bass and 2 of 3 white crappies considered to be large enough for table use (more than 20 cm long) were collected alive. Of the total of 62 bass collected, 60 survived for 24 h.

The Thanite treatment killed all uncollected centrarchids, yellow perch, and some carp up to about 30 cm long. (No attempt was made to collect sedated or dead carp.) A 5-μ g/l antimycin treatment applied 3 days later killed the remaining carp, but no other fish were found.

Barnes Pond, Merrillville, Georgia

Thirteen species of fish were found in this highly fertile pond. The bottom was soft mud, and wading cows had roiled the water. Aquatic macrophytes were virtually absent, but the pond surface was covered by a distinct bloom of blue-green algae. The first Thanite application of 1μ l/l was used to facilitate collection of the more susceptible scalefishes. Two additional 1-μl/l treatments were made within 3 h in an effort to collect brown bullheads, yellow bullheads, and channel catfish. Intermittent showers and winds occurred during the treatment but the pond did not overflow. Three pickup crews, each covering about 0.16 ha, collected fish for 4.5 h.

The combination of excessive turbidity (Secchi disk transparency about 10 cm), intermittent rain, and wind greatly hampered visibility and markedly reduced success in capturing fish (Table 4). The following percentages of bass and sunfish longer than 12.7 cm were collected: largemouth bass, 56; bluegill, 62; redear sunfish, 52; and warmouth, 43. Nearly all were collected after the first treatment (1μ l/l). Percentage mortalities of the fish within 3 h after collection were as follows: bass, 0; bluegill, 4; redear sunfish, 2; and warmouth, 6. We captured two

Table 3.—*Numbers and percentages of fish[a] collected alive or recovered dead in a 0.61-ha section of McGraw Pond after a 1.6-µl/l treatment with Thanite[b] on 16 October 1972.*

| Species | Length range (cm) | Fish captured alive | | Number recovered dead |
		Number	Percent	
Largemouth bass	7–60	62	89	8
White crappie	7–40	540	68	260
Bluegill	3–20	275	75	91
Green sunfish	3–20	46	82	10
Yellow perch	10–20	3	100	0
Total		926	72	369

[a] Carp were killed by Thanite and antimycin treatments, but were not collected.

[b] Three applications of Thanite (0.8, 0.4, and 0.4 µl/l) were made during a period of 2 h.

Table 4.—*Numbers and percentages of fish collected alive or recovered dead in Barnes Pond after a 3.0-µl/l treatment with Thanite[a] on 7 January 1974.*

| Species | Length range (cm) | Fish captured alive | | Number recovered dead |
		Number	Percent	
Largemouth bass	7–40	5	50	5
Bluegill	3–20	810	28	2,053
Redear sunfish	3–20	480	56	374
Warmouth	3–20	2,139	33	4,398
Dollar sunfish	3–12	10	23	34
Chain pickerel	15–20	0	0	6
Redfin pickerel	10–30	2	100	0
Lake chubsucker	20–30	111	68	53
Golden shiner	5–15	640	15	3,659
Mosquitofish	3–5	205	29	512
Channel catfish	40–60	0	0	3
Brown bullhead	10–25	7	3	225
Yellow bullhead	10–20	2	100	0
Total		4,411	28	11,323

[a] Three 1-µl/l applications of Thanite made during a period of 3 h killed the entire fish population.

redfin pickerel, the only ones present, but six small chain pickerel were not collected alive. Dollar sunfish, lake chubsucker, golden shiner, and mosquitofish also were collected alive after the first application. Additional golden shiners, lake chubsuckers, and some small bullheads (both yellow and brown) were collected after the second treatment $(1\mu l/l)$ was applied. The third treatment $(1\mu l/l)$ brought up additional golden shiners and brown bullheads, but no live channel catfish were observed. However, the Thanite treatment killed the entire fish population, as shown by the negative results of a 2-$\mu l/l$ treatment of rotenone several days later.

Watkins Pond, Morganville, Alabama

Five species of fish were present in this pond, and we stocked brown bullheads before the test. The pond was highly turbid and lacked aquatic plants. Again, we applied $1\mu l/l$ at the beginning of the study and a 2-$\mu l/l$ treatment 2.5 h later, in an effort to collect the catfish. Rain and gusty winds struck before the second treatment was applied. Fish were collected by two pickup crews (0.16 ha per crew) over a period of 4.5 h.

Although difficulties with turbidity, wind, and rain were much the same as those encountered during the treatment of Barnes Pond, substantially higher percentages of the scalefishes longer than 20 cm were collected (Table 5). The improvement in success was largely due to the smaller total number of fish in the pond. Of the "catchable" sized fish, 82% of the bass, 89% of the bluegills, 100% of the redear sunfish, and 64% of the channel catfish were captured alive. Nearly all of the scalefishes and about 3% of the brown bullheads were captured after the initial

treatment with $1\mu l/l$ of Thanite. About an equal number of bullheads were collected alive after the 2-$\mu l/l$ treatment and an additional 27.6% were killed by the Thanite. Later treatment with $2\mu l/l$ of rotenone killed no additional scalefish, but killed the remaining 135 brown bullheads.

Scott Ponds A and B, Montgomery, Alabama

The water level in Scott Pond had been lowered by about 1.2 m several months before our trial. The reduction in water level created two isolated ponds identified here as Pond A and Pond B. Both had mud bottoms and lacked aquatic vegetation. Pond A contained only largemouth bass, bluegills, and golden shiners; Pond B contained these species and a few gizzard shad. A 1-$\mu l/l$ Thanite treatment was applied in Pond A at noon, and a similar treatment was added 2 h later to aid in the collection of large golden shiners. Three crews (0.10 ha per crew) collected fish for about 3.5 h. A single 1-$\mu l/l$ treatment was applied in Pond B and four crews (0.33 ha per crew) collected fish for about 2.5 h.

Excellent results were achieved in Scott Pond A, where 99.6% of the total fish population was collected alive (Table 6). The weather was almost ideal—clear, calm, and relatively cool. Furthermore, each pickup crew had to cover an area of only 0.10 ha, the total fish population was low, and the response of the fish was optimal for live collection; many of them swam slowly at the surface or quietly nosed up to the bank as sedation deepened. Most fish were collected alive after the first treatment $(1\mu l/l)$, but a few of the largest bluegills and golden shiners were captured after the second 1-$\mu l/l$ application. Mortality of the fish in the holding tank was negligible during the 6 h

Table 5.—*Numbers and percentages of fish collected alive or recorded dead in Watkins Pond after a 3.0-$\mu l/l$ treatment with Thanite[a] on 28 January 1974.*

Species	Length range (cm)	Fish captured alive		Number recovered dead
		Number	Percent	
Largemouth bass	30–40	9	82	2
Bluegill	3–20	449	21	1,701
Redear sunfish	3–20	63	100	0
Green sunfish	7–15	3	100	0
Channel catfish	20–50	9	64	1[b]
Brown bullhead	12–20	12	6	56[b]
Total		545	22	1,760

[a] Two applications of Thanite (1.0 and 2.0 $\mu l/l$) were made during a period of 3 h.

[b] Number of dead fish does not include 4 channel catfish and 135 brown bullheads killed with rotenone after treatment with Thanite.

Table 6.—*Numbers and percentages of fish collected alive or recovered dead in Scott Pond A after a 2-μl/l treatment with Thanite[a] on 4 February 1974.*

Species	Length range (cm)	Fish captured alive		Number recovered dead
		Number	Percent	
Largemouth bass	40–50	1	100	0
Bluegill	3–20	969	100	0
Golden shiner	5–15	44	92	0[b]
Total		1,014	>99	0

[a] Two 1-μl/l applications of Thanite were made during a period of 2 h.

[b] Number of dead fish does not include four golden shiners that were killed by rotenone after treatment with Thanite.

after collection. No dead fish were found in the pond after the Thanite application, and a 2-μl/l rotenone treatment several days later killed only four adult golden shiners.

The reaction of fish to the 1-μl/l treatment in Scott Pond B appeared to be nearly optimal, but a combination of unfavorable circumstances resulted in live collection of only 43% of the total fish population (Table 7). The numerous shoal areas hampered free movement of the boats, each pickup crew had to cover about 0.33 ha, and collection efforts had to be terminated prematurely because of darkness. We captured 44% of the catchable sized bass. Six of nine bass not collected alive were large fish, not adequately sedated by the 1-μl/l dose. Of the bluegills longer than 20 cm, 43% were collected alive and the rest were killed by the treatment. Fewer than 1% of the golden shiners were collected alive; those that were left apparently were not harmed, as none were found dead until after the rotenone treatment. The entire population of gizzard shad (30 fish) was collected alive, and all survived a 2-h confinement in a heavily loaded recovery tank. Recovery of other

fishes in the holding tank was excellent and mortalities were negligible during the 2 h after collection. Later application of a 2-μl/l treatment of rotenone killed the following percentages of each species originally present: largemouth bass, 37.5; bluegills, 0.5; and golden shiners, 90.9.

Reeves Pond, Greenville, Georgia

We treated this pond primarily to determine what effect extreme turbidity might have on the efficacy of Thanite. Silt washed into the pond by heavy rains had reduced Secchi disk transparency to less than 4 cm. A trickle of water flowed through the pond throughout the field trial. The pond contained no aquatic plants. The first treatment was limited to a 1-μl/l concentration, and 2.5 h later a 3-μl/l treatment was applied to facilitate the collection of catfish. Three crews (0.16 ha per crew) collected fish for 4 h. Conditions for collecting fish were less than ideal; extreme turbidity and glare greatly hampered visibility.

Table 7.—*Numbers and percentages of fish collected alive or recorded dead in Scott Pond B after a 1-μl/l treatment with Thanite on 4 February 1974.*

Species	Length range (cm)	Fish captured alive		Number recovered dead
		Number	Percent	
Largemouth bass	30–50	7	44	3[a]
Bluegill	3–20	2,814	43	3,748
Golden shiner	5–15	3	<1	0[a]
Gizzard shad	10–45	30	100	0
Total		2,854	43	3,751

[a] Number of dead fish does not include 6 largemouth bass, 30 bluegills, and 327 golden shiners that were killed by rotenone after treatment with Thanite.

The live collection of fish longer than 20 cm was regarded as successful despite the turbidity; 69% of the bass, 100% of the bluegills, and 50% of the redear sunfish were captured after the application of the 1-μl/l treatment (Table 8). A few small brown bullheads (100–125 mm) also were taken. The addition of a 3-μl/l treatment 3 h after the first resulted in collection of a 1.8-kg channel catfish, but no other fish were observed. Mortality of fish during a 3-h period in the recovery tank was negligible. The Thanite treatment killed all fish in the pond except seven brown bullheads, which were killed when 2 μl/l of rotenone was applied later. These fish apparently survived in a small flow of spring water at the extreme upper end of the pond. On the day after treatment, a hatch of small mayflies was observed, but many died before they could leave the water.

Ebert Pond, West Salem, Wisconsin

Ebert Pond was chosen to evaluate the activity of Thanite in hard, fertile water against northern strains of fish. Dense, marginal stands of sago pondweed *(Potamogeton pectinatus)* occurred in shallow water, which included about 25% of the total pond area. Moderate amounts of duckweed *(Lemna minor)* and unidentified filamentous algae were interspersed throughout the vegetated area. The pond initially contained a population of black bullheads and bluegills; in addition, 100 chinook salmon, 15 channel catfish, and 109 largemouth bass were stocked 6 weeks before treatment. The pond was treated once with an 80:20 mixture of Thanite and Atlox 1045A surfactant to give a concentration of 1.5 μl/l of Thanite. The chemical was applied to the deeper open water with a pump and weighted hose and to the weedy perimeter with backpack pumps.

Although the application required only a half hour, chinook salmon, bluegills, and largemouth bass were surfacing by the time the application was completed. Bullheads and channel catfish were not seen until 5 h later and none were captured. Salmon and centrarchids were easily captured with dip nets. We did not attempt to capture all of the bluegills because of the large number present, but collected 90 kg (35% of the total weight), of which 51 kg were placed in fresh water for recovery. This amount severely overloaded the two 378-liter recovery tanks and caused significant stress. Data on collection, recovery, and mortality of fish are given in Table 9. The failure of some fish to recover was probably due to oxygen deficiency in the tanks caused by overloading. The high percentage of stocked fish not accounted for indicated that many of them died between stocking and the time of treatment.

After the fish had recovered their equilibrium in the recovery tanks, representative samples of salmon, bluegills, and bass were loaded in an aerated tank and hauled 16 km to the laboratory. This trip required 25 min. Survival of these fish 5 days later was 100% for 6 salmon and 11 bass and 69% for 584 bluegills. The trial in Ebert Pond demonstrated the need for adequate recovery tanks to handle unexpectedly large numbers of fish.

The time required for the 1.5-μl/l concentration of Thanite to degrade was determined by conducting on-site toxicity tests with caged bluegill fingerlings. On the 10th day after the treatment, 10 fingerling bluegills were placed in a live cage in the pond; 7 died by day 12. A second lot of 10 bluegills was placed in the pond on day 12 and a third lot on day 13. Two fish of the second lot, but none of the third, died by day 16. Thus, the Thanite had degraded to a nontoxic level by the 13th day after application.

Table 8.—*Numbers and percentages of fish collected alive or recovered dead from Reeves Pond after a 4-μl/l treatment with Thanite[a] on 12 February 1974.*

Species	Length range (cm)	Fish captured alive		Number recovered dead
		Number	Percent	
Largemouth bass	10–50	39	83	8
Bluegill	3–20	137	1	10,087
Redear sunfish	3–20	208	47	237
Channel catfish	40–50	1	50	1
Brown bullhead	10–20	5	6	67[b]
Total		390	4	10,400

[a] Two applications of Thanite (1.0 and 3.0 μl/l) were made during a period of 3 h.

[b] Number of dead fish does not include seven brown bullheads that were killed by rotenone after treatment with Thanite.

Table 9.—*Sizes and numbers of fish collected alive and recovered dead in Ebert Pond after a 1.5-μl/l treatment with Thanite on 30 October 1974, and survival of fish transported to a laboratory raceway after recovery.*

Species	Length (cm) of fish captured alive		Number of fish in treated pond				Fish hauled to laboratory raceway after recovery	
	Average	Range	Stocked	Collected alive	Recovered dead	Unaccounted for	Number	Survival after 5 days (%)
Chinook salmon	20.2	18.0–23.0	100	12	2	86[b]	6	100
Bluegill	10.1	7.0–14.0	0[a]	4,914	13,925	—[b]	584	69
Largemouth bass	16.5	13.5–21.0	109	19	23	67	11	100
Channel catfish	Not measured		15	0	2	13[b]	0	—
Black bullhead	Not measured		0[a]	0	25	—[b]	0	—

[a] Existing population in pond.

[b] Not known.

The pond was treated with rotenone (3.1 μl/l of 5% formulation) 1 year after the Thanite treatment. This treatment was delayed to accommodate studies on recovery of invertebrate populations. The toxicant killed 363 kg of bullheads 5 to 36 cm long and 1.8 kg of bluegills 3 to 12 cm long, indicating that the 1.5-μl/l Thanite treatment killed less than 1% of the bullheads, but about 99% of the bluegills.

West Sunken Camp Lake, Ashland, Wisconsin

We treated this small lake to assess the efficacy of Thanite for collecting northern species of fish in very soft, acid water. The water was much clearer than other waters studied (Table 1); the bottom was devoid of vegetation. The lake was connected to East Sunken Camp Lake by a narrow neck of shallow water, which was closed with a plastic barrier before treatment to prevent circulation of water. Large numbers of golden shiners (7–12 cm long), yellow perch (8–16 cm), and white suckers (14–20 cm) were resident in the lake. The lake was stocked with the following species, of various sizes (Table 10), by the Wisconsin Department of Natural Resources about 2 weeks before the trial: redhorse, northern pike, bullhead, rock bass, pumpkinseed, bluegill, largemouth bass, black crappie, and walleye. Fish collected during the trial were placed in live cages in the untreated section to assess their survival for 24 h after collection.

A single 1.5-μl/l Thanite treatment was applied, but the chemical formulation and the application procedure were somewhat different from those previously used. The Thanite stock solution consisted of an 80:20 mixture by volume of Thanite and Atlox 3408F, a surfactant used to replace the Atlox 1045A previously employed in making stock solutions. The

Table 10.—*Numbers, sizes, and percentages of stocked fish collected alive and recovered dead in West Sunken Camp Lake after a 1.5-μl/l treatment with Thanite on 10 June 1975.*

Species	Length range (cm)	Number stocked	Captured alive			Recovered dead[b]	
			Number	Percent[a]	Weight (kg)	Number	Weight (kg)
Redhorse	22–24	4	2	50	0.1	2	1.2
Northern pike	22–100	15	9	64	3.8	5	3.6
Rock bass	14–26	54	31	72	3.5	12	1.9
Pumpkinseed	10–15	63	49	83	3.5	10	0.5
Bluegill	10–22	120	89	81	6.7	21	1.1
Largemouth bass	12–40	49	46	95	8.8	2	0.5
Black crappie	13–30	13	7	54	0.7	6	1.4
Walleye	16–71	17	13	81	11.8	3	0.5

[a] Percent of the total number recovered, both living and dead.

[b] Preceding application of antimycin.

stock solution was diluted about 5:1 with water, and was divided into three 115-liter portions. The first was applied in a crisscross pattern at a depth of about 30 to 60 cm; the second was applied to the deeper part of the lake with a weighted hose which distributed the material at depths of 3.0 to 3.7 m; and the third was divided between deep and shallow water. The distribution unit consisted of a small boat, a small outboard engine, a submersible pump with control valve in the chemical container, and a weighted hose that hung over the stern of the boat. Fish were collected by three or four men wading in shallow water and by two two-man crews in boats in the deeper water. The fish were placed in tubs of fresh water and delivered to the recovery cages within 20 min after capture. Fish were placed in four cages, according to the time elapsed after completion of the treatment. The time periods were the first, second, and third half-hours, and from 1.5 to 3.0 h post-treatment. Major effort was devoted to capturing game fish, but large numbers of other fishes were also taken. Weather (clear and calm) and water conditions were ideal for capturing fish.

Application of the Thanite required 1 h, and the first yellow perch were captured within 10 min after completion of the treatment. Shortly thereafter, sunfishes and walleyes were netted, but the more resistant golden shiners, white suckers, and northern pike showed no signs of sedation for nearly 1.5 h. Captures increased with time after treatment; more than twice as many fish were collected during the second 1.5-h period of netting as in the first 1.5 h. All game fishes taken alive were collected within 3 h after treatment. All of 408 fish (except 1 bluegill)

collected in the first 1.5 h survived. After that, survival appeared to decrease with increasing length of exposure. Survival rates of most game species were excellent, ranging from 76% for walleyes to 93% for largemouth bass (Table 11). Northern pike were an exception in that none of nine survived, even though they were collected alive.

Sunfishes, largemouth bass, and walleyes were especially susceptible to capture; the percentages collected ranged from 74 to 94 (Table 10). The range of sensitivity between individuals appeared to be wider in certain other species such as rock bass and black crappie (collection rates near 50%); some were quickly affected and easily netted, whereas others were not seen until they were found dead in shallow water the day after treatment. Some white suckers and golden shiners were still alive in the lake 21 h after treatment, and a number of suckers were seen thrashing about at the surface. However, there was no sign of live fish 24 h after treatment. Except for about a 90% mortality of 1-cm frog tadpoles in the shallow water near the barrier, no mortality of nontarget organisms was noted.

The lake was treated with a 7.5 μ g/l application of antimycin about 4 weeks later. Fish killed by the antimycin included 221 golden shiners, 15 white suckers, 1 bluegill, and 11 yellow perch.

Discussion

Application of 1.0- to 1.6-μl/l treatments of Thanite proved to be efficacious for collecting many sizes of several species of sport fish under a wide variety of

Table 11.—*Fish captured alive at selected time intervals after treatment of West Sunken Camp Lake with Thanite, and percentage survival 24 h after capture.*

Species	Time after treatment (h)								Total survival (%)
	0–0.5		0.5–1.0		1.0–1.5		1.5–3.0		
	Number collected	Survival (%)	Number collected	Survival (%)	Number collected	Survival (%)	Number collected	Survival (%)	
Golden shiner	0	—	0	—	5	100	293	61	61
White sucker	0	—	0	—	6	100	113	83	89
Redhorse	1	100	0	—	1	100	0	—	100
Northern pike	0	—	0	—	1	0	8	0	0
Rock bass	4	100	4	100	7	100	16	81	90
Pumpkinseed	11	100	6	100	8	100	24	71	86
Bluegill	5	80	13	100	25	100	46	80	88
Largemouth bass	2	100	9	100	13	100	22	86	93
Black crappie	2	100	1	100	3	100	1	0	85
Yellow perch	35	100	91	100	45	100	188	47	72
Walleye	3	100	6	100	1	100	3	0	76

conditions. Bluegills, white crappies, dollar sunfish, and yellow perch were the most sensitive to Thanite. Redear sunfish, warmouths, green sunfish, largemouth bass, gizzard shad, lake chubsuckers, and walleyes were somewhat less sensitive. Golden shiners, mosquitofish, carp, chain pickerel, and redfin pickerel were moderately resistant, and catfishes were the most resistant. Channel catfish were less resistant than brown or yellow bullheads; yellow bullheads were the most resistant species tested.

Northern pike were observed in only one test, but their reaction was unique. Of 15 fish stocked in West Sunken Camp Lake, only 9 were captured alive and none survived for 24 h after being transferred to fresh water. Apparently the fish absorbed a lethal dose of chemical before they became sufficiently sedated to be vulnerable to netting.

One recommendation regarding the use of Thanite should be added to the general guidelines suggested by Burress and Bass (1975). If, for example, largemouth bass of widely varying sizes are collected, the application of a 1-µl/l treatement followed by a second application of 0.5 µl/l could apparently be expected to give good results. In our trials, the first treatment was strong enough to produce good sedation and still permit rapid recovery of small fish; the later boost in concentration usually affected the more resistant large fish within a short time. If only large fish are sought, a single application of 1.5 to 2.0 µl/l should suffice.

Channel catfish can be collected alive with high concentrations of Thanite. However, additional research will be required to determine the concentrations needed under different environmental conditions. Bullheads appear to be so resistant to Thanite that their live collection is not economically feasible.

We believe that Thanite has many advantages as an aid in the live collection of fish. It is safe and convenient to handle and apply. Concentrations of 2 µl/l or less are required to effect prompt response of numerous species of fish, and degradation of the compound to nontoxic levels occurred within about 2 weeks in Ebert Pond (the only water in which such test was conducted). Variations in pH, temperature, hardness, conductivity, and turbidity appeared to have little effect on efficacy. However, treatment at low temperatures tended to reduce mortality. Survival of fish (other than northern pike) that were collected and placed in fresh, well aerated water within 2 h after treatment was excellent. Although Thanite was clearly effective for collecting several species of fish, its efficacy for collecting largemouth bass was especially noteworthy: even though weather and water conditions were adverse in four of eight tests, we collected more than 83% of a total of 226 bass (7–60 cm long) alive.

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INVESTIGATIONS IN FISH CONTROL

72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests

United States Department of the Interior

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INVESTIGATIONS IN FISH CONTROL

72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests

By Leif L. Marking

Terry D. Bills

Contents

Toxicity of Rotenone to Fish in Standardized Laboratory Tests

by

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Abstract

Noxfish®, which contains 5% rotenone, was toxic to a variety of freshwater fish at concentrations ranging from 21.5 to 497 μg/l in 96-h laboratory exposures. Goldfish *(Carassius auratus)* and black bullheads *(Ictalurus melas)* were the most resistant species, and the Atlantic salmon *(Salmo salar)* was the most sensitive. Toxicity was influenced little by temperatures of 7 to 22 C, by water hardnesses of 10 to 300 mg/l, or by pH's of 6.5 to 9.5. In exposures of rainbow trout *(Salmo gairdneri)*, newly fertilized eggs were much more resistant than fingerlings. Noxfish detoxified in water solutions; the half-life of biological activity was 22 days at 12 C and 13 days at 17 C. Potassium permanganate was an excellent detoxifier; chlorine was less efficient. Noxfish was consistently more toxic in static tests than in flow-through tests.

Rotenone, a crystalline ketone found in several plants of the Leguminosae, has been used as a toxicant by fishery managers since the 1930's for removing undesired fish populations in lakes (Baker and Cordone 1969). Ideal conditions for the reclamation of static waters with rotenone include temperatures between 16 and 21 C, alkalinities between 150 and 200 mg/l (ppm), pH's of 8 or less, and surface areas of less than 8.1 ha (Spitler 1970). The piscicide has been used extensively under a wide variety of conditions and is relatively harmless to most nontarget organisms (Schnick 1974; Lennon et al. 1970). Twenty-nine formulations of rotenone from 18 companies had been registered for aquatic or agricultural use by 1974 by the Environmental Protection Agency. Because the registrations are old, data supporting the labels must be updated to conform to present requirements (Lennon 1967). A guide or protocol for evaluating the toxicity of candidate fishery chemicals for registration was published by Marking (1975).

The present study was designed to determine (1) the toxicity of rotenone to fish in standardized static and flow-through tests, (2) the toxicity of rotenone to newly fertilized trout eggs, (3) the residual toxicity of rotenone in water after selected periods of aging, (4) the efficiency of two compounds used to detoxify rotenone, and (5) the comparative toxicities of three rotenone formulations.

Materials and Methods

Three formulations of rotenone (furnished by S. B. Penick & Co.) were used in the tests described here: Noxfish®, an emulsifiable concentrate containing 5% rotenone; Pro-Noxfish®, a synergized emulsifiable concentrate containing 2.5% rotenone; and rotenone, a powder containing 33% rotenone. Except for tests in which the toxicities of the different formulations were compared or evaluated, Noxfish was used in all tests, and concentrations were based on the total amount of Noxfish formulation rather than on the amount of rotenone in the formulation. Stock solutions of the toxicants were prepared daily, and the portions needed to yield the desired concentrations were added to test chambers.

Static and flow-through test procedures followed those of Lennon and Walker (1964) and The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static tests, 10 fish were exposed to each concentration in glass jars containing 15 liters of oxygen-saturated test water prepared from deionized water (Marking 1969). Waters of four levels of hardness were used (total hardness as mg/l of $CaCO_3$ in parentheses): very soft (10), soft (44), hard (170), and very hard (300).

In separate tests to assess the effect of pH on toxicity, chemical buffers were added to control the pH (Marking 1975). Test temperatures were regulated

1

by immersing the test jars in constant temperature water baths. In flow-through toxicity tests, 20 fish were exposed to each concentration in a system similar to that described by Mount and Brungs (1967). Modifications included electronic microswitches to control cycling, pressure regulators, and an automatic pipette (Micromedic®) for injecting the toxicants into dilution water. Municipal water used for flow-through exposures was carbon filtered and had a total hardness of 320 mg/l and a pH of 7.5. Flow rates were maintained at about five chamber volumes per 24 h. Temperature was maintained with a water bath around the test chambers.

Fish weighing 1 to 1.5 g each were obtained from Federal hatcheries and maintained according to standardized procedures of the Fish Control Laboratory (Hunn et al. 1968). Scientific names for all species used are listed in Table 1. Fish were acclimated to the test conditions for 4 days before they were exposed to the toxicant. Mortalities were recorded at 1, 3, and 6 h on the first day of exposure and daily thereafter for the remainder of the test. Trout eggs were exposed to Noxfish in a manner similar to that used for fish, except that the static test vessel contained 2.5 liters of test solution. Details of methods for exposing eggs were those outlined by Olson and Marking (1973).

The methods of Litchfield and Wilcoxon (1949) were used in computation of the LC_{50}'s (concentrations producing 50% mortality) and 95% confidence intervals. Regressions were drawn and inspected for each set of data. All data reported fulfilled the chi-square test requirement for acceptability.

Deactivation indices for Noxfish were derived in soft water at temperatures of 12 and 17 C. Aged solutions of the toxicant were bioassayed to determine the biological activity remaining after selected time periods. The deactivation index was determined by dividing the LC_{50} of aged solutions by the LC_{50} of unaged solutions under corresponding test conditions (Marking 1972). The deactivation index was plotted against aging time on semilogarithmic coordinates to estimate the half-life of biological activity. Detoxification procedures with potassium permanganate ($KMnO_4$) and chlorine were those used by Marking and Bills (1975).

Results

Toxicity to Various Species of Fish

Noxfish was toxic to a wide variety of fish at concentrations ranging, in 96-h exposures, from 21.5 μg/l (or parts per billion) for Atlantic salmon to 497 μg/l for goldfish (Table 1). The 96-h LC_{50} was less than 100 μg/l for bowfins, all six salmonids, northern pike, carp, longnose and white suckers, smallmouth bass, yellow perch, and walleyes. The 96-h LC_{50} was greater than 100 μg/l for goldfish, fathead minnows, black bullheads, channel catfish, green sunfish, bluegills, and largemouth bass. Goldfish and black bullheads were most resistant— 10 times as resistant as most other species. Generally, the resistant species required longer exposures than did the sensitive species, before they succumbed. None of the goldfish died in 24 h of exposure to high concentrations of Noxfish and none of the black bullheads in 6 h. On the other hand, most of the sensitive species died in 3-h exposures to much lower concentrations.

Effects of Temperature, Water Hardness, and pH on Toxicity

Noxfish was generally less toxic to rainbow trout, channel catfish, and bluegills at the lower than at the higher temperatures in 3- and 6–h exposures (Tables 2, 3, and 4). After 96 h this trend remained but the differences in toxicity associated with most 5° differences in temperature were insignificant. The difference was significant for rainbow trout, however, at 7 and 12 C. Trout were consistently more sensitive than channel catfish or bluegills.

Water hardness had no effect on toxicity of Noxfish (Tables 2, 3, and 4), with one exception; in 96-h exposures the LC_{50}'s for channel catfish were 277 μg/l in soft water and 328 μg/l in very soft water (Table 3). Water at each hardness contained 384 mg/l of sodium bicarbonate to equalize the pH at about 8.0, and that quantity of bicarbonate in the soft water presumably resulted in slightly decreased toxicity to rainbow trout and channel catfish.

The toxicity of Noxfish was not influenced by differences in pH within the range of 6.5 to 9.5 (Tables 2, 3, and 4). Noxfish appeared to be more toxic to rainbow trout at pH 9.5 than at lower pH's, but the increased sensitivity might have been due to an inability of the trout to acclimate fully to the high pH. Bluegills responded uniformly at the three different pH's in soft water at 12 C; the 96-h LC_{50}'s ranged only from 122 to 138 μg/l.

Toxicity of Noxfish to Green Eggs of Rainbow Trout

Newly fertilized eggs of rainbow trout were 47 to 106 times more resistant than fingerlings to Noxfish; the 96-h LC_{50} ranged from 5.60 mg/l in very soft water to 2.50 mg/l in very hard water (Table 5).

Table 1. *Toxicity of Noxfish® to fish in standardized laboratory tests at 12 C.*

Species	LC$_{50}$ and 95% confidence interval (μg/l) at			
	3 h	6 h	24 h	96 h
Bowfin *Amia calva*	141 114–174	106 82.5–136	57.5 50.4–65.5	30.0 23.7–38.0
Coho salmon *Oncorhynchus kisutch*	358 —	152 105–219	71.6 63.1–81.3	62.0 54.8–70.2
Chinook salmon *O. tshawytscha*	212 171–262	156 137–177	49.0 44.3–54.2	36.9 33.9–40.2
Rainbow trout *Salmo gairdneri*	175 160–191	86.9 —	68.9 56.2–84.4	46.0 32.6–64.9
Atlantic salmon *S. salar*	61.5 53.4–70.8	40.0 33.6–70.8	35.0 29.7–41.2	21.5 15.5–29.8
Brook trout *Salvelinus fontinalis*	141 124–160	79.7 69.2–91.8	47.0 42.2–52.3	44.3 41.1–47.7
Lake trout *S. namaycush*	50.0 38.6–64.7	28.3 21.0–38.0	26.9 19.8–36.5	26.9 19.8–36.5
Northern pike *Esox lucius*	181 160–204	58.2 52.5–64.5	44.9 31.4–64.3	33.0 26.6–41.0
Goldfish *Carassius auratus*	—	—	—	497 412–600
Carp *Cyprinus carpio*	—	270 254–287	84.0 74.7–94.4	50.0 41.1–60.8
Fathead minnow *Pimephales promelas*	—	1,190 917–1,453	400 291–549	142 115–176
Longnose sucker *Catostomus catostomus*	388 332–454	218 141–337	67.2 59.3–76.1	57.0 51.9–62.6
White sucker *C. commersoni*	630 452–878	238 186–304	71.9 64.0–80.8	68.0 54.0–85.6
Black bullhead *Ictalurus melas*	—	—	665 516–856	389 298–507
Channel catfish *I. punctatus*	1,410 1,139–1,745	840 717–984	400 234–684	164 138–196
Green sunfish *Lepomis cyanellus*	389 332–456	332 249–443	218 197–241	141 114–174
Bluegill *L. macrochirus*	424 335–537	336 245–461	149 124–178	141 133–149
Smallmouth bass *Micropterus dolomieui*	277 219–350	165 —	93.2 85.1–102	79.0 70.7–88.2
Largemouth bass *M. salmoides*	514 449–588	360 305–425	200 131–305	142 115–176
Yellow perch *Perca flavescens*	150 126–179	134 120–149	92.0 80.1–106	70.0 59.8–82.0
Walleye *Stizostedion vitreum vitreum*	135 103–176	52.4 46.8–58.7	16.5 15.2–17.9	—

Table 2. *Toxicity of Noxfish® to rainbow trout in water of different temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval ($\mu g/l$) at			
			3 h	6 h	24 h	96 h
7	Soft	7.5	>400	276 237–322	158 134–186	70.0 62.5–78.4
12	Soft	7.5	175 160–191	86.9 —	68.9 56.2–84.4	46.0 32.6–64.9
17	Soft	7.5	73.0 59.8–89.1	73.0 59.8–89.1	43.4 30.9–60.9	43.4 30.9–60.9
12	Very soft	8.0	122 108–138	61.9 54.9–70.2	54.4 45.9–64.4	54.4 45.9–64.4
12	Soft	8.0	90.0 81.1–99.9	62.0 51.6–74.5	56.5 48.2–66.3	56.5 48.2–66.3
12	Hard	8.0	112 95.1–132	81.9 64.5–104	55.1 43.9–69.	55.1 43.9–69.1
12	Very hard	8.0	113 92.7–138	66.9 55.0–81.4	53.0 44.3–63.4	53.0 44.3–63.4
12	Soft	6.5	169 151–189	129 107–155	78.5 69.6–88.6	69.5 63.7–75.9
12	Soft	8.5	133 108–163	98.0 87.4–110	80.0 70.3–91.0	62.1 51.7–74.5
12	Soft	9.5	124 106–145	75.0 63.2–89.0	54.0 45.9–63.6	35.5 28.7–43.9

Table 3. *Toxicity of Noxfish® to channel catfish at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (μg/l) at			
			3 h	6 h	24 h	96 h
12	Soft	7.5	1,720 1,381–2,141	1,000 784–1,276	539 377–770	200 164–244
17	Soft	7.5	1,410 1,139–1,745	840 717–984	400 234–684	164 138–196
22	Soft	7.5	739 672–813	449 352–572	164 137–196	164 137–196
12	Very soft	8.0	1,420 1,080–1,868	640 464–883	476 346–655	328 290–370
12	Soft	8.0	1,640 1,341–2,005	890 677–1,171	450 341–593	237 199–282
12	Hard	8.0	1,220 1,023–1,454	942 756–1,173	400 312–513	308 237–400
12	Very hard	8.0	1,160 996–1,351	1,000 782–1,279	359 282–457	318 240–421
12	Soft	6.5	1,530 1,175–1,991	865 692–1081	500 326–767	200 158–254
12	Soft	8.5	899 689–1,173	735 608–889	565 454–704	309 237–403
12	Soft	9.5	629 548–721	625 546–715	550 444–681	248 184–335

Table 4. *Toxicity of Noxfish® to bluegills at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (μg/l) at			
			3 h	6 h	24 h	96 h
12	Soft	7.5	450 353–573	270 217–335	141 114–174	141 114–174
17	Soft	7.5	424 335–537	336 245–461	149 124–178	141 133–149
22	Soft	7.5	268 240–300	227 194–266	140 107–183	132 122–143
12	Very soft	8.0	334 194–575	219 183–262	142 129–157	132 118–147
12	Soft	8.0	450 317–639	319 241–422	152 135–172	137 123–153
12	Hard	8.0	300 196–460	284 200–403	146 131–162	132 118–147
12	Very hard	8.0	295 211–413	194 148–255	138 125–152	132 121–144
12	Soft	6.5	291 207–409	228 194–267	150 124–181	138 110–173
12	Soft	8.5	255 204–319	192 170–217	122 108–138	122 108–128
12	Soft	9.5	196 162–237	152 134–173	122 108–138	122 108–138

Although the difference in toxicity was not significant at each hardness increment, the difference was significant in very soft as compared to hard or very hard water.

Table 5. *Toxicity of Noxfish® to newly fertilized eggs of rainbow trout in reconstituted water of different hardnesses at 12 C.*

Water hardness	96-h LC$_{50}$ (mg/l) and 96% confidence interval
Very soft	5.60 3.55–8.83
Soft	4.42 3.28–5.96
Hard	3.20 2.31–4.43
Very hard	2.50 2.16–2.90

Persistence of Noxfish in Water

The toxicity to bluegills of Noxfish solutions aged for 1, 2, and 3 weeks decreased through each week of aging. At 12 C the 96-h LC$_{50}$'s were 133 μg/l in freshly prepared solutions and 254 μg/l in solutions aged for 3 weeks (Table 6). The toxicity decreased

Table 6. *Toxicity (96-h LC$_{50}$'s and 95% confidence intervals in μg/l) to bluegills of fresh and aged solutions of Noxfish® in soft water (deactivation indices shown in parentheses).*

Temp (°C)	Aging time (weeks)				Half-life (days)
	0	1	2	3	
12	133 117–151 (1.00)	—	213 193–236 (1.60)	254 215–300 (1.91)	22
17	90.0 64.1–126 (1.00)	117 92.3–148 (1.30)	190 159–228 (2.11)	288 247–336 (3.20)	13
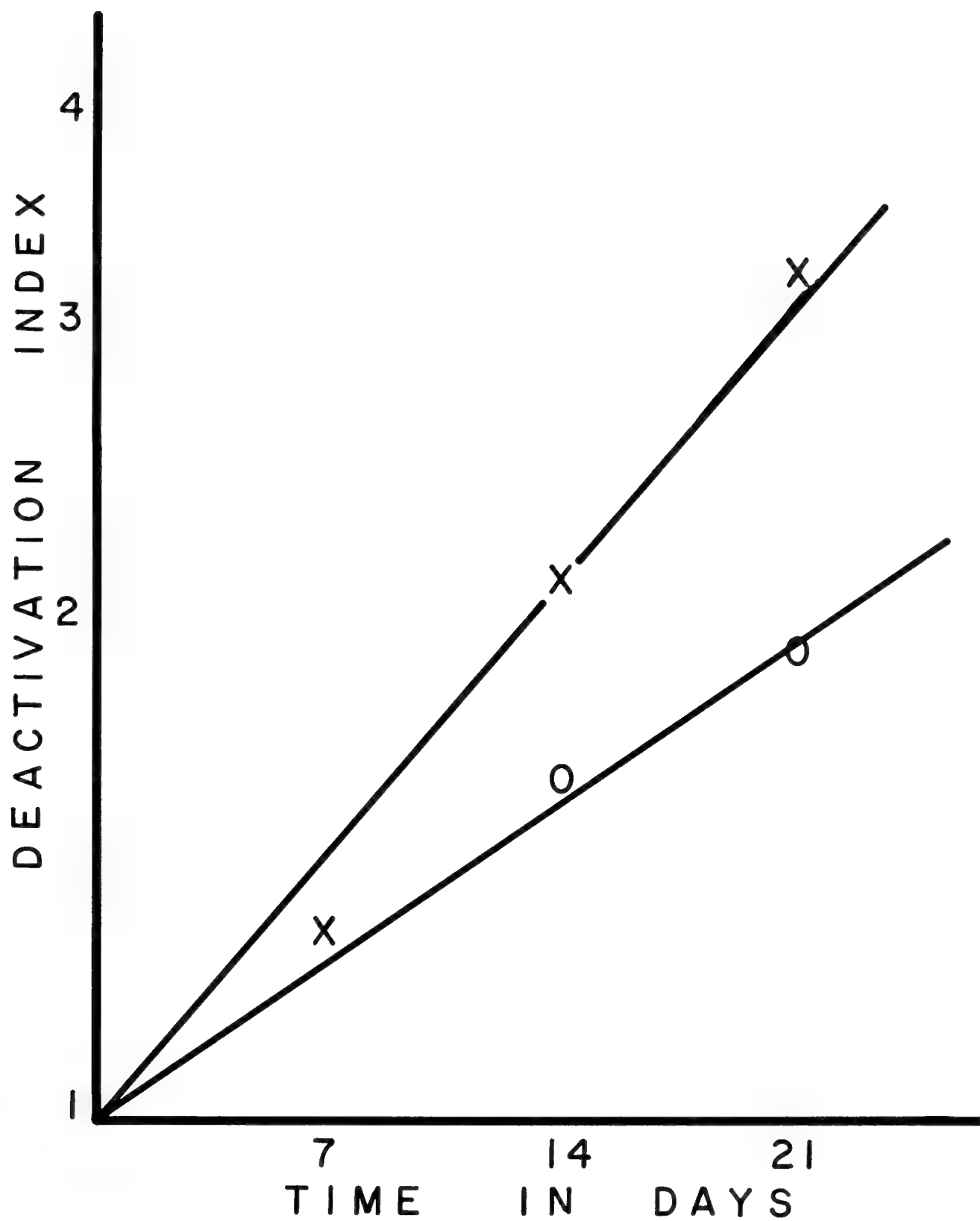
Fig. 1. Detoxification rate for Noxfish® in soft water at 12 C [O] and 17 C [X].

more rapidly at 17 C than at 12 C; the half-lives were 13 and 22 days, respectively (Fig. 1).

Detoxification of Noxfish

Static tests with green sunfish showed that potassium permanganate immediately detoxified Noxfish, as indicated by the marked loss of activity in unaged solutions of Noxfish and $KMnO_4$ (Table 7). For instance, the 96-h LC_{50} at pH 7.5 was 0.241 mg/l for Noxfish alone and 1.71 mg/l for Noxfish plus 1.0 mg/l of $KMnO_4$. The solutions of Noxfish and $KMnO_4$ that were aged for 50 min before the fish were added had lost some additional activity (96-h LC_{50}, 3.09). The immediate detoxification in unaged solutions was probably a reflection of the effective exposure time, i.e., the time required for Noxfish to produce a lethal effect. The $KMnO_4$ detoxified Noxfish in water at all pH's tested.

Chlorine was far less effective than $KMnO_4$ for detoxifying Noxfish in laboratory tests. There was little immediate detoxification in unaged solutions and little detoxification during 6 h of aging. For instance, at pH 7.5 the 96-h LC_{50} was 0.293 mg/l for Noxfish alone and 0.429 mg/l for Noxfish plus 0.5 mg/l of chlorine (Table 8). In a 6-h aging period, Noxfish was detoxified more efficiently at pH 9.5 than at pH's 7.5 and 8.5.

Toxicity of Different Formulations of Rotenone

Pro-Noxfish, a synergized formulation containing 2.5% rotenone, was more toxic to rainbow trout than the Noxfish formulation or powdered rotenone when concentrations were calculated on the basis of rotenone content. The comparative 96-h LC_{50}'s (μg/l) were as follows: Pro-Noxfish, 1.02; Noxfish, 3.05; and powdered rotenone, 4.20 (Table 9). There was no significant difference in toxicity between Noxfish and rotenone powder formulations.

Toxicity in Flow-through Tests

In 4-day flow-through exposures, Noxfish was more toxic to chinook salmon and yellow perch than to carp or white suckers (Table 10). Toxicity did not increase with exposure time after 4 days, except for carp (in which toxicity increased through 20 days). Noxfish

Table 7. *Toxicity and detoxification of Noxfish® in static tests with green sunfish in water containing 1.0 mg/l of $KMnO_4$ at 12 C.*

Compound and (for compounds combined) interaction time (min)[a]	96-h LC_{50} and 95% confidence interval (mg/l) at			
	pH 6.5	pH 7.5	pH 8.5	pH 9.5
$KMnO_4$	3.47 3.12–3.87	3.03 2.69–3.41	1.41 1.14–1.74	3.08 2.32–4.08
Noxfish	0.184 0.161–0.211	0.241 0.203–0.287	0.158 0.114–0.219	0.378 0.317–0.451
Noxfish + $KMnO_4$				
0	1.32 1.04–1.68	1.71 1.49–1.96	2.10 1.91–2.31	1.55 1.16–2.08
10	1.17 0.948–1.44	1.41 1.14–1.74	1.81 1.54–2.13	1.36 1.07–1.70
20	1.41 1.14–1.74	2.89 2.28–3.36	2.38 2.24–2.53	1.64 1.38–1.94
30	1.82 1.54–2.15	2.89 2.28–3.66	3.10 2.69–3.58	1.91 1.59–2.29
40	2.00 1.64–2.44	2.28 1.82–2.85	3.09 2.88–3.32	1.41 1.14–1.74
50	1.93 1.61–2.31	3.09 2.74–3.49	3.59 3.19–4.03	1.81 1.53–2.13

[a] Length of time Noxfish and $KMnO_4$ were added before fish were introduced.

Table 8. *Toxicity and detoxification of Noxfish® in static tests with green sunfish in water containing 0.5 mg/l of chlorine at 12 C.*

Compound and (for compounds combined) interaction time (h)[a]	96-h LC_{50} and 95% confidence interval (mg/l) at		
	pH 7.5	pH 8.5	pH 9.5
Chlorine	0.840 0.703–1.00	0.820 0.588–1.14	0.709 0.567–0.887
Noxfish	0.293 0.264–0.325	0.338 0.304–0.376	0.329 0.294–0.368
Noxfish + chlorine			
0	0.429 0.359–0.512	0.300 0.242–0.372	0.488 0.426–0.559
0.5	0.348 0.299–0.405	0.492 0.427–0.567	— —
1.0	0.483 0.406–0.575	0.380 0.318–0.455	0.900 0.677–1.20
2.0	0.412 0.337–0.504	0.400 0.339–0.473	0.689 0.584–0.813
4.0	0.494 0.438–0.557	0.489 0.432–0.554	0.770 0.689–0.862
6.0	0.494 0.438–0.557	0.454 0.390–0.529	1.37 0.988–1.90

[a] Length of time Noxfish and chlorine were added before fish were introduced.

Table 9. *Toxicity of three formulations of rotenone to rainbow trout in soft water at 12 C.*

Preparation	% active rotenone	LC_{50} and 95% confidence interval (μg/l) at				
		1 h	3 h	6 h	24 h	96 h
Pro-Noxfish®	2.5	13.0 8.15–20.7	4.53 3.28–5.65	2.98 2.43–3.65	1.82 1.60–2.08	1.02 0.917–1.15
Noxfish®	5.0	25.5 16.1–40.5	8.70 6.90–11.0	5.50 4.87–6.20	3.25 2.78–3.81	3.05 2.85–3.27
Powdered rotenone	33.0	16.5 14.3–19.1	8.09 6.37–10.3	6.60 5.41–8.02	3.82 3.30–4.46	3.20 2.09–3.70

Table 10. *Toxicity of Noxfish® to four species of fish in flow-through toxicity tests at 12 C.*

Species	LC$_{50}$ and 95% confidence interval (μg/l) at				
	1 day	4 days	10 days	20 days	30 days
Chinook salmon	112 97.7–128	71.0 55.3–99.1	62.0 52.1–73.7	59.0 49.5–70.3	—
Carp	—	142 122–165	96.0 78.0–118	67.0 57.4–78.3	68.0 57.7–80.1
White sucker	—	144 122–170	129 118–141	112 95.5–131	112 95.5–131
Yellow perch	160 121–211	60.0 53.3–67.6	50.0 36.8–68.0	46.0 32.7–64.8	

was consistently and significantly more toxic to all four species in static than in flow-through tests (Table 11).

Discussion

The literature on toxicity of rotenone to fish suggests that concentrations used in fishery management are generally higher than those known to be lethal in laboratory tests; that toxicity depends on temperature, water hardness, pH, and physical characteristics; and that many different application rates may be effective for the same target species of fish (Schnick 1974; Meyer 1966).

Since laboratory procedures are usually more standardized than field procedures, laboratory data are expected to be more consistent than field data. Although the LC$_{50}$'s of less than 0.2 mg/l for

Table 11. *Comparison of acute toxicities of Noxfish® to four species of fish in 96-h flow-through and static tests in carbon filtered municipal water at 12 C.*

Species	LC$_{50}$ and 95% confidence interval (μg/l)	
	Static	Flow-through
Chinook salmon	34.7 26.9–44.7	71.0 55.3–99.1
Carp	19.0 12.1–29.9	142 122–165
White sucker	17.9 12.8–25.1	144 122–170
Yellow perch	30.0 23.6–38.2	60.0 53.3–67.6

Noxfish against rainbow trout, channel catfish, and bluegills reported by Bridges and Cope (1965) were similar to ours, applications of at least 1 mg/l have been repeatedly recommended for eliminating these species. Spitler (1970) reported that 1.6 mg/l of Noxfish was not effective and that as much as 5 mg/l was sometimes needed. The difference in laboratory and field data is due to several factors. Laboratory data generally indicate concentrations that produce 50% mortality (LC$_{50}$), whereas field concentrations are based on eliminating 100% of the target fish. Organisms, particulate matter, and sunlight contribute to the tendency toward faster detoxification of chemicals in natural waters than in the laboratory. Furthermore, because uniform concentrations are much more difficult to obtain in the field, additional amounts of toxicants are generally applied to ensure a lethal concentration throughout a body of water.

Although some of the reports are conflicting, rotenone is generally more effective at high than at low temperatures (Gersdorff 1943; Almquist 1959; Ball 1948; Hooper 1955), in acid than in alkaline waters (Leonard 1939; Foye 1964), and in soft than in hard water (Foye 1964). In many of these studies, however, efficacy was based on survival time of the fish rather than on the concentration of the toxicant. Our laboratory data show only slight changes in the toxicity of rotenone at different temperatures, hardnesses, or pH's. Consequently, concentrations used in the field should be based on the results of on-site toxicity tests (Burress 1975) rather than on extrapolations of laboratory or field data.

Most studies—in laboratory or field—have shown that goldfish and black bullheads are the species most resistant to rotenone. Individual fish of a species may be exceptionally resistant (Meyer 1966)—an observation that may explain some incomplete fish kills and the need to apply a concentration

greater than that indicated in laboratory tests.

The detoxifiers $KMnO_4$ and chlorine were toxic to fish at concentrations only slightly greater than those needed to detoxify rotenone. For example, against green sunfish in water at pH 8.5, the 96-h LC_{50} for $KMnO_4$ was 1.41 mg/l and that for chlorine was 0.82 mg/l. These results support Engstrom-Heg and Loeb (1968) and Engstrom-Heg (1972), who cautioned that high concentrations of $KMnO_4$ may become toxic and may have to be reduced with sodium thiosulfate or other agents. Therefore, detoxifiers should be used only when necessary and in only the quantities needed.

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INVESTIGATIONS IN FISH CONTROL

Formalin: Its Toxicity to Nontarget Aquatic Organisms, Persistence, and Counteraction

by

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Abstract

The acute toxicity of formalin to selected fishes and aquatic invertebrates was determined in standardized laboratory tests. Fish species exposed were chinook salmon *(Oncorhynchus tshawytscha)*, rainbow trout *(Salmo gairdneri)*, Atlantic salmon *(S. salar)*, lake trout *(Salvelinus namaycush)*, black bullhead *(Ictalurus melas)*, channel catfish *(I. punctatus)*, green sunfish *(Lepomis cyanellus)*, bluegill *(L. macrochirus)*, smallmouth bass *(Micropterus dolomieui)*, and largemouth bass *(M. salmoides)*. Invertebrates exposed were freshwater prawn *(Palaemonetes kadiakensis)*, seed shrimp *(Cypridopsis* sp.), Asiatic clam *(Corbicula leana)*, snail *(Helisoma* sp.), and backswimmer *(Notonecta* sp.). Black bullhead and channel catfish were the fish most sensitive to formalin (96-h LC_{50}'s, 62.1 and 65.8 $\mu l/l$), and Atlantic salmon and green sunfish were the most resistant (96-h LC_{50} for each, 173 $\mu l/l$). The $TILC_{50}$ (lethal concentration producing 50% mortality independent of time) for formalin against rainbow trout was 72.0 $\mu l/l$. Seed shrimp were the most sensitive invertebrates (96-h LC_{50}, 1.05 $\mu l/l$), and backswimmers were the most resistant (96-h LC_{50}, 835 $\mu l/l$). The toxicity of formalin was unchanged in solutions aged as long as 3 weeks; the biological half-life could not be determined. Formalin was not detoxified by oxidation or reduction, and filtration through activated carbon did not significantly reduce toxicity.

Formalin is one of the most effective and widely used compounds in fish culture for therapeutic and prophylactic treatment of fungal infections and external parasites of fish and fish eggs. Uses of formalin in fish culture were reviewed by Schnick (1974). Before about 1967, the registration of chemicals used to treat diseases of fish in hatcheries was not required. Since then the Food and Drug Administration and the Environmental Protection Agency have required specific information about each chemical and its use pattern before registration. Information required for the registration includes toxicity to target and nontarget organisms, efficacy, residues, metabolites, and means of counteraction (Lennon 1967). Standardized tests have been developed for generating toxicity information necessary for the registration of fishery chemicals (Marking 1975).

The purposes of this study were to determine (1) the toxicity of formalin to nontarget aquatic organisms, (2) the toxicity (safety) of maximum use-pattern exposures to formalin, (3) the toxicity of formalin to selected fishes in extended exposures, (4) the effects of certain water characteristics on the toxicity of formalin to fish, (5) the persistence of formalin in water, and (6) the feasibility of counteracting formalin by oxidation or reduction, or removal from water with activated carbon.

Materials and Methods

Stock solutions of commercial grade formalin (37% formaldehyde) obtained from North Central Chemical Co., La Crosse, Wisconsin, were prepared in water (the liquid formulation was measured volumetrically and diluted with water). All concentrations listed are based on the formulated product. To prepare test solutions of the desired concentrations, we pipetted portions of stock solutions into the test vessels, and stirred the resulting mixture to ensure homogeneity. In flow-through toxicity tests, required amounts of the stock solutions were delivered by a solenoid-activated pipetting pump (Micromedic Systems Automatic Pipette Model 2500).

Fish species exposed were chinook salmon *(On-corhynchus tshawytscha)*, rainbow trout *(Salmo gairdneri)*, Atlantic salmon *(S. salar)*, lake trout *(Salvelinus namaycush)*, black bullhead *(Ictalurus melas)*, channel catfish *(I. punctatus)*, green sunfish *(Lepomis cyanellus)*, bluegill *(L. macrochirus)*, smallmouth bass *(Micropterus dolomieui)*, and largemouth bass *(M. salmoides)*. Invertebrates exposed were freshwater prawn *(Palaemonetes kadiakensis)*, seed shrimp *(Cypridopsis* sp.), clam *(Corbicula leana)*, snail *(Helisoma* sp.), and backswimmer *(Notonecta* sp.). The fish were obtained from State and Federal hatcheries and maintained in the laboratory; invertebrates were either cultured outdoors in partly shaded vinyl pools or collected in the field. Organisms collected in the field were held for 7 days in water identical with that used in the toxicity tests, before they were exposed to formalin. Fish and invertebrates were acclimated to test conditions for 24 h before the addition of formalin. Ten or more organisms were exposed at each concentration. Mortalities were recorded at 1, 3, and 6 h the first day and daily thereafter during the 96-h exposure period. Fish were regarded as dead when all opercular movements ceased and invertebrates when they became immobile or failed to respond to physical stimuli.

Laboratory toxicity tests were conducted according to standard procedures described by Lennon and Walker (1964) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Static tests were conducted in 2.5- or 15-liter glass jars, depending on the size of the test organism involved. Flow-through tests were conducted in 45 liters of test solution; the solution was replaced four times daily through a 1-liter dilution apparatus similar to that described by Mount and Brungs (1967).

Temperature was controlled by immersing test vessels in a water bath equipped with a chilling unit. Reconstituted water (Marking 1969) was used in tests with fish and clams and limed spring water (pH 6.5 ±0.1, total hardness 20 mg/l as $CaCO_3$) in tests with the other invertebrates. Chemical buffers were added to soft water in tests of the effect of pH (6.5–9.5), as recommended by Marking and Dawson (1973). The pH's of the test solutions were checked daily and adjusted to within ±0.2 pH units. For determination of the persistence of formalin, aqueous solutions were aged 1, 2, and 3 weeks, after which rainbow trout fingerlings were introduced and 96-h LC_{50}'s computed. Deactivation indices were computed from these data according to the method of Marking (1972).

In counteraction studies, potassium permanganate ($KMnO_4$) at a concentration of 1 mg/l and sodium thiosulfate ($Na_2S_2O_3$) at 10 mg/l were introduced into a series of formalin solutions of selected concentrations 6 h before the introduction of fish. In aeration tests, the solutions were aerated with air stones for 24 h before fish were added. We compared the 96-h LC_{50}'s with a reference standard to assess changes in toxicity. To determine if formalin could be removed from aqueous solutions, we filtered a concentrated solution (150 μl/l) at a flow rate of 100 ml/min through a 15-cm column of activated charcoal (Darco 20 × 40 mesh). Samples of effluent were taken at selected volumes (0–200 ml and 800–1,000 ml). These samples and a sample of the stock solution were bioassayed against rainbow trout and the 96-h LC_{50}'s compared.

We used the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s and 95% confidence intervals, and a modification of the method given by Green (1965) to compute $TILC_{50}$'s.

Results

Toxicity of Formalin to Fish

The 96-h LC_{50}'s for formalin against nine species of fish ranged from 62.1 μl/l for black bullheads to 173 μl/l for green sunfish and Atlantic salmon (Table 1). Toxicity of formalin increased with time; for bluegills, for example, the 3- and 96-h LC_{50}'s were 2,290 μl/l and 100 μl/l, respectively. Ictalurids were twice as sensitive to formalin as the centrarchids or salmonids. Green sunfish were the most resistant centrarchid exposed, followed by largemouth bass, smallmouth bass, and bluegills. Atlantic salmon were the most resistant salmonid, followed by rainbow trout and lake trout (Table 1).

The effects of temperature, water hardness, and pH on toxicity were determined by exposing rainbow trout, channel catfish, and bluegills to formalin. In short exposures, formalin was significantly more toxic to these species at the higher temperatures; however, at 96 h the differences were insignificant except in rainbow trout (Tables 2, 3, and 4). Water hardness had no apparent effect on toxicity. For rainbow trout and channel catfish, formalin was more toxic in waters of pH 9.5 than in waters of pH 6.5, 7.5, or 8.5 (Tables 2 and 3).

In chronic toxicity tests the $TILC_{50}$ for formalin against rainbow trout was 72.0 μl/l as compared with LC_{50}'s of 157 μl/l at 24 h and 131 μl/l at 96 h.

Toxicity of Formalin to Invertebrates

Invertebrates differed widely in their responses to formalin. The 96-h LC_{50}'s ranged from 1.05 μl/l for seed shrimp to 835 μl/l for backswimmers; those for the bivalve and snail—126 and 93 μl/l—were similar

Table 1. *Toxicity of formalin to fingerling fish of nine species in standard toxicity tests at 12 C.*

Species	Average weight (g)	LC_{50} and 95% confidence interval (μl/l) at			
		3 h	6 h	24 h	96 h
Rainbow trout	0.63	1230 957–1581	655 580–740	300 237–380	118 99.7–140
Atlantic salmon	0.60	1410 1049–1896	840 751–939	389 333–455	173 149–201
Lake trout	0.50	—	603 444–819	141 114–174	100 78.2–128
Black bullhead	0.75	—	—	173 123–243	62.1 50.9–75.8
Channel catfish	0.40	495 430–570	232 178–303	122 102–145	65.8 58.1–74.5
Green sunfish	0.70	—	—	323 250–417	173 123–243
Bluegill	0.50	2290 1804–2907	1600 1165–2198	211 171–260	100 80.0–125
Smallmouth bass	0.68	—	—	222 171–288	136 90.2–205
Largemouth bass	1.00	—	1030 928–1140	283 229–350	143 129–159

Table 2. *Toxicity of formalin to fingerling rainbow trout at selected water temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (μl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	>3000	1810 1537–2131	940 762–1160	349 292–418	245 213–282
12	Soft	7.5	2310 1959–2724	1230 957–1581	655 580–740	300 237–380	118 99.7–140
17	Soft	7.5	1210 1015–1443	1000 788–1269	590 532–654	219 174–276	—
12	Very soft	6.6	>2500	1590 1070–2364	729 657–809	230 181–292	—
12	Hard	7.8	1740 1240–2441	1740 1240–2441	925 783–1093	388 332–454	172 108–274
12	Very hard	8.2	1690 1070–2670	1690 1070–2670	910 769–1077	334 272–411	171 122–240
12	Soft	6.5	1730 1233–2427	1730 1233–2427	835 749–931	321 231–446	171 122–240
12	Soft	8.5	1390 1043–1852	1150 917–1442	645 523–796	300 220–408	172 123–241
12	Soft	9.5	1740 1240–2441	875 750–1020	500 355–704	135 105–174	100 78.9–127

Table 3. *Toxicity of formalin to channel catfish at selected water temperatures, hardnesses, and pH's.*

Temp. (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (μl/l) at				
			1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	779 666–911	495 430–570	232 178–303	122 102–145	65.8 58.1–74.5
17	Soft	7.5	600 523–689	350 299–409	282 228–349	119 102–138	65.5 58.2–73.7
22	Soft	7.5	559 475–658	284 229–351	234 185–297	100 83.7–119	64.0 58.6–69.9
12	Very soft	6.6	771 660–901	490 425–565	490 425–565	99.0 86.0–114	69.9 63.7–76.7
12	Hard	7.8	1050 798–1382	450 360–563	355 298–422	117 100–137	49.0 43.3–55.5
12	Very hard	8.2	872 703–1081	439 346–557	285 229–355	111 94.5–130	61.9 53.9–71.1
12	Soft	6.5	779 666–912	424 356–505	282 228–349	118 103–135	62.0 54.4–70.7
12	Soft	8.5	630 554–717	455 365–567	285 230–353	94.0 84.0–105	56.5 51.4–62.1
12	Soft	9.5	559 474–659	282 228–349	235 185–298	63.9 54.7–74.7	42.9 36.2–50.9

Table 4. *Toxicity of formalin to fingerling bluegills at selected water temperatures, hardnesses, and pH's.*

Temp. (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (μl/l) at			
			3 h	6 h	24 h	96 h
12	Soft	7.5	2290 1804–2907	1600 1165–2198	211 171–260	100 80.0–125
17	Soft	7.5	2300 1822–2904	780 670–908	189 153–234	73.5 63.5–85.0
22	Soft	7.5	1750 925–3312	469 403–545	142 115–176	91.0 81.2–102
12	Very soft	6.6	1800 1532–2115	1230 1071–1412	369 315–433	88.4 75.1–104
12	Hard	7.8	1720 1458–2029	1190 1027–1379	249 166–373	106 84.0–134
12	Very hard	8.2	1740 1499–2019	1310 1038–1654	233 181–300	117 101–136
12	Soft	6.5	2310 1961–2721	2290 1944–2697	335 284–395	125 89.1–175
12	Soft	8.5	2300 1822–2904	1650 1401–1943	230 182–290	86.2 72.6–102
12	Soft	9.5	2300 1950–2712	1055 883–1260	232 174–309	100 72.6–138

to those for fish. The freshwater prawn was intermediate in resistance to formalin, having a 96-h LC_{50} of 465 μl/l (Table 5).

Toxicity of Formalin at Use-Pattern Concentrations

Recommended use-pattern concentrations of formalin range as high as 250 μl/l for 1 h in tanks or raceways and are 15 to 25 μl/l for indefinite periods in earthen ponds. Exposure to use-pattern concentrations caused no mortality in chinook salmon, rainbow trout, Atlantic salmon, lake trout, black bullhead, channel catfish, green sunfish, bluegill, smallmouth bass, or largemouth bass. The seed shrimp was the only invertebrate affected; 99% mortality could be expected at a 25-μl/l indefinite treatment level.

Persistence of Formalin in Aqueous Solutions

The toxicity to rainbow trout fingerlings of formalin solutions that had been aged for 1, 2, and 3 weeks was not substantially different from that of fresh solutions (Table 6).

Formalin solutions were not detoxified by either oxidation or reduction. The 96-h LC_{50}'s for the formalin reference solution, an aerated solution, and a solution to which thiosulfate had been added were not significantly different. However, the 96-h LC_{50} for the formalin:potassium permanganate solution was

Table 6. *Effect of aging on the toxicity to rainbow trout of formalin in soft water at 12 C.*

Aging period (weeks)	96-h LC_{50} (μl/l) and 95% confidence interval	Deactivation index
0	119 91.3–155	1.00
1	111 94.5–130	0.933
2	141 114–174	1.18
3	122 87.5–170	1.03

60.0 μl/l as compared with 107 μl/l for the formalin reference (Table 7).

When the first and last 200-ml portions of the filtrate of a 150-μl/l formalin stock solution filtered through a 15-cm column of activated carbon were bioassayed against rainbow trout along with a sample of the stock solution, the 96-h LC_{50}'s were 210 μl/l for the first 200-ml sample, 132 μl/l for the 800- to 1,000-ml sample, and 121 μl/l for the reference solution. Although this difference indicates some removal of formalin, the removal was insignificant when the relative amounts of formalin and carbon involved (1 mg formalin/1 g carbon) are considered (Table 8).

Table 5.—*Toxicity of formalin to selected aquatic invertebrates in soft water at 16 C.*

Species	LC_{50} and 95% confidence interval (μl/l) at				
	1 h	3 h	6 h	24 h	96 h
Seed shrimp (ostracods)[a] *Cypridopsis* sp.	9.00 6.83–11.9	6.40 4.91–8.34	1.20 0.664–2.17	1.15 0.690–1.97	1.05 0.590–1.87
Freshwater prawn[a] *Palaemonetes kadiakensis*	—	2150 1948–2373	1900 1588–2273	1105 896–1362	465 368–588
Bivalves[b] *Corbicula* sp.	--	—	—	800 638–1003	126 80.9–196
Snail[c] *Helisoma* sp.	3525 3201–3881	1340 953–1883	780 629–967	710 544–925	93.0 69.5–124
Backswimmer[c] *Notonecta* sp.	—	—	—	4500 3006–6735	835 652–1069

[a] Toxicity based on immobility.

[b] Toxicity based on ability to resist attempts to open valves and respond to tactile stimulus.

[c] Toxicity based on ability to respond to tactile stimulus.

Table 7. *Toxicity of formalin solutions containing selected oxidizing and reducing agents to fingerling rainbow trout[a].*

Chemical	Concentration (mg/l)	96-h LC$_{50}$ (μl/l) and 95% confidence interval
Formalin (reference)	—	107 89.9–127
Formalin:aeration[b]		117 90.0–152
Formalin:thiosulfate	10	99.0 81.4–120
Formalin:KMnO$_4$	1	60.0 53.8–66.9

[a] Fish were added to the reference, thiosulfate, and KMnO$_4$ solutions 6 h after the chemicals were added.

[b] Solutions were aerated vigorously for 24 h before addition of fish.

Discussion

Information regarding the toxicity of formalin to various aquatic organisms is abundant. However, the varied test conditions under which the data were developed make comparisons difficult, and some of the data are unacceptable for use in the evaluation of formalin for registration (Schnick 1974). Usually no reference has been made to temperature, pH, hardness, or other characteristics of water that directly affect toxicity and efficacy of other chemicals used in fisheries (Marking and Olson 1975; McKee and Wolf 1963).

