SYNERGISM OF 5, 2'-DICHLORO-4'-NITRO-SALICYLANILIDE AND 3-TRIFLUORMETHYL-4-NITROPHENOL IN A SELECTIVE LAMPREY LARVICIDE

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SYNERGISM OF 5, 2'-DICHLORO-4'-NITRO-SALICYLANILIDE AND 3-TRIFLUORMETHYL-4-NITROPHENOL IN A SELECTIVE LAMPREY LARVICIDE

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CONTENTS

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ABSTRACT

The molluscicide 5,2'-dichloro-4'-nitro-salicylanilide improves the action of the selective lamprey larvicide 3-trifluormethyl -4-nitrophenol (TFM). Laboratory bioassays with mixtures containing various percentages of the two chemicals indicated that up to 3 percent of the salicylanilide could be added to TFM without destroying its selective toxicity, but other considerations made use of a Z-percent mixture most advisable. Tests of mixtures containing 2 percent of the salicy-anilide in waters from 26 different sources brought out the effect of chemical condition of the water on the toxicity, selectivity, and relative degree of improvement over TFM alone. Simulated stream treatments, in concrete raceways, and six treatments of streams tributary to Lake Michigan substantiated the laboratory findings. Field use of the mixture reduces treatment costs by approximately 50 percent.

Introduction

The discovery that chemicals belonging to the class of mononitrophenols containing halogens were selectively toxic for larvae of sea lamprey, *Petromyzon marinus,* was a significant advance in the attempt to control this species in the upper Great Lakes (Applegate, Howell, and Smith, 1958). One of these nitrophenols, 3-trifluormethyl-4-nitrophenol (TFM), was studied extensively and subsequently developed for field use (Applegate, *et al.,* 1961).

The first applications of TFM were made in streams tributary to Lake Superior in 1958. It has since been widely used in the Lake Superior and Lake Michigan basins in the treatment of streams containing sea lamprey larvae. Results of these treatments indicate that selective toxicants can reduce the number of sea lamprey. [1] The large amount of TFM required and its high cost have prompted a search for materials which might supplement or replace it in the control program.

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[1] Report of Annual Meeting, Great Lakes Fishery Commission, June 26 and 27, 1963, Ann Arbor, Michigan.

In the screening of various chemicals, it was discovered that 5,2'-dichloro-4'-nitro-salicylanilide was extremely toxic to sea lamprey larvae. Tests revealed that small amounts of 5, 2'-dichloro-4'-nitro-salicylanilide improved the toxicity of TFM without affecting significantly its selectivity toward lamprey larvae. Synergism, as used in this report, is considered to be the simultaneous action of separate agencies which, together, have greater total effect than the sum of their individual effects. This synergy and its effect on the toxicity and selectivity to lamprey larvae and certain fishes are the subject of this report.

5,2'-dichloro-4'-nitro-salicylanilide is commercially available, under the trade name Bayluscide: as a 'IO-percent wettable powder of its ethanolamine salt. It is used principally as a molluscicide for the control of freshwater snails. A considerable amount of literature is available on Bayluscide dealing with its physical and chemical properties, influence of environmental factors on its efficiency, acute and chronic mammalian toxicity, mode of toxic action, and results of field trials as a molluscicide. Some of the more important and comprehensive studies have been published as a single volume?

Bioassays

Laboratory tests were conducted with Bayluscide and a commercially available, 30-percent (by weight) aqueous formulation of the sodium salt of TFM. Various percentage mixtures of the two compounds were prepared by suspending an appropriate amount of Bayluscide in the TFM formulation. The low solubility of Bayluscide in distilled water, 230 ppm $\pm$ 50 ppm at room temperature, required that these suspensions be agitated until their final dilution to test concentrations.

Laboratory facilities, equipment, and procedures were similar to those described by Applegate, *et al.,* (1957, 1961). Larval sea lampreys and fingerling rainbow trout, Salmo *gaird*-neri, from 3.5 to 5.0 inches in total length, served as test animals. Lampreys were collected from streams tributary to Lakes Michigan and Huron. Rainbow trout were hatchery-reared fish provided by the Michigan Department of Conservation.