Schnick (1974) pointed out the wide range of sensitivity for different species of fish, salmonids and centrarchids being the most resistant and ictalurids the most sensitive. Data from our study follow this pattern. Schnick also stated that, although various

Table 8. *Toxicity of selected eluates of a 150- μl/l formalin stock solution filtered through a 15-cm column of activated carbon to rainbow trout in soft water at 12 C.*

Eluate	96-h LC$_{50}$ (μl/l) and 95% confidence interval
Reference[a]	121 105–140
0 to 200 ml	210 189–233
800 to 1,000 ml	132 111–157

[a] Toxicity of stock solution before filtration.

chemical characteristics of the water and physical condition of the fish appear to influence the toxicity of formalin, variations in sensitivity within a species may be due to genetic composition.

The effect of water chemistry on the toxicity of formalin to fish is somewhat controversial. Birdsong and Avault (1971) reported that the toxicity of formalin to pompano *(Trachinotus carolinus)* was not affected by different salinity levels. Piper and Smith (1973) reported that water chemistry has no effect on the toxicity of formalin to fish; however, their data were based on questionnaires received from various hatcheries rather than on experimental data. Marking et al. (1972) also reported that the toxicity of formalin was not affected by water hardness or pH. Bills (1974) first demonstrated that formalin was more toxic to fish and fish eggs in alkaline than in acid water. This conclusion is further supported by data from the present study, which show that in soft water formalin was more toxic to channel catfish and rainbow trout at pH 9.5 than at lower pH's.

Formalin is frequently used at concentrations of 15 to 25 μl/l for control of parasites on fish in earthen ponds. Much information is available on the efficacy of formalin as a parasiticide; however, the effects of formalin on pond flora and fauna, particularly on aquatic invertebrates, have not been determined. Schnick (1974) reported few data on the toxicity of formalin to invertebrates. Our data show a wide range of sensitivities for invertebrates; the 96-h LC$_{50}$'s ranged from 1.05 μl/l for seed shrimp to 835 μl/l for backswimmers. Our data also show formalin to be persistent under laboratory conditions, and at use-pattern concentrations some invertebrates could be affected. Present governmental controls on the use of chemicals in the environment necessitate

counteraction of persistent compounds after they have accomplished their purpose (Dawson 1976); however, the two most commonly used techniques for removal of such compounds (chemical oxidation/reduction or adsorption on activated carbon) failed to neutralize the toxicity of formalin. In fact, under oxidative conditions the solutions became more toxic.

Although some formalin may be removed by activated carbon, the amount is insignificant and the technique probably would not be applicable to hatchery operations.

Conclusions

1. Black bullheads were the species most sensitive to formalin (96-h LC_{50}'s = 62.1 $\mu l/l$).

2. Atlantic salmon and green sunfish were the most resistant species (96-h LC_{50}'s = 173 $\mu l/l$).

3. Lake trout were the most sensitive salmonids and bluegills were the most sensitive centrarchids.

4. The toxicity of formalin was not influenced by water hardness, but in soft water the chemical was more toxic to rainbow trout and channel catfish at pH 9.5 than at pH 6.5 or 8.5.

5. Formalin was more toxic to rainbow trout, channel catfish, and bluegills in warm than in cold water in 3-h exposures, but after 96 h the difference continued to be statistically significant only in rainbow trout.

6. Formalin was about twice as toxic in chronic exposures as in acute exposures.

7. Seed shrimp were the only organisms exposed that were affected by formalin at use-pattern concentrations.

8. Seed shrimp were the most sensitive invertebrates and backswimmers the most resistant; the 96-h LC_{50}'s were 1.05 $\mu l/l$ and 835 $\mu l/l$.

9. The toxicity of formalin solutions persisted after 3 weeks of aging.

10. Formalin solutions were not detoxified by oxidation or reduction; in fact, they became more toxic under oxidative conditions.

11. Vigorous aeration for 24 h did not significantly change the toxicity of formalin solutions.

12. Only a small proportion of formalin was removed by filtration through activated carbon.

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Chlorine: Its Toxicity to Fish and Detoxification of Antimycin

by

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Abstract

The 96-h LC_{50}'s for chlorine ranged from 0.156 mg/l for channel catfish *(Ictalurus punctatus)* to 1.41 mg/l for black bullheads *(I. melas)* in standardized laboratory tests. The toxicity of chlorine was influenced little by pH, temperature, or water hardness. Chlorine at 0.25 and 0.5 mg/l effectively detoxified antimycin. The half-life of biological activity for antimycin with chlorine ranged from 1.1 h at pH 6.5 to 1.5 h at pH 9.5. Chlorine readily detoxified antimycin at pH 6.5, 7.5, and 8.5, but not at pH 9.5.

Chlorine is used extensively for disinfection of municipal water supplies and effluents (Fair et al. 1948; Chambers 1971) and occasionally for sterilization of fish hatchery water supplies (Hagen 1940; Bedell 1971) and culture ponds. Due to the widespread use of chlorine, precautions must be considered for protecting nontarget organisms such as fish, aquatic invertebrates, and plants. Brungs (1973), who summarized acute and chronic effects of residual chlorine on some aquatic organisms, recommended that total residual chlorine not exceed 0.2 mg/l for a period of 2 h/day for the more resistant species of fish. The evaluation of toxicity data in the literature is complicated by lack of information on the water chemistry, temperature, chlorine demand, solubility conditions, formation of chlorine complexes, and exposure time. Merkens (1958) stated that toxicity of chlorine depended on the amount of chlorine that remained in solution rather than on the amount added. Therefore the exact conditions of toxicity tests with aquatic organisms should be reported, and standardized procedures followed.

Because chlorine is a strong oxidizing agent, it can be used to detoxify chemicals that are subject to oxidation reactions. Preliminary studies on the fish toxicant antimycin indicated that it is effectively detoxified with 0.5 mg/l of chlorine in soft water at pH 7.5 (Dawson and Marking 1974); a concentration of 10 μg/l of antimycin was decreased to less than 0.2 μg/l after 2 h. The preliminary results were based on sensitivity bioassays with green sunfish *(Lepomis cyanellus)*. Although antimycin is nonpersistent in the aquatic environment (Marking and Dawson 1972), additional measures to accelerate its detoxification are needed when the possibility exists that treated water will enter municipal water supplies.

This study was designed to determine the toxicity of chlorine to fish and to establish the efficiency of chlorine for detoxifying the fish toxicant antimycin. Factors influencing toxicity of chemicals in water were included in the evaluation.

Materials and Methods

Technical grade antimycin was obtained from Ayerst Laboratories, Rouses Point, New York. Stock solutions were prepared by dissolving weighed portions in acetone and further diluting them in aqueous stock solutions just before use. Portions of the aqueous stock solutions delivered to the static test chambers yielded selected concentrations over a range which produced mortality at high concentrations but permitted survival at low concentrations. Aqueous stock solutions of chlorine were prepared from calcium hypochlorite (commercial grade HTH) containing 70% available chlorine. Test concentrations were based on active chlorine.

Procedures for the static toxicity tests followed those described by Lennon and Walker (1964), with some modifications. Test waters were prepared by adding mineral salts to deionized water in prescribed proportions to yield total hardness (as mg/l of $CaCO_3$) of 12 for very soft water, 44 for soft, 160 for hard, and 300 for very hard water (Marking 1969). The pH of reconstituted water was altered and stabilized by adding chemical buffers to soft water to yield pH's of 6.5, 7.5, 8.5, and 9.5 (Marking and Dawson 1973). The pH of water of different hardnesses was stabilized with sodium bicarbonate. The pH's of test solutions were checked daily and adjusted when necessary. Soft water was used in

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standardized tests for the determination of the toxicity of chlorine to various species of fish.

Fish were obtained from Federal fish hatcheries and maintained by a trained fish culturist (Hunn et al. 1968). Ten fish, 2 to 5 cm in total length, were exposed to each concentration. Fish loading rates did not exceed 1 g per liter of water. Common and scientific names for fish used are listed in Table 1.

Mortalities were observed and recorded at 1, 3, and 6 h during the first day and at least daily thereafter. We analyzed the data by the methods of Litchfield and Wilcoxon (1949) to obtain LC_{50}'s (concentrations calculated to produce 50% mortality) and 95% confidence intervals. Chi-square tests indicated acceptability of all data reported. The half-life of antimycin:chlorine solutions was estimated by plotting deactivation indices against time on semilogarithmic coordinates (Marking and Dawson 1972).

Results

Toxicity of Chlorine to Fish

The toxicity of chlorine to fish varied with the species; 96-h LC_{50}'s ranged from 0.156 mg/l for channel catfish to 1.41 mg/l for black bullheads (Table 1). The three coldwater species (coho salmon rainbow trout, and lake trout) were more sensitive than the warmwater species (except for channel catfish). At 7 or 8 mg/l of chlorine, mortality occurred within 1 h of exposure for all species except goldfish, carp, fathead minnows, and black bullheads. The LC_{50}'s changed little after 24 h of exposure.

In rainbow trout exposed at different temperatures, chlorine was more toxic at 17 and 12 C than at 7 C, after 1 h of exposure (Table 2). This trend was

Table 1. *Toxicity of chlorine (from commercial grade HTH) to 12 species of fish in soft, reconstituted water at 12 C.*

Species	LC_{50} and 95% confidence interval (mg/l) at				
	1 h	3 h	6 h	24 h	96 h
Coho salmon	4.25	0.599	0.434	0.310	0.289
Oncorhynchus kisutch	3.43–5.26	0.541–0.664	0.383–0.492	0.247–0.389	0.226–0.370
Rainbow trout	0.969	0.640	0.550	0.236	0.172
Salmo gairdneri	0.886–1.06	0.533–0.769	—	0.199–0.280	0.148–0.200
Lake trout	1.19	0.615	0.450	0.246	0.200
Salvelinus namaycush	0.974–1.45	0.554–0.682	0.371–0.545	0.177–0.343	0.147–0.272
Goldfish	>7.00	—	2.39	1.42	1.18
Carassius auratus		—	1.87–3.06	1.14–1.76	0.902–1.54
Carp	>8.00	3.65	2.83	0.825	0.800
Cyprinus carpio		2.91–4.57	2.56–3.13	—	—
Fathead minnow	>7.00	2.43	1.38	1.00	0.998
Pimephales promelas		1.57–3.77	0.964–1.98	0.843–1.19	0.841–1.18
White sucker	2.00	0.880	0.631	0.379	0.379
Catostomus commersoni	1.65–2.42	0.830–0.933	0.538–0.740	0.318–0.452	0.318–0.452
Black bullhead	>8.00	>8.00	2.21	1.41	1.41
Ictalurus melas			—	1.14–1.74	1.14–1.74
Channel catfish	1.38	0.346	0.286	0.156	0.156
I. punctatus	1.06–1.79	0.273–0.438	0.231–0.354	0.106–0.229	0.106–0.229
Green sunfish	9.42	3.00	1.72	1.28	1.28
Lepomis cyanellus	6.84–13.0	2.21–4.08	1.23–2.41	1.01–1.62	1.01–1.62
Bluegill	10.8	1.32	1.11	0.569	0.555
L. macrochirus	6.41–18.2	0.996–1.75	0.797–1.55	0.484–0.669	0.468–0.658
Yellow perch	1.32	1.16	0.735	0.570	0.558
Perca flavescens	0.994–1.75	0.842–1.19	0.658–0.821	0.489–0.665	0.474–0.657

Table 2. *Toxicity of chlorine (from commercial grade HTH) to rainbow trout at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (mg/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	3.00 2.34–3.85	1.11 0.912–1.35	0.695 0.594–0.814	0.310 0.224–0.428	0.141 0.114–0.174
12	Soft	7.5	0.969 0.886–1.06	0.640 0.533–0.769	0.550 —	0.236 0.199–0.280	0.172 0.148–0.200
17	Soft	7.5	0.800 0.721–0.888	0.619 0.561–0.683	0.434 0.348–0.541	0.320 0.245–0.419	0.192 0.145–0.254
12	Very soft	8.0	3.00 2.20–4.10	0.900 0.763–1.06	0.830 0.757–0.910	0.362 0.270–0.485	0.200 0.144–0.278
12	Soft	8.0	2.87 2.12–3.89	1.00 0.859–1.16	0.815 0.768–0.865	0.590 —	0.211 0.168–0.265
12	Hard	8.0	2.81 2.28–3.46	1.24 1.03–1.49	0.879 0.806–0.958	0.510 0.414–0.628	0.350 —
12	Very hard	8.0	2.75 2.24–3.37	0.900 0.839–0.965	0.851 0.806–0.898	0.439 0.327–0.590	0.228 —
12	Soft	6.5	0.999 0.871–1.15	0.476 0.436–0.520	0.405 0.372–0.441	0.250 0.218–0.287	0.143 0.115–0.178
12	Soft	8.5	3.30 2.36–4.62	1.23 0.957–1.58	0.900 0.833–0.972	0.350 0.321–0.382	0.189 0.158–0.226
12	Soft	9.5	>3.00	1.65 1.39–1.96	1.00 0.901–1.11	0.308 0.274–0.346	0.200 0.164–0.244

reversed after 24 h, however, and at 96 h chlorine was most toxic in the coldest water—probably because chlorine is more residual at low than at high temperatures.

Water hardness at a constant pH of 8.0 (maintained by adding equal amounts of bicarbonate to water at each level of hardness) did not affect the toxicity of chlorine to rainbow trout (Table 2). Although chlorine toxicity was influenced little by pH, the general trend was toward decreasing toxicity with increasing pH.

Detoxification of Antimycin

Green sunfish were exposed to antimycin, chlorine, and a mixture of antimycin and chlorine to determine the detoxification efficiency of chlorine. Detoxification was represented by a deactivation index, which is the quotient of the LC$_{50}$ for antimycin and chlorine in aged and in fresh solutions. For example, the deactivation index, at pH 7.5 after 1 h of interaction time, was 11.5/6.90, or 1.67 (Table 3). The time required for the deactivation index to reach 2.0—indicating that the toxicity has been decreased by one-half—coincides with the half-life of the toxicant. The half-life of antimycin in combination with chlorine at pH 7.5 was estimated at 1.3 h by plotting the deactivation indices against time on

semilogarithmic coordinates (Fig. 1). Similar curves prepared for the data at other pH's yielded estimated half-lives of 1.1 h at pH 6.5 and 1.5 h at pH's 8.5 and 9.5.
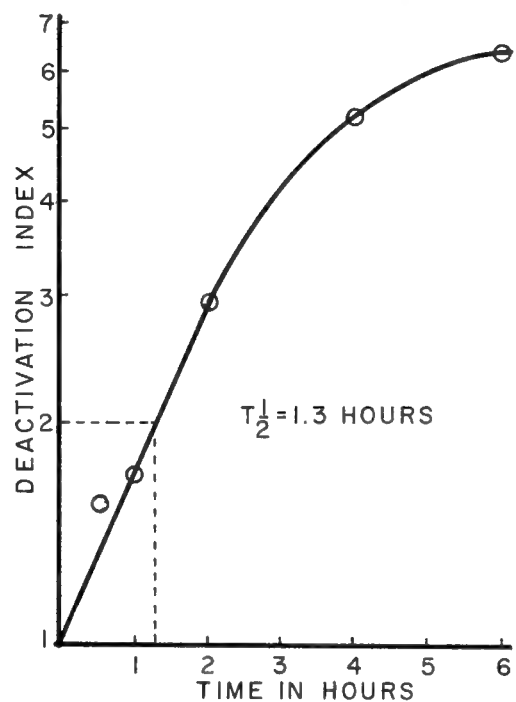
Fig. 1. Half-life (T½) curve for antimycin and 0.5 mg/l of chlorine in soft water at pH 7.5 and 12 C.

Table 3. *Toxicity and deactivation of antimycin in static tests at 12 C with green sunfish in water containing 0.25 mg/l of chlorine at pH 6.5 and 0.50 mg/l of chlorine at pH's 7.5, 8.5, and 9.5.*

Compound, and interaction time[a] of chlorine and antimycin (h)	96-h LC_{50}, 95% confidence interval, and (in parentheses) deactivation index at			
	pH 6.5	pH 7.5	pH 8.5	pH 9.5
Chlorine (mg/l)	0.745 0.623–0.891	0.949 0.837–1.08	0.840 0.688–1.03	0.715 0.578–0.885
Antimycin (μg/l)	0.107 0.084–0.136	0.164 0.138–0.194	0.640 0.539–0.761	16.2 14.1–18.6
Antimycin (μg/l) plus chlorine				
0	0.779 0.644–0.942 (1.00)	6.90 4.69–10.1 (1.00)	5.19 4.44–6.07 (1.00)	4.45 2.93–6.76 (1.00)
0.5	1.72 1.23–2.41 (2.21)	10.7 8.73–13.1 (1.55)	6.00 4.99–7.22 (1.16)	7.09 5.47–9.18 (1.59)
1.0	1.39 1.03–1.87 (1.78)	11.5 8.64–15.1 (1.67)	9.00 7.40–10.9 (1.73)	8.69 6.86–11.0 (1.95)
2.0	2.87 2.15–3.84 (3.68)	20.0 16.9–23.7 (2.90)	13.3 — (2.56)	7.05 5.29–9.39 (1.58)
4.0	4.28 3.38–5.41 (5.49)	36.0 30.0–43.2 (5.22)	15.2 11.6–19.9 (2.93)	15.0 11.3–19.9 (3.37)
6.0	8.51 7.36–9.83 (10.9)	44.8 — (6.49)	25.5 20.6–31.5 (4.91)	23.4 18.5–29.5 (5.26)
8.0	7.60 7.05–8.19 (9.76)	— — —	34.5 29.9–40.0 (6.65)	28.0 25.4–30.8 (6.29)

[a] Time antimycin and chlorine were in solution before fish were introduced.

Toxicity of antimycin decreased significantly at successively higher pH's; the 96-h LC_{50}'s ranged from 0.107 μg/l at pH 6.5 to 16.2 μg/l at pH 9.5 (Table 3). The drop was particularly sharp between pH's 8.5 and 9.5—as previously observed by Marking and Dawson (1972). As in the earlier tests, the toxicity of chlorine was affected little by pH.

The toxicity of antimycin decreased significantly immediately after chlorine was added to the solution at pH 6.5, 7.5, and 8.5—with no interaction time (Table 3). For example, at pH 7.5 the 96-h LC_{50} for antimycin was 0.164 μg/l and that for antimycin plus 0.5 mg/l of chlorine was 6.90 μg/l. The difference was perhaps due to the time required for antimycin to produce a lethal effect, commonly called the effective exposure time (Gilderhus 1972). Therefore the biological measure of concentrations

remaining after aging was delayed by the latent response of fish.

Toxicity to fish increased when chlorine was added to the antimycin solution at pH 9.5 (Table 3). The 96-h LC_{50} was 16.2 μg/l for antimycin alone, and 4.45 μg/l after the addition of 0.5 mg/l of chlorine. Most likely the 0.5 mg/l of chlorine contributed toxicity, rather than detoxifying the antimycin, and its effect was additive rather than antagonistic (Marking and Dawson 1975). As the interaction time increased, however, antimycin was detoxified; its half-life was estimated to be 1.5 h.

Marking and Dawson (1972) demonstrated that antimycin detoxifies in water without the addition of chemical detoxifiers (half-lives for antimycin alone ranged from 310 h at pH 6.5 to 1.5 h at pH 10.0); however, the chemicals greatly increase the rate of

detoxification. Although chlorine detoxifies antimycin much less rapidly than does potassium permanganate (Marking and Bills 1975), it destroys the biological activity of antimycin within a suitably short time.

The detoxification of antimycin without detoxifiers followed a first order decay curve; with detoxifiers, however, the curve was nonlinear. The nonlinearity results from loss of chlorine during the interaction time due to reactions such as reduction, volatilization, and adsorption. The initial detoxification rate is most important, however, because antimycin is generally nontoxic after a time equal to one or two of the initial half-life periods.

When chlorine concentrations were monitored for 96 h to ascertain the dissipation rate during a typical toxicity test, the concentrations measured (Taras et al. 1971) in solutions containing fish were significantly lower than those measured in solutions without fish (Table 4). Little, if any, chlorine remained after 24 h in the fish assays.

Table 4. *Dissipation of chlorine (calcium hypochlorite, 70% active chlorine) in water without fish and in water with green sunfish (0.75 g/l) in soft water at 12 C.*

Time after addition (h)	Chlorine (mg/l)			
	Without fish		With fish	
	0.5	1.0	0.5	1.0
0	0.525	1.075	0.275	0.725
24	0.350	0.825	0.050	0.075
48	0.350	0.925	<0.01	0.025
72	0.325	0.975	<0.01	<0.01
96	0.300	0.925	<0.01	<0.01

Conclusions

1. The 96-h LC_{50}'s for chlorine at pH 7.5 ranged from 0.156 mg/l for channel catfish to 1.41 mg/l for black bullheads.

2. Toxicity of chlorine to fish was influenced little by pH.

3. Toxicity of antimycin to green sunfish decreased as pH increased; the 96-h LC_{50}'s ranged from 0.107 μg/l at pH 6.5 to 16.2 μg/l at pH 9.5.

4. Chlorine effectively detoxified antimycin. Half-lives of antimycin with chlorine ranged from 1.1 h at pH 6.5 to 1.5 h at pH 9.5.

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Malachite Green: Its Toxicity to Aquatic Organisms, Persistence and Removal with Activated Carbon

by

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Abstract

The acute toxicity of malachite green was determined in standardized laboratory tests for chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout *(S. gairdneri)*, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass *(Micropterus salmoides)*, smallmouth bass *(M. dolomieui)*, bluegill *(Lepomis macrochirus)*, snails *(Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods *(Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies *(Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads *(Bufo* sp.). Bluegills were the most sensitive (96-h LC_{50}, 0.0305 mg/l), and coho salmon the most resistant (0.383 mg/l). The $TILC_{50}$ (lethal concentration producing 50% mortality independent of time) for rainbow trout was 0.0998 mg/l. The responses of frog and toad larvae (96-h LC_{50}, 0.173 and 0.0680 mg/l) were similar to those of fish, whereas adult newts were more resistant (1.03 mg/l). The invertebrates exposed were generally more resistant than the fish and amphibians; the 96-h LC_{50}'s ranged from 0.510 to 3.45 mg/l, except for the Asiatic clam, which was extremely resistant (122 mg/l), and the mayfly naiad, which was very sensitive (0.0790 mg/l). The toxicity of malachite green to fish was not affected by water hardness or pH, except bluegills, in which toxicity was about half as great at pH 6.5 as at pH 7.5 to 9.5, and was increased only slightly by increases in water temperatures. Malachite green was very persistent in aqueous solutions; it did not detoxify after 3 weeks of aging in glass containers. The chemical is readily absorbed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C) by filtration through activated carbon; the capacity was 23.4 mg of malachite green per gram of carbon.

Malachite green has been used in fish culture as a fungicide and parasiticide for about 40 years. It was first used as a dip treatment by Foster and Woodbury (1936) to treat fungal infections of four species of trout and largemouth bass *(Micropterus salmoides)*. More recently it has been used in combination with formalin to treat *Ichthyophthirius*, a serious parasite of catfishes (Leteux and Meyer 1972).

Although the use of malachite green as a therapeutant in fish culture has many advantages, it also poses various potential problems (Nelson 1974): toxicity to fishes (Willford 1967); possible teratogenic and mutagenic effects (Lieder 1961; Nelson 1974; T.D. Bills and L.L. Marking, in preparation); and stress induced during and after the treatment of fry of certain fishes (Glagoleva and Malikova 1968; Bills and Hunn 1976).

Malachite green is not registered for aquatic use by either the Food and Drug Administration or Environmental Protection Agency, because information required for registration—toxicity, efficacy, residues, metabolites, and counteraction—is incomplete. The purpose of the present study was to contribute laboratory data on (1) the toxicity of malachite green to nontarget aquatic organisms; (2) its toxicity to rainbow trout *(Salmo gairdneri)* and bluegills *(Lepomis macrochirus)* in extended exposures; (3) the effects of certain water characteristics on its toxicity to fish; (4) its persistence in water; and (5) its possible removal from water with activated carbon.

Materials and Methods

Concentrated stock solutions of commercial grade zinc-free malachite green (4-[P-(dimethylamino)-α-phenylbenzylidene]-2,5-cyclohexadien-1-ylidene dimethyl-ammonium chloride) manufactured by MCB Manufacturing Chemists, Norwood, Ohio, were prepared by mixing weighed portions with water. To prepare test solutions of the desired concentrations, we pipetted portions of stock solutions into test vessels and stirred the resulting mixture to ensure homogeneity. In flow-through toxicity tests, the required amounts of the stock solution were delivered by a solenoid-activated pipette pump (Micromedic Systems Automatic Pipette Model 2500).

Tests were conducted according to the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the protocol described by Marking (1975). Glass jars of 3.78 or 18.9 liters were used, depending on the size of the test organism. Reconstituted water was used in tests with fish (Marking 1969), and limed spring water (pH, 7.5 ± 0.1; total hardness, 20 mg/l as $CaCO_3$) in the tests with amphibians and invertebrates. Chemical buffers were added to soft water to adjust the pH (6.5–9.5), as described by Marking and Dawson (1973).

Flow-through tests were conducted in a proportional diluter similar to that of Mount and Brungs (1967). Test vessels were 45-liter glass aquariums supplied with a flow sufficient to replace the entire volume at least four times daily. Carbon-filtered, municipal well water (total hardness 300 mg/l, pH 7.5) was used in the flow-through system. Temperature was maintained by immersing test vessels in a water bath equipped with a chilling unit.

Fish species exposed were chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass, smallmouth bass *(Micropterus dolomieui)*, and bluegills. Test fishes weighed 0.5 to 1.5 g. Other aquatic organisms exposed were snails *(Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods *(Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies *(Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads *(Bufo* sp.).

In tests for the determination of persistence of malachite green, aqueous solutions were aged for 1, 2, and 3 weeks in glass containers. Rainbow trout were introduced concurrently to these and a freshly prepared reference solution for comparison of mortality. Deactivation indices were computed from these data according to the method of Marking (1972).

We used the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s and 95% confidence intervals, and a modification of the method published by Green (1965) for computing $TILC_{50}$'s (lethal) concentration producing 50% mortality independent of time).

To determine if malachite green could be removed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C), we filtered a concentrated solution (2 mg/l) at a flow rate of 100 ml/min through a glass 2.7 cm ID column containing 15 cm (35.5 g dry weight) of activated carbon (Darco 20 × 40 mesh). Samples were taken periodically and concentrations in the effluent determined colorimetrically (620 nm). The carbon bed was considered saturated when the concentration in the effluent reached 10% of that in the original stock solution (0.2 mg/l). The capacity of activated carbon for the chemical was determined by the following formula:

$$\text{Milligrams of malachite green adsorbed per gram of carbon} = \frac{\text{Concentration (mg/l)} \times \text{liters passed through filter}}{\text{Grams of carbon (dry weight)}}$$

Results

Toxicity to Fish

Malachite green was toxic to all species of fish exposed; LC_{50}'s ranged from 0.0305 to 0.383 mg/l in 96-h exposures in soft water at 12 C (Table 1). Centrarchids were 1.5 to 3.5 times more sensitive to the chemical than the ictalurids and 3 to 7 times more sensitive than the salmonids. The bluegill was the most sensitive species (96-h LC_{50}, 0.0305 mg/l) and the coho salmon the most resistant (0.383 mg/l). The toxicity of the chemical increased as exposures lengthened in all species; for bluegills the LC_{50} was 6.00 mg/l at 3 h and 0.0305 mg/l at 96 h.

Toxicity to Other Aquatic Organisms

In 96-h exposures, the LC_{50}'s for malachite green to frog larvae (0.173 mg/l) and toad larvae (0.0680 mg/l) were similar to those for fish (Table 2). Adult newts were more resistant than frog or toad larvae (96-h LC_{50}, 1.03 mg/l), but about equally or less resistant than most of the invertebrates exposed. Mayfly naiads were the most sensitive invertebrate

Table 1. *Toxicity of malachite green to fingerling fish in soft water at 12 C.*

Species	LC$_{50}$ and 95% confidence interval (mg/l) at			
	3 h	6 h	24 h	96 h
Chinook salmon	1.72	1.38	0.292	0.224
(*Oncorhynchus tshawytscha*)	1.22–2.42	1.04–1.82	0.245–0.348	0.209–0.240
Coho salmon	—	>3.00	0.569	0.383
(*O. kisutch*)	—		0.486–0.662	0.327–0.449
Atlantic salmon	3.56	1.09	0.497	0.283
(*Salmo salar*)	2.77–4.58	0.929–1.28	0.415–0.595	0.229–0.350
Brown trout	1.73	1.27	0.352	0.237
(*S. trutta*)	1.23–2.43	0.991–1.63	0.280–0.443	0.209–0.268
Rainbow trout	1.41	0.760	0.360	0.248
(*S. gairdneri*)	1.14–1.74	0.649–0.890	0.305–0.425	0.193–0.319
Brook trout	3.00	1.44	0.300	0.220
(*Salvelinus fontinalis*)	2.06–4.37	1.05–1.98	0.259–0.348	0.188–0.257
Channel catfish	>3.00	1.10	0.181	0.112
(*Ictalurus punctatus*)		0.904–1.34	0.123–0.266	0.0893–0.140
Largemouth bass	—	—	0.282	0.0728
(*Micropterus salmoides*)	—	—	0.211–0.376	0.0604–0.0877
Smallmouth bass	1.36	—	0.154	0.0453
(*M. dolomieui*)	1.09–1.70	—	0.117–0.202	0.0366–0.0561
Bluegill	6.00	2.19	0.231	0.0305
(*Lepomis macrochirus*)	4.41–8.17	1.66–2.89	0.184–0.290	0.0218–0.0427

Table 2. *Toxicity of malachite green to selected nontarget aquatic organisms in limed water at 16 C.*

Organism	LC$_{50}$ and 95% confidence interval (mg/l) at		
	6 h	24 h	96 h
Snail	—	—	0.720
(*Pleurocera* sp.)	—	—	0.483–1.07
Asiatic clam	—	—	122
(*Corbicula leana*)	—	—	93.8–159
Ostracod	5.85	5.85	3.45
(*Cypridopsis* sp.)	4.00–8.57	4.29–7.97	2.49–4.80
Freshwater prawn	—	9.10	1.90
(*Palaemonetes kadiakensis*)	—	7.29–11.3	1.76–2.06
Midge (larvae)	5.00	1.00	0.510
(*Tanytarsus dissimilis*)	3.13–7.99	0.636–1.57	0.295–1.10
Mayfly naiads	5.75	2.75	0.0790
(*Callibaetis* sp.)	4.95–6.69	2.07–3.65	0.0442–0.141
Newts (adult)	—	3.90	1.03
(*Notophthalmus viridiscens*)	—	3.47–4.38	0.672–1.58
Leopard frog (larvae)	1.00	0.380	0.173
(*Rana pipiens*)	0.875–1.14	0.351–0.412	0.149–0.200
Toad (larvae)	1.70	0.355	0.0680
(*Bufo* sp.)	1.54–1.87	0.235–0.276	0.0530–0.0860

exposed (96-h LC_{50}, 0.0790 mg/l), and the Asiatic clam was by far the most resistant to the chemical; it tolerated concentrations in excess of 100 mg/l. The other invertebrates exposed were more resistant than fish or amphibians, but less resistant than the Asiatic clam. The 96-h LC_{50}'s for these organisms were between 0.510 and 3.45 mg/l.

Effects of Temperature, Water Hardness, and pH on Toxicity

In short exposures of 3 or 6 h, malachite green was more toxic to rainbow trout, channel catfish, and bluegills in warm water (17 and 22 C) than in cool water (7 and 12 C), but at 96 h the LC_{50}'s at different temperatures were not significantly different, except for channel catfish (Tables 3, 4, 5). Neither water hardness nor pH influenced the toxicity of the chemical to any species except bluegills, in which toxicity was about half as great as pH 6.5 as at pH 7.5 to 9.5.

Chronic Toxicity

Rainbow trout and bluegills were exposed simultaneously to the chemical in a flow-through toxicity test to determine the $TILC_{50}$. Mortality increased with time in both species. The 24-h LC_{50} for bluegills was 0.151 mg/l. A $TILC_{50}$ could not be calculated because mortality continued until all organisms succumbed at the lowest concentration (0.0316 mg/l) after 16 days of exposure. The 24-h LC_{50} was 0.230 mg/l for rainbow trout, and mortality continued through 30 days. A $TILC_{50}$ of 0.0998 mg/l was determined after 36 days of exposure.

Persistence of Malachite Green in Water

Bioassays with rainbow trout of aqueous solutions of malachite green aged for 1, 2, and 3 weeks in glass jars indicated no significant loss of activity. The LC_{50} was 0.173 mg/l for the freshly prepared reference solution and 0.179 mg/l for the solution aged for 3 weeks.

Table 3. *Toxicity of malachite green to fingerling rainbow trout at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at			
			3 h	6 h	24 h	96 h
7	Soft	7.5	>2.00	2.30 1.71–3.10	0.400 0.330–0.486	0.168 0.137–0.206
12	Soft	7.5	1.41 1.14–1.74	0.760 0.649–0.890	0.360 0.305–0.425	0.248 0.193–0.319
17	Soft	7.5	1.42 1.15–1.76	0.567 0.517–0.621	0.569 0.516–0.627	0.284 0.229–0.353
12	Very soft	8.0	2.00 1.55–2.58	0.780 0.726–0.838	0.362 0.307–0.426	0.286 0.230–0.355
12	Soft	8.0	2.31 1.72–3.10	0.800 0.659–0.971	0.280 0.226–0.347	0.234 0.179–0.305
12	Hard	8.0	2.30 1.43–3.71	1.40 1.13–1.73	0.345 0.296–0.403	0.288 0.233–0.356
12	Very hard	8.0	2.35 1.74–3.17	0.820 0.701–0.959	0.280 0.226–0.347	0.249 0.195–0.318
12	Soft	6.5	>2.00	1.01 0.764–1.34	0.279 0.207–0.375	0.280 0.227–0.345
12	Soft	8.5	2.60 1.86–3.63	0.980 0.851–1.13	0.284 0.229–0.351	0.212 0.172–0.262
12	Soft	9.5	>2.00	1.26 0.978–1.62	0.367 0.311–0.434	0.173 0.136–0.220

Table 4. *Toxicity of malachite green to fingerling channel catfish at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (mg/l at		
			6 h	24 h	96 h
12	Soft	7.5	1.10 0.904–1.34	0.181 0.123–0.266	0.112 0.0893–0.140
17	Soft	7.5	0.552 0.499–0.610	0.222 0.168–0.293	0.0940 0.0860–0.103
22	Soft	7.5	0.400 0.331–0.483	0.0691 0.0576–0.0831	0.0535 0.0442–0.0647
12	Very soft	8.0	0.600 0.440–0.818	0.106 0.0935–0.120	0.0750 0.0555–0.101
12	Soft	8.0	1.30 1.01–1.67	0.285 0.232–0.350	0.117 0.0972–0.140
12	Hard	8.0	1.72 1.23–2.41	0.284 0.229–0.351	0.142 0.115–0.176
12	Very hard	8.0	1.71 1.22–2.40	0.286 0.232–0.353	0.142 0.115–0.176
12	Soft	6.5	0.960 0.717–1.29	0.236 0.181–0.308	0.0975 0.0937–0.101
12	Soft	8.5	0.835 0.665–1.05	0.835 0.665–1.05	0.237 0.182–0.309
12	Soft	9.5	0.519 0.377–0.714	0.191 0.155–0.236	0.162 0.135–0.194

Table 5. *Toxicity of malachite green to fingerling bluegill at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (mg/l) at			
			3 h	6 h	24 h	96 h
12	Soft	7.5	6.00 4.41–8.17	2.19 1.66–2.89	0.231 0.184–0.290	0.0305 0.0218–0.0427
17	Soft	7.5	2.17 1.63–2.88	0.656 0.584–0.737	0.0920 0.0663–0.128	0.0340 0.0242–0.0477
22	Soft	7.5	0.860 0.737–1.00	0.238 0.184–0.308	0.0780 0.0594–0.102	0.0308 0.0221–0.0430
12	Very soft	8.0	2.30 1.72–3.08	2.00 1.54–2.59	0.117 0.0967–0.142	0.0413 0.0343–0.0497
12	Soft	8.0	>2.00	1.52 1.15–2.00	0.122 0.100–0.149	0.0400 0.0330–0.0486
12	Hard	8.0	>2.00	1.41 1.14–1.74	0.141 0.114–0.174	0.0450 0.0384–0.0528
12	Very hard	8.0	>2.00	1.42 1.10–1.83	0.141 0.114–0.174	0.0440 0.0370–0.0523
12	Soft	6.5	7.43 5.76–9.59	2.18 1.64–2.90	0.282 0.219–0.394	0.0780 0.0594–0.102
12	Soft	8.5	4.68 3.77–5.80	2.18 1.64–2.89	0.123 0.0955–0.158	0.0339 0.0241–0.0476
12	Soft	9.5	3.70 2.81–4.87	2.20 1.67–2.89	0.0810 0.0562–0.117	0.0340 0.0242–0.0477

Counteraction with Activated Carbon

Aqueous solutions of malachite green (2.0 mg/l) were filtered through a bed of activated carbon. In three runs the activated carbon adsorbed the chemical from 420, 401, and 425 liters of solution before the endpoint was reached (0.2 mg/l), an average of 23.4 mg of malachite green per gram of carbon. Activated carbon thus is an excellent means for removing this chemical from water.

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Toxicity of Furanace to Fish, Aquatic Invertebrates, and Frog Eggs and Larvae

by

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Abstract

Furanace (6-hydroxymethyl-2[2-(5-nitro-2-furyl)vinyl]pyridine), a nitrofuran originally designated as furpirinol, nifurpirinol, or P-7138, is effective against certain bacterial infections in fish, especially myxobacteriosis. The toxicity of the drug to fish, frog eggs and larvae, and aquatic invertebrates was determined in standardized laboratory toxicity tests and in use pattern exposures. Additional tests were conducted in aged solutions of Furanace to determine the persistence in water. This research was done to broaden existing toxicity data and to help fulfill drug registration requirements. Furanace was not toxic to any test species in use pattern exposures of 1 mg/l for 1 h daily for up to three treatments. In 96-h exposures, LC_{50}'s ranged from 0.820 to 3.00 mg/l for six species of fish and from 1.13 to 20 mg/l for six species of invertebrates, and was 0.770 mg/l for larvae of the leopard frog *(Rana pipiens)*. Toxicity increased with elevated temperatures in tests with rainbow trout *(Salmo gairdneri)*, channel catfish *(Ictalurus punctatus)*, and green sunfish *(Lepomis cyanellus)*. Increased water hardness and pH decreased toxicity to rainbow trout, but did not influence toxicity to channel catfish and green sunfish.

Furanace (6-hydroxymethyl-2[2-(5-nitro-2-furyl)vinyl]pyridine), a nitrofuran originally designated as P-7138, furpirinol, or nifurpirinol, has proved to be therapeutic against certain fish diseases. It was developed as a fish bactericide by Dainippon Pharmaceutical Co., Ltd. in Japan (Shimizu and Takase 1967) and was tested further against fish diseases in the United States (Amend and Ross 1970; Ross 1972). A review of literature on the drug was provided by Herman (1974). U.S. studies were conducted with the intention of registering the drug for fishery use as outlined by Lennon (1967).

Amend and Ross (1970) demonstrated that experimentally induced columnaris disease in coho salmon *(Oncorhynchus kisutch)* can be controlled with Furanace, that the drug is readily absorbed and eliminated, and that it is nontoxic to juvenile salmon. Additional studies by Amend (1972) showed that furunculosis in coho salmon was partially controlled by 8 or 10 mg/l of Furanace with two daily 1-h baths, but not by feeding the drug because fish refused to eat the medicated feed. He also found that one or two 1-h treatments of 1 mg/l of Furanace in static baths resulted in excellent control of myxobacteriosis and that the drug had low toxicity to fish and posed no residue problems 9 days after the last treatment.

We expanded the information on Furanace toxicity by (1) including more species of fish and additional test conditions, (2) including six species of aquatic invertebrates, and frog eggs and larvae, (3) determining the toxicity of use pattern exposures, and (4) determining the persistence of Furanace in water. The sequence of test procedures and conditions were standardized as suggested in the toxicological protocol of Marking (1975).

Materials and Methods

Purified Furanace, furnished by Abbott Laboratories, North Chicago, Ill., was dissolved in dimethylformamide or acetone to prepare stock solutions, portions of which were then added to static test chambers to yield desired concentrations. Toxicity was calculated on the basis of 100% activity for the added quantity of drug.

The static test procedures were those recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Ten fish were exposed to each concentration in glass jars containing 15 liters of oxygenated, reconstituted water prepared from deionized water (Marking 1969). Test waters were of

1

four different total hardnesses (mg/l as $CaCO_3$ in parentheses): very soft (10), soft (44), hard (170), and very hard (300). In separate tests, chemically buffered solutions (Marking 1975) were used to assess the effects of pH on toxicity. Test temperatures were regulated by immersing the test jars in constant temperature water baths.

Fish weighing 1 to 1.5 g each were obtained from National fish hatcheries and maintained according to standardized procedures of the Fish Control Laboratory (Hunn et al. 1968). Fish were acclimated to the test conditions before they were exposed to the drug. Mortalities were recorded at 1, 3, and 6 h on the first day of exposure and daily thereafter for the remainder of the test. Scientific names of the fishes are listed in Table 1.

Six species of invertebrates (scientific names listed later in Table 5) and eggs and larvae of the leopard frog *(Rana pipiens)* were collected from the wild. These organisms were exposed to Furanace in a manner similar to that for exposing fish except that exposures were in 3 liters of limed spring water (21 mg/l of total hardness) in glass jars. Frog eggs (2- to 8-cell stage) were placed in concentrations of Furanace ranging from 0.01 to 5.0 mg/l and allowed to complete their development. After 7 days in the test vessels, the various stages of development were assessed. Invertebrates and frog larvae were exposed for 96 h to Furanace concentrations ranging from 0.1 to 20 mg/l or to the use pattern concentration of 1.0 mg/l. Higher concentrations of Furanace were not used because such solutions approached saturation and often precipitated. The observation period was extended to 60 days for a group of clams that were

exposed to Furanace for 4 and 24 h and then placed in floating cages in a pond.

Computations of LC_{50}'s (concentrations calculated to produce 50% mortality) and 95% confidence intervals were computed according to the methods of Litchfield and Wilcoxon (1949). All data reported fulfilled the chi-square test requirement for acceptability. LC_{50}'s were considered significantly different when their confidence intervals did not overlap.

Residues of Furanace in water were determined by Abbott Laboratories, North Chicago, Ill., by gas chromatography (R.E. Crutcher and J. T. Manneback, in preparation).

Results

Toxicity to Fish

The toxicity of Furanace was not significantly different in 24-h exposures at 12 C to Atlantic salmon, rainbow trout, fathead minnows, and channel catfish; the 24-h LC_{50}'s ranged only from 4.27 to 4.50 mg/l (Table 1). The 96-h LC_{50}'s for green sunfish and the 96-h LC_{50} for bluegills were significantly greater than those for the other species.

Table 1. *Toxicity of Furanace to selected species of fish in soft water at 12 C.*

Species	LC$_{50}$ and 95% confidence interval (mg/l) at	
	24 h	96 h
Atlantic salmon *(Salmo salar)*	4.50 3.84–5.28	1.41 1.14–1.74
Rainbow trout *(Salmo gairdneri)*	4.32 3.64–5.13	1.00 0.824–1.21
Fathead minnow *(Pimephales promelas)*	4.39 3.96–4.87	0.820 0.717–0.938
Channel catfish *(Ictalurus punctatus)*	4.27 3.40–5.36	1.07 0.854–1.34
Green sunfish *(Lepomis cyanellus)*	6.60 6.16–7.07	2.48 2.07–2.97
Bluegill *(Lepomis macrochirus)*	— —	3.00 2.45–3.68

Table 2. *Toxicity of Furanace to rainbow trout at selected water temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (mg/l) at	
			24 h	96 h
7	Soft	7.5	9.60 8.02–11.5	1.73 1.23–2.44
12	Soft	7.5	4.32 3.64–5.13	1.00 0.824–1.21
17	Soft	7.5	3.69 3.11–4.38	0.795 0.661–0.956
12	Very soft	8.0	2.45 1.90–3.16	0.569 0.450–0.719
12	Soft	8.0	2.83 2.29–3.50	0.618 0.490–0.779
12	Hard	8.0	2.82 2.28–3.99	0.760 0.615–0.939
12	Very hard	8.0	2.82 2.28–3.49	1.18 0.913–1.52
12	Soft	6.5	3.28 2.76–3.90	0.489 0.432–0.554
12	Soft	8.5	3.82 3.20–4.55	0.800 0.696–0.917
12	Soft	9.5	3.29 2.77–3.90	1.42 1.05–1.92

Elevated temperatures increased the toxicity of Furanace to some species. The LC$_{50}$'s were significantly different at the lowest and highest water temperatures at which rainbow trout and channel catfish were exposed (Tables 2 and 3).

Furanace was significantly less toxic to rainbow trout at 96 h in very hard than in soft or very soft water (Table 2). However, the toxicity to channel catfish and green sunfish at 96 h was not significantly different at the four different water hardnesses (Tables 3 and 4).

Toxicity of Furanace to rainbow trout was influenced by pH in 96-h exposures, and the drug was more toxic at pH 6.5 than at pH 8.5 and at pH 8.5 than at pH 9.5 (Table 2). However, toxicity of Furanace at 96 h to channel catfish and green sunfish was not affected by pH (Tables 3 and 4).

Toxicity of Use Pattern Exposures to Fish

The suggested use pattern of Furanace as a fish therapeutant is a 1-h exposure daily to a 1-mg/l solution for up to 3 consecutive days, as necessary. Accordingly, rainbow trout were exposed to concentrations ranging from 0 to 3.0 mg/l of Furanace for 3 consecutive days and then observed for 10 additional days. No mortality occurred and no stress was observed during the 13-day period at any of the exposure concentrations.

Exposure of leopard frog larvae to concentrations of 0.2 to 20 mg/l of Furanace for 3 consecutive days resulted in no mortality after 7 days. Many of the larvae were immobilized in the 10- and 20-mg/l concentrations during the exposure, but they recovered when placed in fresh water. Furanace in the use pattern exposure was nontoxic to fish, frog larvae, and aquatic invertebrates.

Toxicity to Invertebrates

Invertebrates were more resistant than fish to Furanace; the 96-h LC$_{50}$'s ranged from 1.13 mg/l for snails to more than 20 mg/l for water fleas, freshwater prawns, and backswimmers (Table 5).

Asiatic clams that were exposed for 4 or 24 h and placed in floating cages in a pond also survived concentrations of Furanace greater than the therapeutant treatment level. The 4-h exposure produced less mortality than the 24-h exposure. Mortality from most of the exposures increased during the 60-day holding period. However, there was some survival among those exposed to concentrations as high as 20 mg/l.

Table 3. *Toxicity of Furanace to channel catfish at selected water temperatures, hardnesses, and pH's.*

| Temp (°C) | Water hardness | pH | LC$_{50}$ and 95% confidence interval (mg/l) at | | |
			6 h	24 h	96 h
12	Soft	7.5	32.6 23.4–45.5	6.19 5.78–6.63	1.72 1.48–2.00
17	Soft	7.5	— —	5.25 4.48–6.15	1.90 1.55–2.33
22	Soft	7.5	14.2 11.5–17.6	2.90 2.41–3.49	1.22 1.05–1.42
12	Very soft	8.0	27.6 22.5–33.9	2.90 2.62–3.21	0.945 0.791–1.13
12	Soft	8.0	24.6 20.6–29.4	4.00 3.59–4.46	1.00 0.821–1.22
12	Hard	8.0	24.6 20.6–29.4	4.00 3.57–4.48	0.960 0.784–1.18
12	Very hard	8.0	22.8 18.2–28.6	4.38 3.96–4.84	0.969 0.810–1.16
12	Soft	6.5	— —	8.25 7.76–8.77	1.82 1.62–2.05
12	Soft	8.5	— —	7.59 7.20–8.01	1.80 1.60–2.03
12	Soft	9.5	— —	6.55 5.95–7.21	1.42 1.15–1.76

Table 4. *Toxicity of Furanace to green sunfish at selected water temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (mg/l) at	
			24 h	96 h
12	Soft	7.5	6.30 5.55–7.16	2.42 2.04–2.86
17	Soft	7.5	5.88 5.29–6.54	2.00 1.59–2.52
22	Soft	7.5	4.25 3.84–4.70	1.42 0.980–2.04
12	Very soft	8.0	7.00 6.10–8.04	2.44 2.06–2.89
12	Soft	8.0	6.28 5.88–6.71	2.20 1.79–2.70
12	Hard	8.0	6.60 6.16–7.07	2.48 2.07–2.97
12	Very hard	8.0	7.85 7.32–8.42	2.50 2.20–2.84
12	Soft	6.5	6.00 5.38–6.69	1.73 1.23–2.43
12	Soft	8.5	7.00 6.71–7.31	2.00 1.10–3.63
12	Soft	9.5	4.64 3.53–6.09	2.00 1.08–3.69

Effects of Furanace on Frog Eggs and Larvae

Fertilized eggs at the 2- to 8-cell stage were exposed to Furanace at concentrations of 0.2 to 20 mg/l for 1 h on 3 consecutive days and then held in fresh water. After 7 days, there was no apparent effect of 0.2 to 4.0 mg/l of Furanace on the development of embryos. At concentrations of 6.0 to 20 mg/l, however, fewer embryos survived; some developed a head and tail and then died, and others appeared to develop normally but failed to escape the egg mass.

Frog larvae were considerably more sensitive to Furanace than were frog eggs, invertebrates, or fish. The 96-h LC$_{50}$ was 0.770 mg/l in limed water at 16 C (Table 5).

Persistence of Furanace in Water

The persistence of Furanace in water was measured biologically by determining the toxicity to rainbow trout of solutions that had been aged for periods as long as 5 weeks. Additionally, Furanace residues in the aged solutions were determined analytically. Aging for 5 weeks reduced the toxicity of Furanace (i.e., increased the 96-h LC$_{50}$) by about 50% (Table 6). The same waters showed a similar loss of drug as determined by gas chromatographic analysis. Therefore the half-life of biological activity corresponded with the half-life of chemical integrity, i.e., about 5 weeks (Table 7).

Table 5. *Toxicity of Furanace to selected species of aquatic invertebrates and frog larvae in limed water at 16 C.*

Species	LC$_{50}$ and 95% confidence interval (mg/l) at	
	24 h	96 h
Snail (*Physa* sp.)	8.00 6.98–9.17	1.13 0.860–1.47
Asiatic clam (*Corbicula leana*)	>20.0	11.6 8.75–15.4
Water flea (*Daphnia magna*)	>20.0	>20.0
Amphipods (*Hyalella azteca*)	16.0 11.3–22.7	13.6 9.43–19.6
Freshwater prawn (*Palaemonetes kadiakensis*)	>20.0	>20.0
Backswimmer (*Notonecta* sp.)	>20.0	>20.0
Leopard frog (larva) (*Rana pipiens*)	6.90 5.55–8.57	0.770 0.590–1.01

Table 6.   *Deactivation of Furanace in soft water at 12 C, as determined by changes in 96-h LC$_{50}$'s of rainbow trout.*

Aging period (weeks)	96-h LC$_{50}$'s (mg/l) and 95% confidence interval	Deactivation index[a]
0	0.705 0.570–0.872	1.0
1	0.810 0.677–0.969	1.1
2	0.900 0.763–1.06	1.3
3	0.900 0.763–1.06	1.3
4	1.33 1.19–1.48	1.9
5	1.46 1.31–1.62	2.1

[a] Deactivation index $= \dfrac{\text{LC}_{50} \text{ of aged solution}}{\text{LC}_{50} \text{ of fresh solution}}$

Table 7.   *Concentrations of Furanace (mg/l) detected by colorimetry and gas chromatography in water solutions aged for 0 to 5 weeks.*

Concentration before aging (calculated)	Aging period (weeks)	Concentration after aging	
		Colorimetric	GC–eC[a]
1.0	0	1.0	1.15
3.0	0	3.11	2.56
1.0	1	0.67	0.654
3.0	1	2.89	1.82
1.0	2	0.67	0.602
3.0	2	2.89	0.807
1.0	3	0.78	0.575
3.0	3	2.22	2.52
1.0	4	0.56	0.466
3.0	4	2.56	1.74
1.0	5	0.78	0.485
3.0	5	2.50	1.58

[a] Gas chromatography-electron capture—average of two samples.

Discussion and Conclusions

The toxicity of Furanace was relatively low to fish and aquatic invertebrates. Saturated solutions (about 10–20 mg/l) usually did not cause sufficient mortality to permit the derivation of LC_{50}'s in exposures shorter than 24 h. No frog eggs or rainbow trout succumbed after use pattern exposures of 1 mg/l for 1 h daily for up to three treatments, and none of the invertebrates died after a single use pattern treatment. In fact, most fish and invertebrates survived 96-h exposures to 1 mg/l of Furanace.

Toxicity of Furanace was influenced by certain physical and chemical characteristics of water. Toxicity increased with increases in water temperatures in tests with rainbow trout and channel catfish. Increasing water hardness and pH decreased the toxicity of Furanace to rainbow trout but had no effect on its toxicity to channel catfish and green sunfish.

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INVESTIGATIONS IN FISH CONTROL

77. Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM), 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture as Lampricides in Laboratory Studies

By Verdel K. Dawson, Kenneth B. Cumming, and Philip A. Gilderhus

78. Toxicity of the Molluscicide Bayer 73 and Residue Dynamics of Bayer 2353 in Aquatic Invertebrates

By Herman O. Sanders

79. Accumulation, Elimination, and Biotransformation of the Lampricide 2′,5-Dichloro-4′-nitrosalicylanilide by *Chironomus tentans*

By Joseph A. Kawatski and Ann E. Zittel

Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM), 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture as Lampricides in Laboratory Studies

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Abstract

The lampricidal effects of 3-trifluoromethyl-4-nitrophenol (TFM), 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 mixture of the two (TFM:2B) were tested against larvae of the sea lamprey *(Petromyzon marinus)* under controlled laboratory conditions. The lampricides were tested in water at temperatures of 7, 12, and 17 C; total hardnesses of 44, 170, and 300 mg/l as $CaCO_3$; and pH's of 6.5, 7.5, and 8.5. Temperature had little influence on the toxicity of the lampricides, but the effect of Bayer 73 was slowed in cold water. Water hardness did not significantly influence the activity of the 98:2 mixture. The toxicities of TFM, Bayer 73, and TFM:2B were significantly reduced in water of high pH. Burrowed sea lamprey larvae were less vulnerable to TFM, Bayer 73, and TFM:2B than were free-swimming larvae. TFM and TFM:2B were selective for free-swimming lampreys over the nontarget organisms used for comparison, but the margin of safety for nontarget organisms over burrowed sea lampreys was narrow.

Although a number of methods have been used to control the parasitic sea lamprey *(Petromyzon marinus)* in the Great Lakes, the most widely used and most successful method has been application of chemical lampricides. Applegate et al. (1958) reported that 3-trifluoromethyl-4-nitrophenol (TFM) was selective against the sea lamprey, and in 1964 the compound was registered by the Pesticide Registration Division of the U.S. Department of Agriculture for limited use to control sea lamprey larvae in tributaries of the Great Lakes.

In 1963, 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73 or Bayluscide) was found to be extremely toxic to sea lampreys, and the addition of small amounts of Bayer 73 to TFM greatly reduced the amount of TFM required for effective treatment of lamprey populations (Howell et al. 1964). Because Bayer 73 is also very toxic to other fish and is virtually nonselective for lampreys over rainbow trout *(Salmo gairdneri),* not more than 3% by weight can be added to TFM without losing the selective toxicity of TFM (Howell et al. 1964). Since 1963, mixtures of TFM and Bayer 73 have been used to control lampreys in tributaries of the Great Lakes by both the United States and Canadian governments (Hamilton 1974).

Use of the mixture has produced occasional fish kills that could not be predicted on the basis of pretreatment toxicity tests (U.S. Bureau of Commercial Fisheries 1968). These fish kills have been attributed to problems with formulation, application, water chemistry, or a combination of these factors (U.S. Bureau of Commercial Fisheries 1964, 1968; Smith 1966). Numerous tests on the lampricidal activity of the combination have been conducted in waters of various hardnesses, pH's, and temperatures. However, most of the studies were conducted in water from natural sources, where the presence of several variables and undetermined factors made it difficult to evaluate the influence of individual water characteristics (Erkkila 1964; Howell et al. 1964; U.S. Bureau of Commercial Fisheries 1964; Davis et al. 1965; Smith 1966). Recent studies supporting continued registration of the lampricide mixture have defined the influence of water chemistry on the toxicity to nontarget aquatic organisms (Kawatski et al. 1974; Kawatski et al. 1975; Bills and Marking 1976). Additional information on the efficacy of the mixture is needed to support its continued registration as a lampricide.

The purpose of the present study was to determine: (1) the toxicities of TFM, Bayer 73, and the TFM:2B mixture to sea lamprey larvae; (2) the individual and combined influences of temperature, water hardness, and pH on toxicity; (3) the toxicities to burrowed and exposed (free-swimming) lamprey larvae; and (4) the safety to nontarget organisms (selectivity).

1

Materials and Methods

Lamprey larvae collected from the Jordan River (Michigan) with electrofishing gear with anesthetized with MS-222 (100 mg/l) and sorted according to species and size. Sea lamprey larvae used as test organisms (average length, 8 cm; range, 5-10 cm) were held for at least 2 weeks before testing in troughs containing 12 C well water flowing over a 10-cm-deep sand substrate.

Field grade TFM (35.7% in dimethylformamide) was obtained from American Hoechst Corporation, and Bayer 73 (70% wettable powder) from Chemagro Corporation. Bayer 73 was applied at concentrations corresponding to 2% of the TFM concentrations, based on active ingredients of each, as recommended by Howell et al. (1964). The chemicals were tested simultaneously, both singly and in combination, at each temperature, hardness, and pH. The effect of combining the two chemicals was evaluated by the use of an additive index (Marking and Dawson 1975).

The toxicants were added to the test vessels 20 h after the introduction of lampreys. Ten lamprey larvae were exposed to each concentration in 15-liter glass jars according to the method of Lennon and Walker (1964). Test waters of different quality were produced by adding selected reconstituting salts to deionized water. The pH in the various tests was adjusted and maintained with chemical buffers, as suggested by Dawson et al. (1975). Water temperatures of 7, 12, and 17 C were controlled by water baths.

Dead larvae were counted and removed at 1, 3, 6, and 12 h, and daily thereafter, during the 96-h tests. Observations are reported at 12 h because that period approximates the average duration of chemical treatments of streams. LC_{50}'s, LC_{01}'s, LC_{99}'s, and 95% confidence intervals were computed according to the method of Litchfield and Wilcoxon (1949). The LC_{99}'s computed for lampreys statistically approximate the minimum concentrations needed for complete kills of the test organisms. Concentrations of both toxicants were reported on the basis of active ingredient. A P value of 0.05 was used to evaluate significance.

Because larvae usually live in burrows in the substrate of streams, the toxicity of TFM to burrowed lampreys, as well as lampreys confined without substrate, was determined. To minimize the effect of adsorption of the chemical by the substrate, we conducted tests in a flow-through test apparatus similar to that used by Marking et al. (1975). Burrowed and free-swimming sea lamprey larvae were held in separate screened compartments in the same test vessel. In addition to the sea lamprey larvae, we held rainbow trout, brook trout (Salvelinus fontinalis), and crayfish (Procambarus sp.) in the test chambers during the flow-through toxicity tests to accurately assess the selectivity of the lampricides. Selectivity was defined by a safety index (LC_{50} for brook trout divided by LC_{50} for sea lampreys) and a maximum safety index (LC_{01} for brook trout divided by LC_{99} for sea lampreys) similar to those employed by Marking (1967).

Results

Effect of Temperature

The 12-h LC_{99}'s of each compound—TFM, Bayer 73, or TFM:2B—against sea lamprey larvae differed little at different temperatures (7, 12, and 17 C; Table 1). Temperature did not significantly influence the toxicity of any of these compounds at exposure periods ranging from 3 to 96 h (Appendix 1), with one exception: the activity of Bayer 73 was slightly reduced in cold water (7 C) after 3 h of exposure, but not after 6 h or longer.

Effect of Water Hardness

Water hardnesses of 44, 170, and 300 mg/l as $CaCO_3$ did not significantly influence the toxicity of TFM, Bayer 73, or the mixture (Table 1), regardless of the period of exposure (Appendix 1).

Effect of pH

The toxicity of TFM was significantly decreased by increases in pH, as indicated by the 12-h LC_{99}'s (mg/l) of 0.660 at pH 6.5, 1.70 at 7.5, and 4.65 at 8.5 (Table 1). Although not as pronounced, the toxicity of Bayer 73 also was decreased at high pH's; the 12-h LC_{99}'s at pH 6.5, 7.5, and 8.5 were 0.0828, 0.145, and 0.165 mg/l, respectively (Table 1). Thus Bayer 73 was about twice as toxic and TFM about seven times as toxic at pH 6.5 as at 8.5. This decreased biological activity at high pH's was also evident in data reported as LC_{50}'s at all exposure periods (Appendix 1). As expected, the activity of the TFM:2B combination was also reduced at high pH (Table 1).

Effect of Substrate

The lampricide TFM was less toxic to sea lampreys burrowed in sand than to larvae confined without a substrate. A concentration of 5.63 mg/l killed all free-swimming larvae but none of the burrowed larvae in 6 h. In a concentration of 1.83 mg/l, all of the free-swimming larvae, but none of the burrowed larvae,

Table 1. *Toxicity (LC$_{99}$ and 95% confidence interval)[a] of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and additive indices for the mixture) to 8-cm sea lamprey larvae after 12 h of exposure in waters of selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness (mg/l as CaCO$_3$)	pH	Individual		Mixture		Additive index and range
			TFM	Bayer 73	TFM	Bayer 73	
7	44	7.5	1.60	0.0680	1.14	0.0233	-0.0551
			1.24-2.07	0.0407-0.114	0.694-1.87	0.0142-0.0383	-1.45 to 1.17
12	44	7.5	1.70	0.145	1.80	0.0367	-0.312
			1.11-2.59	0.0886-0.237	1.12-2.90	0.0231-0.0582	-2.27 to 0.887
17	44	7.5	1.20	0.120	1.22	0.0249	-0.224
			0.835-1.72	0.0754-0.191	0.782-1.90	0.0159-0.0391	-1.79 to 0.859
12	170	7.5	1.59	0.125	1.46	0.0298	-0.157
			1.01-2.51	0.0741-0.211	0.945-2.26	0.0193-0.0459	-1.86 to 1.14
12	300	7.5	1.58	0.108	1.48	0.0302	-0.216
			1.00-2.49	0.0614-0.190	0.989-2.22	0.0203-0.0449	-1.95 to 0.984
12	44	6.5	0.660	0.0828	0.425	0.00867	0.336
			0.387-1.13	0.0487-0.141	0.277-0.652	0.00565-0.0133	-0.958 to 2.51
12	44	8.5	4.65	0.165	3.74	0.0763	-0.267
			3.02-7.15	0.0971-0.280	2.47-5.65	0.0505-0.115	-2.06 to 0.902

[a] Concentrations based on mg/l of active ingredient.

Table 2. *Toxicity (12-h LC$_{99}$ for sea lampreys and 12-h LC$_{01}$ for rainbow trout, brook trout, and crayfish, and 95% confidence interval)[a] of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and the additive indices for the mixture) in flow-through toxicity tests in carbon filtered city water at 12 C.*

Species	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Sea lamprey (burrowed)	5.39	0.280	12.5	0.255	-2.23
	3.80-7.64	0.249-0.314	7.51-20.8	0.153-0.425	-6.18 to -0.470
Sea lamprey (free-swimming)	3.00	0.0920	1.64	0.0335	0.0979
	2.11-4.26	0.0662-0.128	1.03-2.61	0.0211-0.0533	-1.04 to 1.46
Rainbow trout	3.95	0.0255	1.83	0.0374	-0.930
	3.43-4.55	0.0228-0.0286	1.63-2.05	0.0333-0.0420	-1.44 to -0.523
Brook trout	4.00	0.0245	2.10	0.0428	-1.27
	3.48-4.60	0.0218-0.0275	1.83-2.40	0.0373-0.0491	-1.94 to -0.754
Crayfish	8.20	>0.150[b]	>7.00	>0.143	—
	7.06-9.53				

[a] Concentrations based on mg/l of active ingredient.

[b] No mortality at highest concentration tested.

were dead after 12 h, and 20% of the burrowed larvae were still alive after 96 h. The 12-h LC_{99}'s (mg/l) for TFM were 5.39 against burrowed and 3.00 against free-swimming sea lamprey (Table 2).

In comparison with burrowed lampreys, free-swimming larvae were about three times more vulnerable to Bayer 73 and more than seven times more vulnerable to TFM:2B. The greater sensitivity of free-swimming larvae was evident for each chemical individually and in combination, at all exposure periods tested (Appendix 2).

Safety to Nontarget Organisms (Selectivity)

On the basis of the 12-h LC_{50}'s (Appendix 2), free-swimming (1.88 mg/l) and burrowed (3.39 mg/l) sea lamprey larvae were less resistant to TFM than were rainbow trout (6.10 mg/l), brook trout (6.00 mg/l), or crayfish (12.9 mg/l). However, to show ideal selectivity the lampricide should kill all lamprey larvae without harming nontarget organisms. A comparison of the LC_{99}'s for sea lampreys and the LC_{01}'s for nontarget species showed TFM to have a rather narrow margin of safety. The 12-h LC_{99}'s for burrowed (5.39 mg/l) and free-swimming (3.00 mg/l) sea lampreys were not significantly different from the 12-h LC_{01}'s for rainbow trout (3.95 mg/l) or brook trout (4.00 mg/l). The crayfish (8.20 mg/l), however, were significantly more resistant than free-swimming lampreys (Table 2).

The selectivity of the lampricides can also be represented by a safety index (Marking 1967), in which a value greater than 1.0 indicates selectivity for the target species, and a value less than 1.0 indicates that nontarget species could be harmed by concentrations effective against target species. The

safety index (LC_{50} for trout divided by LC_{50} for lampreys) of 1.77 for TFM indicates selectivity for sea lampreys; however, the maximum safety index (LC_{01} for trout divided by LC_{99} for lampreys) of 0.742 indicates that some mortality of sensitive fishes could be expected (Table 3). On the basis of the safety indices, Bayer 73 (0.188) and the TFM:2B combination (0.952) did not demonstrate selectivity for burrowed sea lampreys in these tests. However, the maximum safety indices comparing exposures of free-swimming sea lampreys and brook trout to TFM (1.33) and TFM:2B (1.28) do show selectivity.

Discussion

The toxicities of TFM, computed on the basis of active ingredient, did not differ between the sea lamprey larvae used in the present study, which were collected in 1973 from the Jordan River (Michigan), and those used in a previous study (Dawson et al. 1975), which were collected in 1972 from the watershed of the Rifle River (Michigan).

Howell et al. (1964) interpreted the activity of a mixture of TFM and Bayer 73 as synergistic if all the sea lamprey larvae were killed at concentrations which were nontoxic when the chemicals were applied singly. Bills and Marking (1976) reported the toxicity of the mixture to be additive or less than additive (not synergistic) when it was tested against fish. The additive indices computed from our data support the conclusion that the toxicity of the mixture is additive or less than additive. However, Howell et al. (1964) and Smith et al. (1974) demonstrated an economic advantage of applying the mixture, i.e., the amount of TFM required to produce toxicosis was reduced while the selectivity was maintained.

Table 3. *Safety and maximum safety indices of TFM (35.7%), Bayer 73 (70%), and TFM:2B in flow-through toxicity tests against fingerling brook trout and burrowed sea lamprey larvae in carbon filtered city water at 12 C.*

Chemical	Sea lamprey		Brook trout		Safety index and range[a]	Maximum safety index and range[b]
	12-h LC_{50}	12-h LC_{99}	12-h LC_{50}	12-h LC_{01}		
TFM	3.39	5.39	6.00	4.00	1.77	0.742
	2.87–4.01	3.80–7.64	5.21–6.91	3.48–4.60	1.29–2.41	0.455–1.21
Bayer 73	0.180	0.280	0.0338	0.0245	0.188	0.0875
	0.114–0.285	0.249–0.314	0.0301–0.0379	0.0218–0.0275	0.106–0.332	0.0694–0.110
TFM:2B	3.15	12.5	3.00	2.10	0.952	0.168
	2.27–4.37	7.51–20.8	2.63–3.42	1.83–2.40	0.602–1.51	0.0880–0.320

[a] LC_{50} for brook trout/LC_{50} for sea lamprey.

[b] LC_{01} for brook trout/LC_{99} for sea lamprey.

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; Dawson et al. 1975). 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 to near freezing.

Lowering the temperature has been reported to reduce the activity of Bayer 73 (Strufe and Gönnert 1962; Tibbles 1967). Our study indicated reduced activity of this compound in cold water after 3 h of exposure, but not after longer exposures. Apparently the effective contact time is extended at low temperatures. Generally, the influence of temperature on the lampricides is insignificant when compared with influences of other factors.

The reduced activity of TFM at high 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 activity of Bayer 73 was slightly reduced at higher pH's. This reduction, which is consistent with results from previous studies (Gillett and Bruaux 1962; Marking and Hogan 1967; Farringer 1972), may result from ionization of the molecule at higher pH's. Meredith (1971) reported some loss of activity of Bayer 73 at pH's below 7, due to precipitation. We did not observe this phenomenon, probably because of the extremely low concentrations used in the toxicity tests.

We found that free-swimming lampreys were more vulnerable than burrowed lampreys to TFM, Bayer 73, and TFM:2B. Possibly the free-swimming lampreys are more excited and have a faster rate of metabolism and uptake than the burrowed lampreys. Also, the burrowed lampreys may be somewhat protected from exposure to the lampricides in the water. Field use concentrations of the lampricides for each stream are routinely determined in on-site toxicity tests against free-swimming lampreys. Results of these tests could indicate treatment concentrations which are insufficient to eliminate all burrowed lampreys. Our 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.

A comparison of the LC_{99}'s for sea lampreys and the LC_{01}'s for nontarget species in our tests showed TFM to have a narrow margin of safety (working range). However, Howell and Marquette (1962) showed that the working range (minimum lethal to maximum allowable concentration) varied from time to time in a particular stream and that optimum conditions and time for stream treatment could be determined by conducting a number of bioassays in a stream.

Conclusions

1. The TFM:2B combination was effective for controlling sea lamprey larvae.
2. Temperature had little influence on the toxicity of TFM or TFM:2B to lampreys.
3. The rate of action of Bayer 73 was only slightly reduced at low temperatures.
4. Water hardness did not significantly influence the activity of the TFM:2B combination.
5. The toxicities of TFM, Bayer 73, and TFM:2B were significantly reduced in water of high pH.
6. Burrowed sea lamprey larvae were less vulnerable than free-swimming sea lamprey larvae to TFM, Bayer 73, and TFM:2B.
7. TFM and TFM:2B are selective for free-swimming sea lamprey larvae, but the margin of safety for sensitive nontarget organisms over burrowed sea lampreys is comparatively narrow.

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Appendix 1. *Toxicity (LC$_{50}$ and 95% confidence interval)[a] of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and additive indices for the mixture) against 8-cm larval sea lampreys in static toxicity test waters of selected temperatures, hardnesses[b], and pH's.*

Exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	

Water temp 7 C, hardness 44, pH 7.5

Exposure time (h)	TFM	Bayer 73	TFM	Bayer 73	Additive index and range
3	2.60 1.97–3.43	0.0970 0.0765–0.123	1.05 0.814–1.35	0.0214 0.0166–0.0275	0.601 -0.0448 to 1.69
6	1.40 1.13–1.73	0.0550 0.0393–0.0771	1.00 0.745–1.34	0.0204 0.0152–0.0273	-0.0852 -0.881 to 0.592
12	0.800 0.743–0.861	0.0370 0.0266–0.0515	0.490 0.358–0.671	0.0100 0.00730–0.0137	0.133 -0.418 to 0.794
24	0.770 0.637–0.931	0.0276 0.0208–0.0366	0.455 0.347–0.597	0.00928 0.00708–0.0122	0.0786 -0.524 to 0.766
48	0.510 0.394–0.660	0.0220 0.0158–0.0306	0.455 0.347–0.597	0.00928 0.00708–0.0122	-0.314 -1.29 to 0.321
96	0.345 0.246–0.484	0.0220 0.0158–0.0306	0.440 0.345–0.561	0.00898 0.00704–0.0114	-0.684 -2.00 to 0.0606

Water temp 12 C, hardness 44, pH 7.5

Exposure time (h)	TFM	Bayer 73	TFM	Bayer 73	Additive index and range
3	>1.6[c]	0.0500 0.0340–0.0740	1.00 0.787–1.27	0.0204 0.0161–0.0259	>-0.0330
6	1.66 1.11–2.50	0.0500 0.0340–0.0740	1.00 0.787–1.27	0.0204 0.0161–0.0259	-0.0104 -0.912 to 0.876
12	0.900 0.708–1.15	0.0450 0.0330–0.0608	0.820 0.610–1.10	0.0164 0.0124–0.0225	-0.276 -1.24 to 0.357
24	0.730 0.612–0.870	0.0450 0.0330–0.0608	0.540 0.407–0.717	0.0110 0.00830–0.0146	0.0161 -0.614 to 0.655
48	0.730 0.612–0.870	0.0450 0.0330–0.0608	0.450 0.347–0.584	0.00918 0.00708–0.0119	0.219 -0.315 to 0.941
96	0.730 0.612–0.870	0.0400 0.0268–0.0598	0.375 0.255–0.551	0.00765 0.00520–0.0112	0.419 -0.318 to 1.63

Water temp 17 C, hardness 44, pH 7.5

Exposure time (h)	TFM	Bayer 73	TFM	Bayer 73	Additive index and range
3	1.20 0.880–1.64	0.045 0.0333–0.0608	0.833 0.621–1.12	0.0170 0.0127–0.0228	-0.0719 -0.962 to 0.700
6	0.850 0.687–1.05	0.0450 0.0330–0.0608	0.485 0.351–0.670	0.00990 0.00720–0.0137	0.265 -0.390 to 1.21
12	0.740 0.618–0.885	0.0410 0.0309–0.0540	0.455 0.350–0.591	0.00928 0.00710–0.0121	0.189 -0.348 to 0.898
24	0.560 0.452–0.693	0.0410 0.0309–0.0540	0.455 0.350–0.591	0.00928 0.00715–0.0121	-0.0388 -0.699 to 0.571
48	0.508 0.423–0.610	0.0410 0.0309–0.0540	0.455 0.350–0.591	0.00928 0.00715–0.0121	-0.122 -0.789 to 0.416
96	0.496 0.415–0.593	0.0410 0.0309–0.0540	0.455 0.350–0.591	0.00928 0.00715–0.0121	-0.144 -0.816 to 0.384

Appendix 1.—Continued

Exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Water temp 12 C, hardness 170, pH 7.5					
3	1.69	0.0550	1.04	0.0212	0.00280
	1.33–2.15	0.0416–0.0726	0.710–1.52	0.0145–0.0310	−0.892 to 0.887
6	1.25	0.0380	0.630	0.0129	0.186
	0.907–1.72	0.0259–0.0550	0.466–0.852	0.00950–0.0174	−0.612 to 1.26
12	0.770	0.0350	0.560	0.0114	−0.0530
	0.586–1.01	0.0249–0.0491	0.435–0.721	0.00888–0.0174	−0.929 to 0.637
24	0.625	0.0350	0.560	0.0114	−0.222
	0.488–0.802	0.0249–0.0491	0.435–0.721	0.00888–0.0147	−1.07 to 0.382
48	0.625	0.0350	0.560	0.0114	−0.222
	0.488–0.802	0.0249–0.0491	0.435–0.721	0.00888–0.0147	−1.07 to 0.382
96	0.625	0.0350	0.560	0.0114	−0.222
	0.488–0.802	0.0249–0.0491	0.435–0.721	0.00888–0.0147	−1.07 to 0.382
Water temp 12 C, hardness 300, pH 7.5					
3	2.62	0.900	1.25	0.0255	0.979
	1.98–3.46	0.142–5.71	0.946–1.65	0.0193–0.0340	−0.0724 to 2.61
6	1.54	0.0440	0.860	0.0175	0.0458
	1.18–2.02	0.0371–0.0603	0.651–1.14	0.0133–0.0232	−0.591 to 0.840
12	0.765	0.0390	0.560	0.0114	−0.0243
	0.582–1.01	0.0266–0.0571	0.449–0.699	0.00920–0.0143	−0.739 to 0.645
24	0.765	0.0390	0.560	0.0114	−0.0243
	0.582–1.01	0.0266–0.0571	0.449–0.699	0.00920–0.0143	−0.739 to 0.645
48	0.710	0.0390	0.560	0.0114	−0.0810
	0.530–0.951	0.0266–0.0571	0.449–0.699	0.00920–0.0143	−0.856 to 0.579
96	0.710	0.0390	0.560	0.0114	−0.0810
	0.530–0.951	0.0266–0.0571	0.449–0.699	0.00920–0.0143	−0.856 to 0.579
Water temp 12 C, hardness 44, pH 6.5					
3	0.450	0.0480	0.381	0.00777	−0.00850
	0.301–0.673	0.0360–0.0640	0.308–0.472	0.00628–0.00960	−0.835 to 0.799
6	0.300	0.0450	0.232	0.00473	0.138
	0.224–0.401	0.0337–0.0602	0.182–0.295	0.00371–0.00602	−0.496 to 0.940
12	0.172	0.0330	0.225	0.00459	−0.447
	0.121–0.245	0.0233–0.0467	0.176–0.288	0.00359–0.00588	−1.63 to 0.257
24	0.172	0.0310	0.170	0.00350	−0.101
	0.121–0.245	0.0227–0.0423	0.121–0.239	0.00250–0.00490	−1.19 to 0.808
48	0.172	0.0300	0.170	0.00350	−0.105
	0.121–0.245	0.0222–0.0406	0.121–0.239	0.00250–0.00490	−1.20 to 0.800
96	0.172	0.0300	0.170	0.00350	−0.105
	0.121–0.245	0.0222–0.0406	0.121–0.239	0.00250–0.00490	−1.20 to 0.800

Appendix 1.—Continued

Exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Water temp 12 C, hardness 44, pH 8.5					
3	>4.00	0.120 0.0855–0.168	3.60 1.91–6.76	0.0734 0.0390–0.138	>-0.512
6	2.80 2.26–3.47	0.0810 0.0541–0.121	1.26 1.00–1.59	0.0257 0.0204–0.0324	0.303 -0.300 to 1.19
12	2.37 1.85–3.04	0.0660 0.0466–0.0930	1.26 1.00–1.59	0.0257 0.0204–0.0324	0.0857 -0.553 to 0.821
24	1.40 1.13–1.74	0.0440 0.0310–0.0620	1.26 1.00–1.59	0.0257 0.0204–0.0324	-0.484 -1.45 to 0.104
48	1.30 0.957–1.77	0.0440 0.0310–0.0620	0.580 0.453–0.743	0.0118 0.00920–0.0152	0.400 -0.267 to 1.47
96	1.30 0.957–1.77	0.0390 0.0266–0.0570	0.580 0.453–0.743	0.0118 0.00920–0.0152	0.336 -0.348 to 1.39

[a] Concentrations based on mg/l of active ingredient.

[b] Water hardness = mg/l as $CaCO_3$.

[c] No mortality at highest concentration tested.

Appendix 2. *Toxicity (LC_{50} and 95% confidence interval)[a] of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and additive indices for the mixture) in flow-through toxicity tests against several aquatic organisms in carbon filtered city water at 12 C.*

Organism and exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Sea lamprey (burrowed)					
3	>11.9[b]	>0.15	>7.80	>0.159	—
6	9.40 6.80–13.0	>0.15	7.80 5.32–11.4	0.159 0.109–0.233	>-0.890
12	3.39 2.87–4.01	0.180 0.114–0.285	3.15 2.27–4.37	0.0643 0.0463–0.0891	-0.286 -1.30 to 0.373
24	1.68 1.45–1.95	0.180 0.114–0.285	2.35 1.87–2.95	0.0479 0.0381–0.0602	-0.665 -1.56 to -0.0927
48	1.68 1.45–1.95	0.180 0.114–0.285	2.35 1.87–2.95	0.0479 0.0381–0.0602	-0.665 -1.56 to -0.0927
72	1.68 1.45–1.95	0.129 0.0995–0.167	2.35 1.87–2.95	0.0479 0.0381–0.0602	-0.770 -1.64 to -0.187
96	1.68 1.45–1.95	0.129 0.0995–0.167	2.35 1.87–2.95	0.0479 0.0381–0.0602	-0.770 -1.64 to -0.187

Appendix 2.—Continued

Organism and exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Sea lamprey (free-swimming)					
3	16.6 12.2–22.5	>0.0800	>7.00	>0.140	—
6	3.60 2.98–4.35	>0.0800	2.31 2.10–2.54	0.0471 0.0427–0.0518	>–0.230
12	1.88 1.59–2.23	0.0625 0.0540–0.0724	0.760 0.573–1.01	0.0155 0.0117–0.0206	0.533 –0.0222 to 1.39
24	<1.48[c]	0.0350 0.0254–0.0482	0.760 0.573–1.01	0.0155 0.0117–0.0206	<0.0456
48	<1.48	0.0335 0.0275–0.0409	0.700 0.544–0.900	0.0143 0.0111–0.0184	<0.111
72	<1.48	0.0335 0.0275–0.0409	0.700 0.544–0.900	0.0143 0.0111–0.0184	<0.111
96	<1.48	0.0335 0.0275–0.0409	0.700 0.544–0.900	0.0143 0.0111–0.0184	<0.111
Brook trout					
3	9.65 8.41–11.1	>0.0800	8.10 6.29–10.4	0.165 0.128–0.212	>–1.71
6	6.00 4.98–7.23	0.0880 0.0696–0.111	3.00 2.63–3.42	0.0612 0.0537–0.0698	–0.195 –0.690 to 0.180
12	6.00 5.21–6.91	0.0338 0.0301–0.0399	3.00 2.63–3.42	0.0612 0.0537–0.0698	–1.31 –1.98 to –0.797
24	6.00 5.21–6.91	0.0338 0.0300–0.0380	3.00 2.63–3.42	0.0612 0.0537–0.0698	–1.31 –1.98 to –0.794
48	5.95 5.18–6.83	0.0338 0.0300–0.0380	3.00 2.63–3.42	0.0612 0.0537–0.0698	–1.31 –1.99 to –0.798
72	5.95 5.18–6.83	0.0338 0.0300–0.0380	3.00 2.63–3.42	0.0612 0.0537–0.0698	–1.31 –1.99 to –0.798
96	5.95 5.18–6.83	0.0338 0.0300–0.0380	3.00 2.63–3.42	0.0612 0.0537–0.0698	–1.31 –1.99 to –0.798
Rainbow trout					
3	16.8 12.3–22.9	0.0720 0.0604–0.0858	4.10 3.57–4.72	0.0836 0.0728–0.0963	–0.405 –0.978 to –0.00438
6	6.40 5.67–7.23	0.0415 0.0351–0.0490	3.45 2.74–4.34	0.0704 0.0559–0.0885	–1.24 –2.29 to –0.520
12	6.10 5.19–7.18	0.0353 0.0315–0.0396	2.47 2.20–2.77	0.0504 0.0449–0.0565	–0.833 –1.33 to –0.440
24	6.10 5.19–7.18	0.0345 0.0309–0.0386	2.47 2.20–2.77	0.0504 0.0449–0.0565	–0.866 –1.36 to –0.470
48	6.10 5.19–7.18	0.0179 0.0145–0.0221	2.47 2.20–2.77	0.0504 0.0449–0.0565	–2.22 –3.43 to –1.34
72	6.10 5.19–7.18	0.0179 0.0145–0.0221	2.47 2.20–2.77	0.0504 0.0449–0.0565	–2.22 –3.43 to –1.34
96	6.10 5.19–7.18	0.0179 0.0145–0.0221	2.47 2.20–2.77	0.0504 0.0449–0.0565	–2.22 –3.43 to –1.34

Appendix 2.—Continued

Organism and exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Crayfish					
3	>16.5	>0.15	>7.00	>0.140	—
6	16.5 12.2–22.4	>0.15	>7.00	>0.140	—
12	12.9 11.0–15.1	>0.15	>7.00	>0.140	—
24	12.9 11.0–15.1	>0.15	>7.00	>0.140	—
48	12.6 10.5–15.2	>0.15	>7.00	>0.140	—
72	12.6 10.5–15.2	>0.15	>7.00	>0.140	—
96	12.6 10.5–15.2	>0.15	>7.00	>0.140	—

[a] Concentrations based on mg/l of active ingredient.

[b] No mortality at highest concentration tested.

[c] Total mortality at lowest concentration tested.

Toxicity of the Molluscicide Bayer 73 and Residue Dynamics of Bayer 2353 in Aquatic Invertebrates

by

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Abstract

The molluscicide Bayer 73 (2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide), a chemical used as a synergist in conjunction with 3-trifluoromethyl-4-nitrophenol (TFM) to control the sea lamprey *(Petromyzon marinus)* in tributaries of the Great Lakes, was tested against five species of crustaceans and two species of aquatic insects: daphnids *(Daphnia magna)*, aquatic sowbugs *(Asellus brevicaudus)*, scuds *(Gammarus pseudolimnaeus)*, glass shrimp *(Palaemonetes kadiakensis)*, crayfish *(Orconectes nais)*, damselfly nymphs *(Ischnura verticalis)*, and midge larvae *(Chironomus plumosus)*. The acute toxicities ranged from a 48-h EC_{50} (median effective concentration causing immobilization) of 0.2 mg/l for daphnids to a 48-h LC_{50} (concentration causing 50% mortality) of 25 mg/l for crayfish. In daphnids exposed continuously to Bayer 73, reproduction was not impaired at concentrations of 0.018 and 0.032 mg/l, but was significantly ($P<0.01$) reduced at concentrations of 0.056, 0.10, and 0.18 mg/l. Exposure to Bayer concentrations of 0.56, 1.0, and 1.8 mg/l significantly ($P<0.01$) reduced emergency of midges. All organisms exposed to Bayer 2353 (chlorosalicylic acid ring UL ^{14}C) accumulated radioactive residues in 24 h that ranged from 4 to 80 times (based on wet weight of whole organism) the water concentration of 1.2 ± 0.2 μg/l. Scuds and midge larvae eliminated 90% of the residues in 48 h.

The molluscicide Bayer 73 (2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide), which is sold commercially as Bayluscide, is especially toxic to freshwater snails (Chemagro Corporation 1970). It has been used in Africa, southeastern Asia, and portions of South America to control several species of snails that are intermediate hosts of organisms causing schistosomiasis in man (Gönnert 1962). Bayer 73 is effective as an ovicide against snail eggs (Gillet and Bruaux 1962), as a herbicide to control the tropical water fern, *Salvinia aurticulata* (Wild and Mitchell 1970), and as a piscicide for controlling fish populations (Brynildson 1970). This molluscicide is also extremely toxic to larvae of the sea lamprey, *Petromyzon marinus* (Howell et al. 1964), and has been used by the U.S. Fish and Wildlife Service and the Canadian Department of Environment for sampling lamprey populations in tributaries of the upper Great Lakes. It is also used as a synergist with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) for lamprey control (Howell et al. 1964).

Authorization for lampricidal uses of TFM and the TFM-Bayer 73 mixture was jeopardized when the U.S. Department of Agriculture, Pesticide Regulation Division, gave notice on 13 May 1970 that registration of TFM would be cancelled unless tolerances were obtained in fish and water. The U.S. Fish and Wildlife Service was granted an extension from the then new regulatory agency, the U.S. Environmental Protection Agency (EPA), on 22 February 1971, to obtain additional data on methodology for application, and toxicology of, TFM, Bayer 73, and their combination. Submissions for an Amended Registration and Petition for Exemption from Tolerance for TFM were filed with EPA in February 1976.

Laboratory and field studies have shown that a 0.1-mg/l concentration of Bayer 73, which is effective for controlling lamprey larvae (Howell et al. 1964; Smith 1967), could have an adverse effect on nontarget aquatic organisms, such as planarians, tubificids, and daphnids (Hunn 1973), mollusks (Gönnert 1962), and fishes (Marking and Hogan 1967). However, aquatic invertebrates with a hard exoskeleton, such as ostracods (Kawatski 1973), aquatic sowbugs, crayfish, dragonflies, and dobsonflies (Hunn 1973), and stonefly naiads (Sanders and Cope 1968) were not severely affected by exposure to Bayer 73. Meyer and Howell (1975) reported that nymphs of burrowing

mayflies (*Hexagenia* sp.) exposed to Bayer 73 in Lake Huron water at 12 C were 160 times more resistant to this chemical than were larval lampreys.

Although the effects of TFM on aquatic invertebrates have been documented (Smith 1967; Chandler and Marking 1975; Fremling 1975; Kawatski et al. 1975; Sanders and Walsh 1975), a recent review of the literature (Hamilton 1974) indicated a lack of information for evaluating the safety of Bayer 73 to aquatic invertebrates. The objectives of the study were to determine the acute toxicities of Bayer 73 to aquatic invertebrates in static tests and to determine the effect of continuous exposure of Bayer 73 on reproduction in daphnids (*Daphnia magna*) and emergence of midges (*Chironomus plumosus*). In addition, the accumulation of ^{14}C-Bayer 2353 from water by seven aquatic invertebrates was determined.

Materials and Methods

Test animals included five species of crustaceans and two species of aquatic insects: early instar and mature daphnids; mature aquatic sowbugs (*Asellus brevicaudus*); mature scud (*Gammarus pseudolimnaeus*); mature glass shrimp (*Palaemonetes kadiakensis*); juvenile crayfish (*Orconectes nais*); early instar damselfly nymphs (*Ischnura verticalis*); and first and early fourth instars of midge larvae. Daphnids, scuds, and midge larvae were from laboratory cultures and the other invertebrates were collected from streams or ponds near Columbia, Missouri. All organisms were acclimated to laboratory conditions by rearing or holding them in the dilution water at the test temperature. A combination of Duro-test and wide spectrum Grow-lux bulbs provided light for the cultures and all tests. The light cycle was controlled for a 16-h photoperiod.

The water used for cultures and all experiments was from a deep well; it had a pH of 7.4 and a total hardness of 270 mg/l as $CaCO_3$. Crayfish and midge larvae were exposed at 22 ± 1 C and all other organisms at 18 ± 1 C.

The Bayer 73 (Chemagro Corp., Lot No. 8059410) was supplied by the Fish Control Laboratory, LaCrosse, Wisconsin, as a wettable powder containing 70% 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide. Concentrations were based on active ingredient.

Acute toxicity tests were conducted under static conditions; methods used were those recommended for standardized laboratory toxicity tests (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). Flow-through tests were performed in a system modeled after Mount and Brungs (1967). The method of Litchfield and Wilcoxon (1949) was used to estimate the LC_{50}'s (concentrations causing 50% mortality) or EC_{50}'s (median effective concentrations causing immobilization) and 95% confidence intervals. In flow-through tests, the incipient LC_{50} or lethal threshold concentration (Sprague 1969) was determined when the asymptote had been reached in the toxicity curve. This value was determined when the mortality in each aquarium in any 5-day period dropped to 10% of the original number of animals.

Reproductive studies were conducted with daphnids in a flow-through system, with a chemical delivery apparatus designed by Chandler et al. (1974). Ten first-instar daphnids up to 24 h old were placed in duplicate exposure vessels that contained 1 liter of water. Thus, 20 daphnids per group were exposed continuously through a complete life cycle (21 days) to concentrations of 0 (the control), 0.018, 0.032, 0.056, 0.10, and 0.18 mg/l of Bayer 73. The test solutions and controls contained 0.1 ml/l of ethanol. Test organisms were fed a suspension of yeast in sufficient amounts to support a stable population. Reproductive success was assessed by counting the offspring produced in each concentration and the control after the parent daphnids had been exposed for 21 days. The mean number of young produced per adult was determined by averaging the number of young produced in replicate tests. Data were analyzed by analysis of variance, and significant differences among treatments were determined by a multiple means comparison test (least significant difference, Snedecor and Cochran 1974).

Use of the rearing technique described by Biever (1965) for the colonization of chironomid larvae maintained a continuously reproducing population of midges. Before the experiments were begun, eggs collected from a rearing unit were hatched in a stender dish. One hundred first-instar larvae (1.5 mm long and up to 24 h old) were transferred into exposure vessels that had been previously prepared by adding 13 g of washed sand to 1 liter of water. During the test, larvae were fed a commercial dog food called Dog Kisses (Biever 1965).

A flow-through system as described in the daphnid studies was used to determine the effects of Bayer 73 on larval survival, pupation, and emergency of midges. Cast pupal skins at the water surface in test containers were counted and removed daily to determine emergence. The test was terminated at 30 days, when 80–90% of the control larvae had emerged. The effects of Bayer 73 on emergence were analyzed by analysis of variance on the arcsin transformation for proportions (angle = $\arcsin\sqrt{\text{percentage}}$) followed by a least significant difference test (Snedecor and Cochran 1974).

The radioactive Bayer 2353 (chlorosalicylic acid ring UL ^{14}C) used in this study was prepared by the

American Radiochemical Corporation, Sanford, Florida, and was obtained through the Fish Control Laboratory, LaCrosse, Wisconsin. The specific activity of 10.0 mCi/mM given for this labeled compound was confirmed by gas chromatographic analysis of the stock solutions.

The concentration used in the accumulation studies (1.2 ± 0.2 μg/l) was selected partly on the basis of the acute toxicity determined for the most sensitive species exposed. Johnson and Schoettger (1975) suggested that a concentration between 1/10 and 1/1000 of the EC_{50}, depending on the slope of the toxicity curve, be selected as the test concentration. On the basis of use pattern, field evaluations, and toxicity of the chemical, a factor of 0.01 was applied to the lowest confidence limit of the EC_{50} for *D. magna*.

Accumulation studies were conducted in a flow-through system that included the chemical delivery apparatus designed by Chandler et al. (1974). Exposure vessels were 2-liter glass aquaria containing 1 liter of well water. Stock solutions of the ^{14}C-Bayer 2353 were prepared in water and further diluted in the flow-through system. Organisms were not fed during the 5-day exposure.

Invertebrate samples were taken in triplicate, weighed, and prepared directly for radiometric analysis by homogenizing the whole organism. The homogenate was obtained by adding 6 ml of Triton X-100:toluene (2:3 v/v) emulsifier to each sample before grinding it (Johnson et al. 1971). The concentration of labeled compound in water was monitored radiometrically by taking triplicate 1-ml samples entering the exposure chambers and then adding 14 ml of Triton:toluene-fluor mixture. The radioactivity of the samples was measured with a Beckman 200-L liquid scintillation counter. All samples were corrected for quench and counted until a statistical counting error of 5% or less had been reached. Residue values and accumulation factors (residue concentration in organism/residue concentration in exposure water) were computed on a whole-body, wet weight basis.

Elimination of ^{14}C-Bayer 2353 residues in several of the invertebrates was measured by exposing the organisms to the compound until a plateau concentration was reached. The organisms were then transferred to clean flowing water and analyzed at 8, 24, and 48 h to measure the decline in whole-body residues.

A method proposed by Mount and Stephan (1967) for establishing acceptable toxicant limits for aquatic organisms under continuous exposure conditions was used to calculate an application factor for determining a toxicant concentration that has no adverse effect on reproduction in daphnids or life

cycle alterations in chironomids. The application factor consists of the laboratory determined maximum acceptable toxicant concentration (MATC) that has no effect on the test animals during chronic exposure, divided by the 48-h EC_{50}.

Results

Toxicity

The acute toxicities of Bayer 73 varied greatly among aquatic invertebrates, and ranged from a 48-h EC_{50} of 0.2 mg/l for daphnids to a 48-h LC_{50} of 25 mg/l for crayfish (Table 1). The invertebrates with a soft integument (daphnids and midge larvae) were more susceptible than those with a highly chitinized exoskeleton (scud, glass shrimp, and crayfish).

Flow-through tests indicated that the compound was not toxic to early instar crayfish in a concentration of 50 mg/l for 24 h. However, continuous exposure produced toxic effects, and at 4 days the LC_{50} was 35 mg/l. Toxicity reached an asymptote at 10 days and a time-independent LC_{50} of 16 mg/l was estimated. The toxicity to scuds was not substantially affected by continuous exposure, and 24-h LC_{50}'s were similar in static and flow-through tests.

Daphnid Reproduction

Continuous exposure of daphnids through a complete life cycle (21 days) to 0.056, 0.10, or 0.18 mg/l of Bayer 73 in a flow-through system

Table 1. *Acute toxicity of Bayer 73 to five species of aquatic invertebrates in static tests.*

Organism and stage	LC_{50} (mg/l) and 95% confidence limits[a]	
	24 h	96 h
Daphnid, 1st instar	—	(0.2)[b] 0.14–0.27
Scud, mature	(5.6) 4.7–6.7	(2.4) 1.8–3.1
Glass shrimp, mature	(19) 12–29	(10) 7–15
Crayfish, juvenile	(32) 23–45	(25) 19–33
Midge, 4th instar larva	(2.1) 1.6–2.9	1.5[b] 1.1–2.2

[a] Toxicities are expressed in terms of EC_{50} for daphnid and midge larva and LC_{50} for the other organisms.
[b] 48-h values.

Table 2. *Survival and reproduction of* Daphnia magna *after a 21-day exposure to Bayer 73 at 18 ± 1 C.*

Concentration (mg/l)	Percent survival of adults[a]	Total young produced per surviving adult
0.0	95	1,586
0.018	95	1,545
0.032	85	1,553
0.056	90	726[b]
0.10	85	244[b]
0.18	45	101[b]

[a] Twenty young exposed at each concentration.

[b] Significantly different from controls ($P<0.01$), $n = 2$.

significantly reduced ($P<0.01$) reproduction (Table 2). Survival of the adult daphnids at the termination of the tests was 85–95% in all Bayer 73 concentrations and controls, except at 0.18 mg/l, in which adult survival was 45%. The number of young produced per parent daphnid ranged from 5 at 0.18 mg/l to 77 at concentrations of 0.032 and 0.018 mg/l. The mean number of young produced in the controls was 79.

The MATC for daphnids was estimated to be between 0.032 and 0.056 mg/l. The application factor (MATC/EC$_{50}$) was between 0.16 and 0.28 (Table 3).

Midge Emergence

Emergence of adult midges was significantly reduced ($P<0.01$) after 30 days exposure to Bayer 73 at concentrations of 0.56, 1.0, and 1.8 mg/l (Table 4). Emergence was significantly delayed ($P<0.05$) in the 0.32 mg/l concentration at 15 days; however, at 30 days the emergence time was similar to that in the controls. The pupal stage seems to be the most

Table 3. *Ranges of application factors and safe concentrations for Bayer 73, continuous exposure, for* Daphnia magna *and the midge* Chironomus plumosus.

Organism	48 h EC$_{50}$ (mg/l)[a]	MATC[b] (mg/l)	Application factor[c]
Daphnid	0.2 (0.14–0.27)	0.032–0.056	0.16–0.28
Midge larva	1.5 (1.1–2.2)	0.32–0.56	0.21–0.36

[a] Values in parentheses are 95% confidence limits of the EC$_{50}$.

[b] Highest concentration producing no observed effect and the lowest concentration in which an effect was observed.

[c] Limits of the maximum acceptable toxicant concentration (MATC), divided by 48-h EC$_{50}$.

sensitive stage in the midge life cycle; some pupal mortality was noted in control chambers. The highest pupal mortality was in the 1.8-mg/l concentration; only 33% of the organisms emerged.

The MATC for midges was estimated to be between 0.32 and 0.56 mg/l and the application factor was between 0.21 and 0.36 (Table 3).

Accumulation and Elimination

The accumulation of ^{14}C-Bayer 2353 was relatively low in the seven species of aquatic invertebrates, ranging from 4 to 80 times the water concentration of 1.2 ± 0.2 μg/l (Table 5). Accumulation of radioactive residues was greater in soft-bodied invertebrates (daphnids and midge larvae) than in organisms with a hard exoskeleton (glass shrimp and crayfish). Most invertebrates accumulated maximum residues

Table 4. *Cumulative percentages of midges* (Chironomus plumosus) *that emerged after continuous exposure of the larvae to different concentrations of Bayer 73 at 22 ± 1 C.*

Days of exposure	Bayer 73 concentration (mg/l)					
	0 (Control)	0.1	0.32	0.56	1.0	1.8
15	10	5	3[a]	4[a]	3[a]	0[a]
20	57	49	47	34[b]	15[b]	5[b]
25	84	79	78	45[b]	24[b]	27[b]
30	87	85	86	47[b]	53[b]	33[b]

[a] Significantly different from controls ($P<0.05$).

[b] Significantly different from controls ($P<0.01$).

Table 5. *Whole-body residues of ^{14}C-Bayer 2353 accumulated from water by seven aquatic invertebrates.*

Organism and stage	Water concentration (μg/l and SE)	Whole-body residues (μg/kg) and accumulation factors[a]			
		24 h	48 h	72 h	120 h
Daphnid, 1st instar	1.4 (0.02)	75 (4) [53]	78 (2) [56]	80 (2) [57]	75 (3) [53]
Aquatic sowbug, mature	1.1 (0.08)	25 (4) [23]	32 (2) [29]	30 (1) [27]	28 (1) [25]
Scud, mature	1.2 (0.25)	80 (4) [67]	88 (2) [73]	83 (4) [69]	85 (2) [71]
Glass shrimp, mature	1.0 (0.09)	4 (2) [4]	6 (2) [6]	9 (1) [9]	9 (1) [9]
Crayfish, juvenile	1.0 (0.17)	4 (2) [4]	8 (2) [8]	9 (1) [9]	11 (1) [11]
Damselfly, mature nymph	1.2 (0.10)	8 (2) [7]	12 (1) [10]	10 (1) [8]	8 (2) [7]
Midge, 4th instar larva	1.1 (0.03)	87 (7) [80]	90 (5) [82]	89 (2) [81]	80 (6) [73]

[a] Residue values are means of three samples (SE in parentheses). Accumulation factor, in brackets, is expressed as the ratio of the concentration in the organism to the concentration in water.

between 24 h and 48 h of continuous exposure and changed little after an additional exposure (up to 120 h).

Residues in crustaceans after a 24-h exposure to ^{14}C-Bayer 2353 ranged from 4 μg/kg in glass shrimp and crayfish to 80 μg/kg in scuds. Concentrations of residues in scuds declined by 50% within 8 h and by 90% after 48 h in clean flowing water. Aquatic insects exposed continuously accumulated residues in 24 h that ranged from 8 μg/kg for damselfly nymphs to 87 μg/kg for midge larvae. Midge larvae eliminated 50% of the residues in about 11 h and 90% in 48 h.

Discussion

Bayer 73 and TFM are often applied in combination as a single application in tributaries of the Great Lakes to control larval lampreys. Because of costs and the efficacy of the compounds, continuous application to streams never exceeds 12 h (Applegate and King 1962). Since nontarget organisms would generally be exposed to Bayer 73 for only a short time, the acute toxicities would seemingly be most important in assessing the likelihood of mortalities of aquatic invertebrates within the area of a lampricide application; however, some of the chemical may be adsorbed and retained by bottom sediments (Strufe and Gönnert 1962), and benthic organisms could be exposed for a longer time than the duration of the

lampricide treatment. Results of our midge emergence studies indicate that there was no effect on emergence of midges at a concentration of 0.32 mg/l, a concentration double that normally applied in sea lamprey control (Howell et al. 1964).

The low accumulation and rapid elimination rate of radioactive residues in scuds and midge larvae suggest that Bayer 73 would not accumulate through the food chain to upper-level consumers. Techniques for determination of degradation products of Bayer 2353 in invertebrates are not well defined, but it is assumed that the loss of radioactivity was due to excretion and degradation of the parent compound.

Field observations and laboratory tests have shown that Bayer 73 is less toxic to most aquatic invertebrates than it is to sea lampreys. Smith (1967) reported that the granular formulation applied in a stream at about three times the normal lampricide application rate had little effect on benthic invertebrates. Kawatski (1973) found that an ostracod *(Cypretta kawatai)* would not be adversely affected at concentrations used to control sea lampreys. Daphnids appear to be more sensitive than most other aquatic invertebrates; consequently, a reduction in population could occur during a lamprey treatment. Daphnid populations could also be reduced if a concentration of 0.1 mg/l was maintained for 21 days. This duration seems highly improbable, since use exposures do not exceed 12 h.

Meyer and Howell (1975) indicated that if a theoretical concentration of 0.11 mg/l were applied to the bottom 5 cm of standing water, the concentration would be sufficiently diluted at 10.7 cm above the bottom to be harmless to trout. Inasmuch as trout are more sensitive to Bayer 73 (Marking and Hogan 1967) than are most of the crustaceans and aquatic insects that have been exposed, concentrations used for lamprey control should have little effect on most arthropods. Moreover, a mixture (98:2 ratio) of TFM and Bayer 73 was more toxic to larval sea lampreys than to nontarget fish in comparable laboratory toxicity tests (Bills and Marking 1976).

The results from this study and others indicate that if standard lamprey control procedures are followed and a Bayer 73 concentration of 0.1 mg/l is not exceeded for more than 24 h, no adverse effect on most aquatic invertebrates should occur. However, data derived from controlled laboratory experiments can serve as guidelines for field applications only after differences in water quality, species of organisms, methods of application, and toxicant formulation have been carefully considered.

Conclusions

1. Invertebrates with a soft integument were more susceptible to Bayer 73 than were those with a chitinized exoskeleton.
2. Daphnid reproduction was significantly reduced ($P < 0.01$) in Bayer 73 concentrations of 0.056, 0.10, and 0.18 mg/l.
3. Emergence of midges was significantly reduced ($P < 0.01$) in Bayer 73 concentrations of 0.56, 1.0 and 1.8 mg/l.
4. Equilibria of ^{14}C-Bayer 2353 residues were attained in most invertebrates in 24 h, when total body residues (wet weight of whole organism) ranged from 4 to 80 times the water concentration.
5. Elimination of radioactive residues in scuds and midge larvae was rapid: 90% of the accumulated residues were lost within 48 h after the organisms were transferred to clean flowing water.
6. Concentrations of Bayer 73 that are effective in controlling lamprey should have little adverse effect on most aquatic invertebrates.

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Accumulation, Elimination, and Biotransformation of the Lampricide 2′,5-Dichloro-4′-nitrosalicylanilide by *Chironomus tentans*[1]

by

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Abstract

When exposed to sublethal concentrations of ^{14}C-2′,5-dichloro-4′-nitrosalicylanilide (Bayer 2353), either alone or in combination with 3-trifluoromethyl-4-nitrophenol (TFM), larvae of the midge *Chironomus tentans* accumlated ^{14}C residue rapidly. Uptake was related directly to amount of toxicant present in exposure water and to water hardness. Residues of ^{14}C-Bayer 2353 did not attain a stable uptake equilibrium but were rapidly excreted, both during continuous exposure and during postexposure periods in toxicant free water. Chironomids biotrans formed ^{14}C-Bayer 2353 to ^{14}C-chlorosalicylic acid and an unidentified ^{14}C metabolite.

In 1958, salicylanilides, particularly 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 2353), were discovered to be potent molluscicides (Gönnert 1962). After further investigation, the U.S. Fish and Wildlife Service and the Canadian Department of Environment began in 1966 to use a 5% granular formulation of Bayer 73 (2-aminoethanol salt of Bayer 2353) to control the sea lamprey, *Petromyzon marinus* (Hamilton 1974a, 1974b). Mixtures of Bayer 73 and 3-trifluoromethyl-4-nitrophenol (TFM) in a 2:98 ratio (by weight) have also been used because the addition of small amounts of Bayer 73 substantially reduces the amount of TFM needed for effective treatment of populations of larval lampreys (Howell et al. 1964).

The toxicity of Bayer 73 to nontarget invertebrates varies widely. Bayer 73, at the concentrations used for lamprey control, does not appear to affect many hard-shelled aquatic invertebrates (Rye 1972; Cumming and Dawson 1973; Fish-Pesticide Research Laboratory 1973; Hunn 1973; and Sanders 1973), but some soft-bodied invertebrates are susceptible (Rye 1972). Additional information regarding the dynamics of the toxicant and its residues in non-target invertebrates is required to satisfy regulatory requirements of the U.S. Environmental Protection Agency.

The present study was designed to determine the rates of accumulation and elimination of Bayer 2353 by larvae of the aquatic midge *Chironomus tentans* (Diptera; Chironomidae) during short-term sublethal exposures. In addition, we investigated the ability of the organism to metabolize the toxicant.

Materials and Methods

Radioactive Bayer 2353 (2′,5-dichloro-4′-nitrosalicylanilide) was synthesized with a uniformly labeled ^{14}C-chlorosalicylic acid ring (10mCi/mM, American Radiochemical Corporation, Sanford, Florida). Nonradioactive Bayer 73 (2-aminoethanol salt of 2′-5-dichloro-4′-nitrosalicylanilide, 70% wettable powder) was obtained from the Chemagro Corporation, Kansas City, Kansas. TFM (95.7% 3-trifluoromethyl-4-nitrophenol) was used in a 98:2 (by weight) combination with Bayer 2353; this mixture is designated as TFM-2B. Reconstituted water was prepared as described by Marking (1970) to effect three levels of hardness (mg/l as $CaCO_3$) and (in parentheses) pH: soft, 40–48 (7.2–7.6); hard, 160–180 (7.6–8.0); and very hard, 280–320 (8.0–8.4). These materials were supplied by the Fish Control Laboratory, La Crosse, Wisconsin. All other reagents were of analytical grade unless otherwise specified.

Stock cultures of *Chironomus tentans*, a widely distributed midge whose benthic larva is an important fish food organism, were maintained in continuous laboratory culture in soft water at 21 ± 2 C according to the procedure of Kawatski et al. (1975). Only fourth instar larvae were used in the experiments. Before exposure to the toxicant, the

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animals were removed from the rearing system and acclimated in 900 ml of the toxicant free test water at 20 ± 0.5 C for 24 h.

For accumulation studies, larvae were exposed to ^{14}C-Bayer 2353 in Pyrex beakers containing 900 ml of water (60–80 larvae per beaker). Before a sublethal concentration of the toxicant (54–108 μg/l) was added, samples of larvae and test water were withdrawn for background radiation determinations. The toxicant was added in an acetone-water (1:1) solution; the acetone concentration in test water never exceeded 1 ml/l. Triplicate samples of larvae and test water were employed throughout the study.

Immediately after addition of the toxicant, 0.5-ml samples of the exposure water were collected. The rate of toxicant accumulation by larvae was determined by sampling animals and water at 2-h intervals during the first 8 h of exposure, and after 12, 24, 48, 72, and 96 h of exposure. Three larvae per sample were withdrawn from each exposure vessel, placed on an absorbent towel, blotted dry, and weighed. Dry weights were calculated on the basis of a determined dry weight correction factor. Each sample was then transferred to a scintillation vial where 0.5 ml NCS tissue digestor (Amersham/Searle Corp.) was added. The vials were held at room temperature for 2 h before the addition of 10 ml of scintillation cocktail (4 g of 2,4-diphenyloxozole, 0.10 g of 1,4-bis [2(5-phenyloxozolyl)] benzene, 250 ml of Triton X-100, and 750 ml of toluene). The vials were shaken vigorously and then refrigerated until radioactivity of the contents was determined.

In elimination studies, the larvae were transferred from the exposure vessels to 900 ml of toxicant free water after 24 h of exposure to the toxicant. Samples of larvae and water were withdrawn at 2-h intervals and prepared for radioactivity determinations by the same counting procedure as used in the accumulation studies.

Biotransformation studies were conducted by the following procedures. About 100 larvae were exposed to a sublethal concentration of ^{14}C-Bayer 2353 (108 μg/l). Thirty larvae were withdrawn at 12-h intervals over a 36-h period. The larvae were blotted dry, transferred to a grinding vessel, and homogenized with a motor-driven Teflon pestle. We added small amounts of double distilled water, and then subjected the homogenate to ultransonic vibrations, using the intermediate tip of a Sonic 300 Dismembrator (Artex Systems Corporatton) at 50% of full power for 10 min.

The sonicated homogenate was spotted directly on precoated thin layer plates purchased from Brinkman Instrument Company (Polygram SIL N-HR, 0.2 mm) and Eastman Kodak Company (6061 Silica Gel, 0.1 mm). The developing systems were: methanol/chloroform/ammonium hydroxide (50/25/2.5,vol/vol/vol; J. J. Lech, personal communication) and methanol/chloroform/acetic acid (16/8/1,vol/vol/vol). For visualization of the separation, we superimposed a mixture of nonradioactive Bayer 73 and chlorosalicylic acid on the spotted sonicate. We vertically sectioned developed plates from 0.5 cm below to 10 cm above the spotting points, and by using the standards as horizontal reference points, cut the vertical sections into several pieces (Kawatski and McDonald 1974). The pieces were placed in individual counting vials with 10 ml of scintillation cocktail, and their radioactivity was determined.

The radioactivity in samples was measured with a Nuclear Chicago Mark II scintillation spectrometer. Observed counts per minute were converted to disintegrations per minute (DPM) with the channels ratio-external standard method of quench correction. For accumulation and elimination studies, DPM's were converted to micrograms of toxicant (in terms of the weight of the parent Bayer 2353 molecule) and expressed in terms of the weight or volume of the sample. In biotransformation studies, the DPM's were summed for each vertical section of the thin layer plate and the percentages of the total contributed by individual horizontal sections were determined.

Results

The magnitude of ^{14}C-Bayer 2353 accumulation from water by *Chironomus tentans* during continuous sublethal exposure was in part a function of the Bayer 2353 concentrations in exposure water. The initial rate of uptake was related both to toxicant concentration and to hardness of exposure water (Fig. 1). For example, when animals were exposed to 63 μg/l of Bayer 2353, uptake rates during the first 12 h were 12.8, 11.9, and 9.6 μg/g per hour in soft, hard, and very hard water; when the exposure concentration was 108 μg/l, the initial uptake rates were 46.7, 42.0, and 31.3 μg/g per hour, respectively.

In nearly all uptake experiments where test animals were exposed continuously to Bayer 2353 for up to 96 h, maximum accumulation occurred during the first 24 h, and usually within 12 h. Residue accumulation did not plateau; rather, the elimination rates thereafter exceeded the uptake rates.

When chironomids that had been exposed to Bayer 2353 were placed in toxicant free water, ^{14}C residues continued to be excreted very rapidly, and the rate of excretion was directly proportional to the toxicant concentration during exposure and to the maximum amount of Bayer 2353 accumulated during the exposure (Fig. 2). Biological half-lives (time required
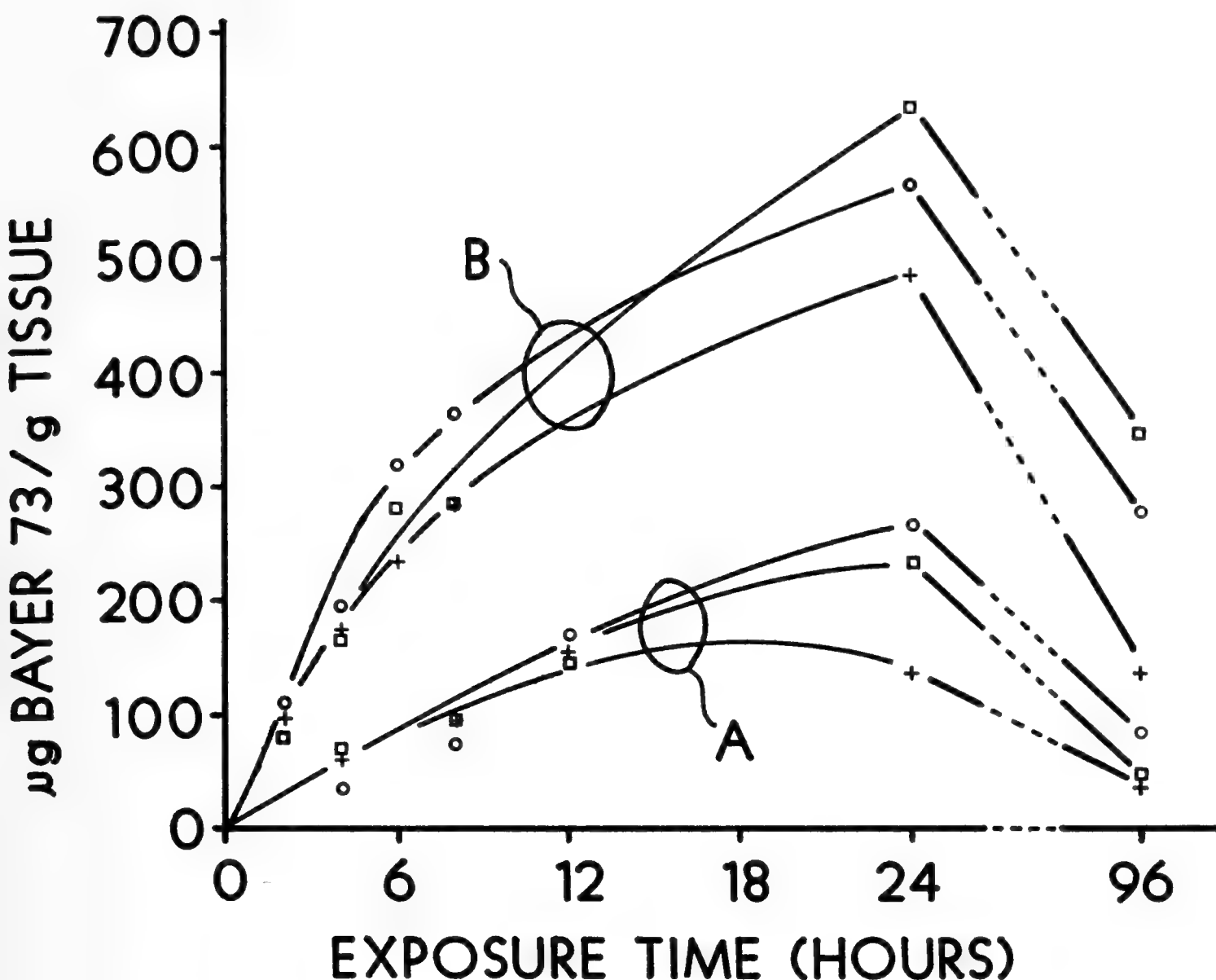
Fig. 1. Uptake of ^{14}C residue (μg/g dry wt) by *Chironomus tentans* larvae from water of three hardnesses (0, soft; □, hard; +, very hard) that initially contained 63(A) or 108(B) μg/l of ^{14}C-Bayer 2353.

for loss of one-half of the accumulated material) of the residue varied from 3.5 to 25.4 h (Table 1), and the rate of elimination was directly proportional to the maximum amount of residue accumulated during the exposure period (Fig. 3).

Uptake of Bayer 2353 by chironomids exposed to TFM-2B was no different from uptake of Bayer 2353 from test water containing only Bayer 2353 (Table 2). When chironomids were exposed to 2.3 μg/l of Bayer 2353 and to TFM-2B (112 μg/l of TFM; 2.3 μg/l of Bayer 2353), they accumulated ^{14}C residue at the rate of 0.59 μg/g per hour during the initial 12 h; thereafter, residue was eliminated even during continuous exposure.

When various substrates (paper, sand, and silt) were placed in exposure vessels, Bayer 2353 concentrations in the water decreased with time at a faster rate than when substrates were absent. Presumably because of the reduction in toxicant concentration, chironomids in the vessels containing substrates absorbed Bayer 2353 residue less rapidly than did control animals in substrate free systems (Table 3).

Chironomids that absorbed ^{14}C-Bayer 2353 were able to cleave the ^{14}C-Bayer 2353 molecule, as evidenced by the recovery of ^{14}C labeled chlorosalicylic acid (Table 4). As exposure continued beyond 8 h, a decreasing percentage of the accumulated residue in chironomid tissue was chlorosalicylic acid and an increasing percentage was Bayer 2353, suggesting that chlorosalicylic acid was being excreted. One other ^{14}C metabolite observed, more polar than either chlorosalicylic acid or Bayer 2353, was probably acidic. A third ^{14}C-labeled component appeared at all exposure times; it did not migrate in

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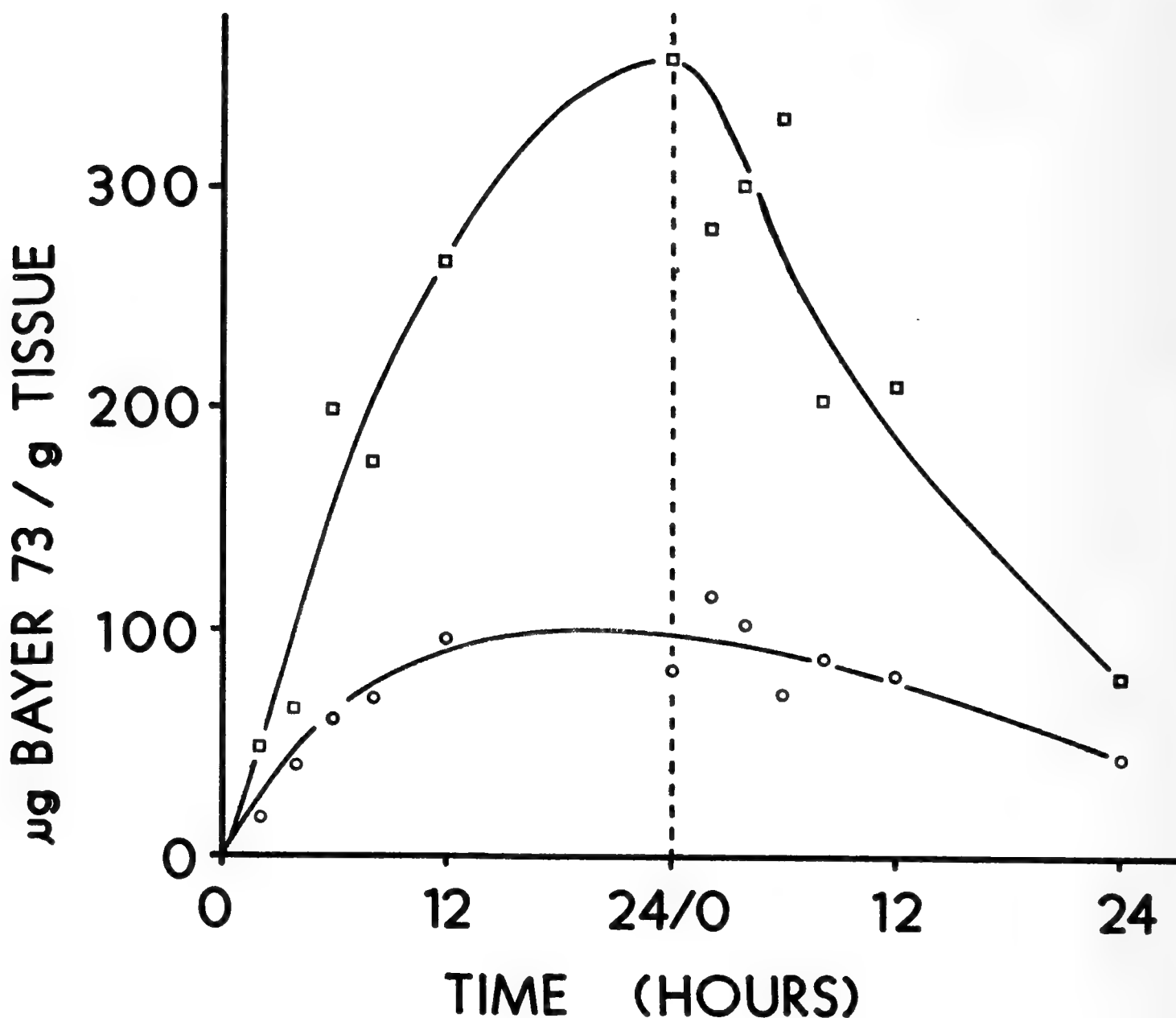
Fig. 2. Uptake of ^{14}C residue (μg/g dry wt) by *Chironomus tentans* larvae from soft water that initially contained 54 (0) or 162 (□) μg/l of ^{14}C-Bayer 2353, and elimination of residue during a 24-h postexposure period in toxicant free water.

our thin layer chromatographic systems, however, and was not present in the ^{14}C-Bayer 2353 standard.

Discussion

The manner of uptake and elimination of Bayer 2353 by *Chironomus tentans* helps to explain the toxic effects of Bayer 73 observed by Kawatski et al. (1975). In 8-h exposures, Bayer 73 was more toxic in soft water than in hard water, but in longer exposures (24-96 h) the differences in toxicity related to water hardness became progressively less, and were statistically nonsignificant. The uptake pattern for

^{14}C-Bayer 2353 is consistent with the toxicity data from previous static tests. During the first 8 h of exposure, the rate of ^{14}C-Bayer 2353 uptake decreased with increasing water hardness, but after about 12 h of exposure, residue levels did not vary significantly in relation to hardness. Furthermore, after 12 h of exposure and during continuous exposure, ^{14}C residue was eliminated regardless of water hardness. The toxicological result of this excretion is that 48-, 72-, and 96-h LC_{50} values do not differ significantly at any given water hardness (Kawatski et al. 1975).

Accumulation of ^{14}C-Bayer 73 is independent of TFM uptake; i.e., Bayer 2353 absorption is identical from solutions of either TFM-2B or Bayer 2353 alone.

Table 1.   *Accumulation of ^{14}C residue ($\mu g/g$ dry wt) by* Chironomus tentans *larvae at 20±1 C under various conditions of exposure to ^{14}C-Bayer 2353, elimination of ^{14}C residue during postexposure in toxicant free water of differing hardness and pH, and biological half-life of accumulated ^{14}C residue [a].*

Initial exposure concn. of Bayer 2353 ($\mu g/l$) [a]	Exposure water hardness [b]	Length of exposure (h)	Total ^{14}C residue accumulated ($\mu g/g$) [c]	Postexposure water hardness [b]	Biological half-life of ^{14}C residue (h)
Experiment 1					
54	S	24	79	S	25.4
162	S	24	356	S	12.6
Experiment 2					
108	H	8	171	H	12.0
108	H	12	164	H	10.7
Experiment 3					
108	S	24	567	S	15.2
108	S	24	567	H	5.0
108	S	24	567	VH	3.7
Experiment 4					
108	H	24	634	S	3.5
108	H	24	634	H	3.5
108	H	24	634	VH	3.5
Experiment 5					
108	VH	24	488	S	5.2
108	VH	24	488	H	5.2
108	VH	24	488	VH	4.2

[a] Results of simultaneous tests are grouped as experiments.

[b] S = soft (hardness, 40–48 mg/l as $CaCO_3$; pH, 7.2–7.6); H = hard (160–180 mg/l; pH, 7.6–8.0); and VH = very hard (280–320 mg/l; pH, 8.0–8.4).

[c] Expressed in terms of the weight and activity of the parent Bayer 2353 molecule.

Kawatski and Bittner (1975) found that accumulation of TFM was likewise independent of Bayer 73 uptake. Therefore the slight synergistic toxicity of TFM and Bayer 73 is not due to potentiated uptake at absorptive surfaces but must be due to other interactions.

The ability of chironomids to metabolize ^{14}C-Bayer 2353 to form at least two compounds (chlorosalicylic acid and a more polar material) probably accounts in part for the rapid excretory rate. The unidentified polar material is probably a conjugated form of either Bayer 2353 itself or of chlorosalicylic acid. Salicylates are readily conjugated with glycine and glucuronic acid in higher organisms (Milne 1963), and chironomids also possess such detoxication systems (Kawatski and Bittner 1975). Since only the chlorosalicylic acid ring of the Bayer 2353 was labeled, the chloronitroaniline ring and possible derivatives of it were not recovered or measured.

The ^{14}C material that did not migrate on thin layer plates (nonmigrating material; NMM) cannot with certainty be identified as a Bayer 2353 metabolite, since it may represent an artifact of the analytical method. If Bayer 2353 or its metabolites are trapped within cell fragments which remain immobile, the material identified as NMM may simply be Bayer 2353, chlorosalicylic acid, or the more polar unidentified derivative. Although we attempted to disrupt subcellular components by sonicating the tissue homogenates before performing thin layer chromatography, some salicylates probably remained bound to proteins (a well-known tendency for salicylates). Thus the identity of the NMM remains undetermined.
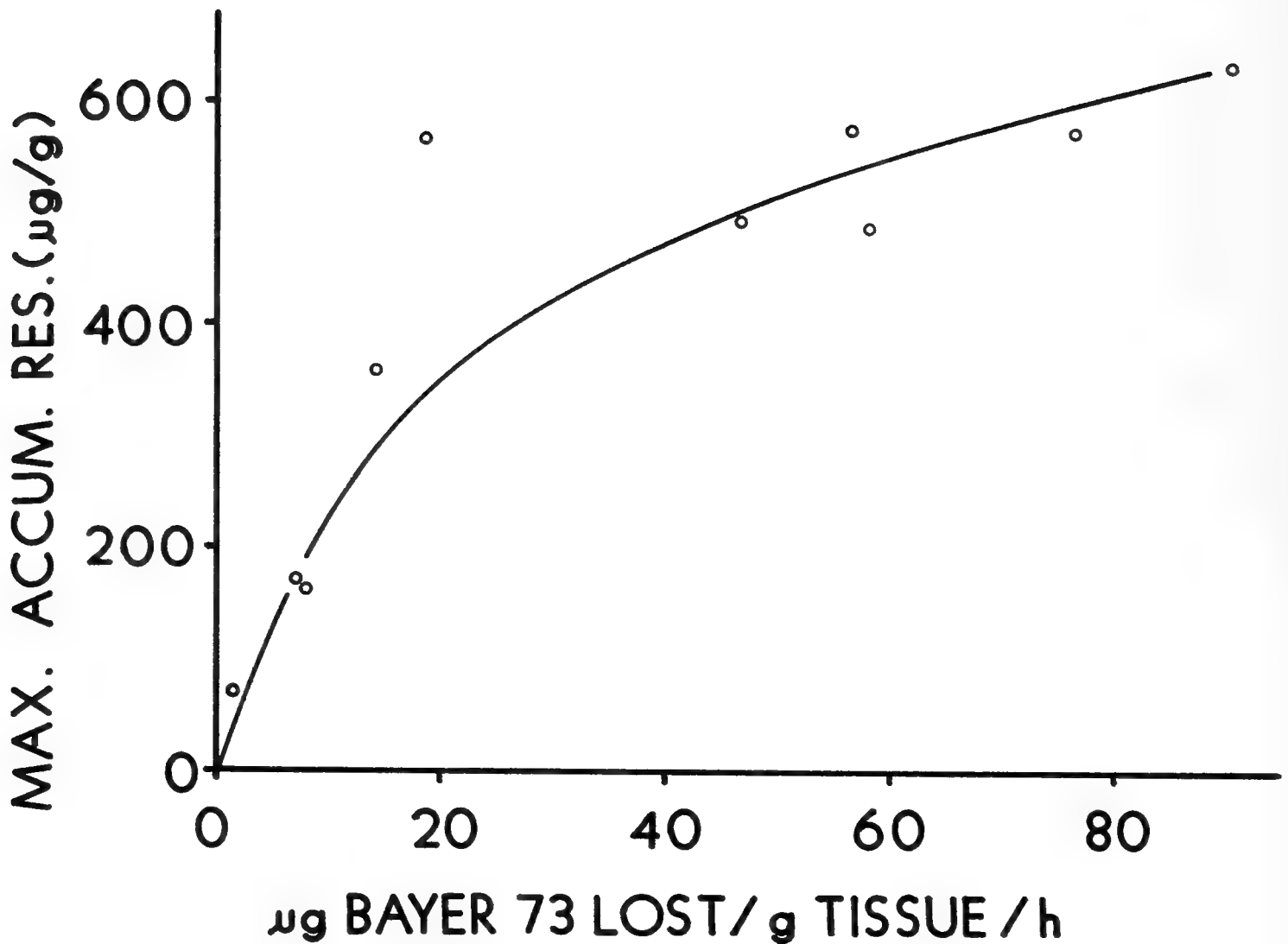
Fig 3. Relation between rate of elimination of accumulated ^{14}C residue and maximum body burden of residue (μg/g dry wt).

Table 2. *Accumulation of ^{14}C residue by* Chironomus tentans *larvae during 96 h of continuous exposure to sublethal concentrations of ^{14}C-Bayer 2353 and TFM-2B[a] in soft water (CaCO$_3$ hardness, 40–48 mg/l; pH, 7.2–7.6) at 20 + 1 C[b]*.

Length of exposure (h)	Exposed to Bayer 2353		Exposed to TFM-2B	
	Exposure water (μg/l)	Tissue (μg/g dry wt)	Exposure water (μg/l)	Tissue (μg/g dry wt)
0	2.52 (0.11)	—	2.52 (0.12)	—
2	2.39 (0.23)	1.55 (0.41)	2.29 (0.04)	2.09 (0.15)
4	2.14 (0.24)	2.32 (0.32)	2.21 (0.11)	2.49 (0.35)
8	1.99 (0.15)	4.84 (1.63)	1.98 (0.19)	4.64 (1.40)
12	1.88 (0.05)	6.37 (1.04)	1.97 (0.29)	6.64 (1.38)
24	1.73 (0.13)	3.72 (1.06)	1.74 (0.03)	5.20 (0.60)
96	1.78 (0.12)	1.69 (0.16)	2.13 (0.06)	1.65 (0.12)

[a] TFM-2B designates a 98:2 (by weight) combination of TFM and Bayer 2353.

[b] Standard deviations are shown in parentheses.

Table 3. *Accumulation of* [14]*C residue (* μ *g/g dry wt) by* Chironomus tentans *larvae during 36 h of continuous exposure to* [14]*C-Bayer 2353 in soft water (CaCO$_3$ hardness, 40-48 mg/l; pH, 7.2-7.6) at 20 + 1 C containing 5 g of the named substrates in 900 ml of test medium*[a].

Substrate and mesh number (where applicable)	Duration of exposure (h)					Water characteristics at end of exposure			
	4	8	12	24	36	Bayer 2353 (μg/l)	Hardness (mg/l as CaCO$_3$)	Dissolved oxygen (mg/l)	pH
None	43.3 (8.7)	121.6 (17.8)	99.5 (6.7)	155.6 (105.9)	185.1 (95.2)	67.1 (1.7)	52	7.7	6.8
Paper toweling	34.3 (11.6)	36.1 (1.0)	74.1 (13.9)	71.0 (22.6)	54.3 (13.6)	40.9 (0.8)	53	7.3	6.8
Sand									
30	36.3 (13.0)	42.6 (12.3)	76.5 (15.9)	41.9 (9.1)	34.4 (1.1)	60.5 (1.3)	57	8.3	6.8
80	22.7 (12.0)	23.7 (1.9)	23.8 (2.7)	30.0 (6.9)	24.4 (5.0)	44.8 (0.8)	78	6.0	6.9
200	28.7 (12.5)	59.2 (13.2)	41.2 (3.1)	74.0 (22.6)	51.4 (8.5)	61.7 (3.4)	70	7.3	6.8
Silt									
30	23.3 (18.8)	45.0 (17.4)	44.5 (22.0)	34.3 (8.8)	39.7 (6.4)	44.1 (1.5)	120	5.3	7.0
80	38.2 (15.3)	95.0 (58.6)	50.1 (34.3)	65.4 (56.1)	26.1 (9.2)	26.4 (1.0)	140	5.0	6.8

[a] Initial concentration of ^{14}C-Bayer 2353: 66.0 μg/l; standard deviations are shown in parentheses.