Tests were set up by first pouring 5 liters of water in lo-liter (lo-inch diameter) glass battery jars placed in a 55°

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[2] Registered trade mark of Farbenfabriken Bayer AG, Leverkusen, Germany.

[3] *Pflanzenschutz Nachrichten "Bayer",* Vol. XV No. 1, 1962; published by Farbenfabriken Bayer AG, Leverkusen, Germany.

F. constant-temperature bath. Two lamprey larvae and 2 rainbow trout were added to each jar. Oxygen levels were maintained by stone aerators at near-saturation during the test. The containers were covered with a sheet of clear glass to prevent the escape of fish. Test animals were left undisturbed for 1 to 2 hours to permit them to adapt to the test environment.

Bioassay concentrations and the final 6-liter test volume were obtained by adding 1 liter of an aqueous stock of appropriate strength to each test jar. The aqueous stocks were prepared from samples of the chemicals weighed to the nearest milligram on a Voland Speedigram balance. The final concentrations in the test containers were checked colorimetrically. The absorption maxima for Bayluscide and TFM, 385 $m\mu$ and 395 $m\mu$ respectively, are sufficiently close to allow mixtures to be analyzed by modifying slightly the method described for TFM by Smith, Applegate, and Johnson (1960).

Dilution waters for test solutions were obtained from several sources depending on the purpose of the experiments. Tests to compare the biological activity of TFM, Bayluscide, and mixtures containing various percentages of the two compounds were made in a single lot of water taken from Hammond Bay, Lake Huron. This water was stored in a covered concrete tank. Daily measurements of the physical and chemical characteristics of this water did not change significantly during storage. Average values over the period were: oxygen, 11.4 ppm; carbon dioxide, 1.0 ppm; methyl-orange alkalinity, 88.0 ppm; phenolphthalein alkalinity, 3.5 ppm; pH, 8.2; conductivity, 180.9 μ mhos/18° C.

Applegate, et al., (1961) noticed that the biological activity of TFM varied with physical and chemical changes in the dilution water. Instances have also been reported where TFM retained its toxicity but lost its selectivity toward larval lampreys (Howell and Marquette, 1962). The extent to which these variations might exist with a mixture of TFM and Bayluscide was determined by assays in waters collected from widely separated sources in the Lake Huron and Lake Michigan basins. Physical and chemical analyses of these dilution waters were made immediately before their use.

Twenty replications, based on a total of 40 specimens of each species, were made at each concentration in the tests to determine the relative toxicity of TFM, Bayluscide, and various percentage mixtures of the two. The responses of the test animals were observed hourly during the 20-hour test period. In assays conducted to test the effect of varying chemical and physical characteristics of the dilution water, 4 replications were made at each concentration. This reduction in the number of tests was necessitated by the limited volume of water that could be transported to the laboratory. Observations of each test in this series were made hourly for the first 8 hours and at the end of 20 hours.

The combined data from the replications at each concentration were tabulated at the completion of the bioassay. Assays were designed to produce data from which two levels of concentration- minimum lethal and maximum allowable-could be determined. The minimum lethal value (MLC_{100}) is the lowest concentration in a series that produces 100-percent mortality among larval lampreys. The maximum allowable concentration (MAC_{25}) is the highest which did not kill more than 25 percent of the rainbow trout. Howell and Marquette (1962) used these two values in applying bioassay data to the treatment of streams. They termed the difference between MLC_{100} and MAC25 as the working range; the ratio of the working range to MLC_{100} is the permissible additional flow. This ratio indicates the factor by which volume of flow at the point of application may increase in a stream treated at the MAC_{25} level before the concentration will be diluted to the MLC_{100}. They considered a permissible additional flow of 1.0 (i.e., a doubling) as the lowest acceptable for stream treatment.

Because of the high toxicity of Bayluscide to larval lampreys, the effect of adding small amounts of the compound to TFM were investigated. Bioassays were conducted with TFM, Bayluscide, and mixtures of the two in which Bayluscide made up 1, 2, 3, 4, and 5 percent of the total weight.