Table 4. *Percentages of total ^{14}C residue retained by* Chironomus tentans *larvae exposed continuously to ^{14}C-Bayer 2353 in soft water (CaCO$_3$ hardness, 40–48 mg/l; pH, 7.2–7.6) at 20 + 1 C^a.*

| Thin layer chromatographic identification | | Exposure period (h)[c] | | |
Component	R_f^b (ranges in parentheses)	8	24	36
Bayer 2353	0.65 (0.55–0.70)	20.7 (3.2)	23.8 (2.9)	39.1[d] (13.1)
Chlorosalicylic acid	0.45 (0.30–0.55)	32.5 (4.0)	29.9 (4.3)	23.7[d] (13.0)
Other[b]	0.25 (0.10–0.30)	18.1 (4.3)	15.9 (3.1)	8.8[d] (2.4)
NMM[e]	0.0 (−0.05–0.10)	28.5 (1.7)	29.2 (2.9)	26.2 (3.9)

[a] Initial concentration of ^{14}C-Bayer 2353: 108 μg/l.
[b] R_f's for both acidic and basic thin layer systems; "other" material appeared to migrate slightly behind Bayer 2353 in the basic system.
[c] Standard deviations given in parentheses.
[d] Change between 8 and 36 h statistically significant ($P = 0.05$).
[e] NMM: nonmigrating material; the amounts of NMM were similar in both basic and acidic thin layer developing systems.

Conclusions

1. Larvae of *Chironomus tentans* accumulated Bayer 2353 rapidly from sublethal exposure concentrations.
2. Accumulation of residue depended in part on water hardness and concentration of the toxicant.
3. Chironomids eliminated residue rapidly, both during continuous exposure and during postexposure periods in toxicant free water.
4. Rates of Bayer 2353 accumulation and elimination were independent of TFM absorption and elimination.
5. *C. tentans* metabolized ^{14}C-Bayer 2353, producing ^{14}C-chlorosalicylic acid and at least one other ^{14}C product.

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60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests, by L. L. Marking and L. E. Olson. 1975. 27 pp.
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INVESTIGATIONS IN FISH CONTROL

80. Effects of Antimycin A and Rotenone on Macrobenthos in Ponds

81. Aquatic Macroinvertebrates in a Small Wisconsin Trout Stream Before, During, and Two Years After Treatment with the Fish Toxicant Antimycin

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

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by

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Abstract

Samples of macrobenthos, collected over a 14-month period from nine 0.03-ha experimental ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri, were analyzed to determine the long- and short-term effects of antimycin A and rotenone. The ponds were characterized by an abundance of bushy pondweed *(Najas guadalupensis)* and by the absence of fish. Treatment concentrations of 0.5 mg/l of rotenone and 20 μg/l of antimycin and concentrations of 2.0 mg/l of rotenone and 40 μg/l of antimycin were applied. There were no effects on species diversity, emergence, seasonal dynamics, abundance, or relative numbers of taxa that could be attributed to either toxicant. Periods of spring emergence, summer buildup, and fall emergence of insects were closely associated with the seasonal development and decline of vegetation.

The short- and long-term effects on invertebrate macrobenthos of treating bodies of water with rotenone and antimycin A lack adequate documentation. Most benthic organisms appear to be unaffected by either toxicant at concentrations applied for fish eradication (Brown and Ball 1942; Walker et al. 1964; Gilderhus et al. 1969; Schoettger et al. 1967; Smith 1972). However, there are reports of mortality among specific taxa exposed to low concentrations of rotenone (Cushing and Olive 1956; Brown and Ball 1942) and to high concentrations of antimycin A (H. Howell et al. unpublished data; Walker et al. 1964; Lesser 1972).

The purposes of this study were to: (1) identify any short- or long-term changes in species abundance in pond benthos resulting from the application of rotenone or antimycin, (2) determine any difference in abundance of benthos which may result from use of different concentrations of toxicants, (3) determine the time required for affected populations of organisms to recover from rotenone and antimycin treatment, and (4) determine whether emergence of benthic organisms is affected by rotenone or antimycin treatment.

The complete qualitative and quantitative analyses of benthic collections, June-October 1971 and April-August 1972, which are the basis for this paper, are presented in an appendix.

The appendix also provides analysis of the seasonal changes in benthic populations: summer and winter development, fall and spring emergence. This description of benthic community dynamics in these experimental ponds is distinctive because it concerns a habitat which is heavily vegetated and devoid of predatory fish populations. Dense vegetation provides unusual cover, abundant food, and more habitat niches than occur in nonvegetated bottom sediments. Vegetation also has the potential for causing low concentrations of dissolved oxygen, either directly through respiration or indirectly through decomposition. The absence of fish may modify species abundance and composition of the benthos because the populations are not subject to predation by fish.

Methods

Description of Study Area

This study was conducted on nine similar ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri. Average dimensions of the standing water mass were 21.4 × 15.6 × 0.6 m; average surface area was 0.03 ha; and average volume was 251.3 m³. Pond bottoms were sloped; water depths ranged from about 0.3 to 1.2 m. The soil type was Mexico silt loam.

1

Experimental Design

The nine experimental ponds, A through I, differed in past use and in length of time they had held water. (Correspondence of pond lettering to the Fish-Pesticide Research Laboratory numbering system is: A, 24; B, 16; C, 23; D, 18; E, 20; F, 19; G, 17; H, 22; and I, 21.) Before the study began, all ponds were drained for 2 weeks of drying, as a means of reducing species differences between ponds and establishing similar successional stages. A random treatment design provided two control ponds, three ponds for rotenone treatment, and four ponds for antimycin treatment. Sample sites were randomized as to location and depth in each pond after the pond was divided into 16 3.0- × 4.5-m sampling rectangles.

Sampling Methods

From 1 June 1971 to 31 August 1972, 121 samples were collected and analyzed. Four samples per pond—two from the shallow area and two from the deep area—were collected at monthly intervals for the 14-month period. From 1 June to 31 August each year, collections were made at 14-day intervals. In addition, more intensive sampling preceded and followed treatment: the interval in days was gradually decreased before treatment in the sequence 14, 7, 3, 2, 1, and treatment; and then gradually increased after treatment in the sequence 1, 2, 3, 7, and 14 days. The sampling device was a modified Ekman dredge, 231 cm^2.

Samples were washed in the field through a screen with 11.8 meshes per linear centimeter. The vegetation, benthic organisms, and debris remaining on the screen were placed in jars containing 10% formalin. In the laboratory the macro-vegetation was removed and examined for benthic organisms, and the remaining detritus with organisms was placed in a saturated aqueous solution of Epsom salts (magnesium sulfate) and stirred so that the organisms floated to the surface. This is a modification of a flotation procedure described by Anderson (1959), in which sugar was replaced with Epsom salts.

Emergence cages—one in each pond—were operated from 7 to 30 April 1972. The wooden and nylon cages, 1.0 m square by 20 cm high, were 1 to 4 m from shore over water about 1 m deep. Adult insects were removed from the covering screens at weekly intervals and preserved in 95% ethanol.

Computation of Species Diversity Index

A species diversity index derived from the information theory (Wilhm and Dorris 1968) was estimated by the formula

$$\bar{d} = - \Sigma \ (n_i/n) \ \log_2 (n_i/n)$$

where $\bar{d}$ = estimate of diversity, n_i = number of individuals in the ith taxon, and n = total number of individuals in the sample.

Application of Toxicants

The ponds were treated in late August, near the usual time of pond treatment (September) in the Midwest. Concentrations of rotenone and the initial concentrations of antimycin were those recommended for fish eradication by Kinney (1968) and Lennon and Berger (1970), respectively.

All ponds were treated on the afternoon of 25 August 1971. Sand formulated antimycin (Fintrol 5) was applied to ponds F, G, H, and I. An initial low treatment concentration of 1.5 μg/l was applied to ponds F and G, and an initial heavy treatment concentration of 10 μg/l to ponds H and I. Applications of emulsifiable rotenone (Noxfish; 5% active ingredient) were made in ponds C, D, and E. Ponds C and D received a low treatment concentration of 0.5 mg/l and Pond E a heavy treatment of 2.0 mg/l. Ponds A and B were untreated controls.

Although rotenone concentrations selected were adequate, the treatments with antimycin were ineffective in eradicating fish held in cages in the ponds with pH values greater than 9.0 (Table 1). After completing toxicity tests to determine adequate concentrations of antimycin (Table 2), we re-treated the ponds on 1 September 1971 at low concentrations of 20 μg/l (F and G) or high concentrations of 40 μg/l (H and I). The low concentration in ponds F and G produced a complete kill of all bluegills *(Lepomis macrochirus)*; however, the high concentration in H and I yielded only a partial kill of bluegills 130 to 180 mm long (Table 3). The lessened effect of the 40 μg/l concentration was possibly related to the higher pH values in ponds H and I. The pH values were 9.0 in pond F and 9.5 in pond G at 1000 h, and 9.6 in pond H and 9.7 in pond I at 1100 h.

Classification

The classification scheme for the Chironomidae follows Sublette and Sublette (1965), except that we assigned generic status to four of the subgenera of Sublette and Sublette: *Dicrotendipes, Harnischia, Endochironomus,* and *Cryptochironomus.*

Table 1. *Numbers of confined bluegills of two sizes killed within 1, 4, and 14 h after treatment of ponds with low and high concentrations of rotenone and antimycin.*

Pond	Treatment	Hours after treatment, total length of fish (mm), and (in parentheses) number of test fish in cages.					
		1		4		14	
		50–100 (20)	130–180 (7)	50–100 (20)	130–180 (7)	50–100 (20)	130–180 (7)
A	Control	0	0	0	1	0	1
B	Control	0	0	1	0	2	1
C	Rotenone, 0.5 mg/l	5	0	20	5	20	6
D	Rotenone, 0.5 mg/l	5	0	16	2	16	2
E	Rotenone, 2.0 mg/l	12	2	20	5	20	7
F	Antimycin, 1.5 μg/l	0	2	5	2	6	2
G	Antimycin, 1.5 μg/l	0	0	1	0	1	0
H	Antimycin, 10.0 μg/l	1	0	2	0	3	0
I	Antimycin, 10.0 μg/l	2	0	3	0	5	0

Results

Water Quality

Chemical analysis of the water from the deep well supplying the ponds used in the present experiment was given by Kennedy et al. (1970). Seasonal fluctuations in pond water chemistry (Table 4) were similar in 1971 and 1972, and did not differ from those expected in small ponds in the Midwest. Seasonal changes in alkalinity were accentuated by the development of dense stands of macrophytes. During both summers, photosynthesis resulted in the elevation of pH to values greater than 9.5 in all ponds. The rotenone and antimycin treatments had no noticeable effect on water chemistry.

Dissolved oxygen concentrations in the experimental ponds were similar to those found in many small ponds in Missouri. Summer stagnation developed in

all ponds (Table 5). Differences in surface values reflect differences in the time of sampling, which varied from about 0900 to 1300. Pond G was the only pond where anaerobiosis was detected; this condition was accompanied by the generation of hydrogen sulfide, the odor of which was evident from the pond margin.

Taxa Identified

A distinctive characteristic of the experimental waters was the dense growth of vegetation

Table 2. *Toxicity test: mortality of bluegills, total length 50–100 mm, exposed to different concentrations of sand formulated antimycin at pH 9.3.*

Concentration (μg/l)	Number of fish	Hours after treatment				
		4	5	6	12	24
5	10	0	0	0	a	a
10	10	0	0	0	0	0
15	10	0	0	0	2	2
20	10	2	2	5	8	8
40	10	a	7	a	10	10

[a] No count made.

Table 3. *Numbers of caged small (50–100 mm long) and large (130–180 mm) bluegills that died after re-treatment of ponds F–I with antimycin.*

Pond	Treatment	Hours after treatment, length of fish (mm), and (in parentheses) numbers of test fish in cages			
		6		20	
		50–100 (20)	130–180 (8)	50–100 (20)	130–180 (8)
A	Control	0	0	0	0
B	Control	0	0	0	0
C	Control	0	0	0	0
D	Control	0	0	0	0
E	Control	0	0	0	0
F	20 μg/l	20	8	20	8
G	20 μg/l	20	8	20	8
H	40 μg/l	19	4	20	5
I	40 μg/l	7	0	20	2

Table 4. *Range of values for water quality determinations, June through August, 1971 and 1972, for five ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri.*[a]

Treatment and pond identification	pH	Temperature (°C)		Dissolved oxygen (mg/l)		Hardness (mg/l as Ca CO$_3$)	Alkalinity (mg/l as Ca CO$_3$)
		Surface	Bottom	Surface	Bottom		
Control							
A	7.8-9.9	24-30	21-28	5-16	0.7-15	80-141	60-150
B	8.3-9.7	24-29	22-28	5-13	1-11	74-169	65-152
Rotenone							
E	8.4-10.0	23-29	21-28	6-16	1-12	75-155	73-145
Antimycin							
H	7.7-10.1	24-29	22-28	4-14	0.8-15	70-132	77-161
I	8.9-10.3	24-28	21-26	4-15	0.6-9	65-122	77-120

[a] Hardness, alkalinity, and pH were determined only from surface samples; pH was measured at midmorning.

Table 5. *Dissolved oxygen concentrations (mg/l) at surface (S) and bottom (B) in six ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri, July-September 1971.*

Date (1971) and site of sample	Ponds					
	A	B	E	H	I	G
July 7						
S	15.3	12.8	15.6	10.4	11.0	12.4
B	2.5	10.0	11.0	11.2	8.2	5.4
July 20						
S	16.0	12.4	14.2	8.8	10.2	10.5
B	1.5	10.7	9.6	8.8	7.9	1.6
August 3						
S	16.0	12.9	13.7	11.4	14.4	15.0
B	1.5	9.7	2.5	11.0	8.6	1.0
August 21						
S	13.0	11.6	9.3	8.5	8.8	8.0
B	1.3	9.6	2.3	6.8	0.6	0.7
August 31						
S	14.0	13.1	14.0	11.2	10.8	5.0
B	0.7	1.6	2.0	1.4	0.8	0.0
September 14						
S	17.2	12.8	11.1	7.7	10.0	1.3
B	0.5	6.0	0.8	6.5	2.1	0.7
September 28						
S	9.9	11.6	13.2	11.6	12.2	7.8
B	6.7	3.2	2.0	3.8	3.9	4.6

throughout all of the ponds. Bushy pondweed *(Najas guadalupensis)* and chara *(Chara* sp.) were the most abundant plants. Others included water hyssop *(Bacopa rotundifolia)*, smartweed *(Polygonum* sp.), arrowhead *(Sagittaria* sp.), spike rush *(Eleocharis* sp.), and sedge *(Carex* sp.).

Seventy-four animal taxa were identified (Table 6). Most abundant members of the communities were herbivorous mayflies *(Caenis simulans* and *Callibaetis fluctuans)*, predaceous dragonflies *(Enallagma civile* and *Ischnura verticalis)*, and predaceous midges *(Sayomyia punctipennis, Ablabesmyia peleenis, and Procladius bellus)*. Other true midges were the filter feeding midges *Tanytarsus* sp. of the tribe Calopsectrini and *Pseudochironomus richardsoni* and *Chironomus attenuatus* of the tribe Chironomini. Other abundant members included ooze transporting oligochaetes and periphyton browsing snails *(Physa* sp., *Gyraulus* sp., and *Helisoma* sp.).

Effects on Abundance of Benthic Organisms

Inasmuch as the effects of treatment on benthic organisms were similar for both heavy and light applications of the toxicants, the data from heavy applications of rotenone (2 mg/l, pond E), and antimycin (4) μg/l, ponds H and I), have been selected as representative.

Rotenon Treatment, 2 mg/l

Short-term effects.—No immediate short-term (August-September 1971) effects from application of

Table 6. *Benthic organisms collected from research ponds A-I, Fish-Pesticide Research Laboratory, Columbia, Missouri, June 1971-August 1972. The numerically dominant members of each group are indicated with an asterisk; dominant groups in number and volume are indicated by two asterisks.*

OLIGOCHAETA (aquatic earthworms)**
INSECTA
  EPHEMEROPTERA (mayflies)**
    Baetidae
      *Caenis simulans**
      *Callibaetis fluctuans**
    Ephemeridae
      Hexagenia bilineata
  ODONATA (dragonflies and damselflies)**
    Libellulidae
      *Tramea carolina**
      Libellula (2 species)*
      *Erythemis simplicicollis**
      Plathemis sp.
    Aeshnidae
      Anax junius
    Coenagrionidae
      *Enallagma civile**
      *Ischnura verticalis**
      Agria sp.
  HEMIPTERA (true bugs)
    Mesoveliidae
      *Mesovelia mulsanti**
    Notonectidae
      Notonecta sp.
    Veliidae
      Microvelia sp.
      Velia sp.
    Hebridae
      Merragata sp.
    Hydrometridae
      Hydrometra martini
    Belostomatidae
      Belostoma
  TRICHOPTERA (caddis flies)
    Leptoceridae
      *Oecetis inconspicua**
      Leptocella sp.*
    Hydroptilidae
      Oxyethira sp.*
    Phryganeidae
      Phryganea
      Agrypnia
  COLEOPTERA (beetles)
    Hydrophilidae
      Berosus sp.*
      Tropisternus
    Dytiscidae
      *Laccophilus maculosa**
      Bidessus lacustris
      Hydroporus sp.
      Agabus sp.
      Ilybius sp.
      Coptotomus

    Haliplidae
      Haliplus sp.*
      Peltodytes
    Gyrinidae
      Dineutus assimilis
  DIPTERA (true flies)**
    Chaoboridae (phantom midges)
      Chaoborus americanus
      Sayomyia punctipennis
    Chironomidae (true midges)
      *Pseudochironomus richardsoni**
      *Ablabesmyia peleenis**
      Tanytarsus (2 species)*
      *Chironomus attenuatus**
      *Procladius bellus**
      P. subletti
      Labrundinia pellosa
      Larsia planesis
      Dicrotendipes nervosus
      D. modestus
      Harnischia collator
      H. monochromus
      H. potamogeti
      Clinotanypus pinquis
      Tanypus punctipennis
      T. neopunctipennis
      Glyptotendipes barbipes
      Endochironomus nigricans
      Polypedilum simulans
      P. trigonus
      Lauterborniella varipennis
      Psectrocladius dyari
      Monopelia sp.
      Cricotopus
      Cryptochironomus fulvus
      Corynoneura
      Paratendipes
    Ceratopogonidae
      Two unidentified species
    Stratiomyiidae
      Odonotomyia sp.
    Tabanidae
      Chrysops sp.
      Tabanus sp.
GASTROPODA (snails)**
    *Gyraulus**
    Physa
    Heliosoma
PELECYPODA (clams)

2 mg/l rotenone in pond E were observed. Major species of mayflies, dragonflies, damselflies, aquatic earthworms, snails, phantom midges (Chaoboridae), and true midges (Chironomidae), present before treatment were also present after treatment. Although most populations of *Caenis simulans, Tramea carolina, Enallagma civile, Ablabesmyia peleenis* and *Pseudochironomus richardsoni* declined in abundance (Fig. 1), these downward trends had started before treatment. Results were similar in the ponds (C and D) treated with 0.5 mg/l rotenone. The declines noted were the result of emergence of insects and summer stagnation, rather than toxicity of the chemical; trends in population density were similar in control and rotenone-treated ponds. Populations of *Sayomyia punctipennis, Libellula* sp., *Erythemis simplicicollis, Ischnura verticalis,* and *Dicrotendipes* showed no density reductions.

Long-term effects.—Long-term effects of a rotenone treatment of 2 mg/l (Pond E) were evaluated by comparing population densities in 1972 with those in 1971. The same species were present in both summers and, generally, in the same relative abundance (Fig. 1).

Although the population densities in 1972 were somewhat variable with respect to the densities measured in 1971, there was no evidence that the rotenone treatment was responsible for these variations. Rather, differences appeared to be due to natural population fluctuations.
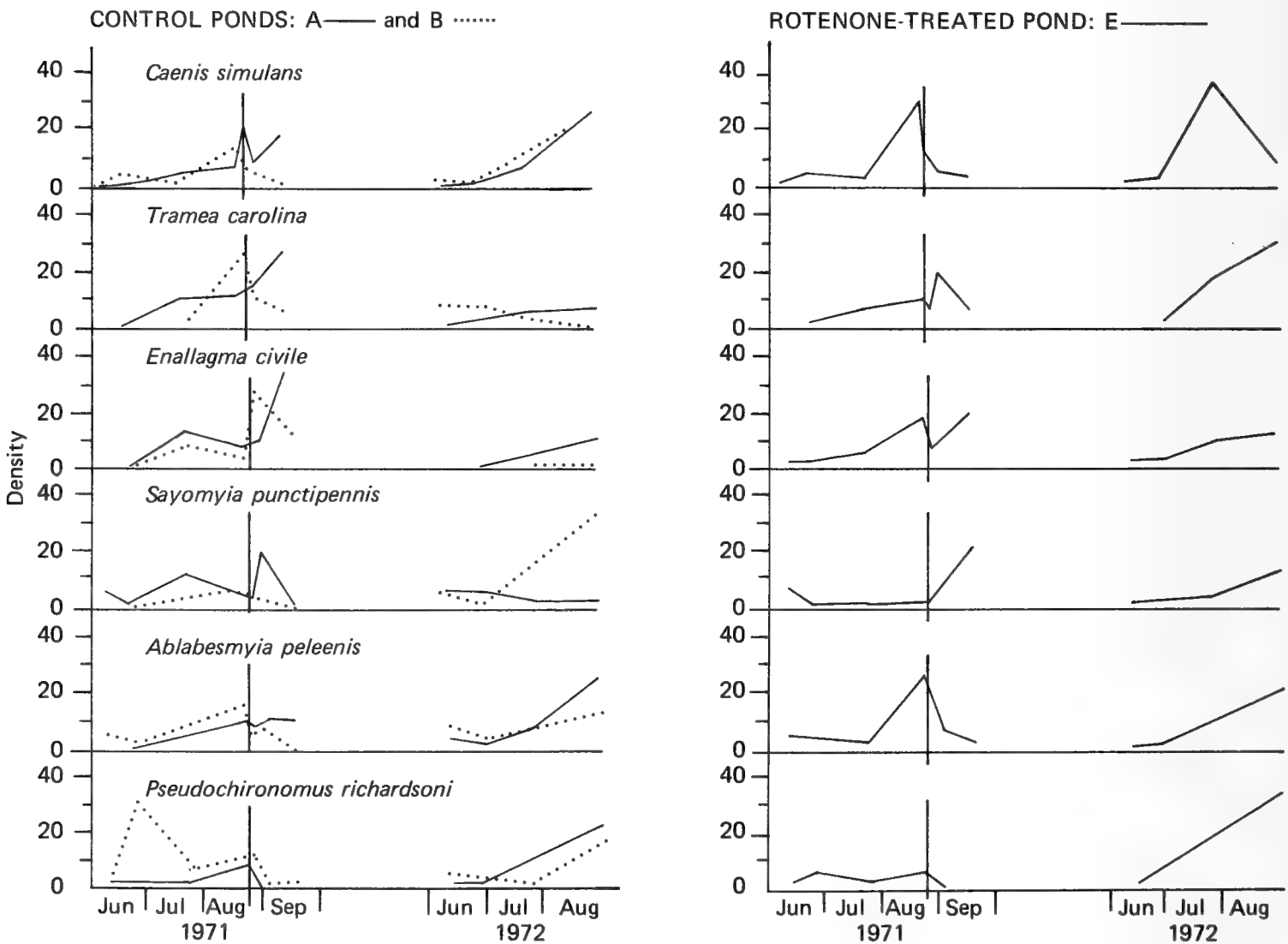
Fig. 1. Changes in population densities of major species of aquatic insects in control ponds A and B, and rotenone-treated (2 mg/l) pond E, 1971 and 1972. Vertical line in August shows treatment date. Density values express numbers collected on any one collection date as a percentage of the total numbers of that species collected throughout the period of collection.

Antimycin Treatment, 40 μg/l

Short-term effects.—No dominant organisms which were present before treatment were eliminated by exposure to heavy (40 μg/l) applications of antimycin in Ponds H and I. These dominant species included representatives of the mayflies, dragonflies, damselflies, phantom midges, true midges, snails, and aquatic earthworms.

In general, a decline in population density was observed during a brief period after treatment, in both treated and control ponds (Fig. 2). In a few organisms this decline preceded treatment. These declining populations are, therefore, not a response to toxicants but to summer stagnation (Table 5) and emergence. Populations of *Caenis simulans, Sayomyia punctipennis,* and *Ablabesmyia peleenis* are examples.

The relatively minor variability among some populations in control ponds and treated ponds—e.g., *Tramea carolina, Enallagma civile,* and *Pseudochironomus richardsoni*—does not negate this general observation.

Long-term effects.—No long-term effects on the bottom fauna were observed after applications of 40 μg/l antimycin in Ponds H and I. This judgment is based on a comparison of the presence or absence and the densities of the major taxa in 1971 and 1972—*Caenis simulans, Tramea carolina, Enallagma civile, Sayomyia punctipennis, Ablabesmyia peleenis,* and *Pseudochironomus richardsoni* (Fig. 2)—which were selected as representative. Species present in 1971 were present in similar numbers in 1972.
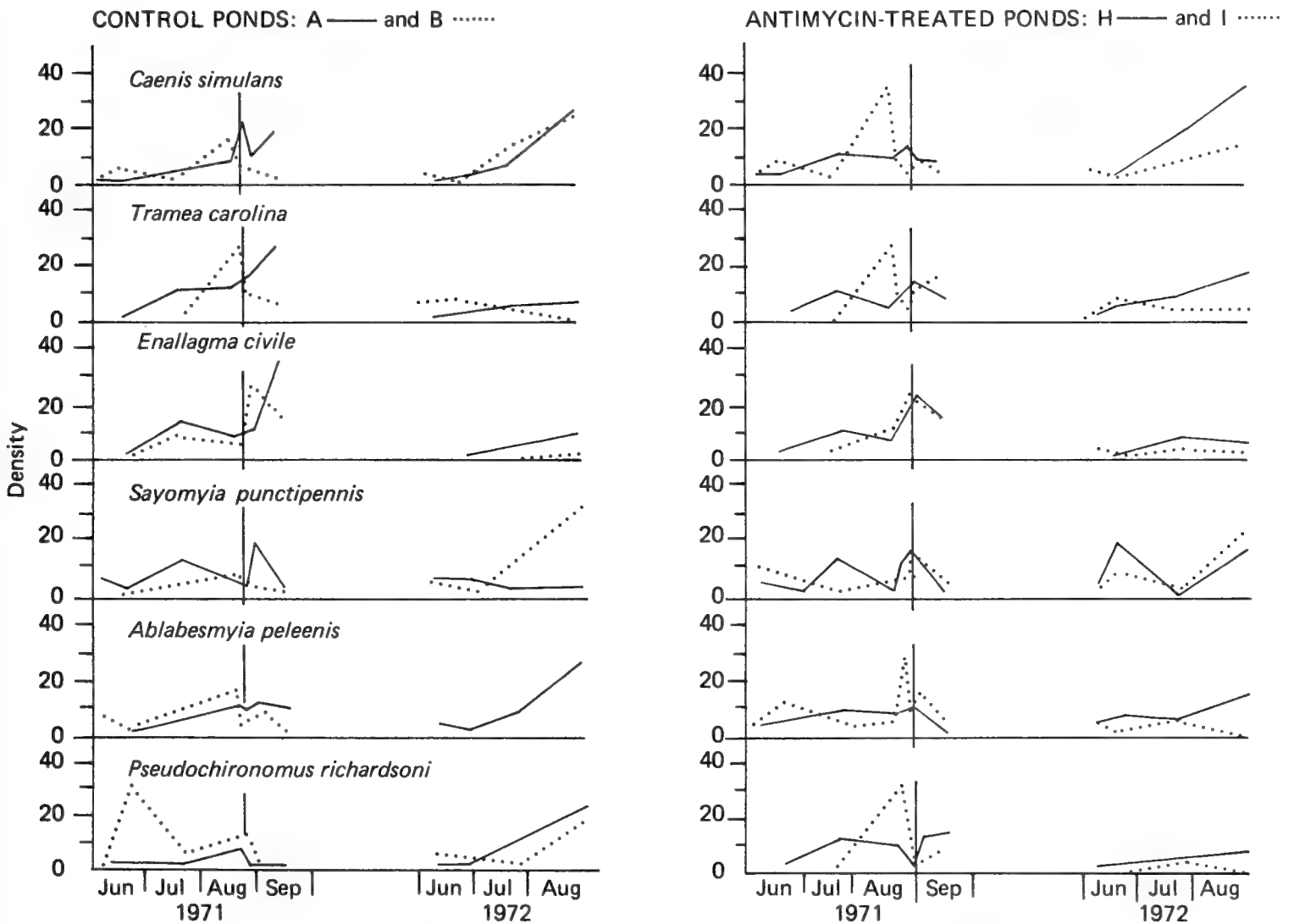
Fig. 2. Changes in population densities of major species of aquatic insects in control ponds A and B and the antimycin-treated (40 μg/l) ponds H and I, 1971 and 1972. Vertical line in August shows treatment date. Density values express numbers collected on any one collection date as a percentage of the total numbers of that species collected throughout the period of collection.

Effects on
Insect Emergence

We conclude that there was no consistent evidence of toxicant interference with insect emergence, on the basis of comparisons of emergences in control ponds and in ponds treated at specified concentrations. The evaluation of emergence was made in 1972, 6 months or more after pond treatment. Emergence behavior was considered to be representative in six selected species: *Caenis simulans, Tramea carolina, Enallagma civile, Sayomyia punctipennis, Pseudochironomus richardsoni,* and *Ablabesmyia peleenis* (Table 7). The total emergence for the study period, April through June, was subdivided to show the percentage occurring in each month for each treatment, so that early or delayed emergence can be identified. Inspection of the table indicates that, although toxicants appeared to have a depressing effect in some treated ponds, comparison with control ponds in the same month shows that the decrease was instead a result of early emergence. For example,

fewer *Pseudochironomus richardsoni* or *Enallagma civile* emerged in June in treated ponds than in control ponds; however, April values indicate that emergence was early in the treated ponds and late in the control ponds.

Effects on
Species Diversity

Species diversity appeared unchanged as a result of pond treatment by toxicants at specified concentrations. Diversity was judged by enumeration of the taxa and by calculation of species diversity indices in control and treated ponds.

The number of taxa present in each pond for 14 samplings in 1971 and 1972 (Table 8) did not differ consistently between (a) pre- and post-treatment samples in 1971, (b) the years 1971 and 1972, or (c) control ponds and ponds treated with antimycin and rotenone. The mean number of taxa for all ponds combined was 21.3. The mean number for all 1971 and 1972 samples for each pond was near this value.

Table 7. *Emergence of insects in ponds treated with rotenone or antimycin, and in control ponds, April-June 1972. Monthly values are expressed as percentage of the total emergence April-June for each species for each treatment.*

Species, and month of emergence	Control ponds	Treatment	
		Rotenone	Antimycin
Caenis simulans			
April	0	0	0
May	0	0	0
June	100	100	100
Tramea carolina			
April	0	0	0
May	0	14	9
June	100	86	91
Enallagma civile			
April	0	0	0
May	0	25	46
June	100	75	54
Sayomyia punctipennis			
April	0	4	1
May	67	19	1
June	33	77	98
Ablabesmyia peleenis			
April	8	16	10
May	50	30	23
June	42	54	67
Pseudochironomus richardsoni			
April	35	70	76
May	11	11	9
June	54	19	15

Table 8. *The number of taxa present in control ponds and in ponds treated with high concentrations of rotenone or antimycin, Fish-Pesticide Research Laboratory, Columbia, Missouri, June 1971-August 1972.*

| Sampling date | Control | | Treatment[a] Rotenone (2 mg/l) | Antimycin (40 μ g/l) | |
	Pond A	Pond B	Pond E	Pond H	Pond I
1971					
June 8	19	16	18	23	19
June 22	9	16	14	24	20
July 20	21	17	16	24	—
August 21	23	21	25	23	25
August 24	20	24	22	27	30
August 25	—	—	T	T	T
August 28	16	16	12	19	14
September 1	—	—	—	RT	RT
September 1	—	—	—	25	23
September 4	20	16	17	17	19
October 12	26	16	28	21	18
1972					
April 15	24	25	27	27	20
June 8	13	13	12	15	25
June 27	21	20	21	26	22
July 25	31	22	23	25	23
August 29	60	17	27	32	20
Mean, all samples	23.3	18.4	20.2	23.4	21.4

[a] T = treatment; RT = re-treatment. Ponds H and I were re-treated with 40 μ g/l antimycin after treatments with 10 μ g/l proved to be insufficient to kill all caged fish in the pond.

Calculated species diversity indices for all sampling periods in 1971 and 1972 for each pond fell between the values 2.00 and 4.12 (Table 9).

Discussion

The results of this study justify the proposal that the toxicants antimycin and rotenone be retained as fish control agents because they are not detrimental to benthic communities when applied in proper dosages. The 14-month investigation of high and low concentrations revealed no short- or long-term effects on species abundance or on insect emergence. These conclusions are generally in agreement with published literature. Other studies have shown that species of major benthic groups have not been seriously affected by recommended treatment concentrations of 10 μg/l antimycin (Walker et al. 1964, Gilderhus et al. 1969) or of 0.5 mg/l of rotenone (Brown and Ball 1942). However, Brown and Ball did identify an initial reduction in the population of certain unidentified species of Chaoboridae. The chaoborid *Sayomyia punctipennis* in the present study was not affected. Penick (1963) described a study by H.S. Swingle showing that *S. punctipennis* was unaffected by rotenone.

Our analysis of species diversity indices showed that treatment with toxicants at specified concentrations did not disturb benthic communities, either immediately after treatment or in the following year. With one exception, our indices fell within the range 2.0 to 4.1. Wilhm (1970) suggested that indices below 1.0 identify unstable, disturbed benthic communities and that indices between 3.0 and 4.0 identify undisturbed communities. Applying these criteria to our indices, we conclude that none of our experimental or control ponds supported disturbed communities. The random occurrence of indices between 2.0 and 4.0 throughout control and treated ponds is further evidence that these ponds contained stable communities.

The value of species diversity indices as monitors of community stability was demonstrated by an inconsistently low index of 1.80 in pond G (treated with 20 μg/l antimycin), which was associated with the development of anaerobic conditions (Table 5) and generation of hydrogen sulfide.

Table 9. *Diversity indices for control ponds and ponds treated with high concentrations of rotenone or antimycin, Fish-Pesticide Research Laboratory, Columbia, Missouri, June 1971-August 1972.*

| Sampling date | Control | | Rotenone 2 mg/l | Antimycin 40 μg/l | |
	Pond A	Pond B	Pond E	Pond H	Pond I
1971					
June 8	2.00	2.38	2.14	2.52	3.31
June 22	2.75	2.25	3.12	2.70	2.93
July 20	2.70	3.19	2.46	3.68	b
August 21	3.60	3.60	3.34	3.62	3.66
August 24	2.81	3.56	3.26	3.80	3.48
August 25	—	—	T	T	T
August 28	3.21	2.40	3.12	3.06	2.66
September 1	—	—	—	RT	RT
September 4	—	—	—	2.66	3.76
September 14	3.05	2.68	3.08	2.93	2.87
October 12	2.45	2.62	3.51	2.74	3.12
1972					
April 15	2.83	3.42	3.74	2.68	3.57
June 8	2.06	2.93	2.24	3.12	2.74
June 27	2.21	2.54	2.54	3.37	2.97
July 25	4.12	2.77	3.24	3.61	2.38
August 29	3.26	3.33	3.56	3.53	3.02

[a] T = treatment; RT = re-treatment. Ponds H and I were retreated with 40 μg/l antimycin after treatments with 10 μg/l proved to be insufficient to kill all caged fish in the pond.

[b] Sample not analyzed.

Two environmental factors that influenced this study were the large beds of vegetation and the absence of fish. The lack of fish may modify species abundance and composition because of the accompanying marked reduction in predation on benthic organisms.

The invasion of all open water of all ponds by vegetation by late summer was accompanied by an increase in number of niches, which was in turn reflected in an increase in the species diversity index. Periods of spring emergence, summer buildup, and fall emergence of insects were closely tied to the seasonal development and decline of vegetation. Photosynthetic activity of vegetation resulted in high pH (above 9.5 in early afternoon), which caused inactivation and subsequent detoxification of antimycin, and made it necessary to increase the concentration of antimycin applied. Decomposition of plant material resulted in low dissolved oxygen concentrations in late summer (Table 5), which, in combination with insect emergence, resulted in a seasonal decline in insect abundance and a decrease in the diversity index.

An advantage of the toxicants antimycin and rotenone is that they are naturally occurring compounds whose persistence is prevented through biodegradation. Thus insects, which have the capacity for rapid recolonization, are not excluded from aquatic ecosystems for long periods even after overdoses of these toxicants.

Conclusions

1. No short- or long-term effects on abundance of dominant benthic species could be attributed to pond treatments with 0.5 and 2.0 mg/l concentrations of rotenone or 20 and 40 μg/l concentrations of antimycin.
2. Species diversity within the benthic community, as evaluated by number of taxa and diversity indices, was not changed by rotenone or antimycin treatment.
3. Insect emergence was not affected by rotenone or antimycin treatments.

Acknowledgments

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Appendices

Appendix I, Community Dynamics in the Experimental Ponds, provides a description of the dynamics of benthic populations in ponds which are heavily vegetated and which lack fish. The absence of fish may modify species abundance and composition because of the accompanying marked reduction in predation on benthic organisms. The dense vegetation provides unusual cover, more niches, and presumably more food than may be available to benthos in nonvegetated habitats. Periods of spring emergence, summer buildup, and fall emergence of insects were closely associated with the seasonal development and decline of vegetation.

Appendix II, Changes in Density of Benthic Organisms, presents the field data from all collections, plotted as number of organisms per square meter, June-October 1971 and April-August 1972, for control and toxicant-treated ponds. Values for representative species from these data (Figs. A3-A13) provided the basis for discussion and conclusions in the text.

Appendix III, Density of Midges Captured in Emergence Cages, shows the rate of capture (no./m²/wk) of two genera of phantom midges and 24 taxa of true midges in emergence cages, April-June 1972, in two control ponds, in one pond treated with 2.0 mg/l of rotenone, and in two ponds treated with 40 μ g/l of antimycin (Figs. A14-A18).

Appendix I

Community Dynamics in the Experimental Ponds

Our observations of seasonal changes in benthic populations describe community dynamics in ponds with extensive beds of vegetation and without fishes. Seasonal changes are presented in the following sequence: summer development, fall emergence, winter development, and spring emergence. These periods were not synchronous in all ponds but were closely approximated.

Summer development was characterized by rapid larval development, as a result of high temperature. This developmental period was disrupted in the first year of the study (1971) by the draining and refilling of the ponds in late April. Draining delayed the growth of macrophytes, increased bottom organic matter and subsequent growth of benthic algae, and later resulted in extremely dense populations of *Chironomus attenuatus* and *Glyptotendipes barbipes*. These species constituted almost the entire community of benthos. Their subsequent reduction followed the decline in benthic algae as developing beds of vegetation limited light penetration.

By mid-July most major genera typical of these pond communities (Table 6) were present, and the vegetation, which developed rapidly because the carbonate reserve was high, provided an abundant food source and a variety of niches for recolonization. This recolonization was hastened by the proximity of the study ponds to nearby ponds that had not been drained.

Distinctive trends occurred in the seasonal patterns of appearance and abundance for major members of the pond communities (Figs. A1 and A2). The mayfly *Caenis simulans*, the dragonfly *Erythemis simplicicollis*, and the damselfly *Ischnura verticalis* showed late summer population increases. Of the true midges, *Ablabesmyia peleenis*, *Pseudochironomus richardsoni*, *Tanytarsus*, and *Procladius subletti* showed two emergence periods and a population buildup in midsummer.

The fall emergence period was characterized by a decline in larval populations. The decline was also associated with a partial decline and decomposition of vegetation, which caused high oxygen demand. The decline in midge larvae *(Ablabesmyia peleenis, Pseudochironomus richardsoni, Procladius subletti, and Tanytarsus)* was due to emergence (Appendix III, Figs. A14-A18). Reduction of other species was linked with stagnation.

The winter development period was characterized by slow larval development resulting from low temperature. Initially, the numbers of mayflies, dragonflies, and damselflies appeared to be large. Later, the numbers declined as larval size increased. A reverse trend characterized the true midges; densities were low in late October and high in early April (Appendix II, Figs. A3-A13).

The spring emergence period was characterized by intensified emergence of genera that pupate throughout the year, and also by the emergence of the genera that pupate once a year. The highly distinctive seasonal emergence pattern of the true midges (Chironomidae) is described for the months of April, May, and June in Table A1. The total emergence resulted in the smallest number of individuals and in the lowest values for species diversity indices found throughout the year.
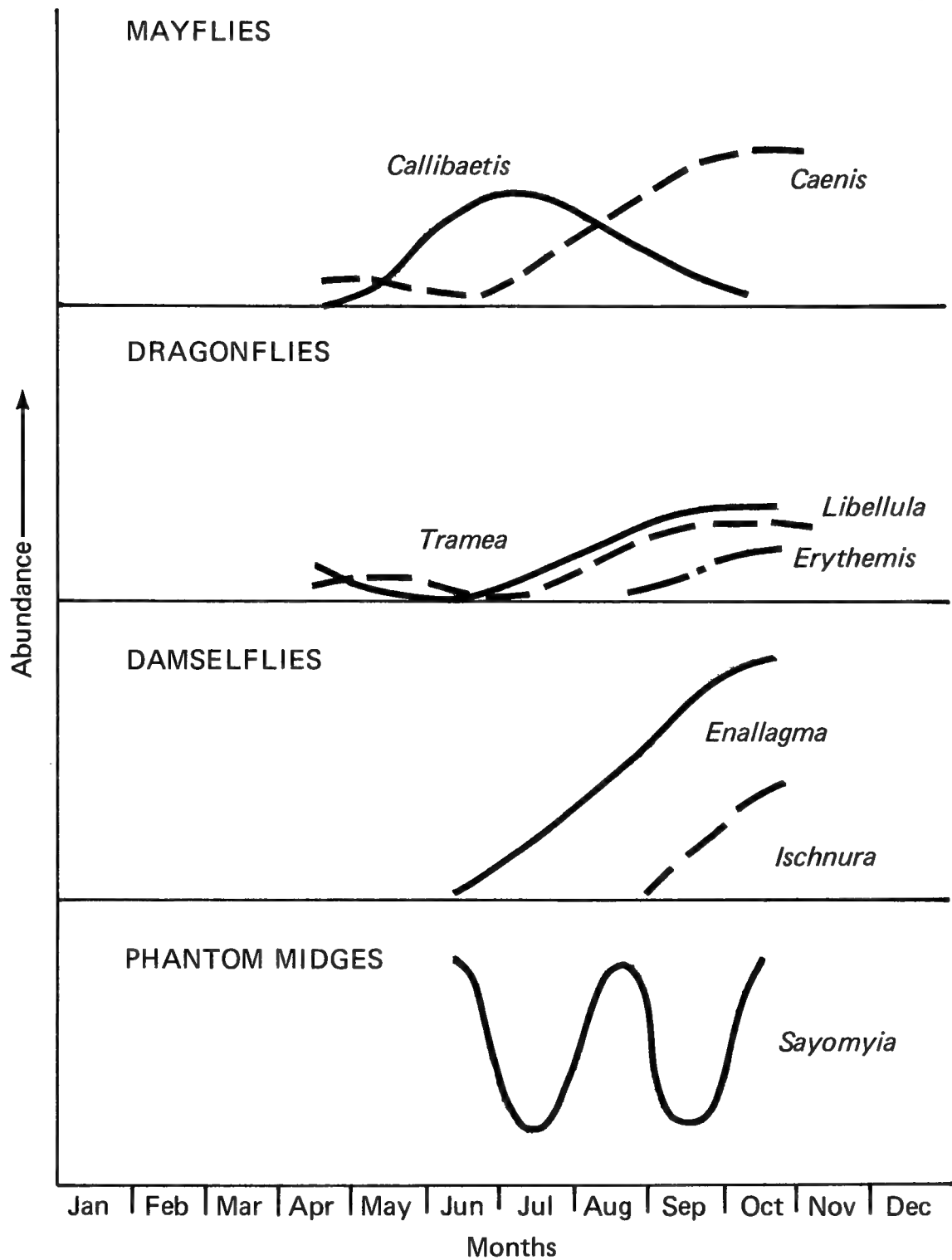
Fig. A1. Schematic presentation of trends in seasonal abundance of the most common dragonflies, damselflies, and phantom midges, collected from nine experimental ponds (A-I) near Columbia, Missouri, in 1971.
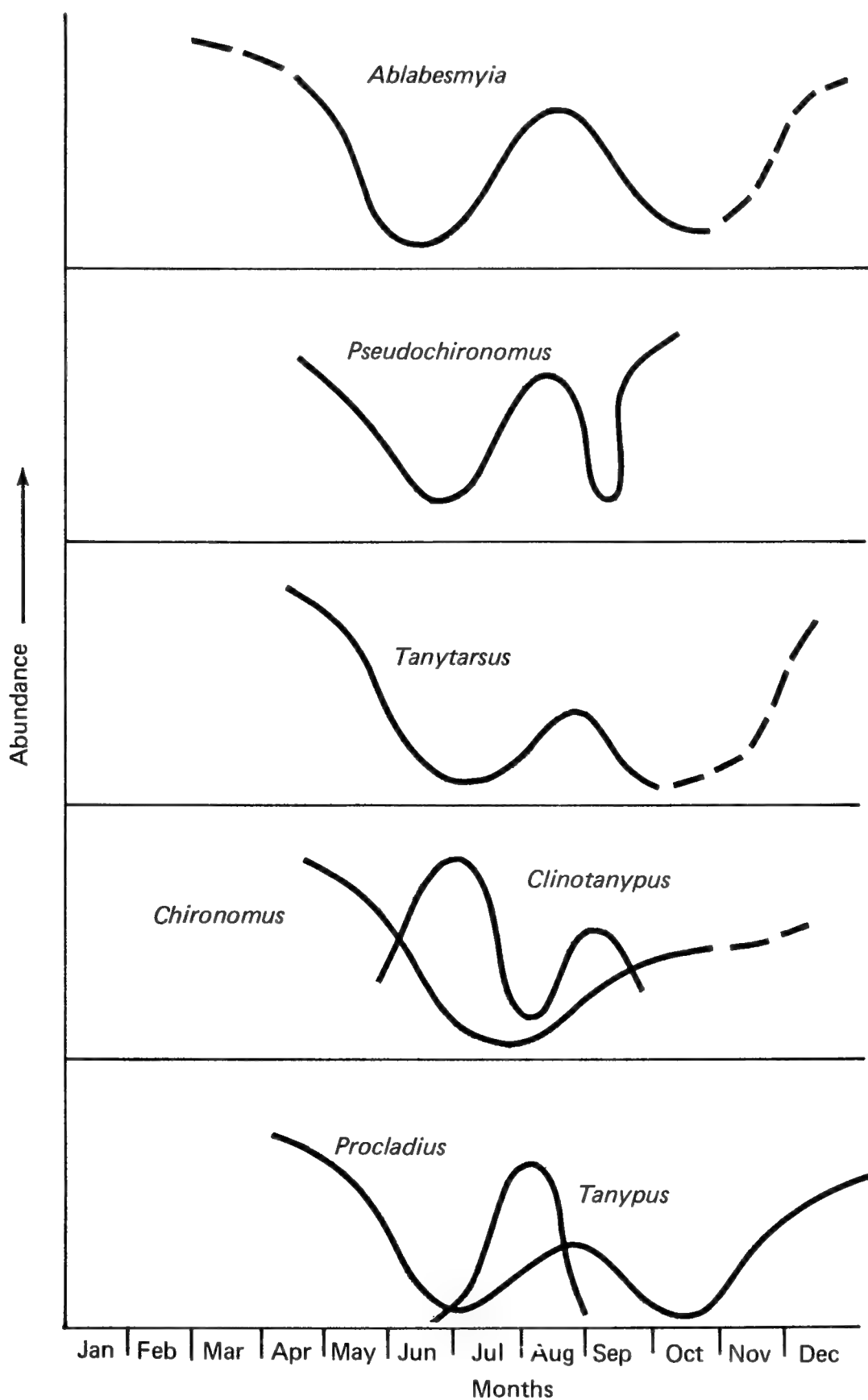
Fig. A2. Schematic presentation of trends in seasonal abundance of seven of the most common midges collected from nine experimental ponds (A-I) near Columbia, Missouri, in 1971.

Table A1. *Seasonal emergence patterns for midges (Chironomidae) in experimental fish ponds A to I, Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. Presence of identifying letter indicates emergence from that pond.*

Species	April	May	June
Pseudochironomus richardsoni	ABEHI	ABEHI	ABEHI
Tanytarsus sp.	ABEHI	ABEHI	A -EHI
Dicrotendipes nervosus	--EHI	A- EHI	ABEHI
Procladius bellus	ABEHI	-BEHI	ABEHI
Ablabesmyia peleenis	ABEHI	ABEHI	ABEHI
Endochironomus nigricans	--E--	A----	A -- H-
Psectrocladius dyari	-B---	-----	-BE--
Monopelia sp.	-----	A -- HI	A - EH-
Lauterborniella varipennis	-----	ABEHI	ABEHI
Larsia planesis	-----	ABEHI	ABEHI
Clinotanypus pinguis	A----	ABEH-	ABEHI
Glyptotendipes barbipes	-----	AB---	A----
Labrundinia pellosa	-----	--EHI	-BEHI
Polypedilum simulans	--E--	--EHI	ABEHI
Chironomus attenuatus			---HI

Appendix II

Changes in Density of Benthic Organisms in Heavily Vegetated Ponds Lacking Fish Populations, June-October 1971 and April-August 1972
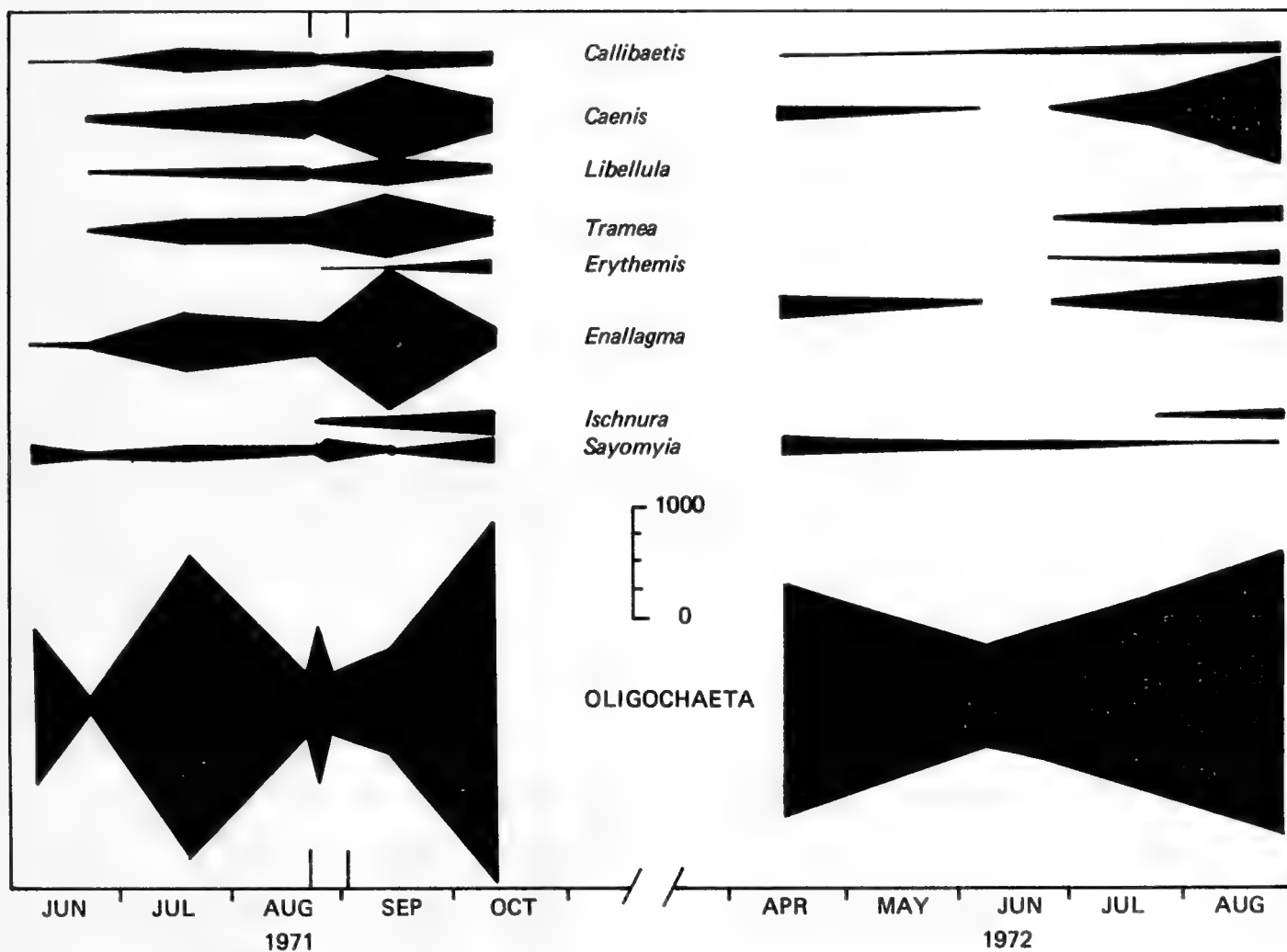
Fig. A3. Changes in density (no./m²) of mayflies *(Callibaetis, Caenis)*, dragonflies *(Libellula, Tramea, Erythemis)*, damselflies *(Enallagma, Ischnura)*, phantom midge *(Sayomyia)*, and aquatic earthworms (Oligochaeta) in control Pond A at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
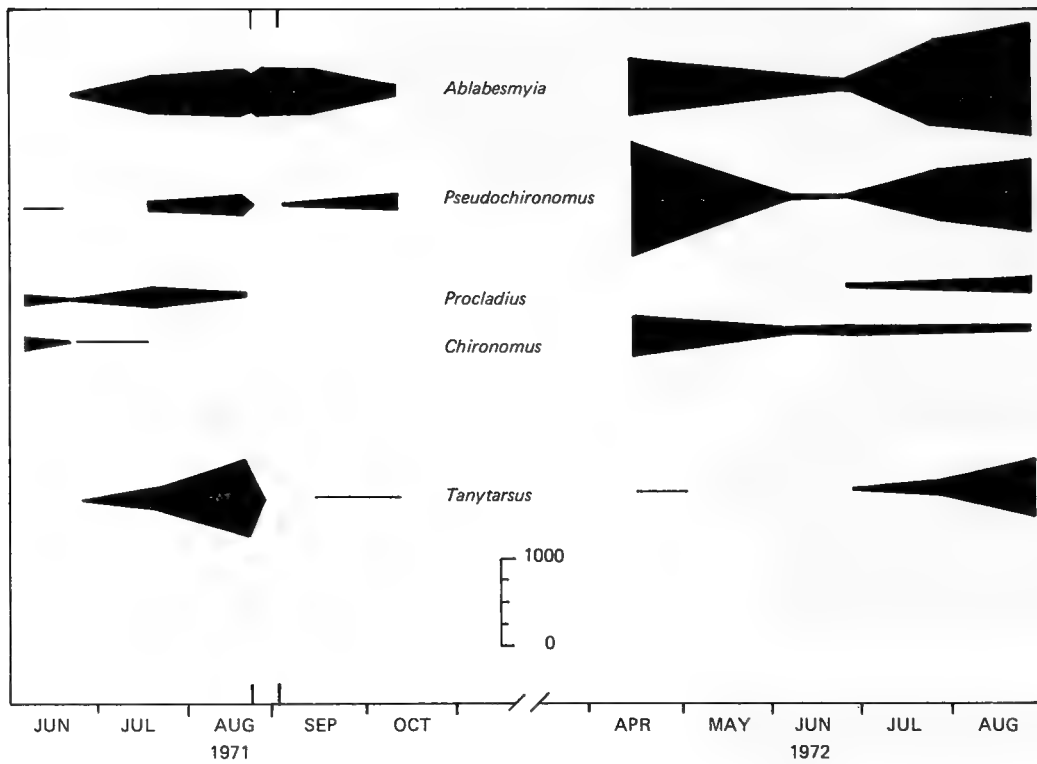
Fig. A4. Changes in density (no./m²) of midges *(Ablabesmyia, Pseudochironomus, Procladius, Chironomus, Tanytarsus)* in control Pond A at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
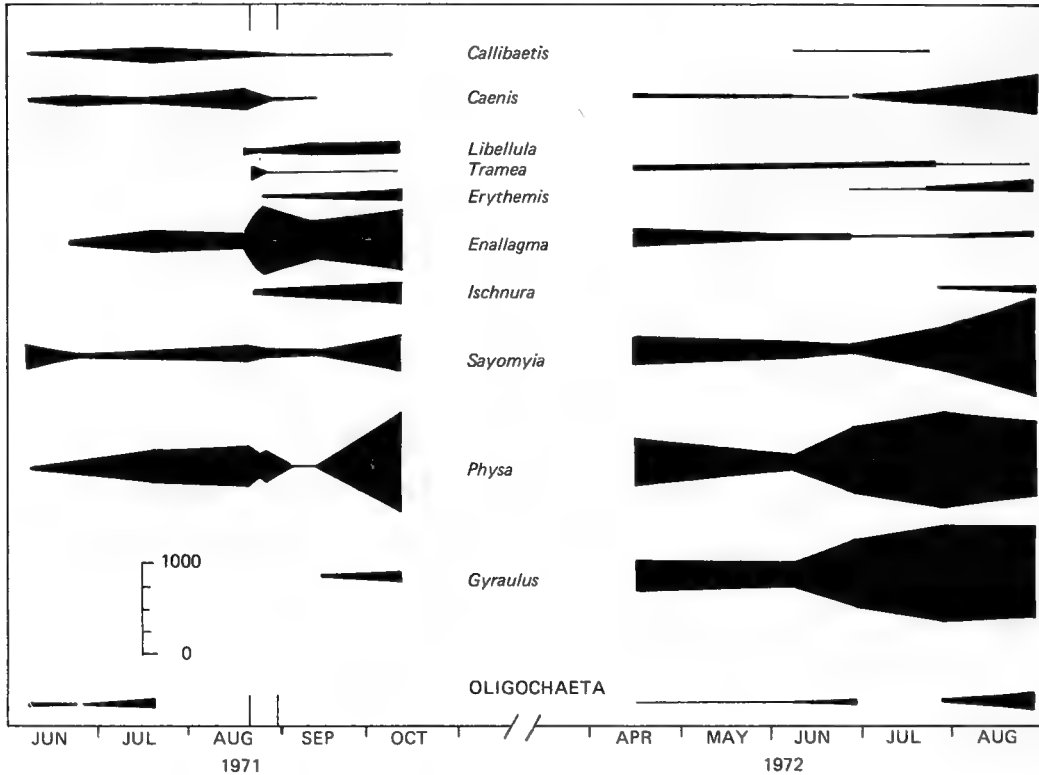
Fig. A5. Changes in density (no./m²) of mayflies *(Callibaetis, Caenis)*, dragonflies *(Libellula, Tramea, Erythemis)*, damselflies *(Enallagma, Ischnura)*, phantom midge *(Sayomyia)*, snails *(Physa, Gyraulus)* and aquatic earthworms (Oligochaeta) in control Pond B at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
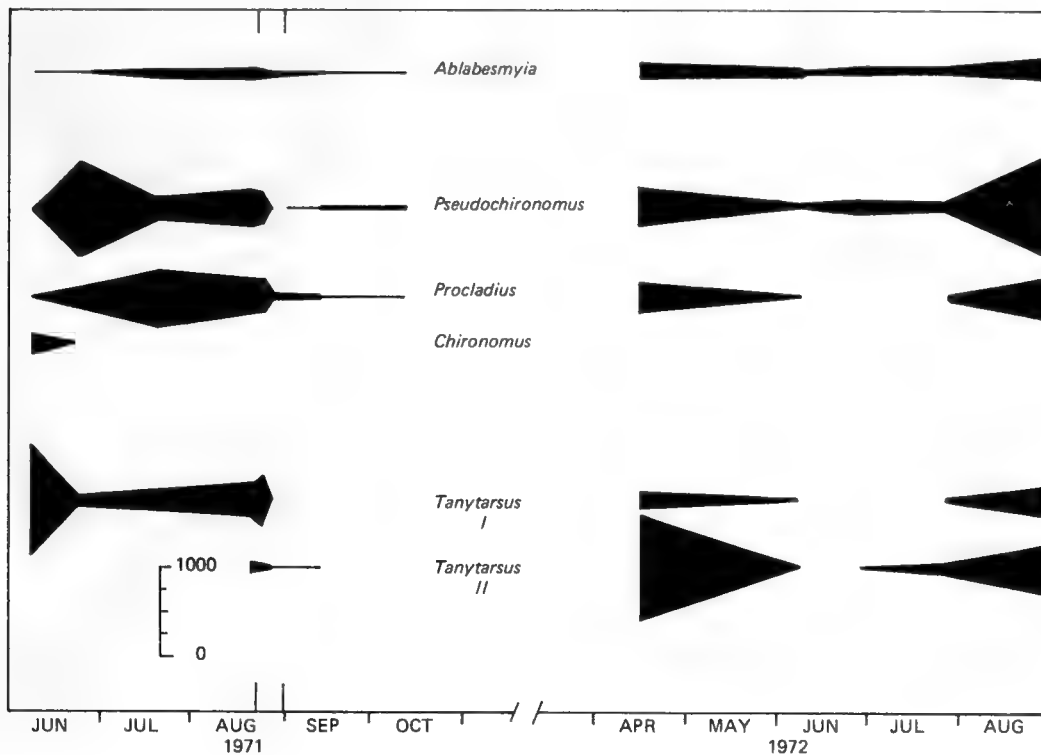
Fig. A6. Changes in density (no./m²) of midges *Ablabesmyia, Pseudochironomus, Procladius, Chironomus,* and two unidentified species of *Tanytarsus* in control Pond B at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
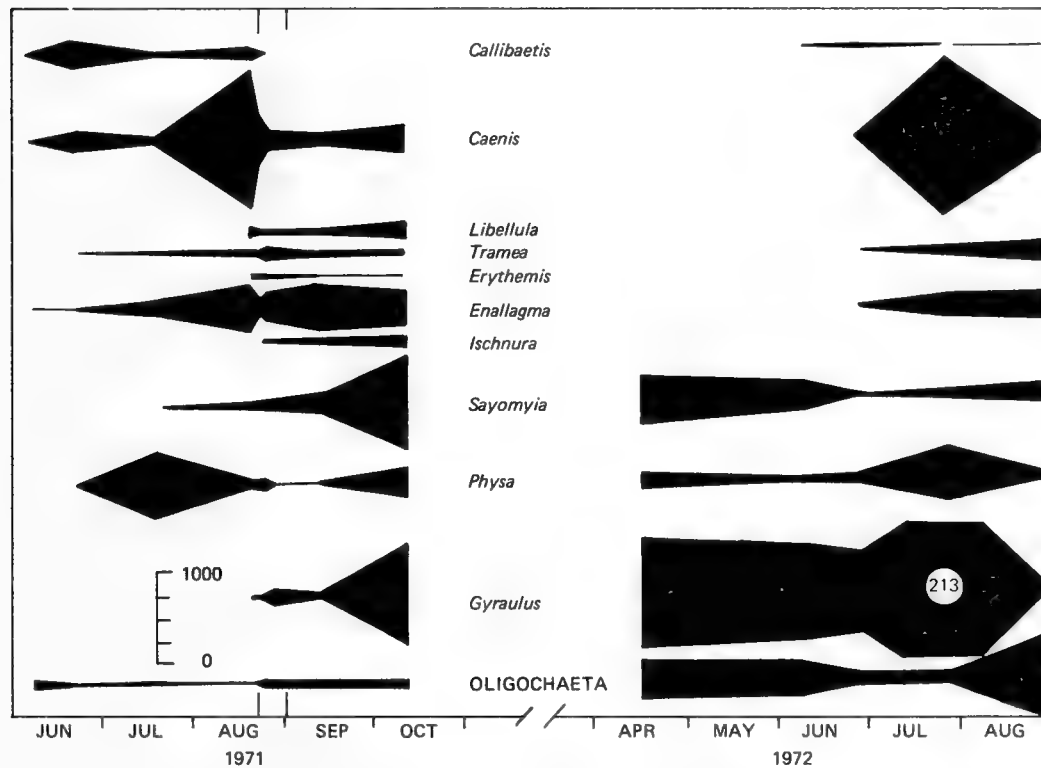
Fig. A7. Changes in density (no./m²) of mayflies *(Callibaetis, Caenis),* dragonflies *(Libellula, Tramea, Erythemis),* damselflies *(Enallagma, Ischnura),* phantom midge *(Sayomyia),* snails *(Physa, Gyraulus)* and aquatic earthworms (Oligochaeta) in Pond E, treated with 2 mg/l of rotenone, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
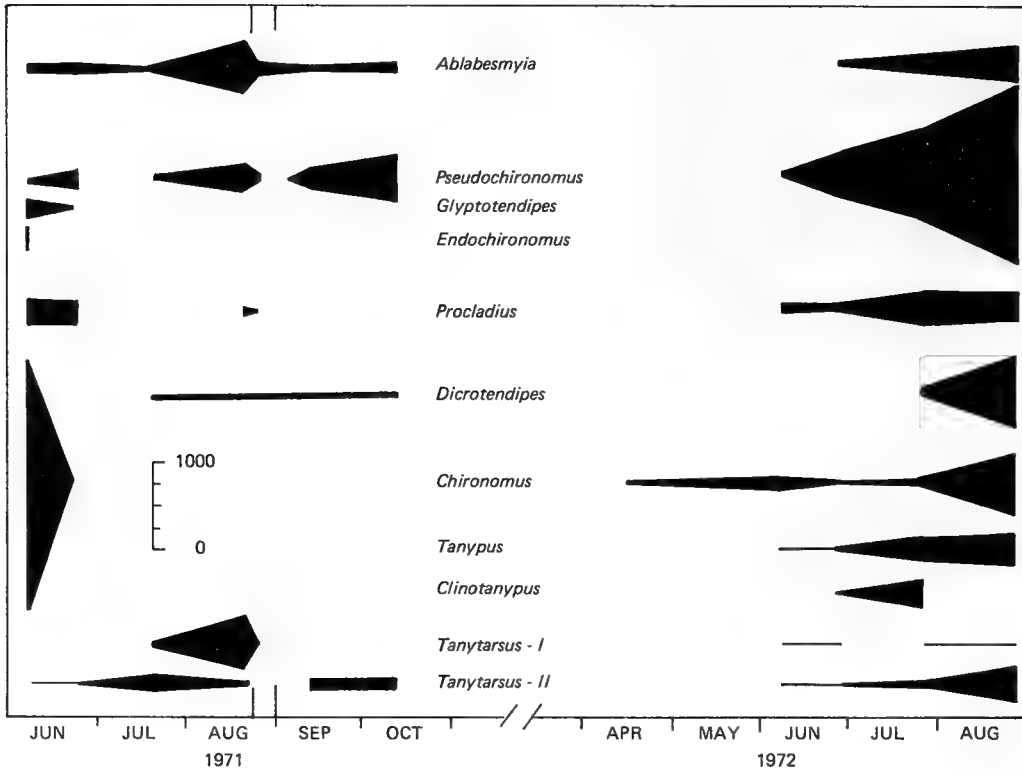
Fig. A8. Changes in density (no./m²) of midges *Ablabesmyia, Pseudochironomus, Glyptotendipes, Endochironomus, Procladius, Dicrotendipes, Chironomus, Tanypus, Clinotanypus,* and two unidentified species of *Tanytarsus* in Pond E, treated with 2 mg/l rotenone, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
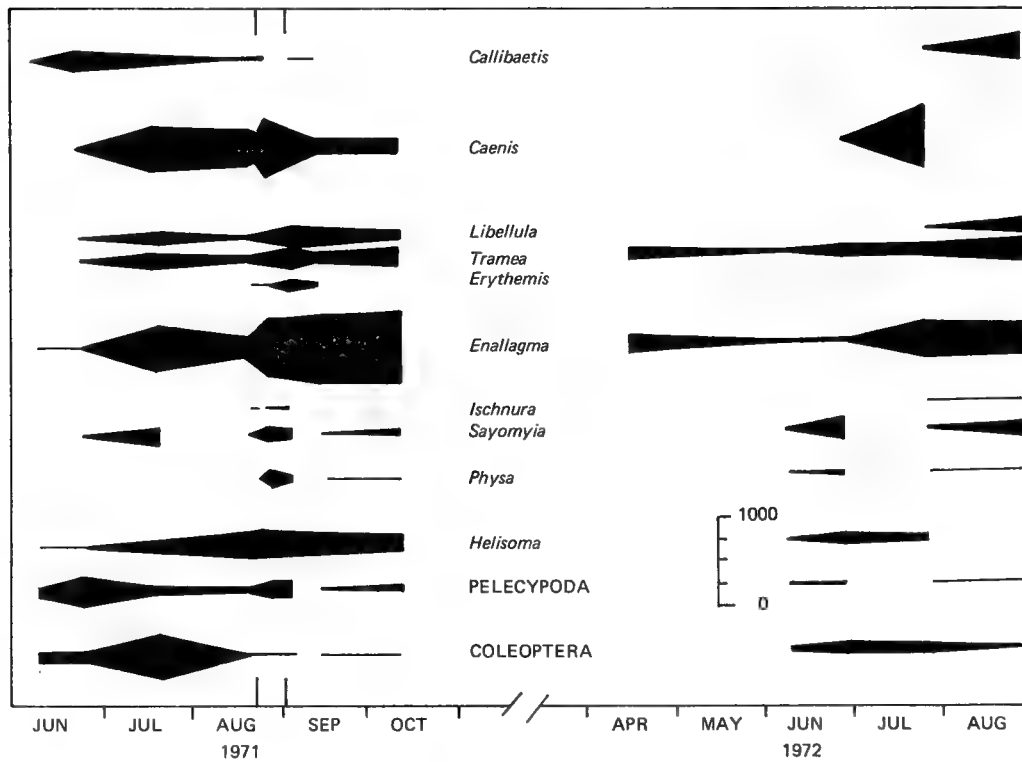
Fig. A9. Changes in density (no./m²) of mayflies *(Callibaetis, Caenis),* dragonflies *(Libellula, Tramea, Erythemis),* damselflies *(Enallagma, Ischnura),* phantom midge *(Sayomyia),* snails *(Physa, Helisoma),* clam (Pelecypoda) and beetles (Coleoptera) in pond H treated with 40 μg/l of antimycin at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
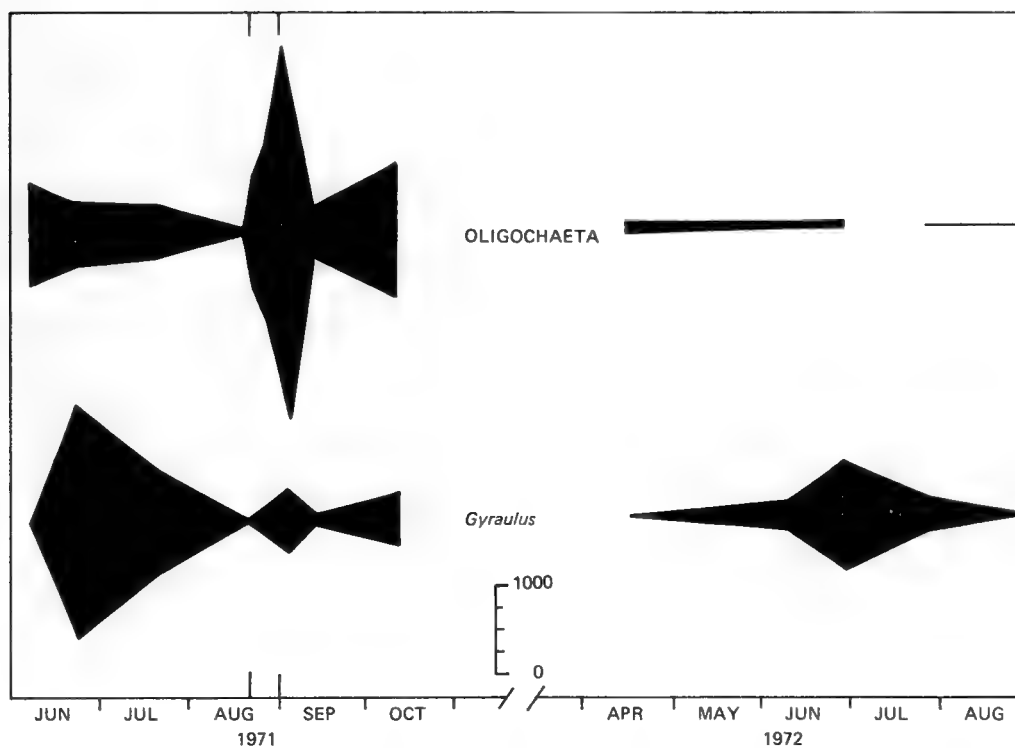
Fig. A10. Changes in density (no./m²) of aquatic earthworms (Oligochaeta) and snails *(Gyraulus)* in Pond H, treated with 40 µg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
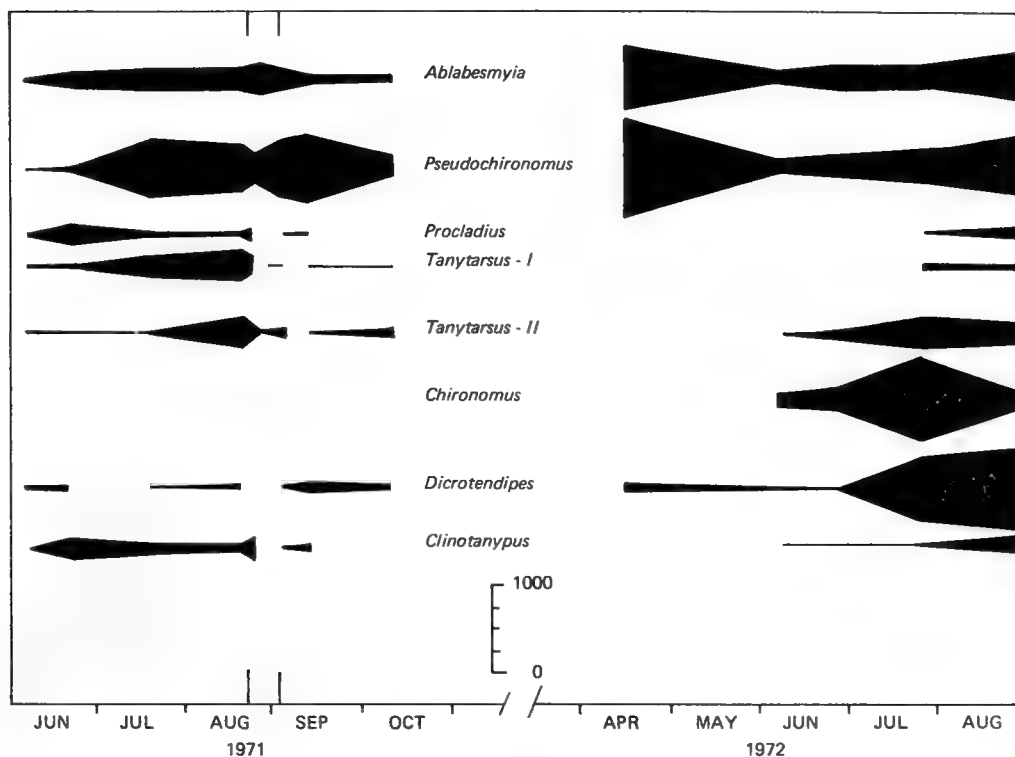
Fig. A11. Changes in density (no./m²) of midges *Ablabesmyia, Pseudochironomus, Procladius, Chironomus, Dicrotendipes, Clinotanypus,* and two unidentified species of *Tanytarsus* in Pond H, treated with 40 µg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars on the baseline indicate time of application of toxicants in the treated ponds.

24
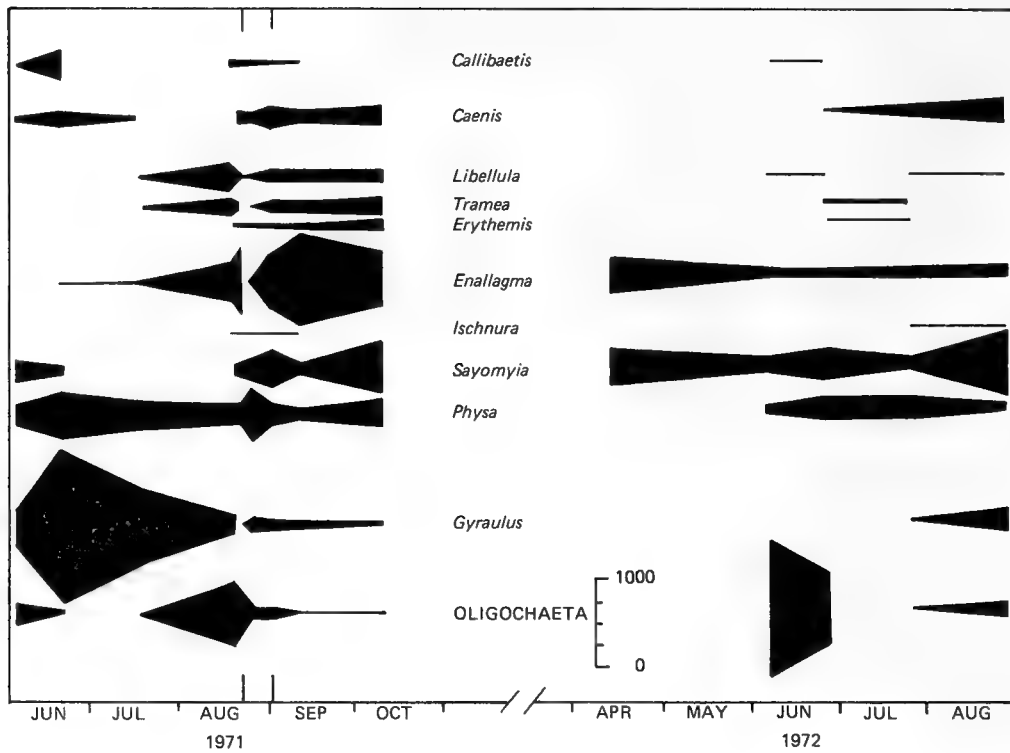
Fig. A12. Changes in density (no./m²) of mayflies *(Callibaetis, Caenis)*, dragonflies *(Libellula, Tramea, Erythemis)*, damselflies *(Enallagma, Ischnura)*, phantom midge *(Sayomyia)*, snails *(Physa, Gyraulus)*, and aquatic earthworms (Oligochaeta) in Pond I, treated with 40 μg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.
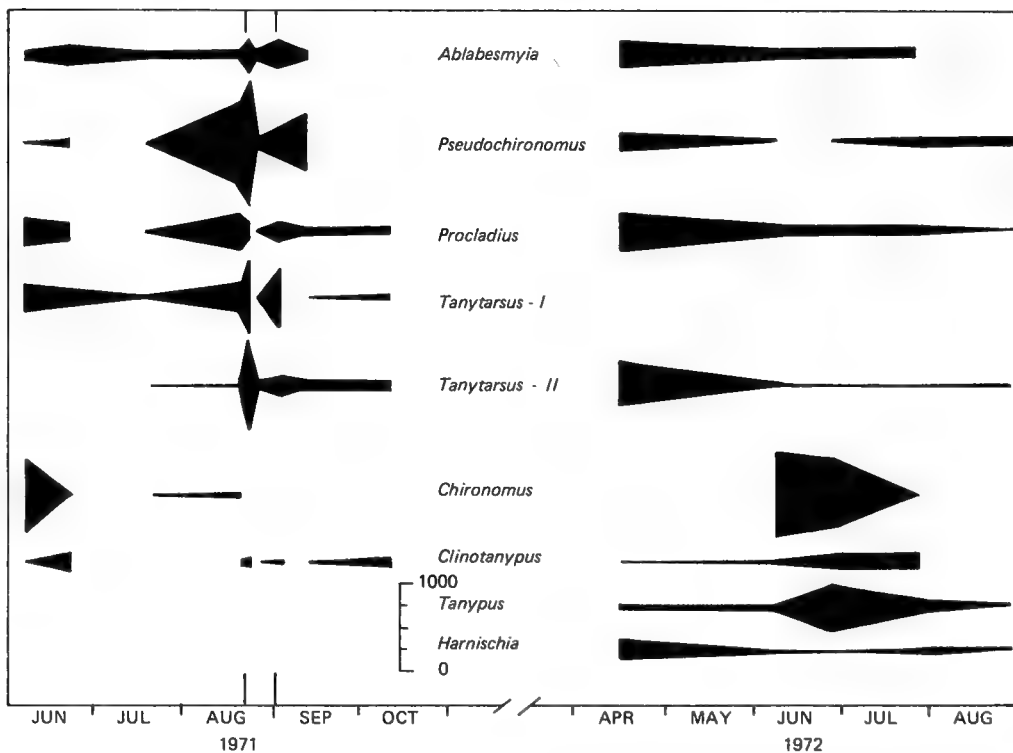
Fig. A13. Changes in density (no./m²) of midges *Ablabesmyia, Pseudochironomus, Procladius, Chironomus, Clinotanypus, Tanypus, Harnischia,* and two unidentified species of *Tanytarsus* in Pond I, treated with 40 μg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

Appendix III

Density of Phantom Midges and True Midges Captured in Emergence Cages in Two Control and Three Treated Ponds, April-June 1972
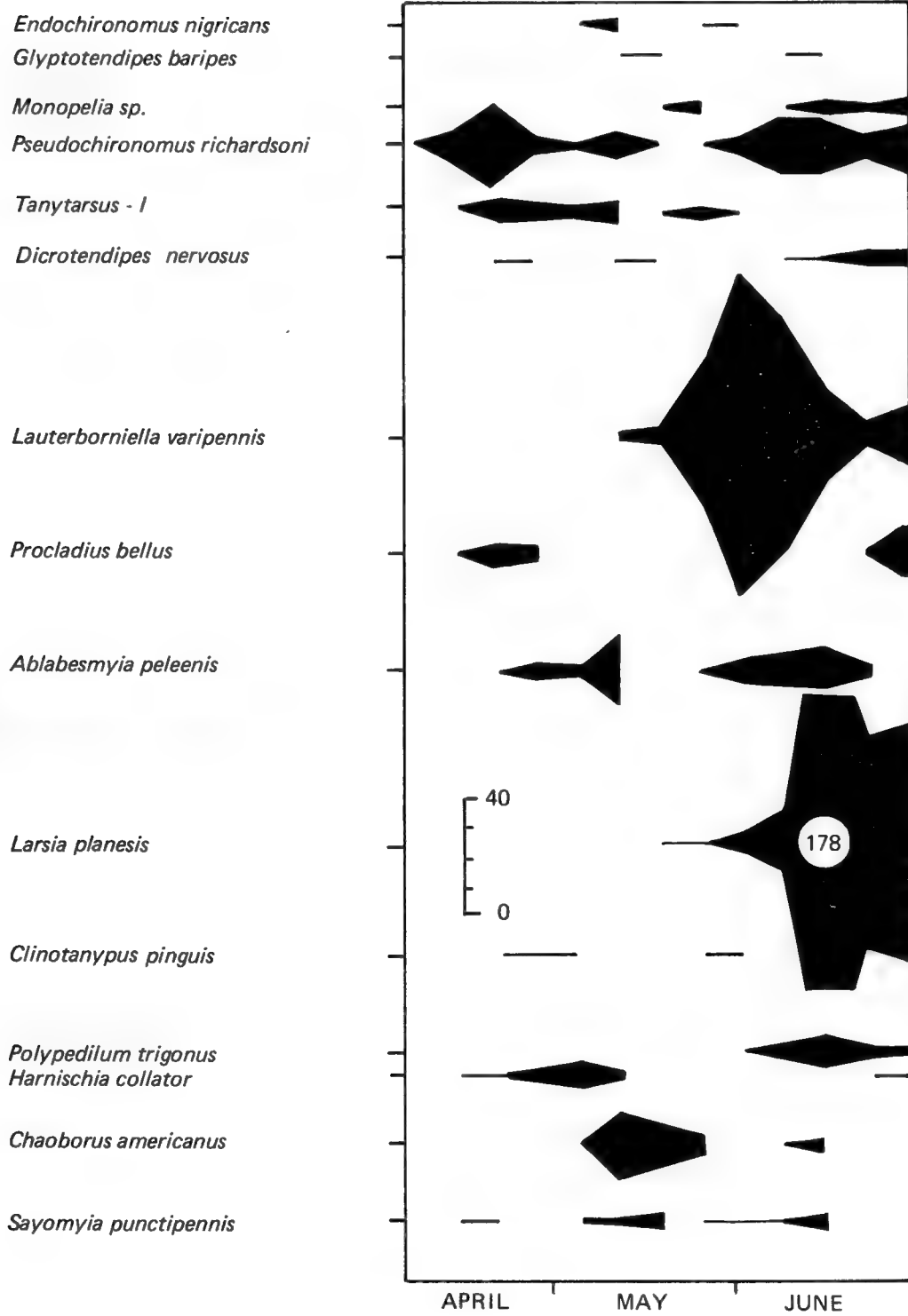
Fig. A14. Density (no./m² per week) of phantom midges *(Chaoborus* and *Sayomyia)* and true midges (other taxa shown) captured in emergence cages in control Pond A at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes one unidentified species of *Tanytarsus.*)
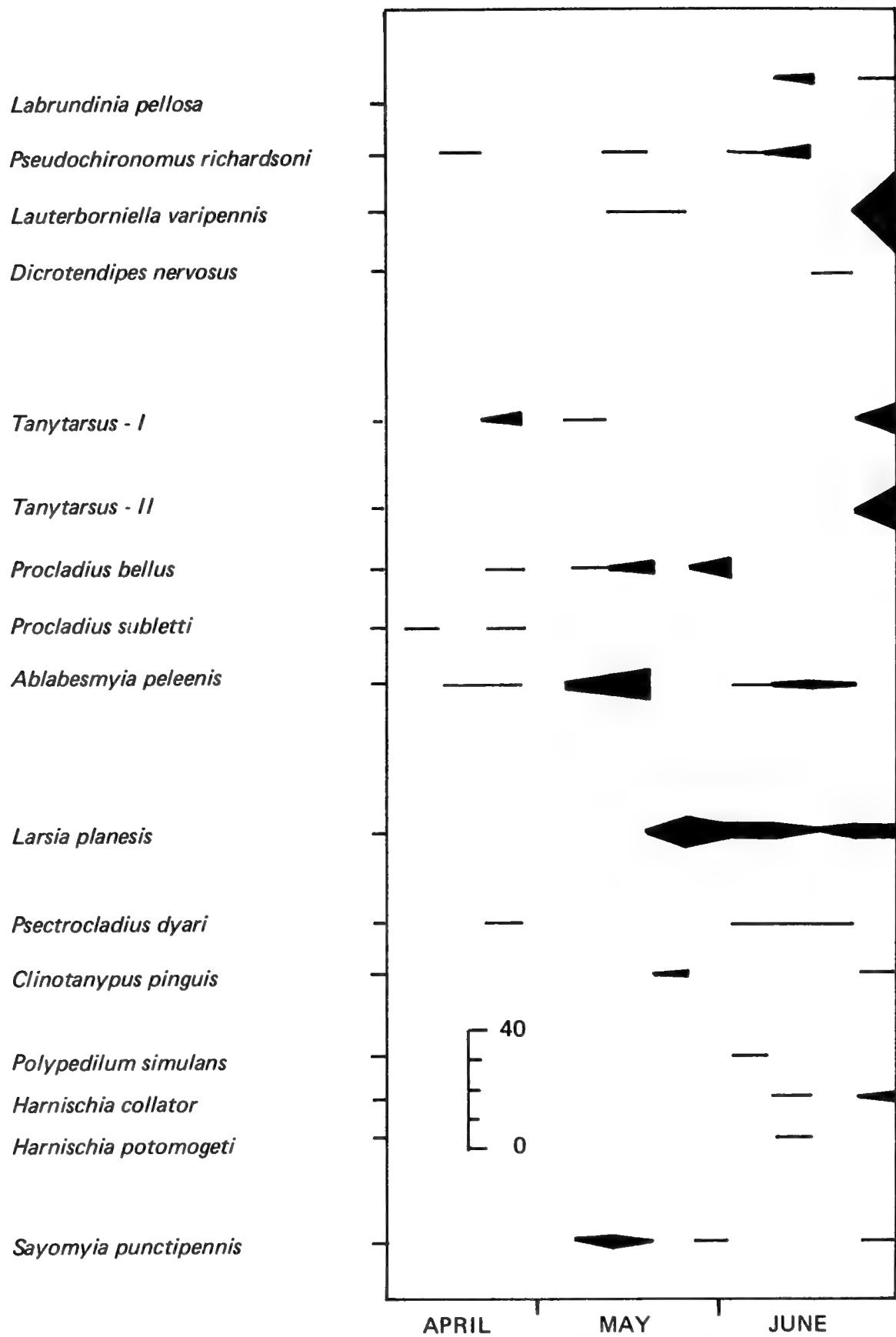
Fig. A15. Density (no./m² per week) of phantom midges *(Sayomyia)* and true midges (other taxa shown) captured in emergence cages in control Pond B at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus*.)
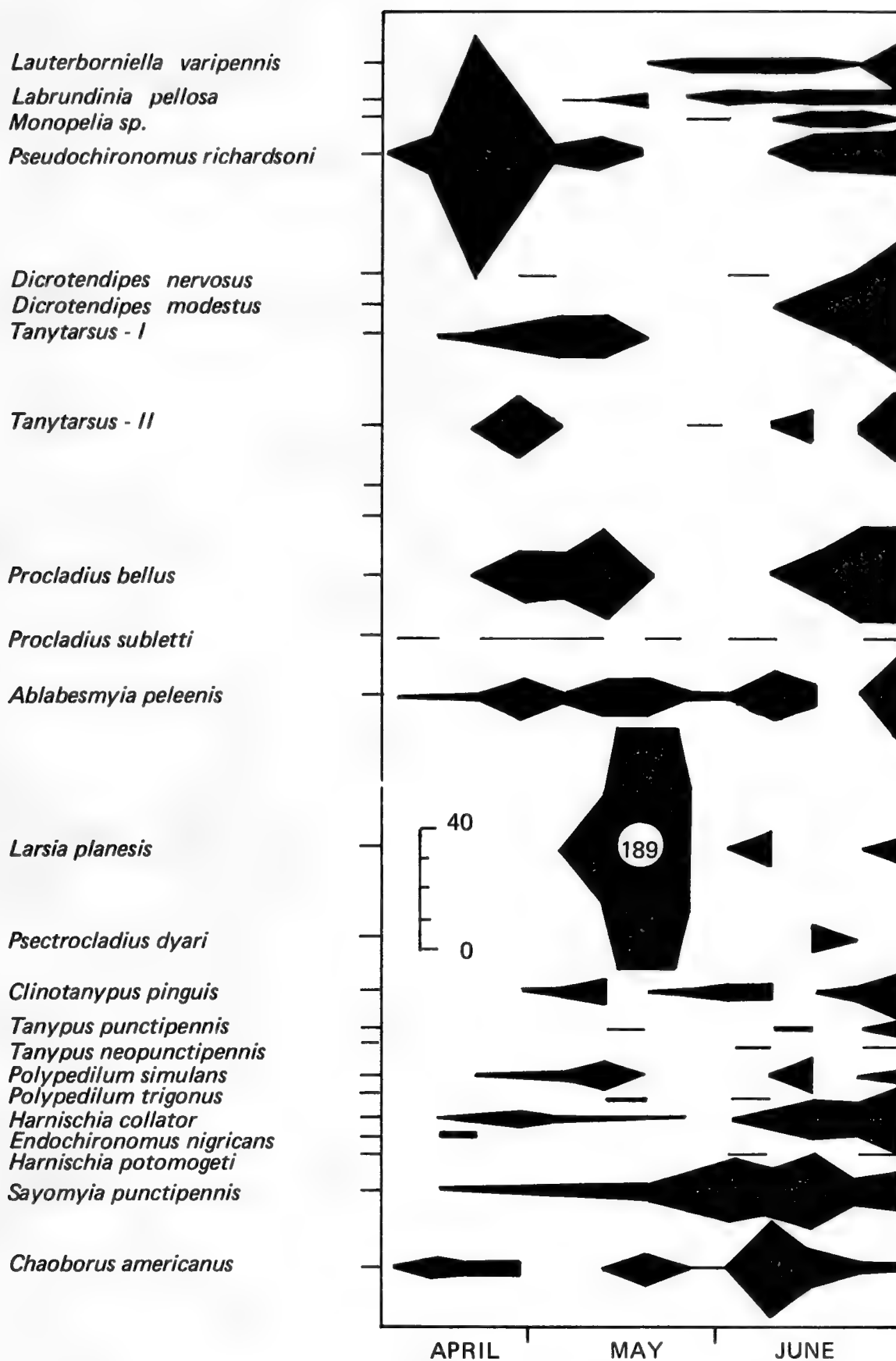
Fig. A16. Density (no./m² per week) of phantom midges *(Sayomyia, Chaoborus)* and true midges (other taxa shown) captured in emergence cages in Pond E, treated with 2.0 mg/l of rotenone, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus.)*

Labrundinia pellosa

Monopelia sp.

Dicrontendipes nervosus
Dicrontendipes modestus
Polypedilum trigonus

Pseudochironomus richardsoni

Chironomus sp.
Tanytarsus - I

Lauterborniella varipennis

Procladius bellus

Polypedilum simulans

Tanytarsus - II

Larsia planesis

Endochironomus nigricans
Clinotanypus pinguis
Tanypus punctipennis
Tanypus neopunctipennis
Harnischia collator
Harnischia monochromus

Ablabesmyia peleenis

Sayomyia punctipennis
Chaoborus americanus

40

0

APRIL   MAY   JUNE

Fig. A17. Density (no./m² per week) of phantom midges *(Sayomyia, Chaoborus)* and true midges (other taxa shown) captured in emergence cages in Pond H, treated with 40 μg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus*.)
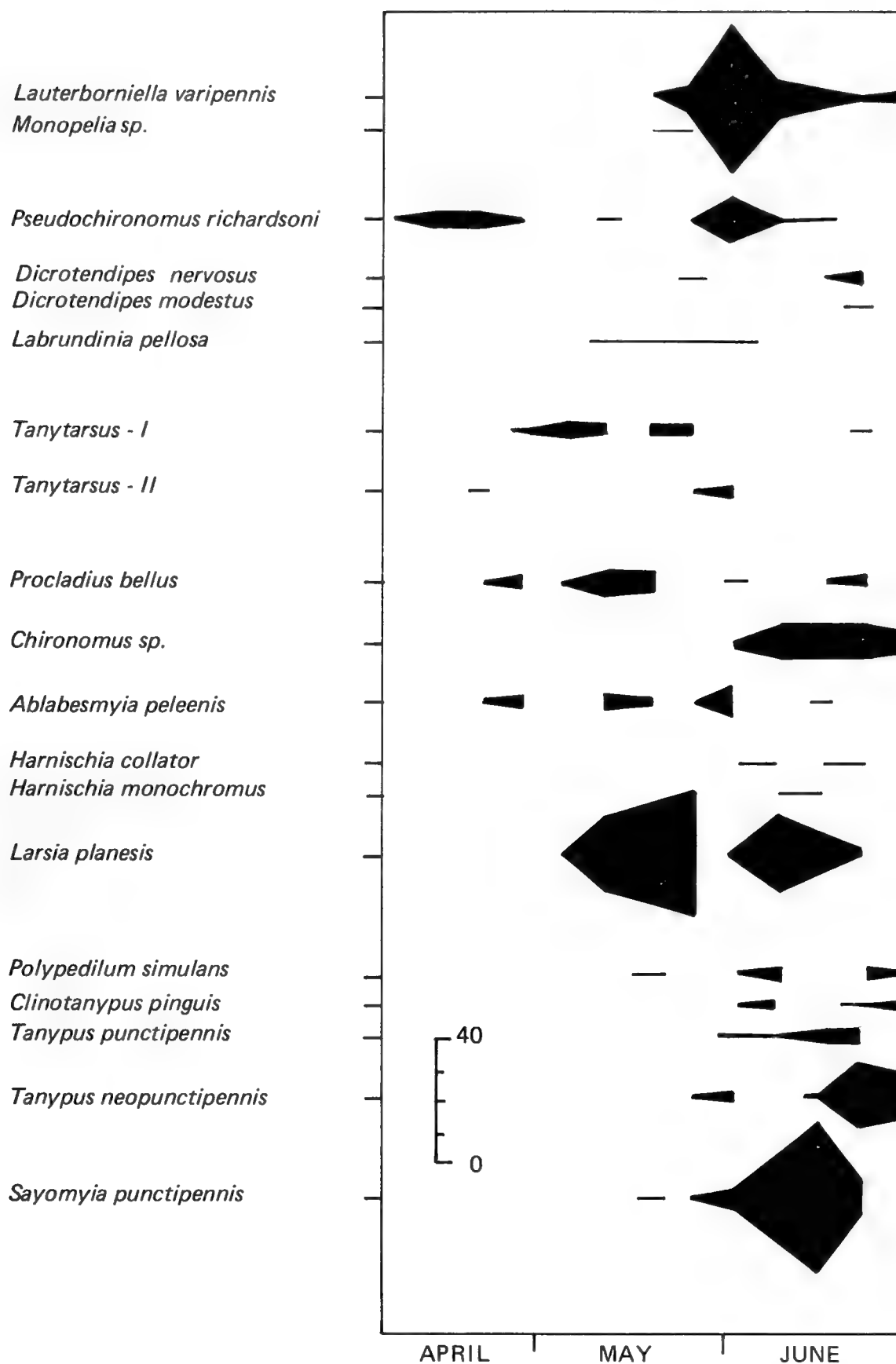
Fig. A18. Density (no./m² per week) of phantom midge *(Sayomyia)* and true midges (other taxa shown) captured in emergence cages in Pond I, treated with 40 μg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus*.)

Aquatic Macroinvertebrates in a Small Wisconsin Trout Stream Before, During, and Two Years After Treatment with the Fish Toxicant Antimycin[1]

by

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Abstract

Benthos and benthic drift were sampled periodically in Seas Branch Creek (Vernon County, Wisconsin) for 5 months before and for 2 years after the stream was treated with antimycin, and over the same period in nearby untreated Maple Dale Creek. During treatment on 4 October 1972, antimycin concentrations varied from 17 to 44 μg/l at the two sampling stations in Seas Branch Creek. Populations of macroinvertebrates were drastically reduced 2 days after treatment, but all common taxa identified before treatment were present in the stream 1 year later. Estimated biomass reductions of living organisms 2 days after treatment were as high as 50% for one caddis fly, *Hydropsyche* sp., and 75% for another, *Brachycentrus americanus;* 70% for a crane fly, *Antocha* sp.; and nearly 100% for a mayfly, *Baetis cingulatus*, and a scud, *Gammarus pseudolimnaeus*. Summer biomass of *Antocha* and *Brachycentrus* did not regain pretreatment levels during the second year. The mortality of the riffle beetle, *Optioservus fastiditus*, was approximately 20% 9 days after treatment. A crayfish, *Orconectes propinquus*, was not affected by the treatment. The biomass of *Gammarus, Prosimulium* (a black fly), *Baetis*, and *Hydropsyche* was high during both summers after treatment. After 1 year, and continuing into the second year, total benthic biomass approached or exceeded that before treatment.

The piscicide antimycin is used for several purposes in fishery management, including eradication of nongame fish species that are suspected of competing with game fish. Antimycin and rotenone are the only two chemicals registered for such use by the Environmental Protection Agency.

In 1972 the Wisconsin Department of Natural Resources, in rehabilitating Seas Branch Creek, used antimycin to eradicate populations of catostomids and cyprinids. After removal of the nongame species, the stream was restocked with brown trout *(Salmo trutta)*. This project afforded us the opportunity to observe the reactions of fish food organisms to antimycin.

The purpose of our study was to observe and document changes in nontarget aquatic macroinvertebrate populations in this small trout stream after the application of antimycin. Short- and long-term effects of treatment were shown by quantitative and qualitative variations in benthic biomass and changes in the composition and abundance of drift organisms.

The effects of antimycin on the invertebrate fauna have been previously investigated in lakes or ponds, but not (to our knowledge) in a natural trout stream. Callaham and Huish (1969) and Rabe and Wissman (1969) reported that 5.0 μg/l applications of antimycin severely reduced populations of zooplankton in lakes and ponds, whereas Walker et al. (1964), Gilderhus et al. (1969), and Houf and Hughey (1973) found that fish-killing concentrations of antimycin had no significant effect on lake plankton and benthos. Snow (1974) observed no gross long-term detrimental effects on zooplankton and benthos 6 years after antimycin treatment in Rush Lake, Wisconsin.

Study Area

Seas Branch Creek is in central Vernon County, in the hilly, unglaciated area of southwestern Wisconsin (Fig. 1). It is an 8-km-long tributary of the West

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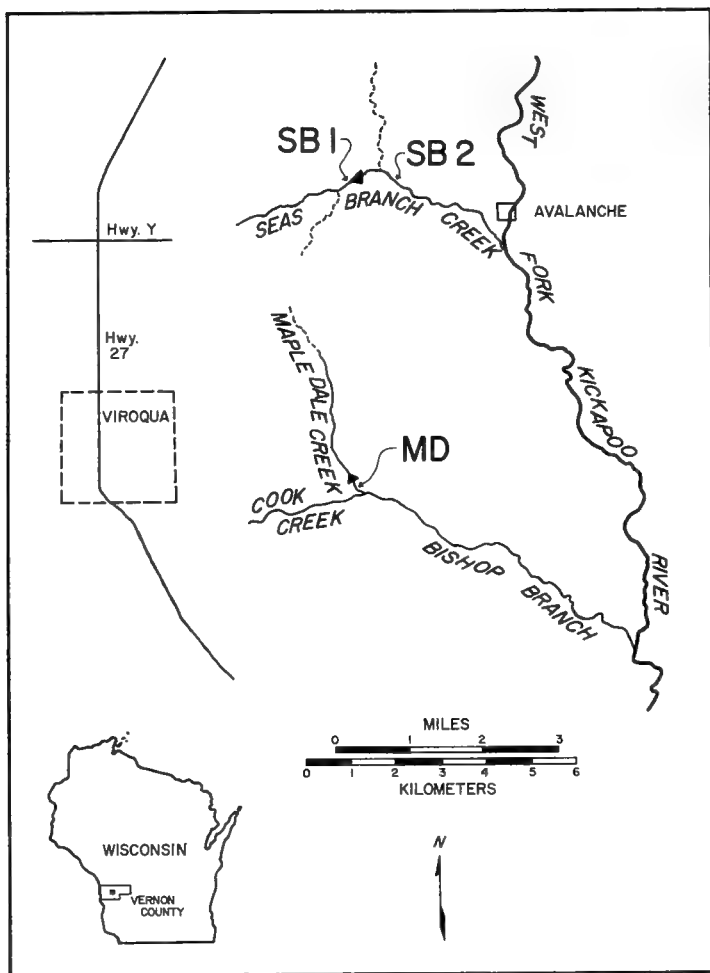
Fig. 1. Locations of study stations SB1 and SB2 on Seas Branch Creek, which was treated with antimycin on 4 October 1972, and control station MD on untreated Maple Dale Creek. The triangles near SB1 and MD indicate flood control reservoirs.

Fork of the Kickapoo River. A 5-ha permanent impoundment on the stream, 4 km above its mouth, serves as a flood control reservoir.

Because Seas Branch Creek was treated with antimycin at its source, a nearby stream, Maple Dale Creek, which has similar physical, chemical, and biological characteristics, was selected as a control. This stream, 4 km long, is a tributary of Bishop Branch Creek, which is also a tributary of the West Fork of the Kickapoo River. The confluence of Bishop Branch Creek with the Kickapoo is 9 km downstream from that of Seas Branch Creek. A flood control structure, with no permanent pool, is about 300 m above the sampling station on Maple Dale Creek.

The upstream Seas Branch Creek station (SB1) was 50 m above the impoundment, in the middle of a riffle 70 m long. At this station the stream averaged 1.5 m in width and 12 cm in depth, and had a mean annual discharge of 0.11 m³/s. The downstream Seas Branch station (SB2) was 800 m below the impoundment and was at the lower end of a riffle 130 m long.

The stream here was 3.3 m wide and 18 cm deep, and had a mean annual discharge of 0.17 m³/s. The Maple Dale Creek control station (MD) was 6 m above the confluence of Maple Dale and Cook Creeks at the lower end of a riffle 40 m long. The stream here was 2.5 m wide and 15 cm deep, and had a mean annual discharge of 0.15 m³/s.

Water quality data were taken at each station throughout the study period (Tables 1, 2, and 3). Temperatures ranged from 0 to 20 C; average temperatures at SB1 were 2 to 4 C lower than at SB2 or MD, or both, during the summer before and the two summers after treatment. Water chemistry differed little at the stations before, during, or after treatment. Dissolved oxygen concentrations were high (8.5 to 15.1 mg/l; 81 to 126% saturation). The calcareous composition of the numerous bluffs along both streams is reflected in the average water quality values: pH, 8.3; total alkalinity, 215 mg/l; and conductivity, 434 μmhos. Turbidity was low, averaging 0.29 Jackson Turbidity Unit (JTU) during periods of normal flow (Tables 1, 2, and 3). Average discharge at all stations was about twice as high in 1973 as in 1972 or 1974. Slow release of cold groundwater after high precipitation in 1973 may have been responsible for the lower water temperatures in 1973 and 1974.

The stream bed at all three stations was composed largely of rough, angulate stones, mostly 5 to 10 cm in diameter (some up to 30 cm). Small amounts of gravel and sand were present; interstitial organic litter was primarily autochthonous plant material. The stones at SB2 and MD were loose, but at SB1 many were imbedded in clay. Water crowfoot *(Ranunculus aquatilis)*, the dominant stream vegetation, covered 8 to 50% of the stream bed throughout the year at all three stations. Limited amounts of pondweed (*Potamogeton* sp.) were present in the control stream, and watercress *(Nasturtium officinale)* along the water's edge in the treatment stream.

Methods

Antimycin (Fintrol-concentrate formulation) was applied to Seas Branch Creek from 0000 to 0920 h on 4 October 1972, under the direction of the Cold Water Research Group of the Wisconsin Department of Natural Resources. Errors in calculating dosages and equipment failure resulted in treatment values much higher than the intended concentrations of 10 μg/l. The concentration was 25 μg/l for the first 3 h and 40 μg/l for the next 6-1/3 h at SB1 and 17 μg/l for 2 h and 44 μg/l for 7 h at SB2. Antimycin drip sites were about 270 m above each of the two stations.

Table 1. *Physical and chemical data from the upper treatment station (SB1) of Seas Branch Creek, 1972-74.*

Date	Temp (°C)		Dissolved O$_2$		pH	Total alkalinity (ppm)	Turbidity (JTU)	Conductance (μ mho/cm)	Discharge (m^3/s)
	Air	Water	ppm	Percent saturation					
1972									
15 May	21	16	11.7	122	8.8	186	0.16	ND	0.08
15 June	15	12	10.1	96	8.5	194	0.27	440	0.08
15 July	32	17	9.2	98	8.1	186	0.12	440	0.08
15 Aug	25	14	10.0	100	8.5	203	0.30	420	0.07
3 Oct	18	11	10.1	95	8.3	215	0.27	520	0.09
6 Oct	10	10	10.2	94	8.4	223	0.57	500	0.08
13 Oct	10	10	11.0	102	8.5	213	0.36	480	0.08
1 Nov	8	8	10.6	93	8.3	215	0.19	420	0.10
1 Dec	–6	4	13.0	102	8.2	207	0.35	400	0.08
1973									
15 Jan	–12	1	14.3	104	8.3	203	0.30	400	0.08
15 March	1	6	13.4	111	8.2	210	0.21	380	0.15
15 May	15	8	12.8	112	8.6	218	0.11	450	0.17
16 July	24	14	11.4	115	8.3	203	0.22	460	0.19
15 Sept	10	9	9.9	90	8.0	198	0.27	440	0.16
6 Oct	11	9	10.3	95	8.1	236	0.17	450	0.14
10 Oct	1	5	12.4	100	8.1	209	0.20	410	0.13
1974									
20 April	10	9	10.2	90	8.1	210	0.20	350	0.11
17 May	16	11	10.4	97	8.2	215	0.50	420	0.04
30 July	21	14	9.8	98	8.0	233	0.07	400	0.09
2 Oct	6	9	10.0	90	7.4	207	0.16	410	0.10
Mean	11.8	9.9	11.0	100	8.2	209	0.25	430	0.11

On 16 August 1972 the gate of the impoundment on Seas Branch Creek was opened and the reservoir drained to an area of 1 ha, where it was maintained until after treatment to reduce the amount of antimycin needed. When the gate was closed 2 days after treatment, the impoundment refilled in about 2 weeks. This refilling reduced the water flow at SB2 by about one-half for most of that period.

Benthic samples were collected a total of 20 times during the 28-month study. On each sampling date, four samples were taken at SB1 and five each at SB2 and MD with a modified Hess circular 0.05-m^2 bottom sampler (with a net of 7.5 meshes/cm), similar to that described by Waters and Knapp (1961). At each station one sample came from vegetation and the rest from the rubble substrate. The biomass of benthos in the vegetation samples was prorated into the total benthic biomass according to the estimated percentage of the riffle area covered with vegetation at each sampling period. This percentage was assigned subjectively on the basis of the estimated change in vegetative cover in each riffle area from one sampling period to another.

Drifting organisms were collected in vertical nets of 7.5 meshes/cm supported by a 0.1-m^2 square frame attached to a board placed on the stream bottom. Three nets were used at SB2 and MD, and two at SB1 to collect samples for 10 min, four or five times in each 24-h period before treatment. Sampling times included sunrise, sunset, midday, and midnight, which represented times of major drift (Waters 1972). Drift samples were taken every 3 h for 24 h during treatment (starting 3 h before application of antimycin) and then every 6 h for 36 h thereafter. Total drift was calculated by the methods of Waters (1962). Current velocities were measured with a Gurley pigmy current meter, no. 625. Velocities were used to calculate discharge, which was then used to calculate drift rates.

Samples of invertebrates were strained with a 0.5-mm mesh soil screen and stored in 70% isopropyl alcohol. Organisms were separated from detritus and

4

Table 2. *Physical and chemical data from the lower treatment station (SB2) of Seas Branch Creek, 1972-74.*

| Date | Temp (°C) | | Dissolved O$_2$ | | pH | Total alkalinity (ppm) | Turbidity (JTU) | Conductance (μmho/cm) | Discharge (m³/s) |
	Air	Water	ppm	Percent saturation					
1972									
15 May	18	14	11.7	115	8.7	186	0.11	ND	0.10
15 June	17	15	9.8	100	8.6	199	0.16	420	0.11
15 July	32	20	10.0	114	8.0	190	0.73	420	0.12
15 Aug	24	18	11.6	125	8.5	201	0.44	400	0.10
3 Oct	17	12	10.3	98	8.3	220	0.52	500	0.16
6 Oct	10	11	10.8	102	8.5	220	0.30	500	0.15
13 Oct	10	10	13.0	119	8.5	240	0.42	520	0.05
1 Nov	8	8	11.6	102	8.5	203	0.20	440	0.20
1 Dec	-6	4	12.5	100	8.1	215	0.19	420	0.15
1973									
15 Jan	-12	1	14.3	103	8.0	224	0.40	460	0.12
15 March	1	6	14.1	117	8.3	185	7.20	320	0.22
15 May	15	12	13.1	125	8.5	197	0.58	425	0.24
16 July	24	17	11.1	118	8.1	204	0.51	420	0.26
15 Sept	10	12	9.1	85	8.0	220	0.42	420	0.29
6 Oct	11	11	10.0	95	8.1	214	0.21	440	0.31
10 Nov	0	4	12.2	98	8.0	214	0.31	420	0.28
1974									
20 April	10	9	9.6	85	7.8	202	0.16	350	0.18
17 May	16	12	10.3	100	8.1	217	0.20	400	0.06
30 July	20	17	8.9	95	8.2	220	0.10	410	0.08
2 Oct	7	10	10.4	95	7.7	232	0.35	400	0.16
Mean	12.1	11.2	11.2	105	8.3	210	0.35	425	0.17

identified, and body length was measured. Identifications were verified by the museum staff of the Smithsonian Institution, Washington, D.C. In estimating the biomass of individual organisms from the length, we followed Hynes (1961), Hynes and Coleman (1968), Hamilton (1969), and Jacobi (1969) in assuming that an insect's shape is that of a cylinder five times as long as wide, that its volume increases by the cube of the length, that its specific gravity is 1.05, and that 3.3×10^{-5} g is the weight of a 1-mm length unit. Insects were not weighed because weight loss varies widely after preservation (Howmiller 1972). Crayfish were wet-weighed after surface water had been removed by blotting.

Additional specimens from some of the major taxa were nonquantitatively collected from the treatment and control stream on 6, 13, and 19 October and 4 November 1972, and the percentages of dead organisms noted (Table 4). We used these values to estimate the percentages of dead organisms in the benthic samples for these periods; biomass was adjusted to show only the weight of living organisms. The taxa collected are given in Table 5; average monthly values for water temperature, discharge, vegetative cover, and total benthic biomass before and after treatment in Table 6; and the estimated biomass (g/m²) for each organism at each station on each collection date in Tables 7-9.

Results

Total Benthos and Drift

The aquatic macroinvertebrates collected included 33 identified to genus or genus and species, 5 to family, and 2 to order (Table 5). The dominant forms on the basis of pretreatment biomass (in order of abundance) were *Hydropsyche* (caddis fly), *Orconectes propinquus* (crayfish), Chironomidae (midges), *Optioservus fastiditus* (riffle beetle), *Antocha* (crane fly), *Brachycentrus americanus* (caddis fly), *Gammarus pseudolimnaeus* (scud),

Table 3. *Physical and chemical data from the control station (MD) of Maple Dale Creek.*

| Date | Temp (°C) | | Dissolved O$_2$ | | pH | Total alkalinity (ppm) | Turbidity (JTU) | Conductance (μ mho/cm) | Discharge (m^3/s) |
	Air	Water	ppm	Percent saturation					
1972									
15 May	18	18	8.5	93	8.6	191	0.40	ND	0.09
15 June	15	13	11.6	113	8.6	216	0.23	420	0.08
15 July	28	20	10.2	116	8.6	219	0.23	470	0.10
15 Aug	28	20	11.0	124	8.5	213	0.13	420	0.07
3 Oct	16	13	10.1	98	8.4	238	ND	ND	0.13
6 Oct	13	11	8.6	81	8.5	235	0.47	560	0.11
13 Oct	6	10	11.0	101	8.5	226	0.30	540	0.09
1 Nov	9	8	10.9	95	8.5	222	0.60	450	0.15
1 Dec	-6	4	13.8	107	8.3	230	0.10	440	0.11
1973									
15 Jan	-11	0	15.1	106	8.2	223	0.30	500	0.10
15 March	1	5	14.5	117	8.4	229	0.21	420	0.33
15 May	15	15	12.4	126	8.7	210	0.21	440	0.25
16 July	24	19	9.9	105	8.1	238	0.55	460	0.16
15 Sept	15	10	10.4	95	8.1	228	0.30	450	0.20
6 Oct	11	10	11.8	108	8.1	206	0.17	480	0.17
10 Nov	1	5	13.1	105	8.1	230	0.19	440	0.17
1974									
20 April	16	13	10.1	98	8.4	224	0.10	345	0.17
17 May	18	13	11.2	110	8.3	223	0.35	420	0.06
30 July	21	17	9.7	102	8.3	247	0.10	425	0.12
2 Oct	7	7	12.3	105	7.6	248	0.20	385	0.10
Mean	12.3	11.6	11.3	105	8.3	225	0.27	447	0.14

Baetis cingulatus (mayfly), and *Prosimulium* sp. (black fly).

Drift rates increased noticeably during treatment at both stations, reaching 50 g/h at SB1 18 h after treatment and nearly 169 g/h at SB2 9 h after treatment (Fig. 2). Other increases in drift rates were associated with increased densities or emergence of the dominant taxa before and after treatment (Fig. 3). The high values for total drift at SB2 in July and October 1974 are attributed largely to scuds, which in these 2 months constituted 67% and 34% of the total benthic biomass.

Total benthic biomass decreased at SB1 and SB2 (as well as at MD) immediately after treatment but attained a peak in the treated stream later in the fall, resuming the generally increasing trend that began in early fall (Fig. 4). One year after treatment, total benthic biomass in Seas Branch Creek approached or exceeded that found before treatment. This trend also was suggested during the second year after treatment, although the order of dominating taxa differed between years.

The decrease in benthos at the control station (MD) during the time of treatment probably reflects a sampling error, rather than a true decrease in density of organisms; the samples were collected from a riffle area which had been disturbed during earlier sampling. Neither drift samples (Fig. 3) nor nonquantitative benthic samples (Table 4) indicated abnormally high values for dead or drifting organisms at MD during the time of treatment.

To compare the pretreatment and posttreatment data, we calculated the average biomass (without crayfish) of samples collected annually at each station in May, July, and October (Table 6). Biomass reached maximum levels during these months, which span the major growing season. Vegetative cover more than doubled during the year after treatment at SB2 and during the second year after treatment at SB1, but changed little at the control station. Benthic biomass also followed this general pattern in the treatment stream but, again, remained nearly constant in the control stream (Table 6).

Table 4. *Summary of total numbers of invertebrates collected and percentage dead at the three study stations on different dates after the antimycin treatment on 4 October 1972. (Dashes indicate no sample taken; P = present, but not counted).*

Date (1972) and taxon (L = larvae)	Station					
	SB1		SB2		MD	
	Total no.	Dead (%)	Total no.	Dead (%)	Total no.	Dead (%)
6 October[a]						
Baetis	77	13	13	85	32	0
Brachycentrus	47	53	31	74	100	1
Gammarus	49	37	19	100	27	4
Hydropsyche	51	12	6	33	48	0
Optioservus (L)	32	16	2	0	27	0
Antocha (L)	41	63	—	—	4	0
Stenonema	11	9	4	0	1	0
Orconectes	0	0	10	0	0	0
13 October						
Baetis	1	100	0	0	16	0
Brachycentrus	12	50	50	98	31	3
Gammarus	17	18	4	50	50	2
Hydropsyche	58	40	100	89	50	0
Optioservus (L)	33	15	35	20	25	0
Antocha (L)	7	43	8	100	25	12
Stenonema	4	25	6	83	4	0
Orconectes	3	0	4	0	3	0
19 October						
Baetis	9	0	0	0	28	0
Brachycentrus	5	40	20	100	55	2
Gammarus	9	0	3	0	69	2
Hydropsyche	19	58	7	100	55	2
Optioservus (L)	26	4	25	0	53	2
Antocha (L)	9	33	2	100	41	7
Stenonema	3	0	2	50	10	0
Orconectes	10	0	4	0	3	0
4 November						
Baetis	0	0	0	0	—	—
Brachycentrus	10	80	P	0	—	—
Gammarus	4	25	P	0	—	—
Hydropsyche	5	0	P	0	—	—
Optioservus (L)	2	0	P	0	—	—
Antocha (L)	2	50	P	0	—	—
Stenonema	2	0	P	0	—	—
Orconectes	0	0	P	0	—	—

[a] Data for SB2 on 6 October were obtained from observations on organisms placed in small containers before treatment.

Amphipoda (Scuds)

Immediately after treatment, *Gammarus pseudolimnaeus* decreased in the benthic samples (Fig. 4), and increased markedly in the drift (Fig. 2). The number of drifting dead and dying organisms reached a maximum 12 h after treatment at SB2 and 18 h after treatment at SB1 (Fig. 2). By the second day after treatment the mortality of scuds was apparently 100% at SB2 but only 37% at SB1 (Table 4). Estimated benthic biomass of scuds at both treatment locations remained low during the winter after treatment but increased in the following summer to values far exceeding those of the previous year (Fig. 4). Scuds were abundant in the benthos during the summer after treatment; they were also dominant in July 1974 at both treatment stations, making up 56% and 67% of the biomass (without crayfish) at SB1 and SB2. A drift rate at SB2 of 5 g/h in September 1973 and about 25 g/h in July and 24 g/h in October 1974 reflected this increased density of organisms (Fig. 3).

In the control stream, the biomass of scuds never varied significantly from one sample period to another (Fig. 4), and drift rates were low throughout the year (Fig. 3).

Diptera (Crane fly, Midges, Black fly)

Benthic biomass of the crane fly *Antocha* was reduced sharply by the treatment at both SB1 and SB2 (Fig. 4), and no live specimens were collected in the drift immediately after treatment. The drift of dead crane flies reached a peak 18 h after treatment at SB1 and 12 h after treatment at SB2 (Fig. 2). No living crane fly larvae were taken in benthic samples for 2 weeks at SB2 (none were found 2 days after treatment), whereas the maximum mortality of 63% at SB1 2 days after treatment decreased gradually to 50% (one of two specimens collected) 1 month later (Table 4). Benthic biomass of crane flies was about four times greater at SB2 than at SB1 before treatment but remained low for 1 year after treatment at both stations. The estimated biomass was high in the samples collected at SB1 in November 1973, but was again low in April and May 1974. Emerging adults were not found at SB1 during May 1974, but were present in drift samples at SB2 and MD. Despite the large numbers of larval crane flies in the samples collected at SB1 in November 1973, the population did

Table 5. *Macroinvertebrate taxa collected in the treatment stream, Seas Branch Creek, and the control stream, Maple Dale Creek.*[a]

Arthropoda
  Insecta
    Diptera
      Chironomidae (midges)
      Tipulidae (crane flies)
        Antocha
        Hexatoma
      Simuliidae (black flies)
        Prosimulium
      Empididae (dance flies)
        Hermerodromia
      Rhagonidae (snipe flies)
        Atherix variegata
      Stratiomyidae (soldier flies)
        Hedriodiscus
      Tabanidae (horseflies)
        Tabanus
        Chrysops
    Ephemeroptera (mayflies)
      Baetidae
        Baetis cingulatus
      Heptageniidae
        Stenonema
      Ephemerellidae
        Ephemerella
    Trichoptera (caddis flies)
      Brachycentridae
        Brachycentrus americanus
      Hydropsychidae
        Hydropsyche
        Cheumatopsyche
      Hydroptilidae
        Ochrotrichia
      Helicopsychidae
        Helicopsyche borealis
      Glossosomatidae
        Protoptila
        Glossosoma
      Limnephilidae
    Plecoptera (stoneflies)
      Perlodidae
        Isoperla
      Nemouridae
    Coleoptera (beetles)
      Elmidae (riffle beetles)
        Optioservus fastiditus
        Stenelmis sandersoni
      Dytiscidae (diving beetles)

Odonata
  Zygoptera (damselflies)
  Hemiptera (bugs)
    Corixidae (water boatmen)
      Sigaria mathesoni
    Belostomatidae (giant water bug)
      Lethocerus
    Gerridae (water striders)
      Gerris
      Trepobates
Crustacea
  Amphipoda
    Gammaridae (scud)
      Gammarus pseudolimnaeus
  Decapoda (crayfish)
    Astacidae
      Orconectes propinquus
Arachnoidea
  Hydracarina (water mites)
Mollusca
  Gastropoda (snails)
    Basommatophora
      Physidae
        Physa obrussoides
  Pelecypoda (clams)
    Heterodonta
      Sphaeriidae
        Pisidium
Annelida
  Hirudinea (leeches)
    Rhynchobdellida
      Glossiphoniidae
        Glossiphonia complanata
    Arhynchobdellida
      Erpobdellidae
        Erpobdella punctata
  Oligochaeta (worms)
    Pleisopora
      Tubificidae
Platyhelminthes
  Turbellaria (flatworms)
    Tricladida
      Planariidae
        Dugesia
Nematomorpha
  Gordiida (horsehair worms)
    Gordiidae
      Gordius

[a] All forms shown were collected in both the treatment and control stream, with four exceptions: *Erpobdella, Helicopsyche,* and *Nemoura* were only in the treatment stream and *Pisidium* only in the control stream.

8

Table 6. *Average monthly (May, July, and October [a]) water temperature, discharge, vegetative cover, and benthic biomass at Seas Branch Creek stations SB1 and SB2 and control station MD before (1972) and after (1973, 1974) the antimycin treatment of Seas Branch Creek.*

Station and year	Water temp (°C)	Discharge (m³/s)	Estimated vegetative cover (%)	Benthic biomass (g/m²)
SB1				
1972	15	0.08	10	56.5
1973	10	0.17	10	49.7
1974	11	0.08	23	114.8
SB2				
1972	15	0.13	11	61.1
1973	13	0.27	27	105.6
1974	13	0.10	17	111.0
MD				
1972	17	0.11	11	96.0
1973	15	0.19	9	93.2
1974	12	0.11	7	100.3

[a] Before treatment on 3 October 1972 for all stations.

not recover from the treatment—as indicated by the sharp (nearly complete) overwinter decline, the lack of adults in the succeeding summer drift, and the near absence of the organisms in October 1974 (2 years after treatment).

The rate of emergence of crane flies was high in spring and decreased from May through September at the control station; the sharp decrease in biomass between March and May 1974 (Fig. 4) was presumably a result of emergence. Larval drift rates were low throughout the year at both treatment stations, except for the increase at the time of treatment (Fig. 3).

Drift rates of Chironomidae at SB2 increased sharply 21 h after treatment, peaked 12 h later, then declined gradually into the next week; drift at SB1 increased slightly 15 h after treatment (Fig. 2). The biomass at both SB1 and SB2 decreased slightly during treatment, then increased sharply in December 1972 (Fig. 5). At this time, midges dominated the biomass (without crayfish) at both treatment stations, contributing 57% at SB1 and 63% at SB2. Apparently the larvae rapidly occupied habitats vacated by more sensitive organisms. Biomass then decreased throughout the year to low levels that approached pretreatment values. High drift rates of midges at SB2 in the year after treatment (Fig. 3) were attributed to overlapping hatches of the various species present.

Benthic biomass values of Chironomidae were low and fluctuated throughout the year at station MD; an increase in biomass similar to that at SB1 and SB2 occurred here after the treatment date, but never reached the levels found at the treated stations (Fig. 5). Drift rates were low throughout the year at MD; the nearly 5 g/h in May 1974 (Fig. 3) reflected the slightly higher benthic biomass present then (Fig. 5).

Antimycin had no direct effect on *Prosimulium* sp. because black flies had emerged before treatment. Biomass at SB2 remained low through November; an increase began in January that reached a maximum of 98 g/m² in July 1973 (Fig. 5), or 65% of the benthic biomass (without crayfish). Drift rates, which previously were low (not illustrated) increased with this large increase of *Prosimulium*. Biomass at SB1 also peaked in July. A residual population was present at MD, throughout the year, but never made up a significant portion of the total benthic biomass (Fig. 5).

The dance fly *Hemerodromia* sp. which was present at all three stations in small numbers (but ranging up to 8 g/m² at station MD in January 1973) throughout the year (Tables 7–9) appeared to be unaffected by the treatment; few specimens were in the drift, and no dead ones were found.

Ephemeroptera (Mayflies)

Many dead nymphs of *Baetis cingulatus* were observed at the time of treatment at SB1, and drift rates doubled (Fig. 6). At SB2, where the water was warmer, a major emergence had taken place before the treatment. Benthic biomass of this species therefore declined at both stations after treatment (Fig. 5). The benthic biomass of *B. cingulatus* increased 20-fold 1 year after treatment at SB2 and also increased greatly at SB1 earlier in the year (Fig. 5). The decrease in biomass at all stations in November 1973 (Fig. 5) was apparently caused largely by earlier increased drift of late instar nymphs and subimagoes (Fig. 7). The very high biomass levels at SB2 during the second summer after treatment were related to increased vegetation and the larger population of the generation in the preceding year. The decrease in biomass in October 1974 at SB1 was presumably due to earlier emergence.

The two periods of maximum emergence of *B. cingulatus* at MD were in May to July and late September to November. Benthic biomass increased here for each generation (Fig. 5), and drift was high at the time of emergence, which coincided with the time of antimycin treatment (Figs. 6 and 7).

The mayfly *Stenonema* sp. was present at the three sampling stations throughout the study; biomass was highest in the second year after treatment.

Mortality during treatment appeared to be initially low or nil at SB2, but five of six organisms (83%) collected 10 days after treatment were dead (Table 4).

Another mayfly, *Ephemerella* sp., was not collected in 1972 or 1973 but appeared in 1974 at SB1 in April, May, and October and at SB2 and MD in May.

Trichoptera (Caddis flies)

Benthic biomass of the caddis fly *Brachycentrus americanus* was reduced immediately after treatment (Fig. 8), and drift increased sharply (Figs. 6 and 7). At SB1, drift did not occur until 12 h after treatment and reached a maximum 3 h later (Fig. 6). Mortality at this station was about 53% 2 days after treatment and 80% 1 month later (Table 4). Drift at SB2 doubled shortly after treatment (Fig. 6) and continued to be high for at least 2 days. Mortality was 74% on the second day after treatment and 100% 2 weeks after treatment (Table 4). This species seemed to become disoriented during the antimycin treatment. At SB2 many organisms moved about sluggishly and crawled onto stream vegetation and stones. About 50% then abandoned their cases and died.

Biomass of *B. americanus* remained low at both treatment stations during the first year after treatment, but increased considerably at SB1 (not at SB2) during the second year after treatment (Fig. 8).

This species overwintered as larvae and emerged in May through August in Maple Dale Creek. An early emergence in May 1973 preceded a rapid increase in biomass of the following generation (Fig. 8).

For the caddis fly *Hydropsyche* sp., the number of dead and dying in the drift at SB2 reached a maximum 9 h after treatment and decreased during the next week (Fig. 6). Mortality then increased gradually to 100% on 19 October (Table 4). Few drift organisms were taken after treatment at SB1, and the number increased only slowly into the next week (Fig. 6). Mortality here was initially low and reached a maximum of only 58% 2 weeks later (Table 4). Biomass of *Hydropsyche* was reduced at both stations during treatment and increased slightly during the months after treatment (Fig. 8). We attributed this increase to recolonization by drift. The population appeared to have recovered during the year after treatment. Biomass levels at both treatment stations during the second summer after treatment exceeded those before treatment.

In 1972, benthic biomass of *Hydropsyche* was markedly lower in samples taken at MD on 6 and 13 October than on 3 October or 1 November (Fig. 8). However, as mentioned earlier, this decrease was attributed to a sampling error as no increase in drift rates or die-off was observed during this period. Larvae in the 3 October and 1 November samples were in the same size group, indicating that no emergence had occurred.

Hydropsyche produced one generation per year; emergence extended from May through August. Drift rates increased at the times of emergence (Fig. 7). Benthic biomass at all three stations showed decreasing trends after emergence, followed by an increase as the new generation developed (Fig. 8). Samples contained a wide range of instars because of the prolonged hatching time, as was observed also by Hynes (1961). Biomass increased in the fall. Medium size specimens (5-10 mm long) dominated the October-November samples and large ones (10-13 mm long) the late winter and early spring collections.

Coleoptera (Riffle beetle)

No noticeable changes in benthic biomass or drift of *Optioservus fastiditus* occurred during treatment, although benthos collections 10 days after treatment suggested a 15% and 20% mortality at stations SB1 and SB2, respectively (Table 4). The biomass of *O. fastiditus*, represented by concurrent populations of larvae and adults, reached a peak at all stations at about the same time in 1972 and 1973 (Fig. 8). After the reduction or disappearance of organisms sensitive to antimycin, the larvae contributed significantly to the total benthic biomass—e.g., up to 70% at SB2 on 13 October 1972 (Table 8).

Decapoda (Crayfish)

No dead or dying *Orconectes propinquus* were observed during or after the antimycin treatment (Table 4). Because this organism is highly mobile, it was difficult to accurately evaluate its population density (Tables 7, 8, and 9). Many young of the year (1.2-2.0 cm long) were found at SB2 from May, June, or July through October in all years (Table 8).

Discussion

The application of high concentrations of antimycin (17-44 µg/l) resulted in an immediate increase in drift rates and a temporary reduction in populations of five of nine major taxa, *Gammarus pseudolimnaeus, Antocha, Baetis cingulatus, Brachycentrus americanus,* and *Hydropsyche. Orconectes propinquus* was not affected by the treatment. The biomass of other organisms, such as Chironomidae, *Optioservus fastiditus,* and *Prosimulium,* increased during the months after treatment. Total benthic biomass (all taxa combined) during the two summers after treatment approached or exceeded that of the summer before treatment.

Table 7. *Benthic biomass (g/m²) for station SB1 of Seas Branch Creek above the impoundment, before and after treatment of the stream with antimycin on October 4, 1972.* [a]

Taxon	1972								
	15 May	15 June	15 July	15 Aug	3 Oct	6 Oct	13 Oct	1 Nov	1 Dec
Diptera									
Chironomidae	11.5	4.0	8.1	2.3	2.2	T	0.4	6.7	102.1
Antocha									
larvae	4.3	1.7	4.6	2.3	3.2	0.9	0.2	0.1	0.3
pupae	2.9	0.8	0.5	0.4	0	0	0	0	0
Prosimulium									
larvae	T	0.3	0.7	0.5	0.1	0	0	0	0
pupae	T	0	T	T	T	T	0	0	0
Hemerodromia	0	0	0.2	0.1	3.7	1.2	1.0	1.1	1.5
Other	0.1	0.2	3.6	2.8	7.1	0.5	1.3	4.0	40.0
Epheremoptera									
Baetis	0.1	1.0	0.9	2.1	5.2	0.3	0	0	0
Stenonema	0	0	0	0.1	0.6	0.9	0	0.2	0.5
Ephemerella	0	0	0	0	0	0	0	0	0
Trichoptera									
Hydropsyche	17.4	17.0	7.0	11.7	54.7	28.3	12.6	9.0	16.9
Brachycentrus americanus	0	0.4	2.8	1.5	1.6	0.4	0.5	0.1	1.3
Ochrotrichia	0.3	0.3	0	0	0	0	0	0	0
Glossosoma	0	0	0	0	0	0	0	0	0
Other	0.2	0.2	0.6	0.1	0.1	0.5	0.2	T	0.1
Plecoptera									
Isoperla	T	0	0	0	T	T	0	0	0.2
Coleoptera									
Optioservus fastiditus									
larvae	1.1	2.4	3.9	7.4	13.4	5.3	7.0	12.7	15.8
adult	0.3	0.1	0.3	1.0	0.9	0.5	0.5	0.3	0.7
Stenelmis sandersoni	0.1	0.1	0.2	0.2	0.7	0.1	0.2	0.3	0.1
Amphipoda									
Gammarus pseudolimnaeus	0.6	6.9	1.9	2.8	1.3	0.4	0.4	0.4	0.1
Mollusca									
Physa obrussoides	0	0	0	0	0	0	0	0.1	0
Hirudinea									
Erpobdella punctata	0	0	0	0.3	0	0.9	0	T	0
Miscellaneous	0.3	T	0.2	0.1	0.1	0	0	T	T
Benthic Biomass									
without *Orconectes*	39.2	35.4	35.5	35.7	94.9	40.2	24.3	35.0	179.6
Decapoda									
Orconectes propinquus	4.2	0.1	16.7	11.9	0	1.4	0	0	4.0
Total Benthic Biomass	43.4	35.5	52.2	47.6	94.9	41.6	24.3	35.0	183.6

[a] T = less than 0.05 g.