Evaluation of the relative merit of TFM, Bayluscide, and mixtures of the two must take into consideration the toxicity to both test species and the margin of selectivity as reflected in the permissible additional flow. Bayluscide is more than 43 times as toxic to larval lampreys as TFM, but its toxicity to rainbow trout is so great that it is practically non-selective between the two. The addition of Bayluscide to TFM progressively reduced MLC,,, , MAC,,, and working range as the percentage of Bayluscide increased from 1 to 5 percent (Table 1). Exceptions were the mixtures containing 3- and 4-percent Bayluscide where the values of MLC,,, were the same. The permissible additional flow was higher at l-percent Bayluscide (1.66) than with TFM alone (1.29), but decreased to 1.00 when Bayluscide made up 2 or 3 percent and less than 1.00 when Bayluscide exceeded 3 percent.

Additions of more than 3 percent of Bayluscide to TFM can not be justified because they result in a permissible

Table 1. - Toxicity of TFM, Bayluscide, and various mixtures of the two compounds to sea lamprey larvae and rainbow trout

[Tests conducted for a 20-hour period at a water temperature of 55° F.; test water was a single lot taken from Hammond Bay, Lake Huron.]

Percentage composition of test material		Minimum lethal concentration for larval lamprey (ppm)	Maximum allowable concentration for rainbow trout (ppm)	Working range (ppm)	Permissible additional flow[1] (cfs)
TFM	Bayluscide				
100	0	3.50	8.00	4.50	1.29
99	1	2.25	6.00	3.75	1.66
98	2	2.00	4.00	2.00	1.00
97	3	1.50	3.00	1.50	1.00
96	4	1.50	2.50	1.00	0.66
95	5	1.13	2.00	0.87	0.77
0	100	0.08	0.09	0.01	0.13

1 Per 1 cfs of flow at point of treatment at the maximum allowable concentration.

additional flow less than the limiting value of 1.00, but lesser percentages produce significant increases in toxicity to larvae without reducing selectivity below a practical level. The MLC $_{100}$ for mixtures containing l-, 2-, and J-percent Bayluscide (2.25, 2.00, and 1.50 ppm) represent reductions of 36, 43, and 57 percent from the value of 3.50 ppm for TFM. Corresponding decreases in MAC,, were 25, 50, and 62 percent.

The greatest saving in the amount of toxicant required to kill larval lampreys without reducing selectivity below a practical limit was with the mixture containing J-percent Bayluscide. Other considerations led us, however, to accept the lesser benefit of the 2-percent mixture for further work.

Preliminary testing with mixtures of TFM and Bayluscide to determine the need for a study such as reported here indicated that in some test waters the 3-percent mixture exceeded the level at which selectivity starts to decline below the acceptable limit. Since the material was to be used in waters with a wide range of chemical and physical characteristics, it seemed desirable to concentrate further study on the a-percent mixture which had consistently produced acceptable selectivity.

A second consideration favoring the use of the a-percent mixture was the physical characteristics of the TFM-Bayluscide combination. Bayluscide is largely in suspension in the aqueous mixture of toxicants. Even though the mixture is agitated mechanically throughout a chemical application the Bayluscide tends to settle out. This tendency results in slight fluctuations in the percentage composition of the toxicants; it seemed discreet, therefore, to use the safer a-percent mixture.

Proof of the synergistic action of Bayluscide appears in the records of the actual amount of TFM and Bayluscide in each of the percentage mixtures at the MLC_{100} and MAC $_{25}$ levels (Table 2). The amount of each compound present in the MLC $_{100}$ levels for the mixtures is considerably below the concentration required to produce the same mortality with the compounds separately. The same synergy is evident in the response of rainbow trout to mixtures containing 1- and 2-percent Bayluscide. Mixtures containing 3, 4, and 5 percent are not synergistic since the amount of Bayluscide is equal to or greater than that required to produce a MAC_{25}.

The evidence of synergistic action of the mixture containing 2-percent Bayluscide and 98-percent TFM is especially significant since this mixture was selected for additional testing and field use. The MLC_{100} for the a-percent mixture is 2.00 ppm- 0.04 ppm Bayluscide and 1.96 ppm TFM. These concentrations individually are non-toxic to lamprey larvae, but they coact to produce loo-percent mortality. Similarly,

Table 2. - Actual amount of TFM and Bayluscide in the minimum lethaland maximum allowable concentrations of various percentage mixtures
[Based on same bioassays as Table 1.]