Table 7—Continued

	1973							1974			
15 Jan	15 Mar	15 May	16 July	15 Sept	6 Oct	10 Nov	20 April	17 May	30 July	2 Oct
23.7	9.9	9.8	11.8	7.1	0.2	0.1	1.1	0.5	8.8	0.1
0.3	0.2	0.1	0.1	1.6	0.4	7.5	0.3	0.2	0.7	0.1
0	0	0	0	0	0	0	0	0	0	0
0.2	0.1	0	24.5	3.5	2.1	10.3	T	T	8.9	0.6
0	0	T	0	T	T	0	0	0	0	0
3.9	0.1	T	0.2	2.2	0.2	0.4	0	0	T	3.2
14.8	15.2	4.8	0	0.5	0	9.0	0	0.5	0	4.0
T	3.9	6.3	1.3	1.6	1.4	1.2	0.3	T	2.5	0.1
0.1	0	1.0	0	0.4	0.4	0.6	0.5	2.9	2.4	0.9
0	0	0	0	0	0	0	4.8	11.4	0	T
25.8	19.1	1.7	0.4	44.8	58.2	13.1	49.7	68.8	17.8	35.5
0.8	0.6	0	1.6	1.4	2.3	11.0	3.0	0	14.3	15.4
0	0	T	0	0	T	0	0	0	T	T
0	0	0	T	0	T	0	3.3	4.7	3.1	4.0
0.4	0.3	1.6	0.2	2.0	0.2	0	1.9	4.5	0.1	T
T	0.7	0.9	0	T	T	0.3	2.1	0.2	0	0
12.2	1.6	1.4	0.8	16.4	6.6	20.1	4.6	6.3	6.6	9.1
1.0	0.2	0.3	0.2	1.8	0.6	0.4	1.0	0.9	1.3	1.1
0.1	0	0	0	0.1	0	0.2	0	T	0	0
0.2	0.2	1.6	0.6	5.6	4.3	9.2	5.2	7.6	86.4	11.2
0.3	0	0	T	T	T	0	T	T	0	0.1
T	0	0	T	3.4	0.4	0.4	0	0.6	0.5	0
T	0	0.3	0	0.1	T	T	0	0	0	0
83.8	52.1	29.8	41.7	92.5	77.3	83.8	77.8	109.1	153.4	85.4
3.6	0	0	0	0	25.9	0	0	39.1	13.2	82.6
87.4	52.1	29.8	41.7	92.5	103.2	83.8	77.8	148.2	166.6	168.0

Table 8. *Benthic biomass (g/m²) for station SB2 of Seas Branch Creek, below the impoundment, before and after treatment of the stream with antimycin on October 4, 1972.*[a]

Taxon	1972								
	15 May	15 June	15 July	15 Aug	3 Oct	6 Oct	13 Oct	1 Nov	1 Dec
Diptera									
Chironomidae	13.8	31.0	15.4	28.6	0.8	2.3	5.2	20.0	111.4
Antocha									
larvae	3.4	1.3	2.9	16.1	12.3	6.7	0	0.3	0.3
pupae	0.5	0.9	0.2	0.4	0	0.1	0	0	0
Prosimulium									
larvae	0	0.5	0.2	T	0.2	0	0	0	0.1
pupae	0	0.1	T	T	T	T	T	0	0
Hemerodromia	0	T	0.5	0	0.3	3.0	0.1	1.2	1.2
Other	0.6	0.2	T	0.2	0	0.4	0.1	16.3	1.8
Ephemeroptera									
Baetis	0.1	1.3	1.5	0.4	T	0	0	0	0
Stenonema	0	0	0	0.1	0.2	0.1	0.1	0.2	0.3
Ephemerella	0	0	0	0	0	0	0	0	0
Trichoptera									
Hydropsyche	6.7	12.1	17.4	30.1	34.2	19.7	4.1	7.2	10.4
Brachycentrus americanus	0	12.4	12.4	11.0	17.0	11.5	0.9	1.6	2.6
Ochrotrichia	T	0.1	0	0.1	0	0	0	0	0
Glossosoma	0	0	0	0	0	0	0	0	0
Other	1.1	0.6	0.3	T	T	0.1	0.3	0.5	0.2
Plecoptera									
Isoperla	0	0	0	0	0	0	0	0	0
Coleoptera									
Optioservus fastiditus									
larvae	0.4	5.4	8.6	7.3	12.3	17.7	40.5	45.7	43.3
adult	T	0.1	T	0.1	0.1	0.1	0.3	0.2	0.2
Stenelmis sandersoni	T	0.1	0.5	0.5	0.6	0.5	1.2	0.5	0.8
Amphipoda									
Gammarus pseudolimnaeus	4.9	4.9	1.7	2.4	2.3	0	0.1	T	0
Mollusca									
Physa obrussoides	0	0.1	0	T	0	0	T	T	0.4
Hirudinea									
Erpobdella punctata	4.6	13.0	0	3.5	3.5	0	4.3	0.4	0.1
Nematomorpha	0	0	0	0	T	T	0	0	T
Miscellaneous	1.8	0.4	T	0	0.1	0.1	0.1	0.3	3.1
Benthic Biomass without *Orconectes*	37.9	84.5	61.6	100.8	83.9	62.3	57.3	94.4	176.2
Decapoda									
Orconectes propinquus	23.3	6.0	7.1	34.0	52.7	21.4	5.3	49.1	0
Total Benthic Biomass	61.2	90.5	68.7	134.8	136.6	83.7	62.6	143.5	176.2

[a] T = less than 0.05 g.

Table 8—Continued

	1973						1974			
15 Jan	15 Mar	15 May	16 July	15 Sept	6 Oct	10 Nov	20 April	17 May	30 July	2 Oct
50.5	39.3	55.3	13.9	8.8	0.1	0.1	0.6	3.2	0.4	0.1
0.1	T	0.5	0.1	0.6	0.5	0.5	0.3	1.1	0.4	1.5
0	0	0	0	T	0	0	0	0	0	0
0.7	3.4	1.9	97.8	0.4	0.1	0.1	T	3.1	1.3	T
0	0	0.1	T	T	T	0	0	0	0	0
2.9	T	0	T	2.2	0.6	1.1	0	0	T	0
3.6	11.0	0.3	0	0.4	0.2	0	1.3	0.4	1.2	9.2
T	0.9	3.4	6.1	13.9	14.0	1.3	23.5	37.7	6.0	7.6
0.1	0.1	0.1	T	1.5	2.6	2.3	8.9	8.4	1.1	1.4
0	0	0	0	0	0	0	0	1.5	0	0
4.7	4.5	3.8	2.5	39.1	39.1	11.9	10.9	50.9	6.6	18.7
0.3	0.5	0	1.1	1.2	0.5	0.3	0.1	0	2.4	3.3
0	0	0	T	T	T	0	0	0	0	0
0	0	0	T	0	0	0	0	0.3	0	0.3
3.0	1.6	0	T	0	0	9.0	0	0.1	0.3	0
0	0	T	0	T	0	0	1.1	0.2	0	0
18.5	9.4	1.5	10.5	33.2	18.9	20.6	7.3	8.2	10.2	14.3
0.1	0.2	0.2	0.2	0.3	0.1	T	0.2	0.4	0.6	0.8
0.2	0.1	T	T	0.1	T	T	T	0.3	0	0
0	T	0.1	11.6	24.0	16.3	14.5	19.7	20.8	71.4	29.3
0.4	T	T	0.2	0.1	T	T	T	0	0.3	0.2
0.1	2.8	20.6	1.0	0.9	1.0	1.6	12.7	2.9	5.1	T
0	0	0	0	0	0	0	0	0	0	0
2.0	0.7	0.4	0	0	T	0	0	0	0	0
87.2	74.5	88.2	145.0	126.7	94.0	63.3	86.6	139.5	107.3	86.7
12.6	8.9	5.3	25.0	45.2	80.7	15.4	15.3	16.7	27.9	13.1
99.8	83.4	93.5	170.0	171.9	174.7	78.7	101.9	156.2	135.2	99.8

Table 9. *Benthic biomass (g/m²) for control station MD of Maple Dale Creek, before and after treatment of Seas Branch Creek with antimycin on October 4, 1972.* [a]

Taxon	1972								
	15 May	15 June	15 July	15 Aug	3 Oct	6 Oct	13 Oct	1 Nov	1 Dec
Diptera									
Chironomidae	5.0	10.7	3.6	6.3	1.0	0.7	1.6	2.0	21.9
Antocha									
larvae	1.6	0.4	12.7	10.9	11.9	13.0	7.1	13.1	7.9
pupae	0.7	0.2	0.3	1.8	0	0	0	0	0
Prosimulium									
larvae	0	0	0.2	0.3	0.1	T	T	0.2	0.2
pupae	0	0	T	T	T	T	0	0	0
Atherix	0	0	0	6.3	4.9	2.3	2.1	2.0	8.3
Hemerodromia	0.4	0.2	0	0.4	1.1	0.9	0.5	2.2	3.4
Other	0	0.7	T	T	0.1	0.2	0.9	0.1	T
Ephemeroptera									
Baetis	T	0.2	0.4	0.2	0.7	0.5	0.1	0.2	0.5
Stenonema	0.4	0	0	T	0.3	T	T	0.2	0.1
Ephemerella	0	0	0	0	0	0	0	0	0
Trichoptera									
Hydropsyche	9.8	6.3	14.7	60.3	172.9	40.2	6.0	164.9	40.3
Brachycentrus americanus	0.1	1.0	1.8	3.3	6.9	6.1	3.9	13.1	2.8
Ochrotrichia	0.1	T	0	0	0	0	0	0	0
Glossosoma	0	0	0	0	0	0	0	0	0
Other	0.8	0.3	T	0.1	0.6	0.4	0.6	0.1	0.1
Plecoptera									
Isoperla	0.3	0	0	0	T	T	0	0.2	0.4
Coleoptera									
Optioservus fastiditus									
larvae	1.4	4.0	4.6	4.2	21.2	13.6	17.8	19.7	11.4
adult	0.1	0.2	0.2	0.4	1.1	0.4	0.2	0.1	0.1
Stenelmis sandersoni	0.1	0.5	0.3	0.4	0.5	0.8	0.6	0.4	0.2
Amphipoda									
Gammarus pseudolimnaeus	0.7	2.3	0.8	0.4	0.5	0.1	0.5	0.2	1.3
Mollusca									
Physa obrussoides	0.3	0.2	T	0	T	0	0	0.1	0
Hirudinea									
Erpobdella punctata	0	7.7	2.6	0	0.1	0	0	0	0
Nematomorpha	0	0	0	0	0	0	T	T	0
Miscellaneous	0.1	0.4	0.1	0.1	0.2	T	T	0.1	0
Benthic Biomass									
without *Orconectes*	21.9	35.3	42.3	95.4	224.1	79.2	41.9	218.9	98.9
Decapoda									
Orconectes propinquus	26.3	32.4	7.2	41.6	30.0	0.2	1.1	0	16.0
Total Benthic Biomass	48.2	67.7	49.5	137.0	254.1	79.4	43.0	218.9	114.9

[a] T = less than 0.05 g.

Table 9. *Benthic biomass (g/m²) for control station MD of Maple Dale Creek, before and after treatment of Seas Branch Creek with antimycin on October 4, 1972.*[a]

		1973						1974		
15 Jan	15 Mar	15 May	16 July	15 Sept	6 Oct	10 Nov	20 April	17 May	30 July	2 Oct
16.8	18.6	5.6	1.6	2.7	0.8	0.2	11.4	24.6	9.4	0.1
7.1	14.9	4.3	5.9	8.8	9.9	6.1	6.6	6.5	13.3	2.1
0	0	0	0	T	0	0	0	0	0	0
0.3	T	0	0.3	1.5	0.4	T	0.4	0.4	2.6	0.2
0	0	0	0	0	0	0	0	0.3	0	0
1.6	2.0	0	0	1.3	0	0	0	0	0	0
8.0	2.0	0.1	0.3	1.5	2.6	1.6	0	T	0	0
0.4	3.4	0.2	0.6	0	0	0	0.4	0	0	0
0.4	5.6	0.5	1.3	3.6	3.3	0.4	0.1	0.3	3.7	7.3
T	0.4	T	0	0.1	T	T	0	1.1	0.1	0
0	0	0	0	0	0	0	0	T	0	0
44.4	82.0	18.5	14.1	79.7	128.2	56.5	62.3	32.4	13.7	94.3
10.4	9.5	0	11.1	32.9	25.6	31.9	3.4	0	2.5	42.5
0	0	0	0	0	0	0	0	0	0	0
0	0	0	T	0	0	0	0.1	0.1	T	0.1
T	1.7	0.3	0.1	0	T	0	0.1	0	0.4	0
0.9	0.4	0.3	0	0	T	0	1.5	0.4	0	0
29.9	2.6	1.5	16.3	50.6	20.7	27.0	9.3	7.9	12.4	17.1
0.1	0.3	0.2	1.0	0.4	1.2	0.4	0.6	0.9	0.5	0.5
0.6	0.1	0	T	T	T	T	0.1	0.3	0.2	0
0.6	1.9	0	1.0	1.6	1.4	2.0	0.3	0.3	1.4	1.0
T	T	0	0.1	T	T	0	0	T	T	0
0	0.8	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0.1	0.1	0	0	T	T	0	0	0	0	0
121.6	146.3	31.5	53.7	184.7	194.1	126.1	96.6	75.5	60.2	165.2
1.2	14.7	0	8.8	42.7	22.8	0	6.6	22.6	128.0	1.0
122.8	161.0	31.5	62.5	227.4	216.9	126.1	103.2	98.1	188.2	166.2
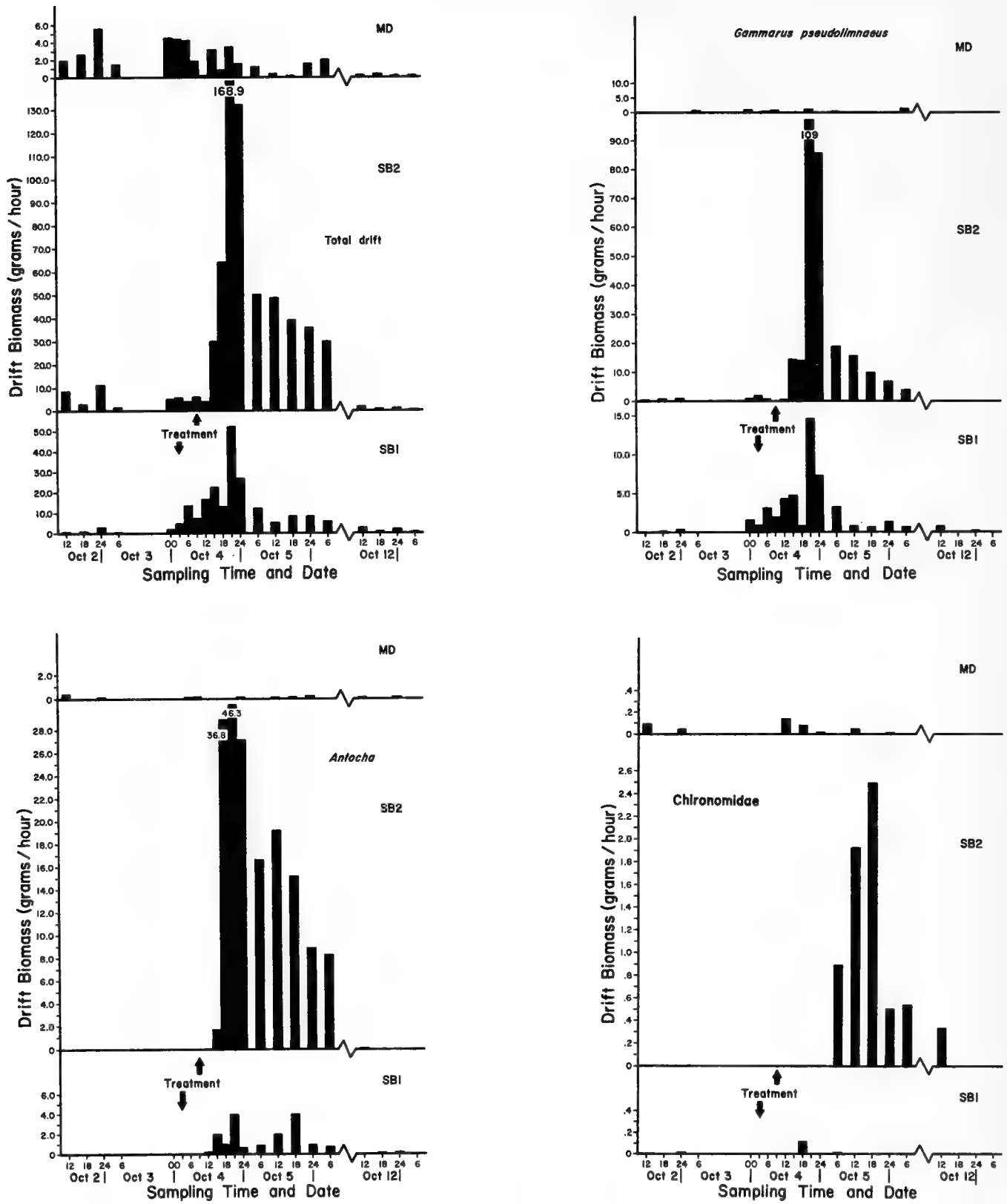
Fig. 2. Drift of benthic macroinvertebrates (total, scuds, crane flies, and midges) at sampling stations in treated Seas Branch Creek (SB1, above impoundment; SB2, below impoundment), and in untreated Maple Dale Creek (MD), October 1972. Numbers along the baseline show sampling times (6 = 0600 h, 12 = 1200 h, . . .) and arrows show time when antimycin reached the station.
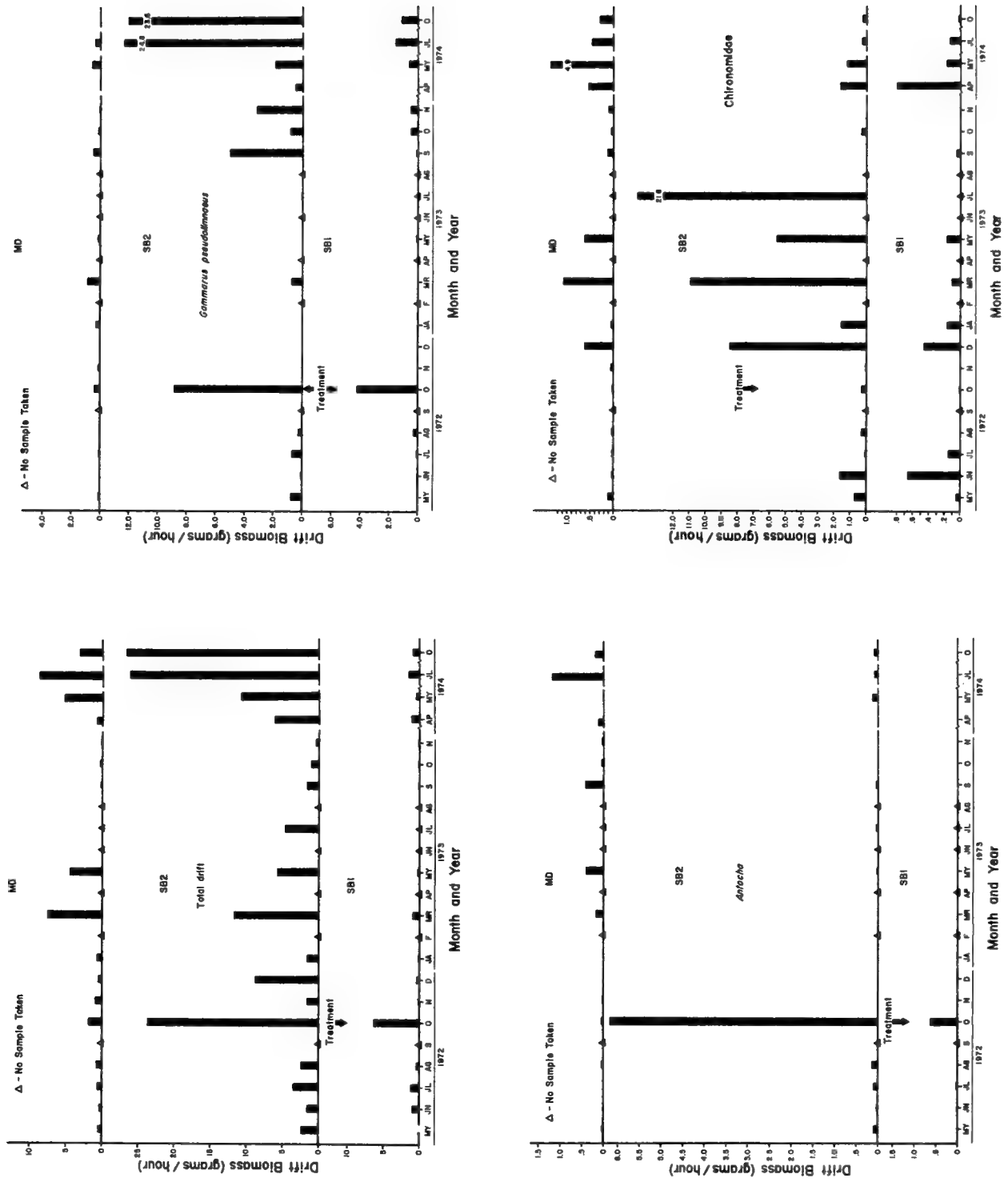
Fig. 3. Drift of benthic macroinvertebrates (total, scuds, crane flies, and midges) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972–74.
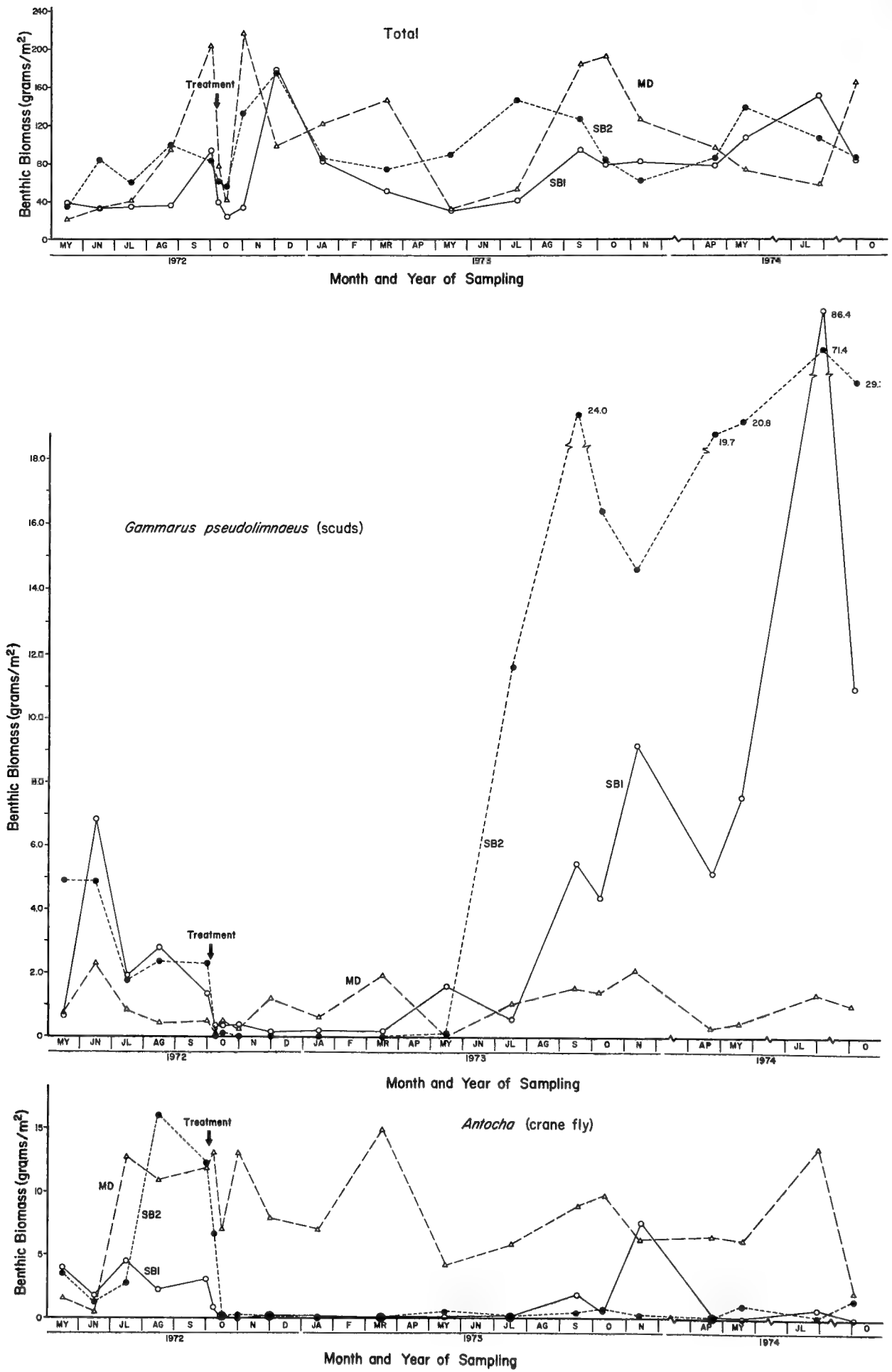
Fig. 4. Biomass of benthic macroinvertebrates (total, scuds, and crane flies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972-74.
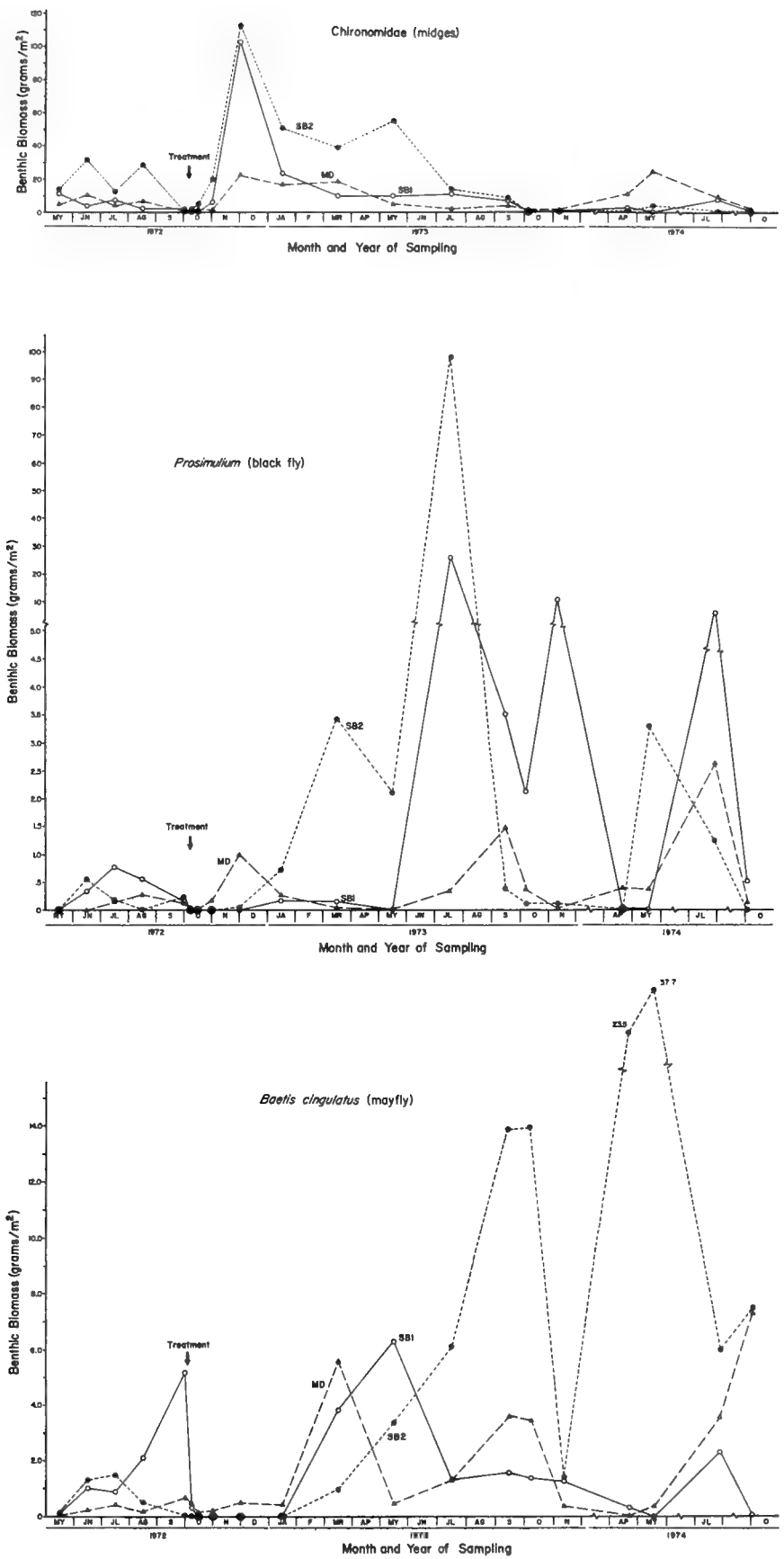
Fig. 5. Biomass of benthic macroinvertebrates (midges, black flies, and mayflies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972-74. (Note the change in scale for biomass of black flies for values larger than 5 g/m²).
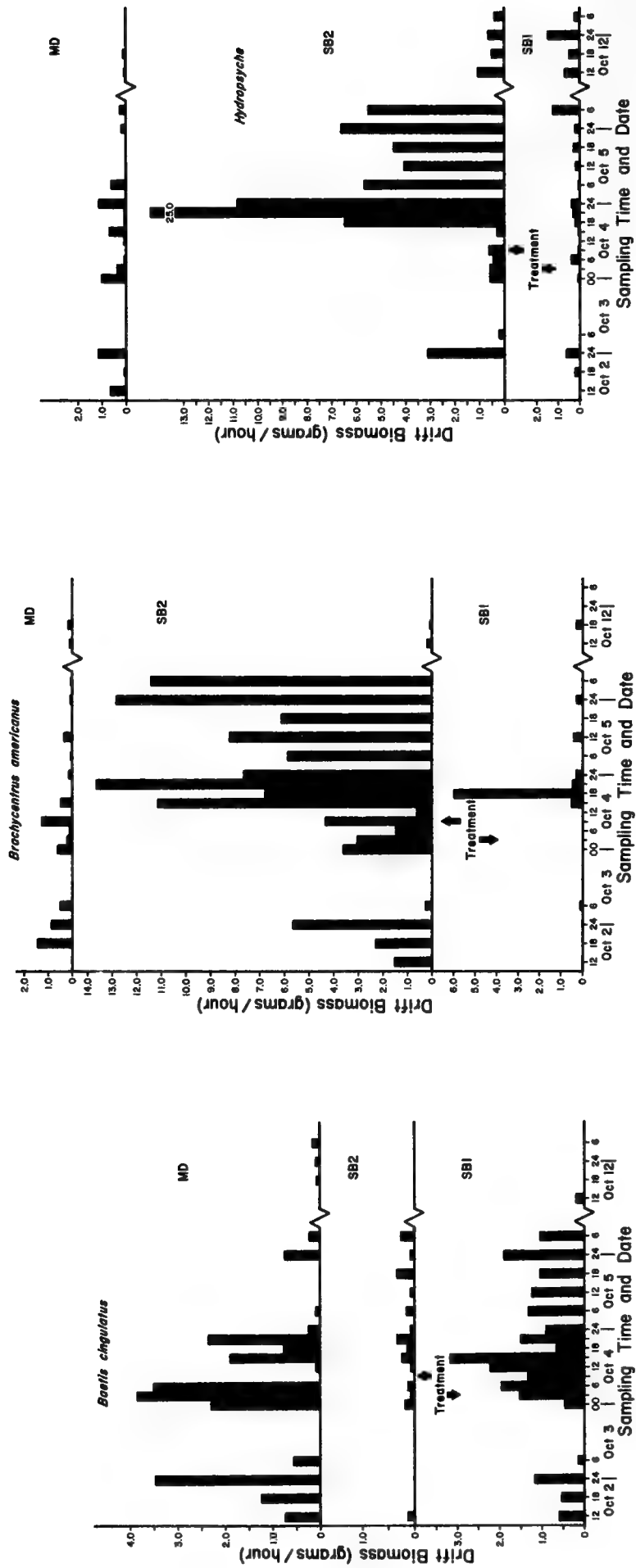
Fig. 6. Drift of benthic macroinvertebrates (a mayfly and two caddis flies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), October 1972.
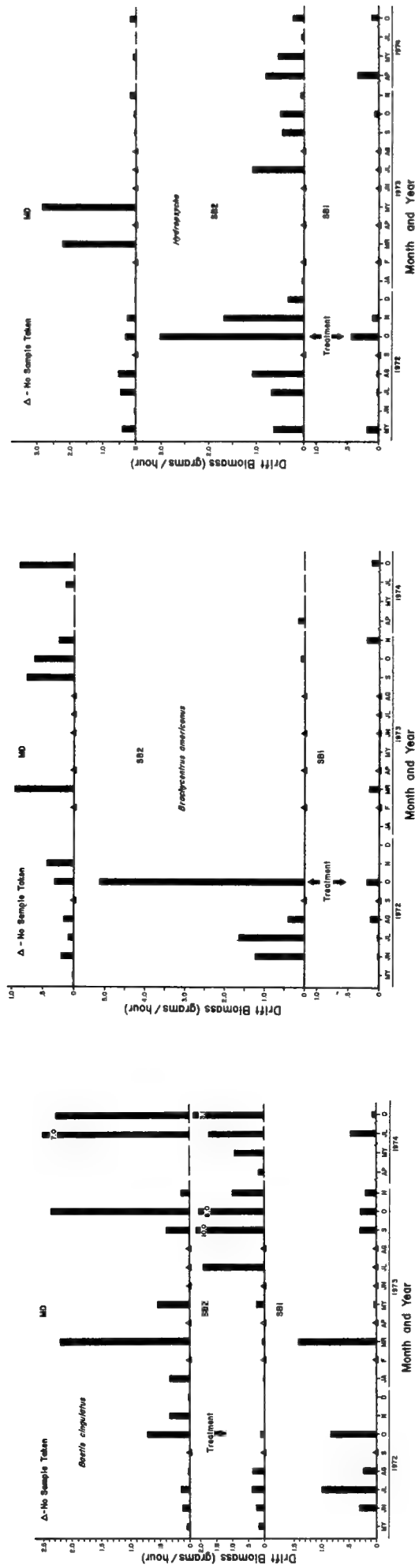
Fig. 7. Drift of benthic macroinvertebrates (a mayfly and two caddis flies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972–74.
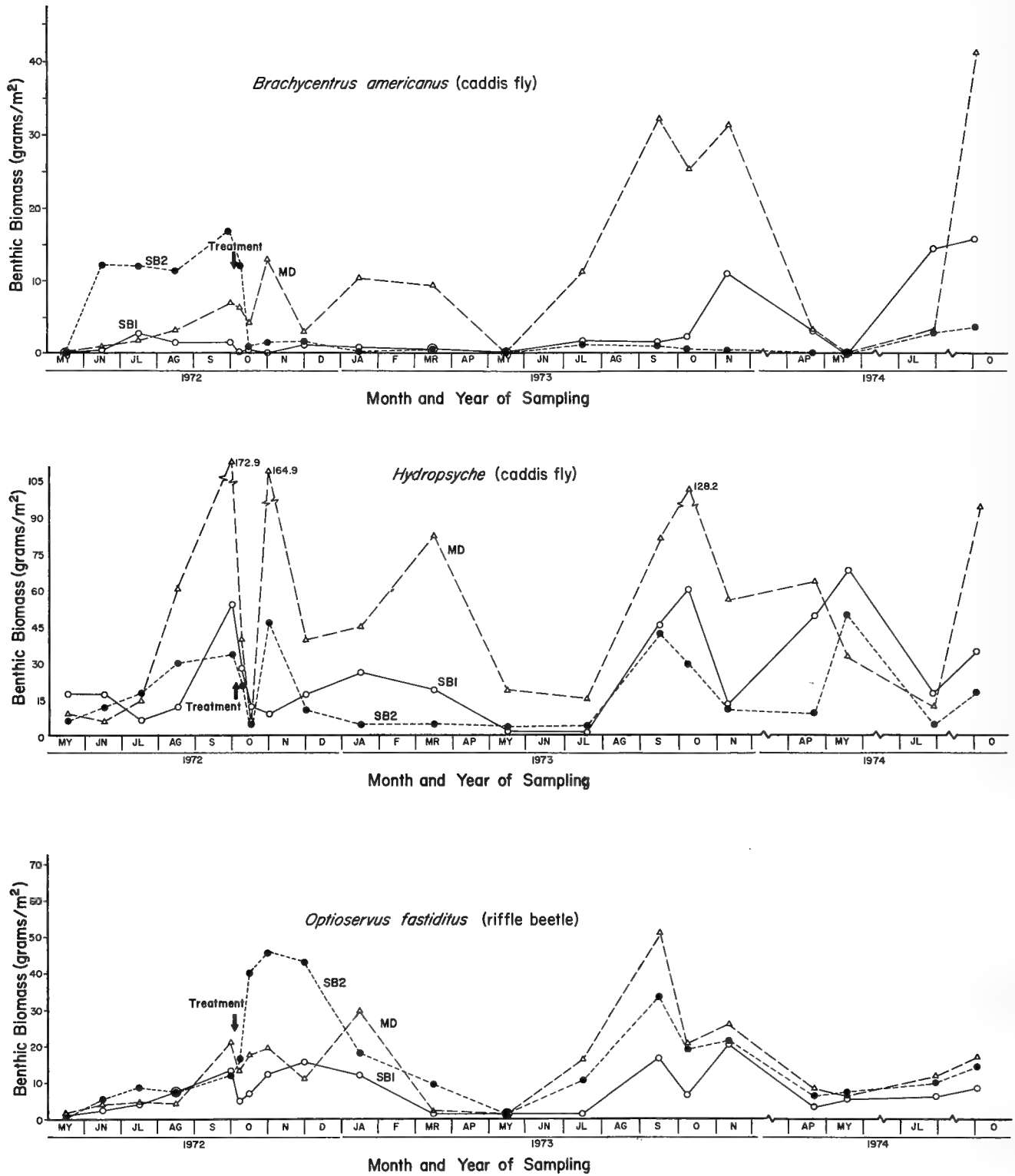
Fig. 8. Biomass of benthic macroinvertebrates (two caddis flies and a riffle beetle) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972-74.

Many benthic organisms are not specialized in food preference, and diets change according to the availability of algae (Chapman and Demory 1963). Additional food and space for Chironomidae, *O. fastiditus*, and *Prosimulium* could result from the reduction of other taxa of invertebrates, and of fish, and an increase in algae and in available plant surface area for attachment. The alga, *Vaucheria* sp., increased noticeably at SB2 1 week after treatment and reappeared in June 1973. *Ranunculus* present in July increased here also from a maximum of 15% stream-bed coverage before treatment to 50% in the year after treatment.

Recovery of invertebrates after treatment may have been hastened by the increase in stream vegetation. Particulate organic matter flushed downstream when the reservoir was draining may have been a source of nutrients. Nutrients also may have been made available by bacterial and fungal degradation of fish carcasses which littered the stream bottom after treatment. An increase in nutrients was observed by Richey et al. (1975) when kokanee salmon *(Oncorhynchus nerka)* died after spawning.

Chironomidae, *Gammarus pseudolimnaeus*, *Baetis cingulatus*, and *Prosimulium*, which had high turnover rates resulting from immature developmental periods of less than 1 year, returned more quickly than most other taxa to pretreatment biomass levels in the year following treatment. Populations of *Antocha* and *Brachycentrus americanus*, which have longer development times, had not recovered to pretreatment levels 1 year after treatment at the downstream station (SB2). Moffett (1936) observed a similar pattern in populations that were decimated by floods. Although both *Antocha* and *B. americanus* showed signs of recovery in November 1973, *Antocha* dropped back to low levels at SB1 during the second year.

Hildebrand (1971), who studied benthos disruptions by salmon spawning, believed that organisms with low drift rates in winter would not repopulate a stream until midsummer, when drift rates increased. Rapid recolonization in Seas Branch Creek could have taken place because treatment of areas adjacent to the mainstream was incomplete; e.g., mortality of *Hydropsyche* was high at SB2 but recovery was rapid after treatment. Repopulation could also have resulted from the insects' normal recolonization cycle which Mueller (1954) found to involve upstream flight of adults, ovoposition, population growth, and a later downstream drift of immatures in response to competition for food and space.

Because antimycin is short-lived, it would be desirable, although somewhat difficult, to treat a stream when adults of dominant or sensitive insects are mating. If ovoposition took place after treatment, survival and perhaps higher biomass levels might result, as observed in *Baetis cingulatus* at the downstream Seas Branch station.

Acknowledgments

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(Reports 60 through 62 are in one cover.)

60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests, by L. L. Marking and L. E. Olson. 1975. 27 pp.

61. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests, by L. L. Marking, T. D. Bills, and J. H. Chandler. 1975. 9 pp.

62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae, by J. H. Chandler and L. L. Marking. 1975. 7 pp.

(Reports 63 through 66 are in one cover.)

63. Laboratory Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM) as a Lampricide, by V. K. Dawson, K. B. Cumming, and P. A. Gilderhus. 1975. 7 pp.

64. Effects of 3-Trifluoromethyl-4-nitrophenol (TFM) on Developmental Stages of the Sea Lamprey, by G. W. Piavis and J. H. Howell. 1975. 4 pp.

65. Accumulation and Loss of Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in Fish Muscle Tissue; Laboratory Studies, by J. B. Sills and J. L. Allen. 1975. 5 pp.

66. Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in a Stream Ecosystem after Treatment for Control of Sea Lampreys, by P. A. Gilderhus, J. B. Sills, and J. L. Allen. 1975. 5 pp.

67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals, by L. L. Marking and V. K. Dawson. 1975. 7 pp.

68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations, by R. M. Burress. 1975. 8 pp.

(Reports 69 and 70 are in one cover.)

69. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2'5,-dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture to Fingerlings of Seven Fish Species and to Eggs and Fry of Coho Salmon, by T. D. Bills and L. L. Marking. 1976. 9 pp.

70. The Freshwater Mussel (*Anodonta* sp.) as an Indicator of Environmental Levels of 3-trifluoromethyl-4-nitrophenol (TFM), by A. W. Maki and H. E. Johnson. 1976. 5 pp.

71. Field Tests of Isobornyl Thiocyanoacetate (Thanite) for Live Collection of Fishes, by R. M. Burress, P. A. Gilderhus, and K. B. Cumming. 1976. 13 pp.

72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests, by L. L. Marking and T. D. Bills. 1976. 11 pp.

(Reports 73 through 76 are in one cover.)

73. Formalin: Its Toxicity to Nontarget Aquatic Organisms, Persistence, and Counteraction, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1976. 7 pp.

74. Chlorine: Its Toxicity to Fish and Detoxification of Antimycin, by L. L. Marking and T. D. Bills. 1977. 6 pp.

75. Malachite Green: Its Toxicity to Aquatic Organisms, Persistence, and Removal with Activated Carbon, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1977. 6 pp.

76. Toxicity of Furanace® to Fish, Aquatic Invertebrates, and Frog Eggs and Larvae, by L. L. Marking, T. D. Bills, and J. H. Chandler, Jr. 1977. 6 pp.

(Reports 77 through 79 are in one cover.)

77. Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM), 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture as Lampricides in Laboratory Studies, by V. K. Dawson, K. B. Cumming, and P. A. Gilderhus. 1977. 11 pp.

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79. Accumulation, Elimination, and Biotransformation of the Lampricide 2',5-Dichloro-4'-nitrosalicylanilide by *Chironomus tentans,* by J. A. Kawatski and A. E. Zittel. 1977. 8 pp.

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INVESTIGATIONS IN FISH CONTROL

82. *Investigations in Fish Control:*
   Index to Numbers 1-72, 1964-76

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Investigations in Fish Control, published by the Fish and Wildlife Service, include reports on the results of work at the Service's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy. [See Investigations in Fish Control 47-50 (in one cover) for list of issues published prior to 1970.]

(Reports 41 through 43 are in one cover.)

41. Identification of MS-222 Residues in Selected Fish Tissues by Thin Layer Chromatography, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1970. 7 pp.
42. Dynamics of MS-222 in the Blood and Brain of Freshwater Fishes During Anesthesia, by Joseph B. Hunn. 1970. 8 pp.
43. Effect of MS-222 on Electrolyte and Water Content in the Brain of Rainbow Trout, by Wayne A. Willford. 1970. 7 pp.
44. A Review of Literature on TFM (3-trifluormethyl-4-nitrophenol) as a Lamprey Larvicide, by Rosalie A. Schnick. 1972. 31 pp.

(Reports 45 and 46 are in one cover.)

45. Residues of MS-222 in Northern Pike, Muskellunge, and Walleye, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1972. 8 pp.
46. Methods of Estimating the Half-Life of Biological Activity of Toxic Chemicals in Water, by Leif L. Marking. 1972. 9 pp.

(Reports 47 through 50 are in one cover.)

47. Preparation and Properties of Quinaldine Sulfate, an Improved Fish Anesthetic, by John L. Allen and Joe B. Sills. 1973. 7 pp.
48. Toxicity of Quinaldine Sulfate to Fish, by Leif L. Marking and Verdel K. Dawson. 1973. 8 pp.
49. The Efficacy of a Quinaldine Sulfate as an Anesthetic for Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.
50. Residue of Quinaldine in Ten Species of Fish Following Anesthesia with Quinaldine Sulfate, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 9 pp.

(Reports 51 and 52 are in one cover.)

51. Methods for Simultaneous Determination and Identification of MS-222 and Metabolites in Fish Tissues, by Charles W. Luhning. 1973. 10 pp.
52. Residues of MS-222, Benzocaine, and Their Metabolites in Striped Bass Following Anesthesia, by Charles W. Luhning. 1973. 11 pp.

(Reports 53 through 55 are in one cover.)

53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish, by Verdel K. Dawson and Leif L. Marking. 1973. 11 pp.
54. The Efficacy of Quinaldine Sulfate:MS-222 Mixtures for the Anesthetization of Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.
55. Residues of Quinaldine and MS-222 in Fish Following Anesthesia with Mixtures of Quinaldine Sulfate:MS-222, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 12 pp.

(Reports 56 through 59 are in one cover.)

56. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to 10 Species of Algae, by A. A. Maki, L. D. Geissel, and H. E. Johnson. 1975. 17 pp.
57. Acute Toxicites of 3-Trifluoromethyl-4-nitrophenol (TFM) and 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73) to Larvae of the Midge *Chironomus tentans,* by J. A. Kawatski, M. M. Ledvina, and C. R. Hansen. 1975. 7 pp.
58. Acute Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nymphs of Mayflies (*Hexagenia* sp.), by C. R. Fremling. 1975. 8 pp.
59. Toxicity and Residue Dynamics of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) in Aquatic Invertebrates, by H. O. Sanders and D. F. Walsh. 1975. 9 pp.

INVESTIGATIONS IN FISH CONTROL

82. *Investigations in Fish Control:* Index to Numbers 1-72, 1964-76

By Rosalie A. Schnick
   Kimberlee A. Graves

Investigations in Fish Control:
Index to Numbers 1-72, 1964-76

by

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Abstract

This index comprises key words from *Investigations in Fish Control,* a publication of the U.S. Fish and Wildlife Service that reports results of research conducted at the Fish Control Laboratories, La Crosse, Wisconsin, and Warm Springs, Georgia, and by certain cooperating investigators. Each number constitutes a separate publication, although several may be issued under a single cover. An appendix lists the titles, authors, and publication dates of *Investigations in Fish Control* included in this index.

This index was constructed from key words and related terms from the text of issues 1-72 of *Investigations in Fish Control.* Items indexed include fish and invertebrate species, plants, chemical names, terminology, and author names. For clarity and convenience, "see also" references are included. Common names of species constitute the main entries; scientific names are referenced to common names. All common and scientific names of species in the list have been verified in the following references:

Aquatic plants: macrophytes, Prescott 1969; algae, Smith 1950, and Whitford and Schumacher 1973.

Invertebrates: insects, Borror and White 1970; others, Pennak 1953.

Fish: aquarium fishes, Axelrod and Schultz 1955; North American fishes, Bailey 1970; others, Grzimek 1973 and 1974, and Sterba 1963.

Amphibians and reptiles: Conant 1958; Smith 1961; frogs, Wright and Wright 1949.

Chemical names are referenced to the common or trade names of the chemicals. Chemical nomenclature conforms to the system suggested by the International Union of Pure and Applied Chemists (IUPAC). Entries are listed alphabetically.

Each indexed term is followed by the issue number(s) in which the term appears.

Index

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Appendix I

Investigations in Fish Control 1-72

1. Laboratories and methods for screening fish-control chemicals. R. E. Lennon and C. R. Walker. 1964. 15 pp. (Also issued as U.S. Fish and Wildlife Service [USFWS] Circular 185)

2. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. C. R. Walker, R. E. Lennon, and B. L. Berger. 1964. 18 pp. (Also issued as USFWS Circular 186)

3. Minimum lethal levels of toxaphene as a piscicide in North Dakota lakes. D. L. Henegar. 1966. 16 pp. (Also issued as USFWS Resource Publication 7)

4. Effects of toxaphene on plankton and aquatic invertebrates in North Dakota lakes. R. G. Needham. 1966. 16 pp. (Also issued as USFWS Resource Publication 8)

5. Growth rates of yellow perch in two North Dakota lakes after population reduction with toxaphene. D. C. Warnick. 1966. 9 pp. (Also issued as USFWS Resource Publication 9)

6. Mortality of some species of fish to toxaphene at three temperatures. M. A. Mahdi. 1966. 10 pp. (Also issued as USFWS Resource Publication 10)

7. Treatment of East Bay, Alger County, Michigan, with toxaphene for control of sea lampreys. W. E. Gaylord and B. R. Smith. 1966. 7 pp. (Also issued as USFWS Resource Publication 11)

8. Effects of toxaphene on fishes and bottom fauna of Big Kitoi Creek, Afognak Island, Alaska. W. R. Meehan and W. L. Sheridan. 1966. 9 pp. (Also issued as USFWS Resource Publication 12)

9. Relation of chemical structure to fish toxicity in nitrosalicylanilides and related compounds. C. R. Walker, R. J. Starkey, and L. L. Marking. 1966. 12 pp. (Also issued as USFWS Resource Publication 13)

10. Evaluation of p,p'-DDT as a reference toxicant in bioassays. L. L. Marking. 1966. 10 pp. (Also issued as USFWS Resource Publication 14)

11. Evaluation of an electronic method of measuring hematocrits of fish. R. A. Schoettger and A. M. Julin. 1966. 11 pp. (Also issued as USFWS Resource Publication 15)

12. Toxicity of MS-222 to selected fishes. L. L. Marking. 1967. 10 pp. (Also issued as USFWS Resource Publication 18)

13. Efficacy of MS-222 as an anesthetic on four salmonids. R. A. Schoettger and A. M. Julin. 1967. 15 pp. (Also issued as USFWS Resource Publication 19)

14. Method for determining MS-222 residues in fish. C. R. Walker and R. A. Schoettger. 1967. 10 pp. (Also issued as USFWS Resource Publication 20)

15. Residues of MS-222 in four salmonids following anesthesia. C. R. Walker and R. A. Schoettger. 1967. 11 pp. (Also issued as USFWS Resource Publication 21)

16. Annotated bibliography on MS-222. R. A. Schoettger. 1967. 15 pp. (Also issued as USFWS Resource Publication 22)

17. MS-222 as an anesthetic for channel catfish: its toxicity, efficacy, and muscle residues. R. A. Schoettger, C. R. Walker, L. L. Marking, and A. M. Julin. 1967. 14 pp. (Also issued as USFWS Resource Publication 33)

18. Toxicity of 22 therapeutic compounds to six fishes. W. A. Willford. 1967. 10 pp. (Also issued as USFWS Resource Publication 35)

19. Toxicity of Bayer 73 to fish. L. L. Marking and J. W. Hogan. 1967. 13 pp. (Also issued as USFWS Resource Publication 36)

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INVESTIGATIONS IN FISH CONTROL

UNITED STATES DEPARTMENT OF THE INTERIOR

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Survival of Two Species of Freshwater Clams,
Corbicula leana and *Magnonaias boykiniana*,
After Exposure to Antimycin

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Abstract

The Asiatic clam, *Corbicula leana* Prime, and a clam native to the southern United States, *Magnonaias boykiniana*, were exposed to the fish toxicant antimycin at several concentrations for various periods and then placed in an untreated earthen pond for posttreatment observation. Both species survived the concentrations and exposure periods usually used in field application. However, latent mortalities were observed in the pond 3 months after a 30–day flow-through exposure of *Corbicula* to 3.6 to 30 μg/l of antimycin. A single treatment (2 μg/l) in an earthen pond did not result in significant mortalities of *Corbicula* during 22 weeks. *Magnonaias* was more sensitive than *Corbicula* to antimycin, but both survived the maximum permissible use-pattern concentrations in flow-through tests.

Asiatic clams (several species of the genus *Corbicula*) introduced into U.S. waters have spread to many important river systems (Sinclair and Isom 1963; Keup et al. 1963; Diaz 1974). Asiatic clams are becoming so numerous that they cause problems in water intakes to industrial plants, in the pipes and plumbing of municipal water supplies, in sand and gravel operations, and as competitors with native species of clams for habitat and food.

The widespread distribution of Asiatic clams suggests hardiness and an ability to tolerate adverse environmental conditions. Sinclair and Isom (1963) indicated that these clams can survive fluctuating environmental conditions, and Chandler and Marking (1975) reported that they are more resistant than native clams to the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM). Burress et al. (1976) found Asiatic clams useful in laboratory toxicity tests because they are hardy (*Corbicula leana* survived for 18 months in outdoor plastic pools with no water exchange) and can be easily collected, transported, handled, and exposed to chemicals.

Magnonaias boykiniana, the other clam used in the present studies, is native to many American river systems, including those in Georgia.

Pond studies with the fish toxicant, antimycin, showed that applications of 5 μg/l severely reduced plankton populations, whereas benthic invertebrates were not severely reduced (Callaham 1968; Callaham and Huish 1968). Field applications of antimycin in Wisconsin were reported to have resulted in delayed mortality of several species of mollusks in the East Branch, Rock River (Bratley and Mathiak 1972) and in the Ashippum River (Flowers et al. 1975). A comprehensive summary of data on the effects of antimycin on nontarget organisms prepared by Schnick (1974) indicated that the 96-h LC_{50} for antimycin against Asiatic clams was 50 μg/l.

Antimycin is generally applied at concentrations ranging from 1 to 10 µg/l in single applications for the control of undesired fish populations in ponds and lakes, or for up to 12 h in streams. The present study was designed to determine the acute toxicity and latent mortality resulting from short- and long-term exposures of the Asiatic clam, *Corbicula leana*, and a native clam, *Magnonaias boykiniana*, to antimycin. Concentrations and exposures investigated equaled or exceeded those currently used for fish control.

Materials and Methods

The clams used in this study were collected from shoals of the Flint River east of Woodbury (Upson County), Georgia. They were transported to the laboratory and kept in limed flowing water until exposed to the toxicant. Deformed organisms or any that appeared weakened by handling were discarded. Mature specimens of uniform size were selected for the tests.

Clams were exposed to antimycin (Fintrol Concentrate formulation) in limed spring water (total hardness 18–22 mg/l as $CaCO_3$ and pH 6.8) in four types of tests.

(1) Standardized static laboratory tests were conducted in three series of 19–liter glass jars containing 15 liters of water. The substrate was mud in one series and sand in a second; one series of jars contained no substrate. Ten Asiatic clams were exposed to 5, 10, or 20 µg/l of antimycin for 1 or 30 days. Antimycin was prepared in concentrated stock solutions and portions were added to the test water to obtain desired concentrations. Tests were conducted according to recommendations of the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Surviving clams were placed in cages in untreated, previously filled earthen ponds for observation over a 112–day period.

(2) An earthen 0.04–ha pond was treated with 2 µg/l of antimycin by dispensing a solution of the toxicant into the propeller wash of an outboard motor. Mortality was recorded among the 300 stocked Asiatic clams and 85 native clams (weight range, 200–500 g) over a 22–week posttreatment observation period. The pond water had the following characteristics: total hardness as $CaCO_3$, 2.0–14.0 mg/l; pH 5.6–7.4; temperature 6.0–19.0 C; and dissolved oxygen 8.7–15.0 mg/l.

(3) Standardized flow-through toxicity tests were conducted in an apparatus similar to that described by Mount and Brungs (1967). Asiatic clams were exposed to 3.6 to 30 µg/l of antimycin in 50–liter glass aquaria for 30 days. Thirty clams were exposed to each concentration. The stock solution of antimycin was metered into a mixing box, and dilutions of the mixture yielded

selected concentrations. Mortalities were recorded daily during exposure.

(4) Flow-through tests were conducted in fiberglass tanks in which 300 Asiatic and 75 or 85 native clams were exposed to 0.2 and 5 µg/l of antimycin for 12 h or to 50 µg/l for 24 h. After exposure, the clams were observed in untreated pond water for 22 weeks. Antimycin solutions were mixed in epoxy-coated steel tanks containing 3,623 liters of limed spring water and pumped through the tanks at a rate of 4.4 liters/min.

Small amounts of boiled trout chow and cereal leaves (*Daphnia* food) were offered weekly to the Asiatic clams in long-term static and flow-through exposures, and excess food was routinely siphoned off.

Survivors of each test were acclimated to holding pond temperatures and placed in cages submersed in the ponds. Each cage contained a 2-cm layer of soil substrate obtained from the holding pond. Cages were placed so that water continuously covered the substrate in the cage to a depth of 41 to 46 cm.

The clams were moved to the holding ponds and examined semimonthly after exposure. Clams that were unable to retract the foot or adduct the valves were considered dead. The period between mortality determinations was usually long enough so that only a casual observation was required because the soft tissues of dead specimens protruded from the valves. All mortality tabulations are cumulative.

Results

Corbicula survived 1–day exposures to 5, 10, and 20 µg/l of antimycin in 19–liter glass jars containing no substrate or substrates of sand or mud (Table 1). During the 29–day posttreatment holding period in jars containing untreated water, no clams died that had been exposed with no substrate, or with mud substrate; however, 70% of the clams died that had been exposed to 20 µg/l of antimycin in jars with sand substrate. During the later 112–day period in the holding pond, a few more died that had been exposed in jars with a sand substrate.

Clams exposed for 30 days to 5, 10, or 20 µg/l of antimycin were affected especially during the posttreatment observation period. By the end of the 112–day holding period in earthen ponds, all clams had died, except for 4 of 10 exposed to 5 µg/l with a sand substrate; thus exposure time was a critical factor.

Antimycin was generally not toxic in 1–day exposures, but latent mortality developed in the 30–day exposures. The 1–day exposures typify field use patterns, and 30–day exposures greatly exceed those used in fishery management.

Table 1. *Cumulative percentage mortality of* Corbicula leana *after static exposures of 1 day or 30 days to se-lected concentrations of antimycin. Each jar contained 10 clams.*

Substrate and concentration of antimycin (μg/l)	Exposure period (days)	Days in jars[a]	Days in untreated holding pond after exposure		
		30	28	70	112
No substrate					
0	1	0	0	0	0
0	30	0	0	10	10
5	1	0	0	0	0
5	30	10	30	80	100
10	1	0	0	0	0
10	30	50	80	100	100
20	1	0	0	0	0
20	30	50	60	100	100
Sand					
0	1	0	0	0	10
0	30	0	0	0	0
5	1	0	0	10	10
5	30	0	20	50	60
10	1	10	10	20	20
10	30	10	20	90	100
20	1	70	70	70	70
20	30	20	20	90	100
Mud					
0	1	0	0	0	0
0	30	10	10	10	10
5	1	0	0	0	0
5	30	20	20	100	100
10	1	0	0	0	0
10	30	50	50	70	100
20	1	0	0	0	0
20	30	40	40	100	100

[a]Clams exposed for 1 day were placed in 15 liters of untreated water in 19-liter jars for 29 days after exposure, and then placed in a holding pond with those exposed in jars for 30 days.

In the static pond application, in which *Corbicula* and *Magnonaias* were exposed to 2 μg/l of antimycin and the toxicant was allowed to dissipate and detoxify with time, 98% of the *Corbicula* and nearly 65% of the *Magnonaias* survived for 22 weeks after the toxicant application (Table 2).

Table 2. *Cumulative percentage mortality after 2 to 22 weeks among 300* Corbicula leana *and 85* Magnonaias boykiniana, *in a pond treated with 2 μg/l of antimycin.*

Species	Weeks			
	0	8	16	22
Corbicula	0	1.7	1.7	2.0
Magnonaias	0	22.4	32.9	35.3

Corbicula survived 30 days of exposure to antimycin concentrations of 3.6 to 30 μg/l in a standardized flow-through system (Table 3). Exceptions were a single mortality at 3.6 μg/l and two at 15.1 μg/l. These mortalities probably resulted from stresses other than the antimycin, since all clams survived at higher concentrations. Few clams died during the first 86 days after they were transferred to the holding pond, but latent mortality became significant thereafter. After 156 days, mortality ranged from 27 to 77% (Table 3). The die-off seemed to stabilize toward the end of the observation period; the increase in mortality from 128 to 156 days was minor. Again, the 30 days of continuous exposure that led to latent mortality far exceeded exposure time in field applications.

Survival of *Corbicula* exposed to 2 and 5 μg/l of antimycin for 12 h was high during exposure and during

Table 3. *Mortality among 30* Corbicula leana *during exposure for 30 days to various concentrations of antimycin in a flow-through system, and cumulative percentage mortality in a holding pond 44 to 156 days posttreatment.*

Concentration (µg/l)	Percentage mortality during exposure	Days after end of exposure period			
		44	86	128	156
0.0	0.0	0.0	0.0	0	0
3.6	3.3	3.3	10.0	47	53
4.4	0.0	0.0	3.3	27	27
5.9	0.0	0.0	0.0	23	30
8.3	0.0	0.0	3.3	50	50
12.4	0.0	0.0	3.3	50	57
15.1	6.6	6.6	16.0	63	67
20.7	0.0	0.0	3.3	63	73
24.1	0.0	0.0	3.3	67	70
30.0	0.0	3.3	6.6	77	77

the later 22-week observation period in an earthen pond; mortality ranged from only 0.7 to 2.7% for exposed clams and was 2.3% for unexposed clams (Table 4). The mortality of *Corbicula* exposed to 50 µg/l of antimycin for 24 h was slightly higher — 4% at 2 weeks and 8.7% after 22 weeks.

Magnonaias was more sensitive than *Corbicula* to antimycin in the 12-h exposures to 2 and 5 µg/l and the 24-h exposure to 50 µg/l in the flow-through tank system. Mortality was low among *Magnonaias* exposed for 12 h during the first 2 weeks of observation, but increased to 20 to 53% after 22 weeks (Table 4). During the 22-week period, however, 20% of the unexposed clams also died. The concentrations of 5 µg/l of antimycin for 12 h and 50 µg/l for 24 h killed more than half of the clams during the 22-week observation period. Despite these losses it seems clear that the population would not have been eliminated, even by 24-h exposures to 50 µg/l.

Discussion

Exposure times longer than 12 h and concentrations of antimycin higher than 10 µg/l are rare in stream treatments with antimycin. The registered label prescribes concentrations of 1 to 10 µg/l, depending on physical and chemical conditions of the water. Our exposures of 30 days were excessive. In streams the toxicant is added to flowing water, where concentrations build up to a peak of short duration and then decrease as a result of dilution and decomposition. Stream organisms are thus exposed to a slug of the toxicant passing downstream for an exposure time that varies with the species of target fish, and with environmental conditions (Gilderhus 1972).

Table 4. *Cumulative percentage mortality among 300* Corbicula leana *and 75 or 85* Magnonaias boykiniana *exposed to antimycin for 12 or 24 h in flow-through tanks and moved to an earthen pond for observation for 22 weeks.*

Species and concentration (µg/l)	Exposure (h)	Weeks			
		2	8	16	22
Corbicula					
0	12	0.3	0.3	1.7	2.3
2	12	0.0	0.7	0.7	0.7
2	12	—	2.7	2.7	2.7
5	12	0.6	1.7	1.7	2.0
50	24	4.0	6.0	8.7	8.7
Magnonaias					
0	12	1.3	13.3	18.7	20.0
2	12	0.0	9.3	17.3	20.0
2	12	—	12.9	23.5	34.1
5	12	3.5	35.3	50.6	52.9
50	24	2.4	30.6	48.2	50.6

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Chronic and Simulated Use-Pattern Exposures
of Brook Trout (*Salvelinus fontinalis*) to
3-Trifluoromethyl-4-nitrophenol (TFM)

by

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Abstract

Effects of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) on brook trout (*Salvelinus fontinalis*) were compared under conditions of continuous (chronic) exposure, and under conditions simulating those used in the application of TFM in tributary streams of the Great Lakes for control of the sea lamprey (*Petromyzon marinus*). Chronic exposure of adult brock trout to concentrations of 4.0 mg/l or higher caused deleterious effects on growth, spawning, survival during spawning, and eye condition. Hatchability and viability of the eggs were reduced. Growth and survival were reduced in the fry at TFM concentrations of 1.6 mg/l or higher. No deleterious effects were noted at lower concentrations. The only effect observed in fish after simulated use-pattern exposure to TFM was a decrease in survival of adults tested at 15 C.

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) was registered in 1964 for control of larval sea lampreys (*Petromyzon marinus*) in selected tributaries of the Great Lakes. The U.S. Environmental Protection Agency (EPA) is now reviewing the registration of TFM. A substantial amount of research has been conducted on TFM toxicity and selectivity to sea lamprey larvae, as indicated by the literature reviewed by Schnick (1972). Later research on toxicity included work with algae (Maki et al. 1975), midge larvae (Kawatski et al. 1975), mayfly nymphs (Fremling 1975), aquatic invertebrates and frog larvae (Chandler and Marking 1975), and early life stages of rainbow trout (Olson and Marking 1973); other studies were on toxicity and residue dynamics in invertebrates (Sanders and Walsh 1975), and included tests on non-target fish — both static (Marking and Olson 1975) and flow-through (Marking et al. 1975).

The accumulation and elimination of TFM residues by fish exposed to TFM after a lamprey control treatment of the East Au Gres River, Michigan, were reported by Gilderhus et al. (1975). Biotransformation and elimination of TFM by fish were elucidated by Lech and Costrini (1972). Hunn and Allen (1974, 1975) reported on the factors affecting the uptake and elimination of the lampricide, and the renal excretion of the compound in fish.

The objectives of the present research were to determine the effects of TFM on brook trout (*Salvelinus fontinalis*) under continuous (chronic) exposure and under exposures simulating a use pattern to which fish would be exposed during a stream treatment. Brook trout are an important and indigenous sport fish in many of the streams treated with TFM. Factors measured were mortality, growth, and reproduction. Samples of fish were analyzed to determine accumulation and elimination of TFM after continuous and simulated use-pattern exposures.

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Materials and Methods

Chronic Study

Yearling brook trout obtained from the Manchester (Iowa) National Fish Hatchery were held in raceways at 16 C and fed a maintenance diet (Brauhn and Schoettger 1975) until the tests were started. On 14 June 1973, 12 fish were placed in each of 12 stainless steel tanks, measuring 137 cm long, 36 cm wide, and 51 cm high, in which water depth was 30 cm. Well water was delivered to the tanks at a rate of 800 ml/min per tank through a proportional diluter system modeled after Mount and Brungs (1967). Each tank was aerated with filtered air to maintain oxygen concentrations above 70% saturation. Adult fish were fed a modification (Mehrle et al. 1977) of the Oregon Test Diet (National Academy of Sciences 1973) ad libitum throughout the study. Fry were fed the commercial trout starter "Ewos."

A diluter system with the modification of McAllister et al. (1972) and a dilution factor of 0.5 between concentrations was used to continuously deliver five concentrations of TFM and a control for the chronic test. The TFM used was a 35.7% field grade formulation manufactured by the American Hoechst Chemical Company; distilled water was the carrier solvent. The measured TFM concentrations (total formulation) averaged 0.94, 1.6, 4.0, 8.8, and 16 mg/l (or 0.34, 0.57, 1.4, 3.1, and 5.7 mg/l active ingredient). Flow-splitting chambers designed by Benoit and Puglisi (1973) were used to thoroughly mix and divide each lampricide concentration for delivery to duplicate exposure tanks. The water temperature in the tanks was controlled by refrigeration units suspended in a circulating water bath. Artificial daylight was provided by the method of Drummond and Dawson (1970), and the water temperature regime and photoperiod were those recommended for brook trout tests (EPA 1972).

The study was conducted according to the recommended procedures for partial chronic tests with brook trout by EPA (1972). The fish were measured (total length) after 60 and 120 days of exposure. After the final growth determinations, the fish were thinned to five to eight per tank and two spawning substrates were placed in each tank. The excess fish above three females and two males were used for artificial reproduction (stripping of eggs and sperm). Spawning began 28 November 1974 and ended 16 January 1975. Two samples of 100 eggs from each spawning, both natural and artificial, were placed in incubator cups to determine hatchability and viability. Viability was determined 10 days after spawning by placing the eggs in 10% acetic acid for several minutes until the neural keel became visible, indicating that the embryo was de-veloping. Upon hatching, 25 fry were randomly selected from each incubation cup, their length was determined by the photographic method of McKim and Benoit (1971), and they were transferred to growth chambers 14 cm deep, 38 cm long, and 15 cm wide, in which water was 10 cm deep. The fry were also measured photographically at 30 and 60 days, and measured directly at 90 days. Mortality of fry was recorded daily.

Use-Pattern Study

The use-pattern study was initiated a year after the chronic study, with 2-year-old fish from the same lot used in the chronic study. The procedures were essentially the same, except that the exposure of fish to TFM was designed to simulate stream treatment for lamprey control. In such treatments, TFM is metered into streams at concentrations of 2 to 20 mg/l (total formulation) for 10 to 12 h, depending on water quality and on-site bioassay. A stream is normally treated once during the summer or fall. In the simulated use-pattern study, eight fish were placed into each of six tanks. TFM was metered into two tanks for 12 h at 18 mg/l on 30 July 1974, and into two other tanks at 16 mg/l on 1 October 1974; the remaining two tanks served as controls. The diluter system used to expose the fish was that of Brungs and Mount (1967). Since egg viability and hatchability did not differ between eggs naturally and artificially spawned in the chronic study, spawning was expedited in the use-pattern study by hand stripping sexually mature brook trout. Egg viability and hatchability, and fry growth were measured as in the chronic study.

Residue Analysis

Residues of TFM were determined on both fillet and offal of four adult fish from each concentration after 137 days of continuous exposure. Six additional fish from each of the 1.6- and 4.0–mg/l exposures were transferred to uncontaminated water at 9 C to determine the elimination rate of TFM; two fish were sampled from each exposure after 3, 7, and 14 days in fresh water. A sample of eggs from each of three spawns within each TFM concentration was also analyzed. Three fish from the controls and the last exposure of the use-pattern study were sampled 45 days after that exposure. Fish and eggs were prepared for residue analysis by the method of Benville and Tindle (1970) and extracted by the column chromatography technique of Hesselberg and Johnson (1972). The extracts were analyzed by gas chromatography (Allen and Sills 1974), in which the minimum detection limit was 1 ng/g. (However, TFM residues below 10 ng/g

were not quantifiable.) Concentrations of TFM in water were periodically determined by the colorimetric method of Olson and Marking (1973).

Experimental Design and Analysis

The design of both tests was a randomized block. Growth data (length gained) were analyzed by analysis of variance (Snedecor 1965). A multiple means comparison test (least significant difference) was used to compare treatments. The effects of TFM on fish mortality and egg hatchability and viability were determined by conducting an analysis of variance on the arcsin transformation for proportions (angle = arcsin $\sqrt{\text{percentage}}$), followed by least significant difference tests.

Results

No differences in adult growth were detected after 60 days of chronic exposure to TFM. However, growth was significantly reduced ($P < 0.05$) after 120 days in fish exposed to 8.8 and 16 mg/l TFM (Table 1). Growth of adult fish under use-pattern conditions was not affected. Chronic exposure to high concentrations of TFM may have caused blindness in some adult fish. The eye was covered with an opaque layer and, in some fish, was filled with blood. After 120 days exposure to 16 mg/l TFM, 10% of the fish were affected in at least one eye; by 180 days, 42% of the fish were affected. Although none of the other fish exposed to lower concentrations showed similar effects at 120 days, a few fish exposed to 8.8 mg/l TFM developed the eye condition at 180 days.

As total spawning activity increased with time, so did the mortality of the adults exposed to 16 mg/l TFM and (to a lesser extent) of those exposed to 8.8 mg/l (Table 2). Apparently the fish died from spawning stress in addition to the TFM exposure. At death, the fish were covered with copious amounts of mucus. Under use-pattern conditions, 19% of the adult fish died during the early exposure, when the water temperature was 15 C. No mortalities occurred at 9 C, just before the fish spawned, in the late exposure.

In the chronic-exposure study, no spawns were obtained from the fish exposed to 16 mg/l TFM, and only one natural spawn occurred at 8.8 mg/l (Table 3). However, these eggs were not viable. Inasmuch as egg viability and hatchability of natural and artificial spawns were not statistically different, the viability and hatchability data were pooled (Table 4). The viability of eggs in the 4.0 mg/l TFM concentration was statistically less ($P < 0.05$) than that of eggs in the lower concentrations and controls. Hatchability of eggs at this concentration was also lower, although

Table 1. *Mean total lengths (mm; standard deviations in parentheses) of adult brook trout before, and 120 days after chronic exposure of yearlings and 100 days after use-pattern exposure of 2-year-olds, to different concentrations of TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Length (mm) of fish:	
	On day 0	On day 120 (chronic) or 100 (use-pattern)
Chronic		
0.0	213 (14)	267 (16)
0.94	215 (12)	265 (17)
1.6	218 (15)	268 (17)
4.0	213 (22)	265 (10)
8.8	220 (13)	264 (15)[b]
16.0	220 (11)	253 (18)[b]
Use-pattern		
0.0	300 (27)	316 (28)
18.0	306 (28)	324 (30)
16.0	312 (36)	320 (30)

[a] Total formulation.

[b] Length gained significantly different ($P < 0.05$) from that of controls.

Table 2. *Mortality of brook trout after chronic and use-pattern exposures to TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Mortality (%)	
	Adults	Fry
Chronic		
0.0	0	27
0.94	0	58
1.6	0	84[b]
4.0	0	100[b]
8.8	34[b]	100[b]
16.0	68[b]	100[b]
Use-pattern		
0.0	0	14
18.0	19[b]	6
16.0	0	9

[a] Total formulation.

[b] Significantly different from controls ($P < 0.05$).

not significantly so. In addition, the eggs exposed to 4.0 mg/l TFM were not consistent in size, shape, and neural keel development, when compared with those exposed to lower concentrations, and the control. The TFM had no effect on viability or hatchability of eggs from adults treated in the use-pattern exposures.

Table 3. *Production of spawn by brook trout after chronic and use-pattern exposures to TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Natural spawns		Artificial spawns (number)
	Number	Eggs per spawn (number)	
Chronic			
0.0	7	309	2
0.94	6	294	4
1.6	8	215	3
4.0	8	236	1
8.8	1	143	0
16.0	0	—	0
Use-pattern[b]			
0.0	—	—	5
18.0	—	—	4
16.0	—	—	5

[a] Total formulation.

[b] Eggs per spawn were not considered because the fish were stripped to obtain eggs and sperm.

Table 4. *Viability and hatchability of brook trout eggs after chronic and use-pattern exposures of TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Viability (%)	Hatch (%)
Chronic		
0.0	82	67
0.94	87	67
1.6	88	74
4.0	50[b]	43
8.8	0[b]	
16.0	—[c]	
Use-pattern		
0.0	92	82
18.0	97	88
16.0	97	85

[a] Total formulation.

[b] Significantly different from controls ($P < 0.05$).

[c] No spawns produced.

For the first 60 days of chronic exposure, no statistical difference existed in the growth of fry continuously exposed to TFM; however, at 90 days growth was significantly reduced ($P < 0.05$) in fry exposed to 1.6 mg/l TFM (Table 5). The length gained in fry exposed for 90 days to 0, 0.94, and 1.6 mg/l TFM was 35.2, 32.7, and 25.8 mm, respectively. All fry exposed to 4.0 mg/l TFM were dead within 30 days (Table 2). No significant differences in mortality of fry exposed to the two lower concentrations and controls were observed at 30 days of exposure. At 60 and 90 days, how-ever, the mortality of fry exposed to 1.6 mg/l TFM was significantly higher ($P < 0.05$) than that of the controls. Growth and survival of fry were not affected in the use-pattern study.

Table 5. *Mean total lengths (mm; standard deviations in parentheses) of brook trout fry 90 days after chronic exposure to different concentrations of TFM or fry from parents exposed to use-pattern concentrations of TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Length (mm) of fish:	
	On day 0	On day 90
Chronic		
0.0	14.8 (0.6)	50 (2)
0.94	14.3 (1.0)	47 (4)
1.6	15.2 (0.9)	41 (6)[b]
4.0	—[c]	—
8.8	—[d]	—
16.0	—[e]	—
Use-pattern		
0.0	16.2 (0.9)	49 (5)
18.0	16.5 (1.1)	51 (4)
16.0	16.7 (0.7)	49 (3)

[a] Total formulation.

[b] Significantly different from controls ($P < 0.05$) based on length gained.

[c] Fry died within 30 days.

[d] No eggs hatched.

[e] No spawns produced.

Table 6. *Mean concentrations of TFM (μg/g; standard error in parentheses) in fillets and offal of adult brook trout and their eggs, after continuous exposure to different concentrations of TFM for 137 days.*

Concentration of TFM (mg/l)[a]	Fillets (n = 4)	Offal (n = 4)	Eggs (n = 3)
0.0	<0.01	<0.01	<0.01
0.94	0.01 (0.003)	0.06 (0.02)	0.18 (0.02)
1.6	0.06 (0.01)	0.43 (0.10)	0.32 (0.03)
4.0	0.09 (0.01)	0.19 (0.03)	0.74 (0.17)
8.8	0.08 (0.04)	0.18 (0.05)	1.9[b]
16.0	0.11 (0.03)	0.38 (0.11)	2.8 (0.49)[c]

[a] Total formulation.

[b] n = 1.

[c] Eggs for samples were removed from females since there were no natural spawns in this exposure.

Residues of TFM in the eggs of brook trout in the chronic-exposure study averaged 0.18 μg/g in fish exposed to 0.94 mg/l TFM and 2.8 μg/g in fish exposed to 16 mg/l (Table 6). The fillet and remaining offal from adult brook trout were analyzed separately. TFM in the fillets ranged from 0.01 μg/g for fish exposed to 0.94 mg/l TFM to 0.11 μg/g for fish exposed to 16 mg/l, indicating an accumulation factor of 0.02 to 0.1, based on the active ingredient. Residues of TFM in the offal from adult brook trout varied considerably, but the indicated accumulation factor was 0.8 or less for each of the exposure concentrations. No residues were found under use-pattern conditions. Results from the residue elimination phase of the experiment indicate that the loss of TFM was rapid (Table 7).

Discussion

Chronic exposure of adult brook trout to TFM concentrations of 4.0 mg/l or less caused no significant differences in growth, but concentrations of 8.8 and 16 mg/l resulted in reduced growth and number of spawns, and adversely affected the eyes. Viability and hatchability of eggs, consistency of egg size and shape, and embryo development were affected by exposure to 4.0 mg/l of the lampricide. Some mortality occurred in the 8.8 and 16 mg/l concentrations, apparently in part as a result of the added stress of spawning. This same response was observed in adult brook trout exposed to toxaphene (Mayer et al. 1975). Effects on growth and mortality were noted in fry exposed to 1.6 mg/l or more of TFM but no significant effect was noted between fry exposed to 0.94 mg/l and controls. No significant differences were found between controls and fish exposed to use-pattern treatments, except for the increased mortality of adults.

The use pattern of TFM as a lamprey larvicide precludes long-term and continuous exposure of fish to concentrations that might cause the effects shown in this study. Gilderhus et al. (1975) showed that the concentration of TFM in water dropped rapidly after termination of the treatment of the East Au Gres River, Michigan. Coburn and Chau (1976) reported 40 μg/l of TFM in a low-flow area of a creek 3 weeks after the treatment for lamprey control. Our studies show no effect on brook trout continuously exposed to about 9.5 times this amount (0.94 mg/l or 0.34 mg/l active ingredient).

The residues in fillets and offal of adults after chronic exposure to TFM showed a low accumulation factor and TFM was rapidly eliminated when the fish were transferred to uncontaminated water. The residue elimination followed a pattern similar to that re-

Table 7. *Elimination of whole-body TFM residues (μg/g) in adult brook trout transferred to uncontaminated water after 137 days of continuous exposure to 1.6 or 4.0 mg/l TFM.*

| Concentration of TFM (mg/l)[a] | Days in uncontaminated water | | |
	3	7	14
1.6	0.06	<0.01	<0.01
4.0	0.04	0.02	<0.01

[a] Total formulation.

ported by Sills and Allen (1975). No residues were found in adult fish at the time of spawning in the use-pattern exposure.

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Hydrolysis and Photolysis of the Lampricide 2', 5-Dichloro-4'-nitrosalicylanilide (Bayer 73)

by

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Abstract

The hydrolysis and photolysis of the lampricide Bayer 73 (aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide) was studied by using ^{14}C-Bayer 2353 (the non-salt form of Bayer 73). No hydrolysis of ^{14}C-Bayer 2353 occurred in pond water or in distilled water buffered at pH 5.0, 6.9, or 8.7 after 56 days. During exposure to long-wave UV light, ^{14}C-Bayer 2353 degraded rapidly on silica gel thin layer chromatographic plates and on glass slides. After exposures of 24 and 168 h, less than 50 and 5%, respectively, of the remaining radioactivity was parent compound. After exposure of an aqueous solution of ^{14}C-Bayer 2353 to long-wave UV light for 14 days, only 5% of the remaining radioactivity was parent compound.

Bayer 73, the aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 2353), is not only toxic to larvae of the sea lamprey (*Petromyzon marinus*) but also synergizes the activity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) and increases its effectiveness (Howell et al. 1964).

The requirement that all piscicides used in the United States must be registered with the Environmental Protection Agency includes the development of data concerning the fate of the chemical in the environment and potential rates of hydrolysis and photolysis. In this study, we investigated the hydrolysis of ^{14}C-Bayer 2353 in water buffered at various pH's, and studied the photolysis of ^{14}C-Bayer 2353 on thin layer chromatographic plates, on glass slides, and in aqueous solutions.

General Materials and Methods

In all tests, materials that had been exposed to UV light were spotted on thin layer chromatographic (TLC) plates (silica gel, F-254, 250 μm; Brinkman Instruments, Westbury, N.Y.). The plates were developed in a solution of chloroform:methanol:ammonium hydroxide (50:20:2.5, v/v/v), dried, and placed on X-ray films. After 3 to 4 weeks of exposure, the X-ray films were developed, the R_f (distance compound moved on TLC plate/distance solvent moved on TLC plate) of each spot was determined, and corresponding radioactive spots on the TLC plates were scraped into scintillation vials for radioactive determination. Nonradioactive scrapings of TLC plates were used to determine quench and background. The scintillation cocktail consisted of Beckman TLA dissolved in toluene.

Hydrolysis of Bayer 73

To investigate the hydrolysis of Bayer 73, we used three 250-ml beakers containing 100 ml of distilled water buffered (buffer tablets, No. 13-640-304-E,K,H, Fisher Scientific, Atlanta, Ga.) to a pH of 5.0, 6.9, or 8.7, and a fourth beaker containing 100 ml of pond water (initial pH, 7.8). The pH of the buffered solutions did not change during the experiment; that of the pond water decreased from pH 7.8 to 7.0. Into each beaker we stirred 1 ml of acetone containing 5 μg of ^{14}C-Bayer 2353 (chlorosalicyclic acid ring UL-^{14}C, specific activity, 10 mCi/mmol, American Radiochemical Corporation, Sanford, Fla.). The acetone was allowed to evaporate, and the beakers were then covered with aluminum foil and kept in the dark at 20 $\pm$ 1 C. Samples (100 μl) were taken at 0, 1, 4, 7, 14, 26, and 56 days, and spotted on thin layer chromatographic plates for separation and quantification of possible degradation products.

No degradation of ^{14}C-Bayer 2353 occurred within the 56 days in either the buffered aqueous solutions or in the pond water (Table 1).

Table 1. *Amounts of ¹⁴C-Bayer 2353 and ¹⁴C-degradation product from TLC plates spotted with aqueous solutions of various pH values, and pond water containing ¹⁴C-Bayer 2353 and aged for 0 to 56 days (values expressed as percentages of total radioactivity from each plate).*

Days	R_f[a]	Buffered water			Pond (pH 7.0)	Standard (¹⁴C-Bayer 2353)
		pH 5.0	pH 6.9	pH 8.7		
0	0	2	5	5	1	1
	0.78	98	95	95	99	99
1	0	4	6	5	1	1
	0.78	96	94	95	99	99
4	0	1	5	3	3	1
	0.78	99	95	97	97	99
7	0	1	2	2	1	1
	0.78	99	98	98	99	99
14	0	1	4	2	7	1
	0.78	99	96	98	93	99
26	0	1	5	3	6	1
	0.78	99	95	97	94	99
46	0	1	1	3	4	1
	0.78	99	99	97	96	99
56	0	6	2	2	4	1
	0.78	95	98	98	96	99

[a]0 = origin; 0.78 = parent compound, ¹⁴C-Bayer 2353.

Photolysis of Bayer 73

Photolysis on Silica Gel Plates

A solution of ¹⁴C-Bayer 2353 (1.31 µg in 10 µl of hexane) was spotted on 18 silica gel TLC plates, 9 of which (the controls) were wrapped in aluminum foil and 9 were left uncovered. The plates were exposed at a distance of 20.3 cm to a long-wave lamp (Blak-Ray, Model X-30, Ultra-Violet Products, Inc., San Gabriel, Calif.) with wave lengths ranging from 290 to 405 nm and a peak emission at 355 nm. The intensity at the surface of the TLC plates, measured with a Blak-Ray UV Meter (Model J221, Ultra-Violet Products, Inc., San Gabriel, Calif.) was 9,800 ergs/s per cm². One control and one exposed plate were removed from under the light at 0, 1, 2, 4, 6, 24, 48, 120, and 168 h and prepared for R_f determination and radiometric quantifications.

The ¹⁴C-Bayer 2353 degraded rapidly on the TLC plates exposed to long-wave UV light (Table 2). About 15% of the parent compound had been degraded after 4 h of exposure, 60% after 24 h, and 95% after 168 h (Fig. 1). Most of the degraded material remained at the origin of the TLC plates; e.g., after a 168-h exposure, more than 70% of the radioactivity remained at the origin. Several other compounds with R_f values of 0.25, 0.34, 0.41, 0.71, 0.74, and 1.0 (R_f of the parent com-

pound was 0.60) were found; their percentages increased up to 24 or 48 h of exposure and then declined. However, none of these compounds ever exceeded 8% of the total radioactivity. The only major compound other than that found at the origin, was a composite of compounds with R_f values ranging from 0.04 to 0.12. These spots were distinct, but overlapped, and could not be separated. The R_f of one other spot (0.25) corresponded to that of standard ¹⁴C-chlorosalicyclic acid. This spot never exceeded 3.8% of the total radioactivity. No attempt was made to check for nonradioactive degradation products such as chloronitroaniline because the amount potentially present was very small.

Photolysis on Glass Slides

In further tests of the photolysis of Bayer 73, we streaked 10 µl (1.31 µg) of ¹⁴C-Bayer 2353 on 18 glass microscope slides. The solvent was evaporated at room temperature in a fume hood, and the slides were then placed under long-wave UV as described for the TLC plates. One group of nine slides was covered with aluminum foil to serve as controls. Individual slides from each set were removed from UV exposure at 0, 1, 2, 4, 6, 24, 48, 120, and 168 h. The radioactive material was washed from the slides with 1 ml of acetone: methanol (50:50, v/v), and the wash concentrated to 0.5 ml and spotted on TLC plates for development, R_f

Table 2. *Amounts of* [14]*C-Bayer 2353 and* [14]*C-degradation products from TLC plates spotted with* [14]*C-Bayer 2353 and exposed to long-wave UV light for up to 168 h (values expressed as percentages of total radioactivity from each plate; values in parentheses are from control plates)*[a].

Time (h)	R_f								
	0	0.1[b]	0.25[c]	0.34	0.41	0.60[d]	0.71	0.74	Solvent front
0	0.2	0.0	0.4	0.0	0.0	99.0	0.0	0.2	0.1
1	1.9 (0.2)	1.6	0.8 (0.4)	0.9	0.8	92.7 (98.7)	0.0	0.9 (0.4)	0.4 (0.4)
2	2.7 (0.2)	2.4	0.8 (0.4)	1.5	1.3	89.3 (98.9)	0.0	1.2 (0.3)	0.9 (0.2)
4	4.2 (0.2)	3.4	0.9 (0.4)	2.4	1.6	84.5 (98.5)	1.0	1.2 (0.7)	0.9 (0.2)
6	8.5 (0.2)	5.6	2.4 (0.4)	4.5	2.6	71.4 (99.1)	1.8	1.6 (0.2)	1.5 (0.1)
24	27.7 (0.3)	10.8	3.3 (0.4)	8.0	3.9	39.9 (99.0)	2.2	2.2 (0.2)	2.0 (0.1)
48	44.0 (0.3)	11.4	3.8 (0.4)	7.5	4.0	22.7 (98.7)	2.8	2.0 (0.3)	1.6 (0.2)
120	66.9 (0.5)	10.4	3.2 (0.5)	5.2	2.9	8.0 (98.4)	1.3	1.0 (0.4)	1.0 (0.2)
168	73.7 (0.4)	11.6	2.3 (0.4)	3.9 (0.2)	1.6	4.4 (98.7)	1.1	1.0 (0.3)	0.3 (0.1)

[a]Plates exposed to 9,800 ergs/s per cm^2.

[b]A composite of spots with R_f's ranging from 0.04 to 0.12.

[c]This R_f corresponds to that of ^{14}C-chlorosalicylic acid.

[d]This R_f corresponds to that of the parent compound, ^{14}C-Bayer 2353.

determination, and radiometric quantification.

The degradation of ^{14}C-Bayer 2353 on glass slides was similar to that on TLC plates (Table 3), except that the degradation occurred more rapidly in the first 24 h. For example, after 4 and 6 h of exposure only 62.3 and 56.7% of the parent compound remained on glass slides, as compared with 84.5 and 71.4% on TLC plates. The more rapid rate of degradation could have resulted from greater penetration of UV light through the chemical on the glass slides, whereas the silica gel could have absorbed or adsorbed ^{14}C-Bayer 2353 and prevented the light from affecting all the molecules on the TLC plates. Material with an R_f of 0.1 increased during the first 24 h and then declined on glass slides whereas the same compound remained nearly constant after 24- to 168-h exposures on TLC plates. The change in the slope of the degradation curve (Fig. 1) may have resulted from incomplete elution of the radioactive compounds from the glass slides.

Photolysis in Buffered Solutions

To examine photolysis in buffered solutions, we added 1 ml of an acetone solution containing 5 μg of

[14]C-Bayer 2353 to two 250-ml beakers containing 100 ml of distilled water buffered to pH 6.9. Depth of the solution was 4.3 cm. The beakers were placed in the dark until the acetone had evaporated. The control beaker was then covered with aluminum foil and the other was left uncovered. The beakers were placed in a constant temperature water bath maintained at 20 ± 1 C and exposed to long-wave UV light. The distance from the lamp to the surface of the solution was 20.3 cm. Samples (100 μl) were taken from each beaker at 0, 1, 4, 7, and 14 days and spotted on silica gel TLC plates for development, R_f determination, and radiometric quantification.

The ^{14}C-Bayer 2353 in the aqueous solution was also degraded by long-wave UV light (Table 4), but the degradation (which was accompanied by a reduction in pH from 6.9 to 6.7) was much slower than on the TLC plates or glass slides. However, only 49% of the parent compound remained after 7 days of exposure and only 5% after 14 days. Most of the degraded material remained at the origin as on the TLC plates and glass slides. Three other compounds with R_f values of 0.13, 0.34, and 0.41 were noticed on the X-ray films from the study of aqueous photolysis. The spot at an R_f of 0.13

Table 3. *Amounts of ¹⁴C-Bayer 2353 and ¹⁴C-Bayer degradation products from TLC plates spotted with eluants from glass slides. Glass slides were streaked with ¹⁴C-Bayer 2353 and then exposed to long-wave UV light up to 168 h (values expressed as percentages of total radioactivity from each plate; values in parentheses are from control plates)[a].*

| Time (h) | R_f | | | | | | | |
	0	0.1[b]	0.25[c]	0.34	0.41	0.60[d]	0.71	Solvent front
0	0.25	0.0	0.4	0.0	0.0	98.9	0.2	0.3
1	1.4 (0.1)	2.5	1.1 (0.3)	2.2	2.6	86.7 (98.8)	1.5	1.9 (0.8)
2	3.2 (0.2)	5.0	1.5 (0.3)	2.6	2.9	81.0 (99.0)	1.4	2.4 (0.5)
4	12.8 (0.2)	9.2	(0.4)	4.8	5.8	62.3 (99.2)	2.4	2.7 (0.3)
6	13.7 (0.2)	11.2	(0.3)	6.1	7.2	56.7 (99.1)	2.7	2.2 (0.3)
24	47.5 (0.2)	12.6	(0.4)	7.3	6.2	22.9 (99.1)	2.2	1.3 (0.3)
48	56.7 (0.2)	10.3	(0.4)	5.3	5.7	19.7 (99.1)	1.7	0.4 (0.3)
120	65.9 (0.2)	9.5	(0.4)	6.2	4.8	12.8 (99.1)	0.0	0.8 (0.3)
168	83.8 (0.4)	4.5	(0.4)	4.8	3.1	3.6 (99.0)	0.0	0.1 (0.2)

[a]Glass slides exposed to 9,800 ergs/s per cm².

[b]A composite of spots with R_f's ranging from 0.04 to 0.12.

[c]This R_f corresponds to that of ¹⁴C-chlorosalicylic acid.

[d]This R_f corresponds to that of the parent compound ¹⁴C-Bayer 2353.

appeared only at the 14-day exposure and was a composite of several inseparable spots with R_f's ranging from 0.09 to 0.16.

Comparison with Other Studies

Strufe and Gönnert (1962) reported that the lowest concentration of Bayer 73 lethal to snails increased from 0.3 to about 1.8 mg/l after exposure of the chemical to UV light for 8 h. Meyling et al. (1962), who exposed 1-mg/l solutions of Bayer 73 to direct sunlight for up to 36 h, found that the concentrations of Bayer 73 in soft water dropped from 1 mg/l at 0 h to 0.9, 0.8, 0.67, and 0.56 mg/l at 8, 16, 24, and 36 h, and in hard water from 1.0 at 0 h to 0.71, 0.58, and 0.57 mg/l at 8, 12, and 16 h. Dawson (1971), who exposed Bayer 73 to UV light found that the LC_{50} to bluegills (*Lepomis macrochirus*) was 0.252 mg/l after 6 h (as compared with a control value of 0.197 mg/l) and 0.348 mg/l after 24 h (as compared with a control value of 0.250). He also reported LC_{50}'s of 0.242 for Bayer 73 exposed to sunlight for 6 h, 0.177 for shielded samples, and 0.150 mg/l for control samples.

Other workers reported no effects of UV light or sunlight on the toxicity of Bayer 73. Farringer (1972) exposed solutions of Bayer 73 to UV light for up to 96 h and found no change in toxicity of the chemical to carp (*Cyprinus carpio*). He believed that the differences between his results and those of Strufe and Gönnert (1962) may have been due to the difference in formulations. Farringer used a 99% technical formulation, whereas Strufe and Gönnert used a 70% wettable powder.

Sturrock (1974), after gathering samples of aquatic vegetation which had been sprayed with Bayer 73, reported no diminution in toxicity to snails from samples exposed to tropical sunlight for up to 8 weeks. Possibly the chemical was "protected" from UV degradation by absorption into, or adsorption onto, the plant material.
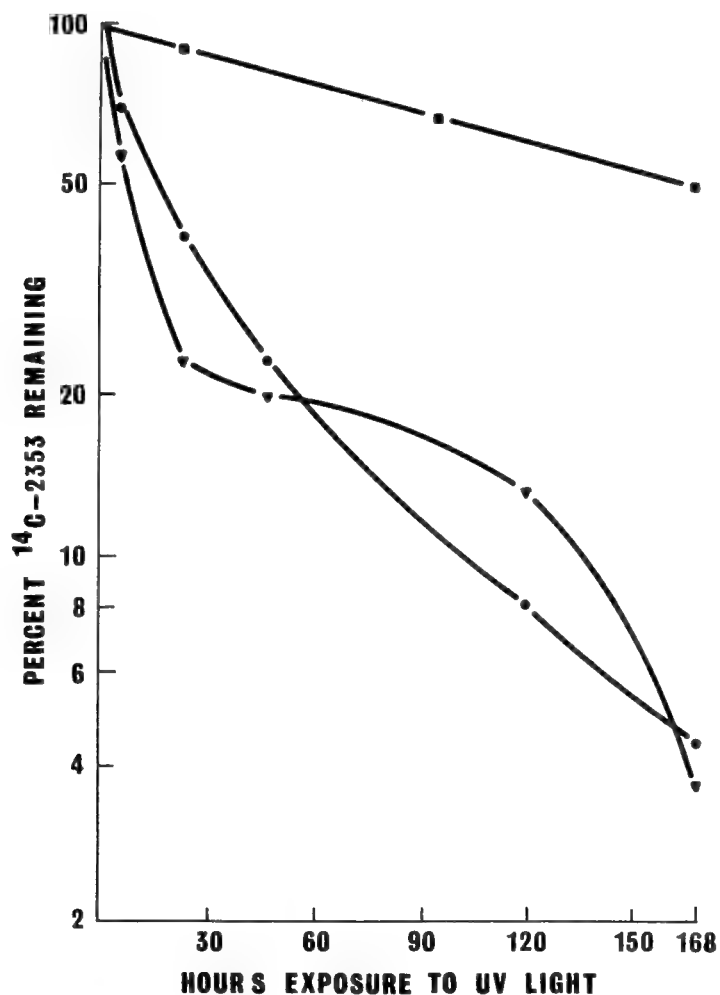
Fig. 1. Percentage of ¹⁴C-Bayer 2353 remaining on thin layer plates, glass slides, and in an aqueous solution after exposure to long-wave UV light. Legend: ■ = aqueous solution, ● = thin layer plates, and ▼ = glass slides.

Table 4. *Amounts of ¹⁴C-Bayer 2353 and ¹⁴C-degradation products from TLC plates spotted with an aqueous solution of ¹⁴C-Bayer 2353 exposed to long-wave UV light for up to 14 days (values expressed as percentages of total radioactivity from each plate)[a].*

Days and treatment	R_f				
	0	0.13[b]	0.34	0.41	0.65[c]
0					
UV	1	—	—	—	99
Control	1	—	—	—	99
1					
UV	9	—	—	—	91
Control	1	—	—	—	99
4					
UV	27	—	4	3	66
Control	2	—	—	—	98
7					
UV	41	—	6	4	49
Control	2	—	—	—	98
14					
UV	84	7	2	2	5
Control	5	—	—	—	95

[a]Solution exposed to 9,800 ergs/s per cm².

[b]These values are a composite of spots with R_f's ranging from 0.09 to 0.16.

[c]This R_f corresponds to that of authentic ¹⁴C-Bayer 2353.

Strufe and Gönnert (1962) are the only workers known to have attempted isolation or separation of degradation products of Bayer 73; they irradiated an ethanolic solution of the chemical for several days and then used paper chromatography to separate the products. Two compounds were found, in addition to the parent compound.

In our tests the ¹⁴C-Bayer 2353 was degraded by UV light into four to seven compounds.

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INVESTIGATIONS IN FISH CONTROL

86. Registration of Thirty-three Fishery Chemicals: Status of Research and Estimated Costs of Required Contract Studies

UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

Investigations in Fish Control, published by the Fish and Wildlife Service, include reports on the results of work at the Service's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy. [See Investigations in Fish Control 47-50 (in one cover) for list of issues published prior to 1970.]

(Reports 47 through 50 are in one cover.)

47. Preparation and Properties of Quinaldine Sulfate, an Improved Fish Anesthetic, by John L. Allen and Joe B. Sills. 1973. 7 pp.

48. Toxicity of Quinaldine Sulfate to Fish, by Leif L. Marking and Verdel K. Dawson. 1973. 8 pp.

49. The Efficacy of Quinaldine Sulfate as an Anesthetic for Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.

50. Residue of Quinaldine in Ten Species of Fish Following Anesthesia with Quinaldine Sulfate, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 9 pp.

(Reports 51 and 52 are in one cover.)

51. Methods for Simultaneous Determination and Identification of MS-222 and Metabolites in Fish Tissues, by Charles W. Luhning. 1973. 10 pp.

52. Residues of MS-222, Benzocaine, and Their Metabolites in Striped Bass Following Anesthesia, by Charles W. Luhning. 1973. 11 pp.

(Reports 53 through 55 are in one cover.)

53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish, by Verdel K. Dawson and Leif L. Marking. 1973. 11 pp.

54. The Efficacy of Quinaldine Sulfate:MS-222 Mixtures for the Anesthetization of Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.

55. Residues of Quinaldine and MS-222 in Fish Following Anesthesia with Mixtures of Quinaldine Sulfate:MS-222, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 12 pp.

(Reports 56 through 59 are in one cover.)

56. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to 10 Species of Algae, by A. A. Maki, L. D. Geissel, and H. E. Johnson. 1975. 17 pp.

57. Acute Toxicites of 3-Trifluoromethyl-4-nitrophenol (TFM) and 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73) to Larvae of the Midge *Chironomus tentans*, by J. A. Kawatski, M. M. Ledvina, and C. R. Hansen. 1975. 7 pp.

58. Acute Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nymphs of Mayflies (*Hexagenia* sp.), by C. R. Fremling. 1975. 8 pp.

59. Toxicity and Residue Dynamics of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) in Aquatic Invertebrates, by H. O. Sanders and D. F. Walsh. 1975. 9 pp.

(Reports 60 through 62 are in one cover.)

60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests, by L. L. Marking and L. E. Olson. 1975. 27 pp.

61. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests, by L. L. Marking, T. D. Bills, and J. H. Chandler Jr.. 1975. 9 pp.

62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae, by J. H. Chandler, Jr. and L. L. Marking. 1975. 7 pp.

(Reports 63 through 66 are in one cover.)

63. Laboratory Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM) as a Lampricide, by V. K. Dawson, K. B. Cumming, and P. A. Gilderhus. 1975. 13 pp.

64. Effects of 3-Trifluoromethyl-4-nitrophenol (TFM) on Developmental Stages of the Sea Lamprey, by G. W. Piavis and J. H. Howell. 1975. 8 pp.

INVESTIGATIONS IN FISH CONTROL

86. Registration of Thirty-three Fishery Chemicals: Status of Research and Estimated Costs of Required Contract Studies

By R. A. Schnick and F. P. Meyer

UNITED STATES DEPARTMENT OF THE INTERIOR

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Contents

Registration of Thirty-three Fishery Chemicals:
Status of Research and Estimated Costs of Required Contract Studies

by

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Abstract

An estimated $8.8 million for contract studies is needed to meet registration requirements for 33 chemicals now used or being considered for use in fish culture and management. Information given for each chemical includes its sponsor, current registration status, research situation in six categories (toxicity to target and nontarget organisms, field testing, physiological studies, analytical methods development, counteraction, and mammalian safety determination), costs of required contract studies, and the prognosis for registration of the use of each compound.

Since Lennon (1967) first issued a warning about the need to register chemicals for fishery use, regulations and guidelines have been developed that require extensive and costly safety evaluation studies (Cumming 1975; U.S. Environmental Protection Agency 1975a, 1975b). Without these studies, many compounds now being used by fish culturists and fishery managers could become unavailable. If this occurred, the impact on fisheries would be far-reaching: The Great Lakes Fishery Commission estimated that 3.5 million angler days spent each year fishing for lake trout (*Salvelinus namaycush*) and Pacific salmon (*Oncorhynchus* sp.) would be lost if the sea lamprey (*Petromyzon marinus*) were not being controlled by lampricide applications; an estimated 4 million hatchery fish intended for stocking in lakes and streams would be lost if chemicals used for disease treatment were unavailable; and it is anticipated that the $200 million bait and commercial fish culture industry would suffer a 50% or $100 million loss if chemicals were not available for use.

When the U.S. Fish and Wildlife Service (FWS) assigned primary responsibility for facilitating registration of fishery compounds to the Fish Control Laboratory in 1972, the Deputy Associate Director for Research and Environment requested, for each priority compound, a summary of current information regarding its use patterns in fisheries, patent position, status of current registration, and cost estimates of research

needed to obtain registration for fishery uses. This information was first presented in status reports on 22 compounds in 1973. Literature reviews on 20 of these compounds were prepared in 1974. Since then, two articles on the registration status of fishery chemicals (Meyer et al. 1976) and on the approaching crisis in the registration of fishery chemicals (Meyer and Schnick 1978) have emphasized the need for mammalian safety data to support registration or reregistration of fishery chemicals. Development of these data requires specialized facilities that are not available within FWS.

We summarize here the research known to have been completed, or yet to be done, on 33 fishery chemicals, based on our interpretation of the requirements and guidelines of regulatory agencies as of January 1978. Information is included on the sponsor of each compound, its current registration status, the research situation in six categories (toxicity to target and nontarget organisms, field testing, physiological studies, analytical methods development, counteraction, and mammalian safety determination), costs for required contract studies, and the prognosis for achieving registration. Requirements for safety evaluation studies on various domestic animals, fish, and wildlife were excerpted from the *Federal Register* (U.S. Environmental Protection Agency 1975a). Costs for required work to be done outside FWS are based on January 1978 prices, but will vary among testing facilities. New

1

rules by FDA governing the laboratory evaluation of the safety of chemicals will probably increase the costs of testing (Smith 1977).

The sequence of the registration procedure is shown in Appendix 1, and the status of research on the various fishery chemicals is summarized in Appendix 2.

Information on each compound was gathered from sponsors, regulatory notices, chemical reference works, literature reviews, and status reports prepared by FWS.

Our estimates of the costs of the contract studies needed to meet registration requirements are $8,839,800, divided as follows:

```
7 piscicides, lampricides, and
    collecting aids . . . . . . . . . . . . . . . . . . $2,954,000
15 therapeutants, disinfectants, pond
    sterilants, oxidizing agents,
    and osmoregulatory enhancers . . . . . . 4,239,650
9 herbicides and algicides. . . . . . . . . . . . 1,010,000
2 anesthetics . . . . . . . . . . . . . . . . . . . . . . 636,150
Total 33 compounds. . . . . . . . . . . . . . . $8,839,800
```

Costs of registering or reregistering chemicals have increased as much as 20-fold in the past 10 years. Under current regulations, registration costs are relatively fixed, whether a product is likely to be widely used and highly profitable or of such limited use that profitability is questionable. FWS encourages industry to accept and bear the major costs of compounds needed in conservation programs, but most chemical companies cannot afford the costs of developing "minor-use" products under present requirements, without outside support.

The Environmental Protection Agency (EPA) recently established a separate committee to give attention to problems concerning registration of minor-use compounds, and the Food and Drug Administration (FDA) is revising the criteria for registration of drugs and biologics needed for minor uses in the production of food animals.

Even though some progress is being made in clarifying procedures and guidelines for registering minor-use compounds, funding has been inadequate to meet the complete research need. In the absence of increased funding for registration research, FWS has had to limit its effort to a few selected priority chemicals at the expense of others. Lack of funds has also made it necessary for FWS to forego the development of new techniques and chemicals.

The following sections provide a synopsis of the current status of the registration for fishery uses of 33 priority chemicals, and the cost of fulfilling existing requirements for safety testing.

Piscicides, Lampricides, and Collecting Aids

Antimycin

Use
  Piscicide.

Sponsor
  Sold by Aquabiotics Corporation, Northbrook, Ill., under license from the Wisconsin Alumni Research Foundation; Aquabiotics Corp. will not assist in the registration effort.

Registration Status
  Registered for nonfood fish use as a piscicide. Studies on antimycin are not being actively pursued by industry. Major studies are needed on mammalian safety, methodology, and residues.

Research Situation
  1. Toxicity to target and nontarget organisms: fish — most requirements met; invertebrates and birds — requirements met; plants — requirements partly met.
  2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
  3. Physiological studies: mode of action, biotransformation, and excretion — requirements partly met.
  4. Analytical methods development: residues, metabolites, and degradation — requirements partly met.
  5. Counteraction: removal and inactivation — most requirements met.
  6. Mammalian safety determination:
      a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); acute dermal, eye, and inhalation studies (rat and rabbit); and 90-day subacute oral (rat) — requirements met.
      b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS
  1. Teratology (rabbit) . . . . $ 35,000
  2. Metabolism (cow) 100,000
  3. Metabolism (rat or dog) 25,000
  4. 2-year oncogenicity (rat) 100,000
  5. 2-year oncogenicity (hamster) 100,000
  6. 6-month feeding (dog) 35,000
  7. Mutagenicity — Ames test or
      equivalent 1,500
  8. Residues — methodology 120,000
  9. Residues — use pattern 40,000
  10. Residues — metabolites 60,000
      Total. $616,500

Prognosis

Reregistration uncertain. Low concentrations (< 10 parts per billion) ordinarily are used and the material degrades so rapidly under most conditions that sensitive analytical methods with detection limits in parts per trillion are required. Technology for such sensitivity is not now available. Entire registration cost will have to be borne by FWS.

Bayer 73, and the Combination of TFM and Bayer 73

Use

Lampricide; survey tool.

Sponsor

FWS and Great Lakes Fishery Commission. Manufactured by Mobay Chemical Corporation, Kansas City, Mo. (formerly Chemagro), specifically for use as a lampricide. Mobay Chemical Corp. cooperates by allowing FWS to use data in its files.

Registration Status

Registered for nonfood fish use in surveys for larval lampreys and for use as a lampricide in combination with 3-trifluoromethyl-4-nitrophenol (TFM) in the Great Lakes.

Research Situation

1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and degradation — requirements met.
4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal and inactivation — requirements met.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); intravenous (iv) or intraperitoneal (ip) injections (rat and mouse); 90-day subacute (rat and hamster); metabolism (cow); and 6-month feeding (dog) — requirements met.
   b. Carcinogenicity and teratology — requirements met.

Costs for Required Work to be Done Outside FWS

None; requirements met.

Prognosis

Reregistration promising. A petition for an exemption from tolerance and an amendment of registration is scheduled to be prepared in fiscal year 1978. Further studies may be needed on the TFM:Bayer 73 mixture.

GD-174

Use

Piscicide.

Sponsor

FWS; owned by McLaughlin Gormley King Co., Minneapolis, Minn. Some technical studies and research are being done by the company.

Registration Status

Not registered for fishery use. GD-174 [2-(digeranylamino)-ethanol] is an experimental compound being tested by FWS as a possible selective control for carp, or as a general piscicide.

Research Situation

1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements partly met.
2. Field testing: geographic areas, ecotypes, and efficacy — requirements partly met; delivery systems — no work done.
3. Physiological studies: mode of action, biotransformation, and excretion — no work done.
4. Analytical methods development: residues, metabolites, and degradation — requirements partly met.
5. Counteraction: removal and inactivation — requirements partly met.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); acute dermal and eye studies (rabbit); 90-day subacute oral (rat and hamster) — requirements met.
   b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS

1.	Acute inhalation (rat)	$ 500
2.	21-day subacute dermal (rabbit)	3,000
3.	Teratology (rabbit)	35,000
4.	Metabolism (cow)	100,000
5.	Metabolism (rat or dog)	25,000
6.	2-year oncogenicity (rat)	100,000
7.	2-year oncogenicity (hamster)	100,000
8.	6-month feeding (dog)	35,000
9.	Avian acute oral (three species)	5,000
10.	1-generation reproduction (bobwhite quail or mallard)	20,000
11.	Residues — methodology	60,000
12.	Residues — use pattern	40,000
13.	Residues — metabolites	60,000
	Total	$583,500

Prognosis

Registration uncertain. Field studies have indicated that GD-174 is an excellent piscicide but have failed to duplicate the selective action against carp noted in laboratory studies. GD-174 has phytotoxic properties but these should not be a serious obstacle to registration. Should either rotenone or antimycin be lost to fishery use, GD-174 is an excellent candidate replacement.

Rotenone

Use

Piscicide.

Sponsor

S. B. Penick & Co., Lyndhurst, N.J., and others.

Registration Status

Registered for nonfood fish use as a piscicide. S. B. Penick & Co. is negotiating with EPA to resolve the problem of a Rebuttable Presumption Against Registration listing caused by a Spanish report which supposedly showed that rotenone is carcinogenic when injected into rats. A study was initiated by EPA to determine whether the Spanish results could be duplicated. Available results to date show no carcinogenicity. A hamster study was terminated because high mortality of control animals made the test results statistically invalid. The master study is being repeated.

Research Situation

1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements partly met.
4. Analytical methods development: residues, metabolites, and degradation — requirements partly met.
5. Counteraction: removal and inactivation — requirements met.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); 2-year feeding (rat); and acute dermal, eye, and inhalation studies — requirements met.
   b. Carcinogenicity and teratology; 2-year oncogenicity (hamster) — in progress.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) . . . . $ 35,000
2. Metabolism (cow) 100,000
3. Metabolism (rat) 25,000

4. 2-year oncogenicity (rat) 100,000
5. 6-month feeding (dog) 35,000
6. Mutagenicity — Ames test or equivalent 1,500
7. Residues — methodology 120,000
8. Residues — use pattern 40,000
9. Residues — metabolites 180,000
   Total $636,500

Prognosis

Reregistration uncertain. S. B. Penick & Co. is highly interested in maintaining the registration of rotenone as a piscicide, and is willing to perform some of the needed residue and safety studies.

Squoxin

Use

Piscicide.

Sponsor

American Cyanamid Co., Princeton, N.J.; assisted by the National Marine Fisheries Service.

Registration Status

Not registered for fishery use. EPA has granted a yearly renewable permit for field tests for use as a selective toxicant for squawfish.

Research Situation

1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements partly met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action and excretion — requirements partly met; biotransformation — no work done.
4. Analytical methods development: residues, metabolites, and degradation — requirements partly met.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); dermal toxicity (rabbit); and 31-day subacute oral (rat) — requirements met.
   b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS
1. 90-day subacute oral (rat) 25,000
2. 90-day subacute oral (dog) 25,000
3. Teratology (rabbit) 35,000
4. Metabolism (cow) 100,000

5. Metabolism (rat)   25,000
6. 2-year oncogenicity (rat). 100,000
7. 2-year oncogenicity (hamster) 100,000
8. 6-month feeding (dog).   35,000
9. Mutagenicity — Ames test
    or equivalent   1,500
10. Avian acute oral (mallard or quail)   1,500
11. 8-day avian subacute
    dietary (bobwhite quail
    or pheasant).   1,000
12. Residues — methodology   15,000
13. Residues — use pattern   10,000
14. Residues — metabolites   60,000
    Total $534,000

Prognosis

Registration uncertain. Currently completed studies are not adequate to meet EPA requirements for registration. Safety evaluation and residue data must be developed before the compound can be registered.

TFM

Use

Lampricide.

Sponsor

FWS and Great Lakes Fishery Commission; manufactured by American Hoechst Corp., Somerville, N.J.

Registration Status

Registered for nonfood fish use as a lampricide.

In February 1976 FWS submitted petitions for an exemption from tolerance and an amendment of registration for use of the sodium salt of TFM (3-trifluoromethyl-4-nitrophenol) as a lampricide. EPA provided preliminary comments on 22 October 1976, to which FWS responded. Further comments from EPA were received in March and April 1977.

Data provided were adequate to support negotiations that eliminated the need for further studies in several categories: acute oral toxicity tests; 2-year hamster feeding study; characterization of residues in milk, cattle kidney, and other edible products of cultured mammals; and an indication of the distribution, retention, or elimination of TFM and its metabolites.

Points still being negotiated include an exemption for the application of dimethylformamide in streams as a part of TFM formulations, residue information in potable waters, possible restrictions on use in irrigation waters, and possible soil binding effects. An Ames test to evaluate potential mutagenicity of TFM was completed and found negative by Wisconsin Alumni Research Foundation. Progress continues toward registration.

Research Situation

All research studies originally required have been completed.

Costs for Required Work to be Done Outside FWS

None; requirements met.

Prognosis

Reregistration highly promising. Outlook is excellent for continued and amended registration and for exemption from tolerance.

Thanite

Use

Collecting aid; piscicide.

Sponsor

FWS; owned by McLaughlin Gormley King Co., Minneapolis, Minn.

Registration Status

Not registered for fishery use. Thanite is an experimental fish collecting aid. EPA will require additional oncogenic data for reregistration of its current label as an insecticide.

Research Situation

1. Toxicity to target and nontarget organisms: fish — most requirements met; invertebrates and birds — requirements partly met; plants — no work done.
2. Field testing: geographic areas and ecotypes — requirements met; efficacy and delivery systems — requirements partly met.
3. Physiological studies: mode of action — requirements met; biotransformation and excretion — no work done.
4. Analytical methods development: residues and metabolites — requirements partly met; degradation — no work done.
5. Counteraction: removal and inactivation — requirements partly met.
6. Mammalian safety determination:
    a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat, rabbit, and guinea pig); 6-month subacute oral (rat and guinea pig); and acute dermal and inhalation studies — requirements met.
    b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS

1. 90-day subacute oral (hamster) . . . . $ 25,000
2. Teratology (rabbit)   35,000
3. Metabolism (cow) 100,000

4. Metabolism (rat) 25,000
5. 2-year oncogenicity (rat). 100,000
6. 2-year oncogenicity (hamster) 100,000
7. 6-month feeding (dog) 35,000
8. Mutagenicity — Ames test
    or equivalent 1,500
9. 8-day avian subacute (mallard) 1,000
10. 8-day avian subacute (quail or
    pheasant) 1,000
11. Residues — methodology 60,000
12. Residues — use pattern 40,000
13. Residues — metabolites 60,000
    Total $583,500

Prognosis
Registration unlikely. Research has been halted. McLaughlin Gormley King Co. has expressed concern over the cost of the mammalian safety tests that will be required and has decided not to continue efforts toward obtaining a registration for fishery use at this time.

Therapeutants, Disinfectants, Pond Sterilants, Oxidizing Agents, and Osmoregulatory Enhancers

Betadine

Use
Therapeutant.

Sponsor
Purdue Frederick Company, Norwalk, Conn.

Registration Status
Not registered for fishery use. National Institute for Occupational Safety and Health has expressed concern because a portion of the molecule (poly[1-vinyl-2-pyrrolidinone], polymer no. 1) has produced tumors in rats in experimental studies.

Research Situation
Requirements are considered to have been met, except perhaps for bird toxicity.

Costs for Required Work to be Done Outside FWS
1. Mutagenicity — Ames test or
    equivalent $1,500
2. 8-day avian subacute dietary
    (mallard). 1,000
3. 8-day avian subacute dietary
    (quail or pheasant). 1,000
    Total $3,500

Prognosis
Registration highly promising. Betadine is registered as a disinfectant for human and animal skin. Purdue Frederick Co., in conjunction with Tavolek, Inc., is preparing a New Animal Drug Application on Betadine for use as a fish egg disinfectant. Most of the required research is considered complete on Betadine.

Calcium Hypochlorite (HTH)

Use
Disinfectant.

Sponsor
Olin Corporation, Stamford, Conn.

Registration Status
Registered for fishery use as a disinfectant; for sanitizing fish tanks, raceways, and utensils; and for controlling algae and bacteria in fish ponds.

Research Situation
No additional research needed.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis
Desired registration has been achieved.

Formalin

Use
Therapeutant.

Sponsor
FWS; manufactured and sold by many companies.

Registration Status
Not registered for fishery use. Formalin is used extensively by fish culturists as a therapeutant for external parasites on fish and fungus on fish eggs. National Institute for Occupational Safety and Health has expressed concern because neoplastic effects were observed in rats.

Research Situation
Required studies completed to date are considered to be adequate. FDA may require that further tests be done.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) $35,000

Prognosis
Registration highly promising. A Not New Drug

Monograph was submitted to FDA in 1973. On the basis of the review of this document, FDA ruled that residue studies based on the use pattern would have to be carried out. The Fish Control Laboratory completed such studies and submitted the information to FDA in 1977.

Furanace

Use
Therapeutant.

Sponsor
Abbott Laboratories, North Chicago, Ill. The company has expressed a willingness to help support some of the required research. Zodiac Pet Products, Inc., Dallas, Texas, currently markets the compound for aquarium use.

Registration Status
Registered for nonfood fish use. Abbott Laboratories obtained an aquarium use registration for Furanace in December 1975. A petition for food fish use submitted by Abbott to FDA in 1976 was denied, pending submission of additional data.

Research Situation
1. Toxicity to target and nontarget organisms: fish and invertebrates — requirements met; birds and plants — not needed.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: host responses and mode of action — requirements met; biotransformation and excretion — no work done.
4. Analytical methods development: residues — most requirements met; metabolites and degradation — no work done.
5. Counteraction: removal and inactivation — requirements met.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); and 120-day subacute oral (mice) — requirements met.
   b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS
1. Acute dermal (rabbit) . . . . $ 300
2. Acute dermal irritation (rabbit)   150
3. Acute eye irritation (rabbit)   200
4. Acute inhalation (rat)   500
5. 90-day subacute oral (dog)   25,000
6. Teratology (rabbit)   35,000

7. 2-year oncogenicity (rat)   100,000
8. 2-year oncogenicity (hamster)   100,000
9. 6-month feeding (dog)   35,000
10. Mutagenicity — Ames test or
    equivalent   1,500
11. Residues — methodology   40,000
12. Residues — use pattern   40,000
13. Residues — metabolites   60,000
    Total $437,650

Prognosis
Food use registration uncertain. Many of the above tests may not be needed if FDA accepts the removal of Furanace by filtration of treated water through carbon, or establishes new minor-use requirements. Current label for use on aquarium fishes could easily be expanded to include nonfood fishes. Inclusion of food fishes will require completion of listed research or changes in FDA position, as indicated above.

Furazolidone

Use
Therapeutant.

Sponsor
Hess and Clark, Division of Rhodia, Inc., Ashland, Ohio.

Registration Status
Not registered for fishery use.

Research Situation
1. Toxicity to target and nontarget organisms: fish — requirements partly met; invertebrates and plants — no work done; birds — requirements met.
2. Field testing: geographic areas, ecotypes — no work done; efficacy — requirements partly met; delivery systems — no work done.
3. Physiological studies: host responses, mode of action, biotransformation, and excretion — no work done.
4. Analytical methods development: residues and metabolites — requirements partly met; degradation — no work done.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); 53-week feeding (rat); and ip injection studies — requirements met.
   b. Carcinogenicity and teratology: 2-year oncogenicity (rat and mouse) — requirements met.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit).................$ 35,000
2. Metabolism (cow)...................100,000
3. Metabolism (rat)...................25,000
4. 6-month feeding (dog) 35,000
5. Residues — methodology 40,000
6. Residues — use pattern............40,000
7. Residues — metabolites............40,000
　　　　　Total........................$315,000

Prognosis

Registration highly unlikely. A notice of intent by FDA to cancel registrations for furazolidone appeared 13 May 1976 in the *Federal Register*. This course of action is being considered because of the potential carcinogenic or mutagenic action of the compound. Chances of having the compound approved for fishery use are nil.

Hyamine 1622

Use
Therapeutant; disinfectant.

Sponsor
Rohm and Haas Co., Philadelphia Pa.

Registration Status

Not registered for fishery use. Hyamine 1622 has potential use as a disinfectant and as a treatment for bacterial gill disease. The compound was dropped from consideration in 1973 because the product is a mixture of compounds, and complex residue studies would be required. Also, Furanace was considered to be a better choice as a control for bacterial gill disease. Research was resumed on the product in 1978 because of renewed interest in the compound by fish culturists.

Research Situation
1. Toxicity to target and nontarget organisms: fish — requirements partly met; invertebrates, birds, and plants — no work done.
2. Field testing: geographic areas and ecotypes — no work done; efficacy and delivery systems — requirements partly met.
3. Physiological studies: host responses, mode of action, biotransformation and excretion — no work done.
4. Analytical methods development: residues — requirements partly met; metabolites and degradation — no work done.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
　　a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat and mouse); 1-year feeding

(dog); 2-year feeding (rat); and acute dermal and eye studies; iv or ip injections — requirements met.
　　b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit).................$ 35,000
2. Metabolism (cow) 100,000
3. Metabolism (rat)...................25,000
4. 2-year oncogenicity (rat)............100,000
5. 2-year oncogenicity (hamster) 100,000
6. Residues — methodology............100,000
7. Residues — use pattern 40,000
8. Residues — metabolites 60,000
　　　　　Total........................$560,000

Prognosis

Registration uncertain. Too few data are available to evaluate potential problems in registering Hyamine 1622. The fact that the product is a mixture of compounds may complicate registration efforts.

Lime (Calcium Carbonate, Calcium Hydroxide, and Calcium Oxide)

Use
Pond sterilant.

Sponsor
FWS.

Registration Status
Registered for fishery use as a pond sterilant under the Generally Recognized As Safe classification.

Research Situation
Requirements met.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis
Desired registration has been achieved.

Malachite Green, and the Combination of Malachite Green and Formalin

Use
Therapeutant.

Sponsor
FWS; produced by American Cyanamid Co., Princeton, N.J., and others.

Registration Status

Not registered for fishery use. Malachite green is used extensively to control fungi and protozoans. In combination with formalin it is very effective against *Ichthyophthirius* infections.

Research Situation

1. Toxicity to target and nontarget organisms: fish and invertebrates — requirements met; birds — not needed; plants — requirements partly met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: host responses and excretion — requirements partly met; mode of action — requirements met; biotransformation — no work done.
4. Analytical methods development: residues — requirements partly met; metabolites and degradation — no work done.
5. Counteraction: removal — requirements met; inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); iv or ip injections — requirements met.
   b. Carcinogenicity and teratology: teratology (rabbit) — requirements met.

Costs for Required Work to be Done Outside FWS

1. 90-day subacute oral (rat) . . . . $ 25,000
2. 90-day subacute oral (dog) 25,500
3. Teratology (F_3 generation rat) 100,000
4. Metabolism (cow) 100,000
5. Metabolism (rat) 25,000
6. 2-year oncogenicity, parent compound (rat) 100,000
7. 2-year oncogenicity, parent compound (mouse) 100,000
8. 3-generation reproduction, parent compound (rat) 100,000
9. 2-year oncogenicity, metabolites (rat) 100,000
10. 2-year oncogenicity, metabolites (mouse) 100,000
11. 3-generation reproduction, metabolites (rat) 100,000
12. 6-month feeding (dog) 35,000
13. Mutagenicity — Ames test or equivalent 1,500
14. Residues — methodology 200,000
15. Residues — use pattern 40,000
16. Residues — metabolites 400,000
      Total $1,552,000

Prognosis

Registration highly unlikely. Malachite green has been implicated as a possible teratogen and carcinogen in fish, and a study in rabbits showed some teratology. Discussions with FDA officials indicated that the full complement of safety tests would be required. Even if all the studies were performed, no guarantee could be given that malachite green could be registered. FWS has halted its efforts to register it.

Masoten (Trichlorfon)

Use

Therapeutant.

Sponsor

Bayvet Division of Mobay Chemical Corp., Kansas City, Mo.

Registration Status

Registered for nonfood fish use. Masoten is used in fisheries as a control for a variety of ectoparasites, especially the anchor parasite, *Lernaea*. National Institute for Occupational Safety and Health has expressed concern because it has produced carcinogenic effects on animals in two studies and teratogenic effects in another.

Research Situation

1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: host responses, mode of action, excretion, and biotransformation — requirements partly met.
4. Analytical methods development: residues and degradation — requirements met; metabolites — no work done.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); ip injections; 90-day subacute (rat); and 2-year feeding (rat and dog) — requirements met.
   b. Carcinogenicity and teratology — requirements met.

Costs for Required Work to be Done Outside FWS

1. Residues — metabolites $75,000

Prognosis

Registration for use on food fish unlikely. Masoten is subject to Rebuttable Presumption Against Registration and its status is uncertain. If it is not canceled for other uses and if a food use is desired, the listed tests will probably be required. The company has shown little interest in extending the label to food fish use.

Nitrofurazone (Furacin)

Use

Therapeutant.

Sponsor

FWS; sold by Norwich Pharmacal Co., Norwich, N.Y.

Registration Status

Not registered for fishery use. Nitrofurazone was listed as a drug of concern in an FDA notice of intent to cancel registration of furazolidone because of its possible carcinogenicity or mutagenicity. Nitrofurazone is considered a close analog of furazolidone.

Research Situation

1. Toxicity to target and nontarget organisms: fish and birds — requirements partly met; invertebrates and plants — no work done.
2. Field testing: geographic areas and ecotypes — no work done; efficacy and delivery systems — requirements partly met.
3. Physiological studies: host responses, mode of action, biotransformation, and excretion — no work done.
4. Analytical methods development: residues and degradation — requirements partly met; metabolites — no work done.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); 53-week feeding (rat); skin tests; and ip injections — requirements met.
   b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS

1. 90-day subacute (dog). . . . . $ 25,000
2. Teratology (rabbit) 35,000
3. Metabolism (cow) 100,000
4. Metabolism (rat) 25,000
5. 2-year oncogenicity (hamster) 100,000
6. 2-year oncogenicity (rat). 100,000
7. 6-month feeding (dog). 35,000
8. Residues — methodology 40,000
9. Residues — use pattern 40,000
10. Residues — metabolites 40,000
    Total. $540,000

Prognosis

Registration highly unlikely. Because of the concern over the possible carcinogenicity of nitrofurans, it is unlikely that any fishery uses of nitrofurazone could be registered unless such concern is favorably resolved.

Potassium Permanganate

Use

Oxidizing agent.

Sponsor

FWS; sold by Carus Chemical Company, Inc., La Salle, Ill.

Registration Status

Not registered for fishery use.

Research Situation

1. Toxicity to target and nontarget organisms: fish — requirements met; invertebrates and plants — requirements partly met; birds — no work done.
2. Field testing: geographic areas and ecotypes — requirements partly met; efficacy — requirements met; delivery systems — requirements partly met.
3. Physiological studies: host responses and mode of action — requirements partly met; biotransformation and excretion — no work done.
4. Analytical methods development: residues — requirements partly met; metabolites — no work done; degradation — requirements met.
5. Counteraction: removal and inactivation — requirements met.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); acute subcutaneous (mouse); and acute dermal and eye studies — requirements met.
   b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS

1. 90-day subacute oral (rat). . . . . $ 25,000
2. 90-day subacute oral (dog) 25,000
3. Teratology (rabbit) 35,000
4. Metabolism (cow) 100,000
5. Metabolism (rat) 25,000
6. 2-year oncogenicity (rat). 100,000
7. 2-year oncogenicity (hamster) 100,000
8. 6-month feeding (dog). 35,000
9. Residues — methodology 40,000
10. Residues — use pattern 40,000
11. Residues — metabolites 60,000
    Total $585,000

Prognosis

Registration promising. Since certain fishery uses of potassium permanganate are not considered to be pesticidal, a petition for exemption from registration has been requested. If the exemption is not allowed, many of the above tests will be required.

R05-0037 (Sulfadimethoxine and Ormetoprim)

Use
Therapeutant.

Sponsor
Hoffmann-La Roche, Inc., Nutley, N.J.

Registration Status
Not registered for fishery use. A decision was made to drop this sulfa drug in 1974 because the potentiator leaves residues in fish skin. A nitrofuran such as furazolidone or nitrofurazone was suggested as a suitable substitute, but these have been dropped because they are considered to be potential carcinogens. R05-0037 was reconsidered for registration in 1977.

Research Situation
1. Toxicity to target and nontarget organisms: fish and birds — requirements met; invertebrates and plants — requirements partly met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements partly met.
3. Physiological studies: host responses and excretion — requirements partly met; mode of action — requirements met; biotransformation — no work done.
4. Analytical methods development: residues and metabolites — requirements partly met; degradation — no work done.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (mouse); 13-week subacute oral (rat and dog); and 9-week subacute oral (pig) — requirements met.
   b. Carcinogenicity and teratology: teratology (dog) — requirements met.

Costs for Required Work to be Done Outside FWS
1. Metabolism (cow) $100,000
2. Metabolism (rat) 25,000
3. Mutagenicity — Ames test
    or equivalent 1,500
4. Residues — use pattern 10,000
       Total $136,500

Prognosis
Registration promising. The sponsoring company apparently is interested in the compound and will pursue its registration. Potential problems exist because skin tissues may retain residues of the drug for extended periods. Withdrawal requirements could be as long as 6 months after use. Efficacy studies are under way to determine if a shorter treatment period would result in a shorter residue retention period.

Sodium Chloride

Use
Osmoregulatory enhancer.

Sponsor
FWS.

Registration Status
Registered for fishery use as an osmoregulatory enhancer under the Generally Recognized as Safe registration.

Research Situation
Requirements met.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis
Desired registration has been achieved.

Sulfamerazine

Use
Therapeutant.

Sponsor
FWS and American Cyanamid Co., Princeton, N.J.

Registration Status
Registered for food fish use. Sulfamerazine is registered for the treatment of furunculosis in trout and salmon only.

Research Situation
No research is under way to extend the use label. Known research needs have been met.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis
Reregistration promising. No problems are anticipated when reregistration is required.

Terramycin

Use
Therapeutant.

Sponsor
FWS and Pfizer, Inc., New York, N.Y.

Registration Status

Registered for food fish use. Terramycin is registered for treatment of bacterial infections in trout, salmon, and catfish and for marking bones or scales of fish in age or identification studies.

Research Situation

No research is under way to extend the label. Known research needs have been met.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis

Reregistration promising. No problems are anticipated when reregistration is required unless Terramycin is restricted to human uses only. Such a restriction has been rumored because of the possible transfer of resistance factors between pathogenic and non-pathogenic bacteria. Current FDA concerns relate only to subtherapeutic uses. Since fishery uses involve only therapeutic levels, it appears that the compound will remain available.

Herbicides and Algicides

Copper Sulfate

Use
Herbicide and algicide.

Sponsor

Cities Service Co., Atlanta, Ga.; Phelps Dodge Refining Corp., New York, N.Y.; 3M Company, St. Paul, Minn.; and others.

Registration Status

Registered for food fish use. Two types of tolerances exist for copper as an active component of algicides: exemptions from tolerance exist for $CuSO_4:5H_2O$ and basic copper carbamate, and finite tolerances of 1 ppm have been established for copper complexes.

Research Situation
1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.
4. Analytical methods development: residues, metabolites, and degradation — requirements met.

5. Counteraction: removal — requirements met; inactivation — requirements partly met.
6. Mammalian safety determination:
    a. Acute, subacute, and chronic toxicity — requirements met.
    b. Carcinogenicity and teratology — requirements met.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis

Desired registration has been achieved. Manufacturers of copper sulfate (Kennecot Chemical, 3M Company, and Phelps Dodge Refining Corp.) indicated to the Fish Control Laboratory that they were not interested in attempting to register copper sulfate for other uses.

2,4-D

Use
Herbicide.

Sponsor

AmChem Products, Inc., Ambler, Pa.; Dow Chemical USA, Midland, Mich.; and others.

Registration Status

Registered for food fish use. It can be used as an herbicide only by Federal, State, or local public agencies.

Research Situation

All requirements are considered met, except counteraction.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis

Extended registration uncertain. Efforts are being made by AmChem Products, Inc. to extend the use of 2,4-D to other than public agencies. Contract studies have been started by the company.

Dichlobenil

Use
Herbicide.

Sponsor

Thompson-Hayward Chemical Co., Kansas City, Kans.

Registration Status

Registered for nonfood fish use. Dichlobenil can be

used in ponds, lakes, and reservoirs with nonflowing waters, but the fish cannot be used for food or feed for 90 days after application. The herbicide cannot be used in waters open to commercial fishing for fish or shellfish. EPA will require additional data on oncogenic properties for reregistration.

Research Situation
1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.
4. Analytical methods development: residues — requirements met; metabolites and degradation — requirements partly met.
5. Counteraction: removal and inactivation — most requirements met.
6. Mammalian safety determination:
    a. Acute, subacute, and chronic toxicity — requirements met.
    b. Carcinogenicity and teratology: 2-year oncogenicity (rat) — requirements met.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) . . . . $ 35,000
2. 2-year oncogenicity (hamster) 100,000
        Total $135,000

Prognosis
Registration for food fish use unlikely. No known effort is under way to extend the label for food fish use.

Diquat

Use
Herbicide.

Sponsor
Chevron Chemical Co., San Francisco, Calif.

Registration Status
Registered for food fish use. EPA allows residues of diquat in potable water during the review of the petition for tolerance. EPA will require additional oncogenic data for reregistration.

Research Situation
1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.

4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal and inactivation — requirements met.
6. Mammalian safety determination:
    a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); 2-year feeding (rat and dog); and acute dermal, eye, and inhalation studies — requirements met.
    b. Carcinogenicity and teratology: teratology (rat); and histopathological and neuropathological studies on chronic test animals — requirements met.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) . . . . $ 35,000
2. 2-year oncogenicity (rat). 100,000
3. 2-year oncogenicity (hamster) 100,000
        Total $235,000

Prognosis
Reregistration promising. No problems are anticipated when reregistration is required.

Diuron

Use
Herbicide.

Sponsor
AmChem Products, Inc., Ambler, Pa.; E. I. Dupont De Nemours & Co., Inc., Wilmington, Del.

Registration Status
Not registered for fishery use. It is registered in the United States for treating irrigation ditches only and has an aquatic use registration in Canada. EPA will require additional oncogenic data for reregistration.

Research Situation
All research has been completed except for counteraction and teratology studies.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) $35,000

Prognosis
Registration unlikely. There is a tolerance in meat of mammals, but diuron accumulates in fish tissues.

Endothall

Use
Herbicide.

Sponsor
Pennwalt Corp., Fresno, Calif.

Registration Status
Registered for food fish use. Endothall was given an interim food additive tolerance covering use in canals, lakes, ponds, or other potential sources of potable water in 1973.

Research Situation
1. Toxicity to target and nontarget organisms: fish, birds, invertebrates, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.
4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal — requirements met; inactivation — requirements partly met.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity — requirements met.
   b. Carcinogenicity and teratology: life-time oncogenicity (mouse); teratology (rat) — requirements met.

Costs for Required Work to be Done Outside FWS
None; requirements met.

Prognosis
Reregistration promising. No problems are anticipated when reregistration is required.

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Fenac

Use
Herbicide.

Sponsor
AmChem Products, Inc., Ambler, Pa.; and others.

Registration Status
Registered for nonfood fish use. Fenac is registered for use in lakes, drainage ditches, ponds, and reservoirs where the water is not used for irrigation or domestic purposes or for livestock. EPA will require additional oncogenic data for reregistration.

Research Situation
1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.

4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat); chronic feeding (rat and dog); and acute dermal, eye, and inhalation studies — requirements met.
   b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) . . . . $ 35,000
2. 2-year oncogenicity (rat). 100,000
3. 2-year oncogenicity (hamster) 100,000
   Total $235,000

Prognosis
Extended registration uncertain. AmChem Products, Inc. would like to obtain a label for more generalized use, but is pursuing an extension of 2,4-D labels first.

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Silvex

Use
Herbicide.

Sponsor
Dow Chemical USA, Midland, Mich.; and others.

Registration Status
Registered for nonfood fish use. Silvex can be used in lakes and ponds to control emergent or submersed vegetation, but cannot be used where it could contaminate water intended for domestic use, irrigation, or crop spraying.

Research Situation
1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.
4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal — most requirements met; inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity — requirements met.

b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) . . . . $ 35,000
2. 2-year oncogenicity (rat). 100,000
3. 2-year oncogenicity (hamster) 100,000
      Total $235,000

Prognosis

Food use registration uncertain. No known research is under way to extend the label for food use. Silvex is a candidate for the Rebuttable Presumption Against Registration list.

Simazine

Use

Herbicide.

Sponsor

Ciba-Geigy Corp., Greensboro, N.C.

Registration Status

Registered for food fish use. Simazine has a tolerance of 0.1 ppm in potable water and 12 ppm in agricultural commodity fish. The registration allows use of simazine in ponds (single owner and little or no outflow). An experimental use permit was granted for experiments on algae in lakes in 1975. EPA will require additional oncogenic data for reregistration.

Research Situation
1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.
4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal — most requirements met; inactivation — requirements partly met.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity — requirements met.
   b. Carcinogenicity and teratology: 2-year oncogenicity (rat) — requirements met.

Costs for Required Work to be Done Outside FWS
1. Teratology (rabbit) . . . . $ 35,000
2. 2-year oncogenicity (hamster) 100,000
      Total $135,000

Prognosis

Extended registration uncertain. The compound may require more research.

Anesthetics

MS-222 (Tricaine Methanesulfonate)

Use

Anesthetic.

Sponsor

Ayerst Laboratories, New York, N.Y., and others.

Registration Status

Registered for food fish use as an anesthetic; 21-day withdrawal period required after use on food fish.

Research Situation
1. Toxicity to target and nontarget organisms: fish, invertebrates, birds, and plants — requirements met.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.
4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal — requirements met; inactivation — not needed.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity — not needed.
   b. Carcinogenicity and teratology — not needed.

Costs for Required Work to be Done Outside FWS
1. Mutagenicity — Ames test or equivalent $1,500

Prognosis

Desired registration has been achieved. Further research will be directed toward reducing the 21-day withdrawal time.

Quinaldine Sulfate, and the Combination of MS-222 and Quinaldine Sulfate

Use

Anesthetic.

Sponsor

FWS; a possible producer is McLaughlin Gormley King Co., Minneapolis, Minn.

Registration Status

Not registered for fishery use.

Research Situation

1. Toxicity to target and nontarget organisms: fish — requirements met; invertebrates and plants — not needed; birds — no work done.
2. Field testing: geographic areas, ecotypes, efficacy, and delivery systems — requirements met.
3. Physiological studies: mode of action, biotransformation, and excretion — requirements met.
4. Analytical methods development: residues, metabolites, and degradation — requirements met.
5. Counteraction: removal and inactivation — no work done.
6. Mammalian safety determination:
   a. Acute, subacute, and chronic toxicity: acute oral LD_{50} (rat) — requirements met.
   b. Carcinogenicity and teratology — no work done.

Costs for Required Work to be Done Outside FWS

1. Acute dermal (rabbit) . . . . $ 300
2. Acute primary dermal irritation (rabbit) 150
3. Acute primary eye irritation (rabbit) 200
4. Acute inhalation (rat) 500
5. 90-day subacute oral (rat) 25,000
6. 90-day subacute oral (dog) 25,000
7. Teratology (rabbit) 35,000
8. Metabolism (cow) 100,000
9. Metabolism (rat) 25,000
10. 2-year oncogenicity (rat) 100,000
11. 2-year oncogenicity (hamster) 100,000
12. 6-month feeding (dog) 35,000
13. Mutagenicity — Ames test or equivalent 1,500
14. Avian acute oral (three species) 5,000
15. 8-day avian subacute dietary (mallard) 1,000
16. 8-day avian subacute dietary (quail or pheasant) 1,000
17. 1-generation reproduction (bobwhite quail or mallard) 20,000
18. Residues — methodology 60,000
19. Residues — use pattern 40,000
20. Residues — metabolites 60,000
    Total $634,650

Prognosis

Registration promising. McLaughlin Gormley King Co. has expressed interest in supporting a limited amount of the needed research. After reviewing New Animal Drug Applications for quinaldine sulfate and the combination of quinaldine sulfate and MS-222 submitted by FWS in 1974, FDA required additional research for consideration of a registration for food fish use. Some contract studies on this compound may not be needed if FDA waives certain of its requirements.

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Appendix 1. *Flow chart of activities required for the registration of fishery chemicals by the Environmental Protection Agency (EPA) or by the Food and Drug Administration (FDA).*
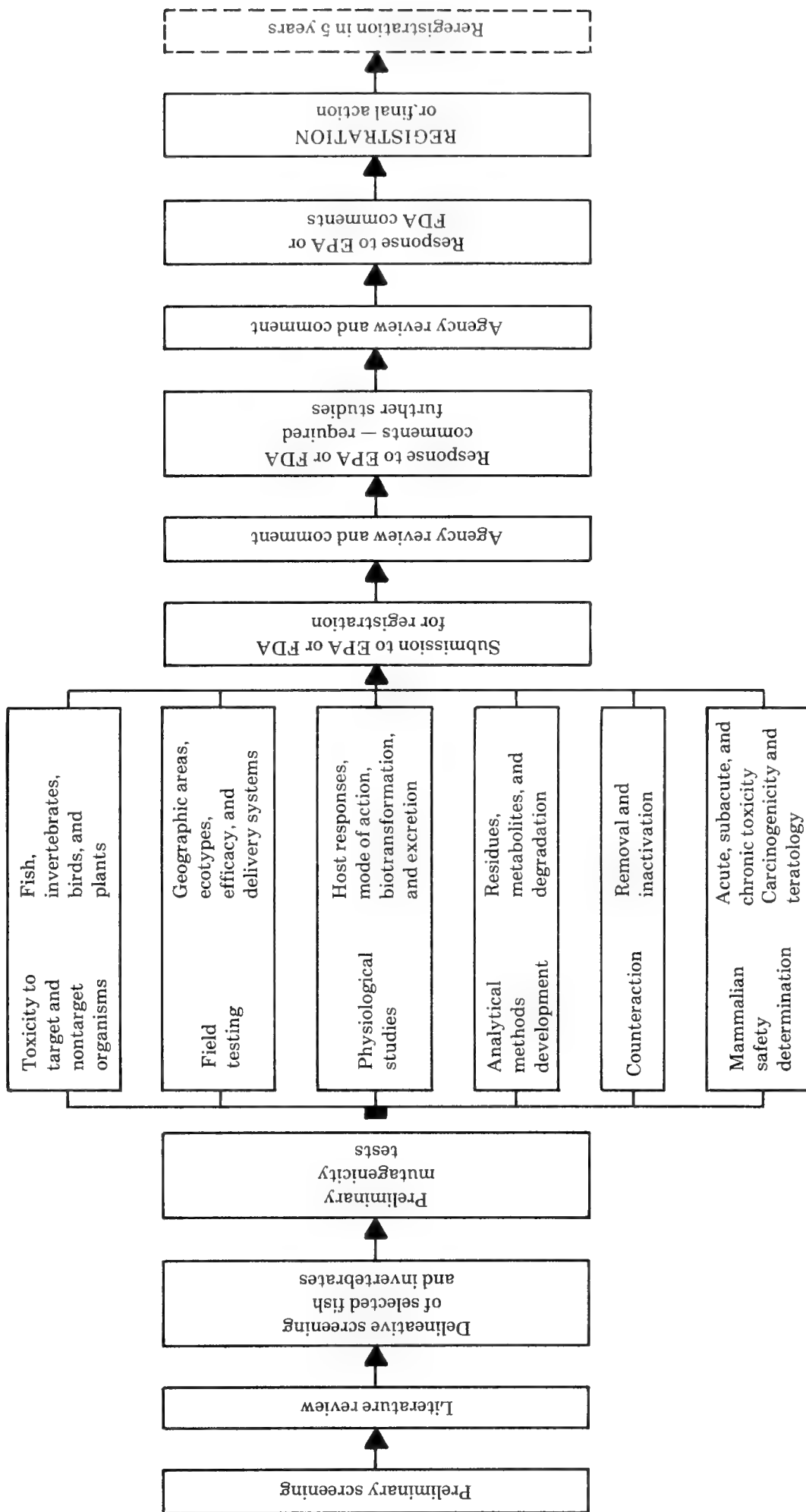
Appendix 2. *Status of research on fishery chemicals, January 1978 (c = complete, pc = partly complete; o = no work done).*

	Preliminary investigations	Preliminary mutagenicity tests	Toxicity to non-target organisms	Field tests	Physiological studies	Analytical methods and residues	Counteraction	Mammalian safety — acute, subacute, chronic toxicity	Mammalian safety — developmental problems	Submissions to FDA or EPA[a]	Comment or situation[a]
Piscicides, lampricides, or collecting aids											
Antimycin	c	o	pc	c	pc	pc	pc	pc	o	c	Registered by EPA
Bayer 73	c	c	c	c	c	c	c	c	c	o	Ready for submission to EPA
GD-174	c	c	pc	pc	o	pc	pc	pc	o	o	Experimental use only
Rotenone	c	c	c	c	pc	pc	c	pc	pc	c	On RPAR list
Squoxin	c	o	pc	c	pc	pc	o	pc	o	o	Being sponsored by NMFS
TFM	c	c	c	c	c	c	c	c	c	c	Awaiting final action by EPA
Thanite	c	o	pc	pc	pc	pc	pc	pc	o	o	Research discontinued
Therapeutants, disinfectants, pond sterilants, oxidizing agents, and osmoregulatory enhancers											
Betadine	c	o	pc	c	c	c	c	c	c	o	Ready for submission to FDA
Calcium hypochlorite	c	c	c	c	c	c	c	c	c	c	Registered by EPA
Formalin	c	c	c	c	c	c	c	c	pc	c	Awaiting final action by FDA
Furanace	c	o	c	c	pc	pc	c	pc	o	o	Responding to FDA comments for use on food fish
Furazolidone	c	c	pc	pc	o	pc	o	pc	pc	o	Notice to cancel filed by FDA
Hyamine 1622	c	c	pc	pc	o	pc	o	pc	o	o	Research renewed in 1977
Lime	c	c	c	c	c	c	c	c	c	c	GRAS
Malachite green	c	o	pc	c	pc	pc	pc	pc	pc	o	Research terminated
Masoten	c	c	c	c	pc	pc	o	pc	c	c	Nonfood use only, on RPAR list
Nitrofurazone	c	c	pc	pc	o	pc	o	pc	o	o	FDA may cancel
Potassium permanganate	c	c	pc	pc	pc	pc	pc	pc	o	c	Awaiting ruling by EPA
R05-0037	c	o	pc	pc	pc	pc	o	pc	c	o	Experimental use only

Appendix 2. Continued. *Status of research on fishery chemicals, January 1978 (c = complete, pc = partly complete; o = no work done).*

	Preliminary investigations	Preliminary mutagenicity tests	Toxicity to non-target organisms	Field tests	Physiological studies	Analytical methods and residues	Counteraction	Mammalian safety — acute, subacute, chronic toxicity	Mammalian safety — developmental problems	Submissions to FDA or EPA[a]	Comment or situation[a]
Sodium chloride	c	c	c	c	c	c	c	c	c	c	GRAS
Sulfamerazine	c	c	c	c	c	c	c	c	c	c	Registered for food fish
Terramycin	c	c	c	c	c	c	c	c	c	c	Registered for food fish
Herbicides and algicides											
Copper sulfate	c	c	c	c	c	c	pc	c	c	c	Registered for food fish
2,4-D	c	c	c	c	c	c	pc	c	c	c	Restricted registration
Dichlobenil	c	c	c	c	c	pc	pc	c	pc	c	Nonfood use only
Diquat	c	c	c	c	c	c	c	c	pc	c	Registered for food fish
Diuron	c	c	c	c	c	c	pc	c	pc	o	Registered for irrigation ditches only
Endothall	c	c	c	c	c	c	pc	c	c	c	Registered for food fish
Fenac	c	c	c	c	c	c	o	c	o	c	Nonfood use only
Silvex	c	c	c	c	c	c	pc	c	o	c	Nonfood use only
Simazine	c	c	c	c	c	c	pc	c	pc	c	Registered for food fish
Anesthetics											
MS-222	c	o	c	c	c	c	c	c	c	c	Registered by FDA
Quinaldine sulfate	c	o	pc	c	c	c	o	pc	o	c	Awaiting final action by FDA

[a]Abbreviations: EPA = Environmental Protection Agency; FDA = Food and Drug Administration; GRAS = Generally Recognized as Safe; NMFS = National Marine Fisheries Service; RPAR = Rebuttable Presumption Against Registration.

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66. Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in a Stream Ecosystem after Treatment for Control of Sea Lampreys, by P. A. Gilderhus, J. B. Sills, and J. L. Allen. 1975. 7 pp.

67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals, by L. L. Marking and V. K. Dawson. 1975. 7 pp.

68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations, by R. M. Burress. 1975. 8 pp.

(Reports 69 and 70 are in one cover.)

69. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2'5,-dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture to Fingerlings of Seven Fish Species and to Eggs and Fry of Coho Salmon, by T. D. Bills and L. L. Marking. 1976. 9 pp.

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71. Field Tests of Isobornyl Thiocyanoacetate (Thanite) for Live Collection of Fishes, by R. M. Burress, P. A. Gilderhus, and K. B. Cumming. 1976. 13 pp.

72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests, by L. L. Marking and T. D. Bills. 1976. 11 pp.

(Reports 73 through 76 are in one cover.)

73. Formalin: Its Toxicity to Nontarget Aquatic Organisms, Persistence, and Counteraction, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1977. 7 pp.

74. Chlorine: Its Toxicity to Fish and Detoxification of Antimycin, by L. L. Marking and T. D. Bills. 1977. 5 pp.

75. Malachite Green: Its Toxicity to Aquatic Organisms, Persistence, and Removal with Activated Carbon, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1977. 6 pp.

76. Toxicity of Furanace to Fish, Aquatic Invertebrates, and Frog Eggs and Larvae, by L. L. Marking, T. D. Bills, and J. H. Chandler, Jr. 1977. 6 pp.

(Reports 77 through 79 are in one cover.)

77. Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM), 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture as Lampricides in Laboratory Studies, by V. K. Dawson, K. B. Cumming, and P. A. Gilderhus. 1977. 11 pp.

78. Toxicity of the Molluscicide Bayer 73 and Residue Dynamics of Bayer 2353 in Aquatic Invertebrates, by H. O. Sanders. 1977. 7 pp.

79. Accumulation, Elimination, and Biotransformation of the Lampricide 2',5-Dichloro-4'-nitrosalicylanilide by *Chironomus tentans*, by J. A. Kawatski and A. E. Zittel. 1977. 8 pp.

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80. Effects of Antimycin A and Rotenone on Macrobenthos in Ponds, by L. J. Houf and R. S. Campbell. 1977. 29 pp.

81. Aquatic Macroinvertebrates in a Small Wisconsin Trout Stream Before, During, and Two Years After Treatment with the Fish Toxicant Antimycin, by G. Z. Jacobi and D. J. Degan. 1977. 24 pp.

82. *Investigations in Fish Control:* Index to Numbers 1–72, 1964–76, by R. A. Schnick and K. A. Graves. 1977. 19 pp.

(Reports 83 through 85 are in one cover.)

83. Survival of Two Species of Freshwater Clams, *Corbicula leana* and *Magnonaias boykiniana*, After Exposure to Antimycin, by L. L. Marking and J. H. Chandler, Jr. 1978. 5 pp.

84. Chronic and Simulated Use-Pattern Exposures of Brook Trout (*Salvelinus fontinalis*) to 3-Trifluoromethyl-4-nitrophenol (TFM), by W. P. Dwyer, F. L. Mayer, J. L. Allen, and D. R. Buckler. 1978. 6 pp.

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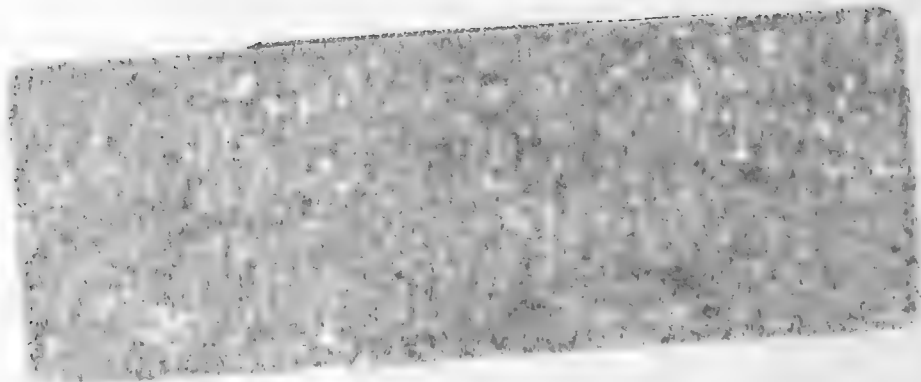
Fish Control Laboratories
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INVESTIGATIONS IN FISH CONTROL

UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

Investigations in Fish Control, published by the Fish and Wildlife Service, include reports on the results of work at the National Fishery Research Laboratory at La Crosse, Wis., and the Southeastern Fish Control Laboratory at Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy. [See Investigations in Fish Control 47-50 (in one cover) for list of issues published prior to 1973.]

(Reports 51 and 52 are in one cover.)
51. Methods for Simultaneous Determination and Identification of MS-222 and Metabolites in Fish Tissues, by Charles W. Luhning. 1973. 10 pp.
52. Residues of MS-222, Benzocaine, and Their Metabolites in Striped Bass Following Anesthesia, by Charles W. Luhning. 1973. 11 pp.

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53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish, by Verdel K. Dawson and Leif L. Marking. 1973. 11 pp.
54. The Efficacy of Quinaldine Sulfate:MS-222 Mixtures for the Anesthetization of Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.
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56. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to 10 Species of Algae, by A. W. Maki, L. D. Geissel, and H. E. Johnson. 1975. 17 pp.
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60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests, by L. L. Marking and L. E. Olson. 1975. 27 pp.
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62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae, by J. H. Chandler, Jr. and L. L. Marking. 1975. 7 pp.

(Reports 63 through 66 are in one cover.)
63. Laboratory Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM) as a Lampricide, by V. K. Dawson, K. B. Cumming, and P. A. Gilderhus. 1975. 13 pp.
64. Effects of 3-Trifluoromethyl-4-nitrophenol (TFM) on Developmental Stages of the Sea Lamprey, by G. W. Piavis and J. H. Howell. 1975. 8 pp.
65. Accumulation and Loss of Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in Fish Muscle Tissue: Laboratory Studies, by J. B. Sills and J. L. Allen. 1975. 10 pp.
66. Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in a Stream Ecosystem after Treatment for Control of Sea Lampreys, by P. A. Gilderhus, J. B. Sills, and J. L. Allen. 1975. 7 pp.
67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals, by L. L. Marking and V. K. Dawson. 1975. 7 pp.
68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations, by R. M. Burress. 1975. 8 pp.

INVESTIGATIONS IN FISH CONTROL

87. Ethyl-*p*-aminobenzoate (Benzocaine): Efficacy as an Anesthetic for Five Species of Freshwater Fish

By V. K. Dawson and P. A. Gilderhus

88. Influences of Selected Environmental Factors on the Activity of a Prospective Fish Toxicant, 2-(Digeranyl-amino)-ethanol, in Laboratory Tests

By C. A. Launer and T. D. Bills

89. Toxicities of the Lampricides 3-Trifluoromethyl-4-nitrophenol (TFM) and the 2-Aminoethanol Salt of 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73) to Four Bird Species

By R. H. Hudson

Ethyl-*p*-aminobenzoate (Benzocaine): Efficacy as an Anesthetic for Five Species of Freshwater Fish

by

V. K. Dawson and P. A. Gilderhus
U.S. Fish and Wildlife Service
National Fishery Research Laboratory
La Crosse, Wisconsin 54601

Abstract

Ethyl-*p*-aminobenzoate (benzocaine) was tested for its efficacy as an anesthetic for rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), northern pike (*Esox lucius*), carp (*Cyprinus carpio*), and largemouth bass (*Micropterus salmoides*). Since benzocaine is not water soluble, it was applied with acetone as a carrier. Concentrations of 100 to 200 mg/l were required for large adult northern pike, compared with 50 to 100 mg/l for small fish. Rates of sedation and recovery were slower in cold water than in warm water. Water hardness had little influence on the activity of benzocaine. Fish were anesthetized faster and recovered more slowly in acid than in alkaline water. Benzocaine produced deep anesthesia, but concentrations that rendered the fish handleable within 5 min were generally not safe for exposures longer than 15 min. Concentrations of benzocaine efficacious for fish were not acutely toxic to eggs of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout, brown trout, or lake trout (*Salvelinus namaycush*). Benzocaine is not registered for fishery use and is neither more effective nor safer than the registered anesthetic, tricaine methanesulfonate (MS-222).

Many substances have been tested for their ability to anesthetize fish (McFarland 1959; Bell 1967; McErlean 1967; Schoettger and Julin 1967; Schoettger and Julin 1969; Howland and Schoettger 1969; Gilderhus et al. 1973). Few of these have been used extensively as anesthetics, however, and only one—tricaine methanesulfonate (MS-222)—is registered for fishery use by the U.S. Food and Drug Administration (Office of the Federal Register 1976).

McErlean (1967) suggested the use of ethyl-*p*-aminobenzoate (benzocaine) as a possible alternative to MS-222, and McErlean and Kennedy (1968) compared some anesthetic properties of the two compounds. Wedemeyer (1970) and Soivio et al. (1977) evaluated physiological effects of anesthesia with MS-222 and benzocaine on rainbow trout (*Salmo gairdneri*). Both studies revealed similarities between the two compounds at pH 7.0.

Benzocaine and MS-222 are similar in structure but differ in the addition of a methanesulfonate group to MS-222, to make it water soluble; the amine group is *meta*-substituted in MS-222 and *para*-substituted in benzocaine.

Although benzocaine is not registered for fishery use, it has been used extensively in veterinary (Windholz 1976) and human medicine (Baker 1976).

The present study was conducted to evaluate the potential of benzocaine as a fish anesthetic, under selected water conditions, for several species and sizes of fish. The investigation involved a laboratory study with subadult fish and a field study with adult northern pike (*Esox lucius*). The toxicity of benzocaine to fish eggs which might be exposed during hatchery spawning operations was also evaluated.

Materials and Methods

Benzocaine (90%) used in the tests was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Stock solutions were made up in acetone and added to the test vessels in the quantities needed to produce the desired concentrations. Fish eggs were exposed in glass jars containing 2.5 liters of soft reconstituted water. Subadult fish were exposed in polyethylene tanks containing 45 liters of water and adults in tanks containing 100 liters.

Hardness of water used in the tests was produced by adding selected amounts of salts to aerated, deionized water as described by Marking and Dawson (1973). Selected pH values (from 6.5 to 9.5) were produced and maintained with chemical buffers according to the method of Dawson et al. (1975). Temperatures of 7, 12,

1

Table 1. *Efficacy of benzocaine as an anesthetic against five species of fish in standard reconstituted water.*

Species and average weight (g)	Temp (°C)	Concn (mg/l)	Time (min) to loss of: Equilibrium[a]	Reflex	Exposure time (min)	Recovery time (min)	Survival (%)
Rainbow trout							
65	12	50	2.8	5.0	10	12.0	60
Brown trout							
1	12	50	2.1	2.5	10	3.4	5
90	7	50[b]	3.9	8.8	10	6.7	100
90	12	50	2.4	4.5	10	7.4	100
90	17	50	2.5	4.3	10	7.5	10
Carp							
50	12	100	2.6	3.7	15	18.0	100
Largemouth bass							
50	12	50[b]	9.3	9.7	15	11.5	100
Northern pike							
6	7	60[b]	7.0	13.3	15	23.5	100
6	12	60[b]	4.0	8.0	15	20.0	100
6	17	60[b]	2.8	7.5	15	8.5	100
6	12	100	2.5	3.5	15	15.0	100
1,543	3	200	5.0	5.0	15	35.0	100
1,543	12	200	3.0	3.0	15	35.0	100

[a]Loss of equilibrium, stage 2 of Schoettger and Julin (1967).
[b]Loss of reflex did not occur within the desired period of 5 min.

and 17 C were maintained with water baths.

We tested the anesthetic against rainbow trout, brown trout (*Salmo trutta*), northern pike, carp (*Cyprinus carpio*), and largemouth bass (*Micropterus salmoides*), and evaluated its toxicity to green eggs of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout, brown trout, and lake trout (*Salvelinus namaycush*). Eggs were exposed to the anesthetic within 24 h after fertilization.

Fish used in the laboratory tests were obtained from Federal or State fish hatcheries, maintained under a fish culturist's care, and acclimated to test waters 2 days before the benzocaine was added. Ten fish or 25 fish eggs were exposed to each concentration of anesthetic.

Field tests were conducted with adult northern pike collected from the Mississippi River with fyke nets in April. Most were females (average length, 61 cm, and weight, 1,543 g) that had been artificially spawned 3 days previously. Most tests were conducted at the State Fish Hatchery, Lansing, Iowa, in water from the Mississippi River (temperature, 3 C; pH, 7.8; total alkalinity, 135 mg/l as $CaCO_3$; and total hardness, 192 mg/l as $CaCO_3$). Tests at the National Fishery Research Laboratory were conducted in well water (temperature, 12 C; pH, 7.8; total alkalinity, 206 mg/l as $CaCO_3$; and total hardness, 224 mg/l as $CaCO_3$). Three fish were exposed to each concentration.

The tests were designed to establish the effective concentration that would result in loss of reflex and thus render the fish handleable within 5 min and still permit survival of all fish after at least 10 min of exposure. We determined the various stages of anesthesia by using characteristics defined by Schoettger and Julin (1967): sedation (decreased reactivity to stimuli); partial loss of equilibrium (swimming ability disrupted); total loss of equilibrium, stage 1 (usually the fish turn over but swimming ability persists); total loss of equilibrium, stage 2 (locomotion ceases but fish respond to pressure on the caudal peduncle); loss of reflex (failure to respond to external stimuli); and medullary collapse (opercular activity ceases).

We analyzed mortality data according to the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s (concentration causing 50% mortality) and 95% confidence intervals. A safe exposure index was obtained by dividing the time required for the first fish to reach medullary collapse by the time required for fish to reach loss of reflex (Schoettger and Julin 1967). An index value of less than 1.0 indicates that the anesthetic causes mortality before it produces the desired level of anesthesia.

Results

Efficacy to Subadult Fish

At 12 C, rainbow trout and brown trout exposed to 50 mg/l of benzocaine showed a total loss of equilibrium (stage 2) within less than 3 min and loss of

reflex within 5 min (Table 1). A 10-min exposure at 50 mg/l caused a mortality of 40% in rainbow trout and 95% in brown trout fry. Brown trout exposed to the 50-mg/l concentration at 7 C did not experience loss of reflex within the desired period of 5 min, and at 17 C, 90% died.

Warmwater fishes consistently withstood longer exposures and higher concentrations than did cold-water species. For example, largemouth bass and northern pike fingerlings exposed to concentrations of 50 and 60 mg/l, respectively, at 12 C did not reach loss of reflex within 5 min; at 100 mg/l, however, the northern pike—as well as carp—were completely anesthetized. However, exposure to the 100-mg/l concentration for 30 min killed subadults of all five species (data not shown). The safe exposure indices for the different species ranged from 1.5 to 2.5.

Efficacy to Adult Northern Pike

Adult northern pike did not progress through the stages of anesthesia shown by small fish. Most remained active until they turned on their sides. They had then lost reflex, having bypassed total loss of equilibrium, stages 1 and 2. Several fish were hyperactive for 20 to 30 s immediately before they turned over. Northern pike exposed to 200 and 300 mg/l in 12 C well water at the Laboratory exhibited some headshaking immediately after being placed in the solution, suggesting that the solution might be mildly irritating.

Anesthetization of adult northern pike at 3 C required exposure for 7 to 8 min to concentrations of 100 to 160 mg/l of benzocaine. A concentration of 200 mg/l rendered them motionless in 4.0 to 6.5 min at 3 C and in 2.5 to 3.5 min at 12 C. Anesthesia was induced in 3.5 to 4.8 min at 3 C and in 2.0 to 3.5 min at 12 C at a concentration of 300 mg/l of benzocaine; however, some mortality occurred at the higher concentration. The length of time required for fish to recover in fresh water varied from 35 to 100 min after 15 min of exposure to 200 mg/l at 3 C.

In the 3 C water at the Lansing (Iowa) tests, there were no mortalities among fish exposed to 200 or 300 mg/l for 30 min. In the 12 C water at the Laboratory, northern pike suffered 67% mortality (two of three fish) after 60 min of exposure to 200 mg/l and after 30 min of exposure to 300 mg/l.

Effect of Temperature

There was almost no difference in the rates of anesthesia at 12 and 17 C for brown trout, and the times to loss of equilibrium and reflex were only slightly longer for subadult northern pike at 12 C than at 17 C. At 7 C, however, the times to loss of equilibrium and reflex were significantly longer for both

species. Little difference was noted in recovery time for brown trout at any of the test temperatures, but northern pike recovered considerably faster at 17 C than at 7 or 12 C (Table 1).

Effect of Water Hardness and pH

Water hardness had little influence on the average time to loss of reflex in subadult brown trout; the rate of anesthesia, however, appeared to be somewhat slower at higher pH's (Table 2). The rate of recovery after anesthesia (10-min exposure to 50 mg/l) was not influenced by water hardness or pH except that recovery tended to be slower at pH 6.5 than at pH 8.0 or 9.5 (Table 3).

Table 2. *Average time (min) for 10 subadult brown trout to reach loss of reflex in 50 mg/l of benzocaine at 12 C in waters of different total hardness and pH.*

	Water hardness		
pH	Soft	Hard	Very hard
6.5	3.3	3.1	4.2
8.0	4.9	3.7	5.0
9.5	5.3	4.3	5.2

Table 3. *Average recovery time (min) for 10 subadult brown trout after a 10-min exposure to 50 mg/l of benzocaine at 12 C in waters of different total hardness and pH.*

	Water hardness		
pH	Soft	Hard	Very hard
6.5	11.2	6.3	9.9
8.0	4.6	5.7	5.7
9.5	4.8	6.4	8.2

Safety to Fish Eggs

The toxicity of benzocaine to green eggs ranged from an LC_{50} of 88.0 mg/l for rainbow trout to 43.0 mg/l for coho salmon after 1 day of exposure (Table 4). The 5-day LC_{50}'s for eggs of these species were 47.0 and 38.4 mg/l, respectively. The most sensitive eggs after 5 days of exposure were those of brown trout (LC_{50} = 17.8 mg/l). Toxicity to eggs of all species tested was not substantially increased by exposures as long as 10 days.

Eggs of rainbow trout were resistant to benzocaine exposures that might be encountered during hatchery spawning operations. No mortalities occurred after a

4

Table 4. *Toxicity (LC$_{50}$ and 95% confidence interval, mg/l) of benzocaine to fish eggs in standard reconstituted water at 12 C.*

| Species | Exposure period (days) | | |
	1	5	10
Rainbow trout	88.0	47.0	42.5
	75.3-103	36.2-61.0	33.0-54.3
Brown trout	62.0	17.8	14.5
	53.9-71.4	14.1-22.4	11.9-17.7
Lake trout	65.0	36.5	29.2
	56.9-74.3	30.1-44.2	23.1-37.0
Coho salmon	43.0	38.4	32.0
	34.0-54.4	32.2-45.8	28.1-36.5
Chinook salmon	64.1	46.0	44.0
	54.6-75.3	37.7-56.1	33.2-58.4

1-h exposure to 500 mg/l of benzocaine or to a 3-h exposure to 100 mg/l (Table 5). Exposure for 3 h to 200 and 500 mg/l of benzocaine resulted in mortalities of 24 and 100%, respectively.

Discussion

The observed progression of adult northern pike exposed to the anesthetic, from partial loss of equilibrum directly to loss of reflex, would present no use problem, since fish in the loss of reflex stage are easier to handle.

The activity of many chemicals is influenced by the pH of the test solution, often as a result of an ionization equilibrium where only the un-ionized form of the molecule is able to penetrate the gill membrane (Sills and Allen 1971; Dawson and Marking 1973; Hunn and Allen 1974; Dawson et al. 1977). However, the ionization constant (pKa) for benzocaine (2.38; Fasman 1976) is so low that little ionization occurs at the pH's of 6.5 to 9.5 used in our tests. Therefore most benzocaine molecules should have been in the lipid-soluble, un-ionized form. The apparent increase in activity at lower pH's may be related to increased stress on the fish in acidic water.

The safe exposure indices for benzocaine (1.5-2.5) are closely similar to those of its structural homologue, MS-222, as reported by Schoettger and Julin (1967).

Benzocaine does not depress the pH of poorly buffered solutions as do the fish anesthetics MS-222 and quinaldine sulfate (Dawson and Marking 1973). Benzocaine, however, is not water soluble and is not registered for fishery use. Furthermore, by comparison with the reported efficacy (Schoettger and Julin 1967) and toxicity (Marking 1967) of the registered anesthetic, MS-222, benzocaine is neither more effective nor safer.

Table 5. *Mortalities (%) of green eggs of rainbow trout after exposures to different concentrations of benzocaine for 1 to 3 h and transfer to fresh water for 96 h.*

| Concentration (mg/l) | Exposure period (h) | | |
	0.5	1	3
100	0	0	0
200	0	0	24
500	0	0	100

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Influences of Selected Environmental Factors on the Activity of a Prospective Fish Toxicant, 2-(Digeranylamino)-ethanol, in Laboratory Tests

by

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Abstract

Fathead minnows (*Pimephales promelas*), brown trout (*Salmo trutta*), and rainbow trout (*S. gairdneri*) were used to assess the influences of temperature, pH, turbidity, ultraviolet light, and aquatic vegetation on the toxicity of an experimental fish toxicant, 2-(digeranylamino)-ethanol (GD-174). Also examined was the feasibility of chemical counteraction by oxidation or reduction. The activity of the compound was reduced in cold water, in acid water, and in turbid water where the turbidities were produced by bentonite or Crowley silt loam, and in water containing waterweed (*Elodea* sp.). The activity was not affected by exposure to ultraviolet light, by the presence of duckweed (*Lemna* sp.), or by turbidities produced by kaolin or barium sulfate. GD-174 can be counteracted by either oxidation or reduction; oxidation by potassium permanganate was the most effective procedure tested.

Rotenone and antimycin are currently the only two piscicides registered for use in the United States. Both are general toxicants. Through an intensive screening program seeking selective chemicals for the control of undesirable fishes, Marking (1974) determined that a terpine derivative, 2-(digeranylamino)-ethanol (GD-174), was more toxic to carp than to other warmwater species under laboratory conditions. Although the chemical has shown great promise in laboratory studies as a general fish toxicant and potential for the selective control of carp, results in small-scale field applications have been inconsistent. We conducted several laboratory studies to assess the effects of water temperature, pH, turbidity, aquatic vegetation, and ultraviolet radiation on the activity of GD-174. We also examined the effectiveness of selected oxidizing and reducing agents for counteracting the chemical.

Materials and Methods

Stock solutions of technical grade GD-174 obtained from Glidden Durkee, Division of SCM Corporation, Jacksonville, Florida, were prepared by solubilizing the chemical in an equal volume of 20% glacial acetic acid and then diluting to the desired volume with water. To prepare test solutions of desired concentrations, we pipetted portions of the stock solutions into test vessels and then stirred the mixture to ensure homogeneity.

Fish species tested were brown trout, *Salmo trutta* (average weight 7.5 g); rainbow trout, *S. gairdneri* (2.5 g); and fathead minnows, *Pimephales promelas* (1.1 g). Fish were either obtained from Federal fish hatcheries or cultured at the National Fishery Research Laboratory, La Crosse, Wisconsin.

Acute toxicity tests for the effects of water temperature and pH on the activity of GD-174 were conducted according to methods described by the Committee on Methods for Acute Toxicity Tests with Aquatic Organisms (1975).

In photodegradation studies, stock solutions of GD-174 were exposed to UV light ($21 \, \mu W/cm^2 \times 100$) from a GE-275W sunlamp at a distance of 25 cm from the surface for periods of 6, 12, or 24 h. These solutions were maintained in a temperature-controlled water bath and continuously stirred to ensure homogeneity. Portions of these solutions were then added to test vessels and their toxicities compared with those of reference solutions aged in darkness for the same period.

In studies on turbidity, GD-174 was added to a series of solutions containing either barium sulfate, kaolin, bentonite, or Crowley silt loam (classified as a Typic Albaqualf; consists of 6% sand, 83% silt, 11% clay) and having turbidities of 25, 50, 75, and 100 nephelometric turbidity units (NTU). Turbidity was measured with an HF DRT-100 model turbidimeter. The solutions were compared to a reference series of clear water (0.53 NTU), and the toxicities compared.

In studies to determine the effects of aquatic vege-

1

tation on the toxicity of GD-174, waterweed (*Elodea* sp.) and duckweed (*Lemna* sp.) collected from the wild were used. In outdoor tests, GD-174 was added to polyethylene tanks containing reconstituted water and either 0, 5, 10, or 15 g/l of waterweed or 0 or 5 g/l of duckweed. In outdoor tests, 0, 2, 4, 8, or 12 g/l of waterweed were used. After 24 h, the vegetation was removed, fish were introduced, and the toxicities were compared with those of reference solutions which had contained no vegetation.

In counteraction studies, potassium permanganate (KMnO₄) at 2 mg/l, chlorine at 0.05 mg/l (active Cl_2 from commercial grade calcium hypochlorite), or sodium thiosulfate ($Na_2S_2O_3$) at 10 mg/l was introduced into a series of GD-174 solutions 6 h before the introduction of fish. The toxicities of these solutions were then compared with that of reference solutions.

The method of Litchfield and Wilcoxon (1949) was used to calculate LC_{50}'s and 95% confidence intervals. Significant differences in all data were based on $P = 0.05$. All reported data met the chi-square test for acceptability.

Results

Water Temperature

Toxicity of GD-174 was greater at higher than at lower water temperatures (Table 1). At 96 h, the LC_{50}'s for fathead minnows were significantly higher at 12 C than at 7 C and at 22 C than at 17 C. Furthermore, the toxic action was more rapid at the higher temperatures: there were no significant differences between the 24- and 96-h LC_{50}'s at either 17 or 22 C, whereas at 7 and 12 C the LC_{50}'s for 96 h equaled only about half those for 24 h (Table 1).

pH

The GD-174 was more toxic to fathead minnows in alkaline than in acid waters (Table 1). At 24 h, there were significant differences between the LC_{50}'s at each pH tested (6.5, 7.5, 8.5, and 9.5), and a fourfold difference between pH 6.5 and pH 9.5. At 96 h, there were no significant differences between pH's 7.5 and 8.5 or between pH's 8.5 and 9.5.

Ultraviolet Light

In tests in which stock solutions of GD-174 exposed to UV light were compared with unexposed reference solutions, no significant differences were found in the toxicities to fathead minnows, based on 96-h LC_{50}'s (Table 2).

Table 1. *Toxicity of 2-(digeranylamino)-ethanol to fathead minnows in water of selected temperatures and pH's.*

Temp (°C)	pH	LC_{50} and 95% confidence interval (µl/l)	
		24 h	96 h
7	7.5	>1.0	0.547
			0.461-0.649
12	7.5	0.467	0.200
		0.377-0.578	0.164-0.244
17	7.5	0.196	0.150
		0.169-0.228	0.126-0.179
22	7.5	0.100	0.0750
		0.0803-0.125	0.0573-0.0982
12	6.5	0.820	0.324
		0.712-0.943	0.274-0.383
12	7.5	0.386	0.137
		0.329-0.452	0.113-0.166
12	8.5	0.243	0.100
		0.225-0.263	0.0788-0.126
12	9.5	0.178	0.0730
		0.146-0.216	0.0653-0.0815

Turbidity

The activity of GD-174 was severely reduced in turbid water where the primary source of turbidity was produced by either bentonite or Crowley silt loam. In fact, no mortality of fathead minnows was observed in solutions where bentonite was the source of turbidity. A slight reduction in toxicity was observed in kaolin turbidities, and barium sulfate turbidities had no significant effect on toxicity (Table 3).

Table 2. *Toxicity of 2-(digeranylamino)-ethanol solutions exposed to ultraviolet light (21 µW/cm² X 100) to fathead minnows in soft water at 12 C.*

Exposure[a] or aging time (h)	LC_{50} and 95% confidence intervals (µl/l)	
	Unexposed solutions[b]	Exposed solutions
6	0.268	0.250
	0.241-0.298	0.219-0.285
12	0.200	0.250
	0.172-0.232	0.220-0.283
24	0.172	0.150
	0.127-0.232	0.121-0.185

[a]Time the solutions were irradiated with ultraviolet light.
[b]Unexposed solutions were shielded with aluminum foil.

Table 3. *Effects of selected turbidities on the toxicity of 2-(digeranylamino)-ethanol to fathead minnows.*

Turbidity (NTU)	Source of turbidity[a], exposure time, and LC$_{50}$'s and 95% confidence intervals (μl/l)							
	Bentonite		Crowley silt loam		Kaolin		Barium sulfate	
	24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h
0.53[b]	0.410	0.142	0.353	0.128	0.500	0.128	0.560	0.141
	0.345-0.487	0.119-0.169	0.285-0.438	0.110-0.149	0.413-0.605	0.108-0.152	0.475-0.660	0.118-0.168
25	>2.0	>2.0	0.353	0.247	0.542	0.194	0.448	0.123
			0.285-0.438	0.234-0.357	0.435-0.675	0.165-0.227	0.320-0.627	0.108-0.139
50	>2.0	>2.0	0.690	0.289	0.560	0.209	0.531	0.140
			0.552-0.862	0.234-0.357	0.477-0.658	0.187-0.234	0.400-0.704	0.109-0.179
75	>2.0	>2.0	0.790	0.290	0.557	0.196	0.474	0.184
			0.681-0.916	0.249-0.338	0.446-0.696	0.165-0.232	0.394-0.570	0.155-0.218
100	>2.0	>2.0	0.750	0.292	0.640	0.221	0.532	0.143
			0.582-0.967	0.250-0.341	0.549-0.745	0.181-0.270	0.430-0.658	0.120-0.171

[a]Grams of substance per 15 liters of solution used to produce turbidities of 25, 50, 75, and 100 NTU's were: bentonite—1.5, 3.75, 7.5, and 15.0; Crowley silt loam—1.8, 3.45, 5.1, and 6.75; kaolin—0.375, 0.75, 1.125, and 1.5; and barium sulfate—0.27, 0.615, 0.99, and 1.35.

[b]Turbidity of standard reconstituted water.

Table 4. *Toxicity of 2-(digeranylamino)-ethanol to three species of fish after 24-h exposure of test solutions to duckweed or waterweed.*

Species of fish, and type and amount (g/l) of vegetation	LC$_{50}$ and 95% confidence interval (μl/l)	
	24 h	96 h
Brown trout		
Duckweed		
0	0.347	0.323
	0.278-0.433	0.270-0.386
5	0.323	0.263
	0.275-0.378	0.198-0.347
Rainbow trout		
Waterweed		
0	0.298	0.264
	0.248-0.358	0.222-0.314
5	0.562	0.562
	0.495-0.638	0.495-0.638
10	0.800	0.800
	0.694-0.921	0.694-0.921
15	1.07	1.07
	0.931-1.23	0.931-1.23
Fathead minnows		
Waterweed		
0	0.728	0.218
	0.688-0.771	0.183-0.259
2	0.800	0.390
	0.736-0.869	0.344-0.440
4	1.06	0.363
	0.889-1.26	0.300-0.439
8	1.00	0.618
	0.871-1.15	0.560-0.681
12	1.40	0.720
	1.05-1.88	0.657-0.789

Aquatic Vegetation

Duckweed appeared to have no effect on the toxicity of GD-174, whereas waterweed reduced the toxicity significantly (Table 4). The reduction was directly proportional to the amount of waterweed in the solutions. In rainbow trout, toxicity decreased 20.1% for each 1 g increase of waterweed per liter of solution, and results with fathead minnows were similar.

Counteraction

Our study indicated that GD-174 was subject to detoxification by both oxidation and reduction. However, sodium thiosulfate and chlorine were considerably less effective than potassium permanganate (Table 5).

Table 5. *Toxicity of 2-(digeranylamino)-ethanol solutions containing selected oxidizing or reducing agents to fathead minnows in soft water at 12 C.*

Agent and concentration	LC$_{50}$ and 95% confidence interval (μl/l)	
	24 h	96 h
Reference solution	0.250	0.0895
	0.192-0.324	0.0688-0.116
KMnO$_4$ (2 mg/l)	2.45	1.42
	2.04-2.97	1.14-1.77
Cl$_2$[a] (0.05 mg/l)	0.970	0.450
	0.844-1.12	0.382-0.529
Na$_2$S$_2$O$_3$ (10 mg/l)	0.635	0.263
	0.560-0.720	0.197-0.351

[a]Commercial grade calcium hypochlorite (65% available Cl$_2$).

Discussion and Conclusions

The compound GD-174, like rotenone (Gersdorff 1943; Spitler 1970) and antimycin (Berger et al. 1969), was more toxic at high than at low temperatures. However, unlike rotenone (Brooks 1961; Spitler 1970) and antimycin (Berger et al. 1969), GD-174 was more toxic in alkaline than in acid waters. Toxic action of GD-174 did not appear to be altered by ultraviolet light, whereas that of both antimycin and rotenone is decreased (Dawson 1973; Cheng et al. 1972).

In tests with plants, the toxicity was reduced by the submerged waterweed, whereas the floating duckweed had no effect. Although it is difficult to assess the cause of the differences, it could be related to differences in metabolism, or to differential sorption of the toxicant onto the surface of the plants. Because submerged vegetation has much more area in contact with the solution than the floating variety, it would reduce activity by a greater degree.

In field situations, the inhibiting effects of aquatic vegetation and turbidity on the activity of GD-174 are likely to be additive. Furthermore, interactions between those factors and pH and temperature, which may themselves either antagonize or enhance the activity, will make difficult the choice of the proper concentration for selective carp control. Therefore, a precise on-site toxicity test will be necessary to prescribe the proper concentration for field application.

The counteraction studies showed that the toxicant could be deactivated rapidly with strong oxidizing or reducing agents. Potassium permanganate was the most effective and would be the chemical of choice for field application. However, no chemicals are currently registered for aquatic use of this type.

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Toxicities of the Lampricides 3-Trifluoromethyl-4-nitrophenol (TFM) and the 2-Aminoethanol Salt of 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73) to Four Bird Species[1]

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Abstract

The acute oral toxicities of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73, Bayluscide) were determined in mallards (*Anas platyrhynchos*), ring-billed gulls (*Larus delawarensis*), bobwhites (*Colinus virginianus*), and California quail (*Lophortyx californicus*). Ring-billed gulls were the most sensitive to both chemicals, alone and in combination. Field grade TFM (35% TFM in N,N-dimethylformamide) was toxic; median lethal dosages (LD_{50}'s) ranged from 250 mg/kg in gulls to 546 mg/kg in California quail. Toxicity of Bayer 73 (70%) was lower; LD_{50} values ranged from 500 mg/kg in gulls to more than 2,000 mg/kg in mallards and bobwhites. Field grade TFM was more toxic than purified TFM (> 96% pure) to mallards. Toxicity of a mixture of Bayer 73 with field grade TFM was additive in gulls and possibly additive in mallards. Mallards exposed for 48 h to drinking and swimming water containing the lampricides showed mild signs of intoxication but no birds became severely ill. On the basis of these studies, use of TFM or Bayer 73, alone or in combination, presents little toxic hazard to birds when applied in the amounts routinely used for lamprey control.

The toxicant 3-trifluoromethyl-4-nitrophenol (TFM) has been used extensively in the Great Lakes region for controlling the sea lamprey (*Petromyzon marinus*). Since Howell et al. (1964) reported that the 2-amino-ethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73, Bayluscide) acts synergistically to increase the toxicity of TFM to sea lamprey larvae, Bayer 73 has sometimes been used in combination with TFM at concentrations corresponding to 2% or less of the TFM concentrations, based on active ingredients of each.

Under ideal conditions TFM is 2 to 10 times more toxic to sea lamprey larvae than to most fish, but it is also toxic to many other organisms (Schnick 1972). Bayer 73, on the other hand, is extremely toxic to a wide variety of aquatic organisms, but appears to have little effect on mammals (Marking and Hogan 1967). Although the toxicity of TFM to sea lampreys is increased by the addition of Bayer 73, its selectivity is decreased; consequently fish kills occur more frequently when the combination of the two chemicals is used (Schnick 1972). The present study was conducted to gather basic data on the toxicity of TFM, Bayer 73, and a mixture of the two chemicals to four bird species—mallards (*Anas platyrhynchos*), ring-billed gulls (*Larus delawarensis*), bobwhites (*Colinus virginianus*), and California quail (*Lophortyx californicus*)—and to assess the toxic hazard of aqueous solutions of these compounds to mallards.

Materials and Methods

The lampricides used in these studies, obtained from the National Fishery Research Laboratory, La Crosse, Wisconsin, consisted of purified TFM (TFM[A]; > 96% pure, Aldrich Chemical Co., Lot No. 060217); field grade TFM (TFM[FG]; 35% TFM in N,N-dimethylformamide); and Bayer 73 (70% pure, Chemagro Corp., Lot No. 8059410). Reagent grade N,N-dimethylformamide (DMF), obtained locally, was tested alone and in combination with TFM(A) to assess its contribution to

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toxicity in the field grade formulation.

Mallards used in this study were raised at the Denver Wildlife Research Center, either from stock lines (for the studies with purified and field grade TFM, and DMF) or from 1-day-old ducklings obtained from the Max McGraw Wildlife Foundation, Dundee, Illinois (for the studies on Bayer 73 and the mixture of Bayer 73 with field grade TFM). Ring-billed gulls were obtained from a captive breeding colony maintained at the Denver Wildlife Research Center, and California quail were raised at the Center, from stock lines. Bobwhites came from Highland Game Bird Farms, Frank-town, Colorado. Age and sex of test birds varied, depending on availability. Previous studies have indicated that, unlike mammals, nonbreeding birds show only minor sex-dependent differences in sensitivity to oral administration of pesticides in acute toxicity tests (Tucker and Crabtree 1970; Tucker and Haegele 1971), and differences between age groups are generally small (Hudson et al. 1972). However, these differences occasionally are significant and should not be ignored. The age and sex of the birds used in the present tests are shown in Table 1.

Table 1. *Acute oral toxicity (LD$_{50}$)[a] of lampricides to four species of birds.*

| Species[b] | Lampricide[c] | | | |
	TFM(A)	TFM(FG)	Bayer 73	TFM(FG) + Bayer 73
Mallard	458 (324-649)	308 (237-400)	>2,000	472 (408-546)
Ring-billed gull		250 (170-368)	500 (77.8-3,210)	154 (118-200)
Bobwhite			>2,000	435 (335-565)
California quail		546 (313-950)		

[a]LD$_{50}$ values are expressed as milligrams of toxicant per kilogram of body weight; 95% confidence limits are shown in parentheses.

[b]Age and sex of birds exposed to the different chemicals follow. Mallards: TFM(A) and TFM(FG), 1-year-old drakes; Bayer 73, 1-year-old hens; TFM(FG) + Bayer 73, 14- to 17-week-old drakes. Ring-billed gulls: TFM(FG), immature males and females; Bayer 73, adult females; TFM(FG) + Bayer 73, immature and adult males and females. Bob-whites: all tests, 18- to 24-week-old cocks. California quail: TFM(FG), 1-year-old hens.

[c]Lampricides: TFM(A) = purified, >96% active 3-trifluoro-methyl-4-nitrophenol; TFM(FG) = field grade, 35% active TFM in the carrier N,N-dimethylformamide (DMF); and Bayer 73 = 70% active 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide. The LD$_{50}$'s of DMF alone (not shown) were >2,000 mg/kg in mallards, >185 in ring-billed gulls, and >460 in bobwhites.

Mallards and ring-billed gulls were exposed in groups of 2 to 16 birds in unheated, concrete-floored pens (2.4 × 3.0 m, 2.4 m high), which were enclosed and covered, and contained a 37-liter swimming pond with about 2,450 cm^2 of water surface. Quail were held individually in indoor cages (30 × 56 cm, 23 cm high) provided with a water trough. All birds were held in the pens for at least 1 day before they were exposed to lampricides. Temperature in the indoor test room was maintained at 20 to 24 C. Mallards and quail were fed a commercially prepared game bird diet, and gulls a commercially prepared dry dog food. Feed was available ad libitum, except during a 20-h fasting period before oral administration of toxicants for the acute toxicity tests. Water was available ad libitum, and fresh water was constantly available in the mallard and gull cages, except during 48-h exposures of mallards to lampricides in the water under static conditions.

For determination of the acute oral median lethal dosage (LD$_{50}$), the test compounds were administered either by stomach tube or in gelatin capsules inserted through glass tubing. Each compound was delivered to the level of the proventriculus in mallards and gulls and to the crop in both species of quail. Gelatin capsules were used for the administration of TFM(A) to mallards; of Bayer 73 to mallards, gulls, and bobwhites; and of DMF to gulls and bobwhites. A stomach tube was used to deliver TFM(FG) to mallards, gulls, and California quail, and DMF to mallards.

In tests to assess the toxicity of a mixture of the lampricides, TFM(FG) and Bayer 73 were administered sequentially (because the chemicals could not be successfully administered simultaneously) to mallards, gulls, and bobwhites as follows: (1) Bayer 73 in gelatin capsules was administered through glass tubing; (2) a small amount of pure DMF was administered by stomach tube; and (3) within 5 to 18 min, TFM(FG) was administered by stomach tube. Dosages of the individual lampricides administered to each animal provided the active ingredients in the ratio of 98 parts TFM to 2 parts Bayer 73 (34.3% active TFM and 0.7% active Bayer 73 in the mixture).

Three to six birds were treated at each of two to four geometrically spaced dosage levels. Acute oral LD$_{50}$ values were computed by the methods of Thompson (1947) and Weil (1952).

The indices used to describe the toxicity of lampricides administered in combination were derived by Marking and Dawson (1975). An additive index greater than 0 indicates greater than additive toxicity (synergism), an index less than 0 indicates less than additive toxicity (antagonism), and an index of 0 indicates simple additive toxicity of the toxicants in a mixture. If the confidence interval for the additive

index overlaps 0, simple additive toxicity is indicated.

Mallards were exposed to lampricide-contaminated water in their swimming ponds for 48 h under static conditions. Experimental groups in these tests were composed of five males and five females. The ages of the ducks exposed to the different chemicals were as follows: TFM(A), 6-8 weeks; TFM(FG) and Bayer 73, 1 year; and the mixture of TFM(FG) with Bayer 73, 17 weeks. Appropriate quantities of TFM(A) were dissolved in 59.2 ml ethanol and then added to the tap water in the swimming ponds to make up treatment levels of 5, 15.8, 50, and 500 mg/l of active TFM. Only the solvent (59.2 ml of ethanol) was added to the swimming pond of the control group. In other swimming ponds TFM(FG) was at concentrations of 57.1 and 286 mg/l (20 and 100 mg/l active TFM); Bayer 73 at concentrations of 0.01, 0.10, and 1.0 mg/l (0.007, 0.07, and 0.70 mg/l of active Bayer 73); and the mixture of TFM(FG) and Bayer 73 (of the same composition as that administered orally) at concentrations of 11.7, 58.4, and 292 mg/l (4.01 and 0.082, 20.0 and 0.409, and 100 and 2.04 mg/l of active TFM and Bayer 73, respectively). At the end of the 48-h exposure, the lampricide-contaminated water was flushed out and replaced with tap water, augmented by a small continuous flow. The swimming ponds of the controls for all tests other than TFM(A) contained untreated tap water.

Mortality, signs of intoxication, and body weight changes were observed for 14 to 21 days after treatment. (In general, the weights of the survivors of all studies were normal by the end of the observation periods.)

Results

Acute Oral Toxicity Tests

Of the species exposed, ring-billed gulls were the most sensitive to all of the compounds (TFM[A] not tested), alone or in combination (Table 1). Median lethal dosages for TFM(FG) ranged from 250 mg/kg in gulls to 546 mg/kg in California quail. Bayer 73 was less toxic than TFM; LD_{50} values ranged from 500 mg/kg in gulls to >2,000 mg/kg in mallards and bobwhites.

The additive toxicity indices of combinations of lampricides and solvent (DMF) are presented in Table 2. TFM(A) and DMF produced an additive index of 1.98 (greater than additive toxicity) with mallards. TFM(FG) and Bayer 73 produced an additive index of −0.506, which suggests less than additive toxicity, although the range for that index could not be calculated because there was no 95% confidence interval for Bayer 73 tested individually. Since the index is close to zero, however, the toxicity possibly was additive. The combination of TFM(FG) and Bayer 73 was additive in

toxicity to ring-billed gulls (the interval for the additive index, −0.179 to 2.17, overlapped zero).

Mallards treated with oral doses of TFM(FG) drank excessive amounts of water and showed a number of other responses: regurgitation, stumbling, excessive sitting, weakness, incoordination, imbalance, labored breathing, immobility, and terminal wing-beat convulsions accompanied by arching of the head and neck over the back. Mallards treated with TFM(A) also displayed these signs of intoxication (except for weakness), in addition to social withdrawal, imperturbability, convulsions, and temporary whole-body rigidity. Signs of intoxication were produced more rapidly by TFM(FG) than by TFM(A): signs of TFM(FG) intoxication appeared as soon as 1 min after treatment, and deaths from 4 to 30 min after treatment, whereas the effects from TFM(A) did not appear for 13 min and deaths occurred 20 to 50 min after treatment. Recovery (when it occurred) usually was complete within 4 h after treatment with TFM(FG), but was prolonged for up to 7 or 8 days after treatment with TFM(A).

The signs of intoxication observed in ring-billed gulls dosed with TFM(FG) were similar to those observed in mallards with the addition of rapid breathing, imperturbability, wing-droop, outspread wings, and temporary whole-body rigidity, but the gulls did not display stumbling, weakness, imbalance, immobility, or terminal wing-beat convulsions. The timing of the appearance of signs and deaths was usually similar to that in mallards; however, one gull died 11 days after treatment. There was no apparent sex-related difference in response among the gulls.

After treatment with TFM(FG), California quail displayed signs of intoxication similar to those observed in gulls and mallards, except that regurgitation did not occur. The timing of the appearance of signs and deaths was also similar.

Bayer 73 produced intoxication in all of the species tested at all of the dosage levels administered. Signs observed in mallards included tenseness of the masseter, regurgitation, high carriage, excessive drinking and sitting, imperturbability, incoordination, imbalance, rapid and labored breathing, tremors, and temporary whole-body rigidity followed by death. Signs observed in bobwhites and gulls were similar; however, regurgitation in gulls was more profuse, especially at the higher dosage levels, and probably accounts for the wide confidence interval (Table 1) calculated for this test (regurgitation did not occur in bobwhites). Deaths occurred at 500, 1,000, and 2,000 mg/kg in mallards (one of the six mallards treated at each of these levels died), and one of three bobwhites treated at 2,000 mg/kg died. Toxic signs in all three species appeared as soon as 15 min after treatment and persisted for up to 24 h; deaths occurred from

4

30 min to 3 h after treatment.

After treatment with the mixture of TFM(FG) and Bayer 73, signs observed in mallards included most of those observed after treatment with the individual chemicals. The timing of the appearance of effects, deaths, and recovery in mallards was similar to that after treatment with TFM(FG) alone. Toxic signs observed after treatment of ring-billed gulls with the mixture were similar to those observed in mallards, although the appearance of signs, deaths, and recovery was more rapid: deaths usually occurred in 2 to 12 min and recovery within 1 h. Signs of intoxication observed after treatment of bobwhites with the mixture were similar to those observed in mallards, except that regurgitation did not occur. Timing of the appearance of signs, deaths, and recovery was also similar to that observed in mallards.

Signs of intoxication in mallards (1-year-old drakes) given DMF at dosages up to 2,000 mg/kg were limited to slowness, incoordination, and falling, which developed within 10 min after treatment. All ducks used in the tests had recovered within about 24 h. Bobwhites (19- to 20-week-old cocks) treated at dosages up to 505 mg/kg and gulls (immature and adult males and females) treated at dosages up to 249 mg/kg showed no signs of intoxication after DMF treatment.

Water Exposure Toxicity Tests

No mortalities occurred in mallards exposed to lampricides in the ponds in their enclosures, and body weights by the end of the observation periods were normal. However, various clinical signs of poisoning were observed.

All of the 6- to 8-week-old mallards exposed to 5 mg/l TFM(A) appeared normal throughout the 48-h exposure and subsequent 12-day observation period. In the group treated with 15.8 mg/l, several birds appeared wobbly and showed slight masseter tenseness about 7 h after exposure began. About 22 h later most of the mallards in this test group may have had slight degrees of incoordination, and 49 h after exposure the group was underactive and all birds tended to sit; one drake showed a moderate degree of incoordination and jerky movements. All birds in the 50-mg/l group were strongly affected after 3 h of exposure. They displayed excessive sitting, underactivity, and weakness of the leg muscles, and some ran and fell with moderate to extreme degrees of incoordination. Although one bird was extremely incoordinated 22 h after exposure began, most birds had begun to recover. All were normal 24 to 48 h after exposure ended. The group exposed to 500 mg/l apparently avoided consumption of the solution after initial exposure. This group sat huddled together more than controls and stumbled and showed slight to moderate degrees of incoor-

dination; they appeared normal 24 h after exposure ended.

The 1-year-old mallards exposed to 57.1 mg/l TFM(FG) appeared normal throughout the study. Those exposed to 286 mg/l displayed incoordination, slowness, and stumbling; however, recovery occurred within 24 h after the end of treatment.

During the water exposure studies of Bayer 73 and the mixture of Bayer 73 with TFM(FG), only mild signs of intoxication were observed. One mallard exposed to 0.10 mg/l Bayer 73 showed slight incoordination 24 to 48 h after the beginning of treatment, and one exposed to 292 mg/l of the mixture showed slight incoordination on the day treatment was begun.

Discussion

The present tests showed that TFM is moderately toxic to birds exposed by acute oral administration, and that it has about the same magnitude of toxicity in birds as in mammals. Bayer 73 appears to have a low order of toxicity in birds, although birds may be more susceptible than mammals to acute oral administration. The analysis for additive toxicity revealed that the mixture of TFM(A) and DMF worked synergistically in mallards (Table 2). Maki et al. (1975), who studied the toxicity of purified and field grade TFM to several species of algae, noted that DMF in the field grade material may augment the toxicity of TFM; Sanders and Walsh (1975) found a synergistic effect of the field grade formulation in crayfish (*Orconectes nais*); and Marking and Olson (1975) noted that the field grade TFM was more toxic to several species of fish. Apparently the carrier (DMF) increases dispersion, reduces particle size, or enhances the ionic state of the TFM molecule (Marking and Olson 1975). The increased activity of TFM(FG) in mallards can probably be attributed to increased absorption of TFM in the gastointestinal tract as a result of the presence of DMF. Whether DMF acts by altering the epithelial lining, or merely by changing the state of the TFM (solution vs. solid) is not known, but DMF is known to increase the epidermal absorption of many substances (Wurster 1972).

In these studies it appears that the mixture of TFM(FG) with Bayer 73 was additive in ring-billed gulls and possibly additive in mallards. Kawatski et al. (1975) noted that the toxicity of TFM and Bayer 73 to larvae of a midge (*Chironomus tentans*) also was usually additive.

Concentrations of the lampricide TFM routinely used for sea lamprey control range from 1 to 15 mg/l (active ingredient), and exposures generally last 12 to 15 h. Concentrations of Bayer 73 routinely used are 2% or less of the TFM concentration. Exposures of mallards to drinking and swimming water containing

Table 2. *Additive toxicity indices of lampricides administered individually and in combination to birds in acute oral tests.*

Species and lampricide[a]	LD$_{50}$[b] and 95% confidence limits		Additive index[c]
	Individually	In combination	
Mallard TFM(A) and	458 (324-649)	108 (83.0-140)	1.98
DMF	> 2,000	200 (154-260)	
Mallard TFM(FG) and	308 (237-400)	463 (400-535)	-0.506
Bayer 73	> 2,000	4.72 (4.08-5.46)	
Ring-billed gull TFM(FG) and	250 (170-368)	151 (116-196)	0.647 (-0.179-2.17)
Bayer 73	500 (77.8-3,210)	1.54 (1.18-2.00)	

[a]Lampricides: TFM(A) = purified, > 96% active 3-trifluoromethyl-4-nitrophenol; DMF = reagent grade N,N-dimethylformamide; TFM(FG) = field grade, 35% active TFM in DMF; and Bayer 73 = 70% active 2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicylanilide.

[b]LD$_{50}$ values are expressed as milligrams of toxicant per kilogram of body weight.

[c]For discussion of derivation, see Marking and Dawson (1975).

lampricides lasted longer than those used in field treatments; no mortalities occurred at any of the concentrations tested; and toxic responses were lacking or mild at the concentrations used in sea lamprey control.

Conclusions

- The lampricide TFM was toxic to birds in acute oral tests, and apparently has about the same magnitude of toxicity in birds as in mammals.
- Bayer 73 appeared to have a low order of toxicity in birds, but may be more toxic to birds than to mammals.
- Field grade TFM appeared to be more toxic than purified TFM to mallards.
- Mixtures of Bayer 73 and TFM (2:98) appeared to have additive toxic action in ring-billed gulls and possibly additive action in mallards.
- Among mallards exposed to lampricides in the drinking and swimming water, none died at the concentrations tested, and toxic responses to use-pattern concentrations were lacking or mild.

- The addition of TFM or Bayer 73, alone or in combination, to the environment in the amounts routinely used for lamprey control appears to present little toxic hazard to birds.

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