Percentage composition of test material		Minimum lethal concentration for larval lamprey (ppm)	Amount in minimum lethal concentration (ppm)		Maximum allowable concentration for rainbow trout (Ppm)	Amount in maximum allowable concentration (ppm)	
TFM	Bayluscide		TFM	Bayluscide		TFM	Bayluscide
100	0	3.50	3.50	. . .	8.00	8.00	. . .
99	1	2.25	2.23	0.02	6.00	5.94	0.06
98	2	2.00	1.96	0.04	4.00	3.92	0.08
97	3	1.50	1.46	0.05	3.00	2.91	0.09
96	4	1.50	1.44	0.06	2.50	2.40	0.10
95	5	1.13	1.07	0.06	2.00	1.90	0.10
0	100	0.08	. . .	0.08	0.09	. . .	0.09

the MAC,, value for the same mixture is 4.00 ppm, of which 0.08 ppm is Bayluscide and 3.92 ppm is TFM. Neither of these concentrations is toxic to rainbow trout; nevertheless, they coact to produce 25-percent trout mortality.

Chemical and physical characteristics of natural waters strongly influence the toxicity of TFM (Applegate, *et al.,* 1961). Kanayama (1963) described a method for estimating the MLC_{100} and MAC,, for TFM from the alkalinity and conductivity of the test waters. To determine the effect of these factors on the relative toxicity of TFM and TFM plus 2-percent Bayluscide (TFM-2B) comparative bioassays were run in dilution waters from 26 different sources. These waters varied widely enough in pH, conductivity, and alkalinity to be representative of most streams infested with sea lamprey larvae. No detailed analysis was made of these bioassays to determine if the relation of alkalinity and conductivity to biological activity which Kanayama (1963) noted for TFM also applies to TFM-2B. The results indicate, however, that a similar correlation exists.

The data on physical and chemical properties of the 26 dilution waters and the results of the 26 assays (Table 3) bring out the influence of the quality of the dilution water. Both TFM and TFM-2B were more effective in the softer waters with the lower pH values. As conductivity, alkalinity, and pH increased, the MLC,,, value became greater. A comparison of the MLC_{100} values of TFM to the same values for TFM-2B indicates that their relative toxicity is also influenced by water quality. In the softest water tested, Milligan Creek, Presque Isle County, Michigan, the two materials were equally toxic to larval lampreys. As water hardness increased, the differential toxicity increased to approximately a 2:1 ratio of TFM to TFM-2B.

The values of MLC,,,, MAC,,, and working range in all 26 dilution waters (Table 4) were lower for TFM-2B than for TFM. Differences in the permissible additional flowvaried randomly. The values were the same for TFM and TFM-2B in 7 streams. TFM had the higher value in 11 streams and TFM-2B in 8. No complete loss of selectivity of the kind noted by Howell and Marquette (1962) appeared. The lowest permissible additional flows for both TFM and TFM-2B (1.00 and 1.33 cfs) were for water from Good Harbor Creek, Leelanau County, Michigan. The highest values were 5.00 with TFM in water from Lake Huron, Presque Isle County, Michigan, and 4.67 with TFM-2B in water from Devils River, Alpena County, Michigan.

Table 3. - Variations in the biological activity of TFM and TFM-2B in waters of differing physical and chemical characteristics

[Tests conducted for a 20-hour period at a water temperature of 55° F.; all streams in State of Michigan.]

Source of test water	Minimum lethal concentration (ppm)		Maximum allowable concentration (ppm)		Properties of test water			
	TFM	TFM-2B	TFM	TFM-2B	Conductivity (μmhos/18° C.)	CC2 (Ppm)	Alkalinity (ppm $CaCO_3$)	pH
Milligan Creek Presque Isle County	1.0	1.0	5.0	4.0	99.3	3.4	67.5	7.3
Lake Huron Presque Isle County	1.5	1.25	9.0	4.5	181.6	1.3	104.0	7.9
Devils River Alpena County	3.0	1.5	13.0	8.5	265.4	2.1	171.0	7.9
Little Fishdam River Delta County	4.0	2.0	18.0	9.0	214.4	3.0	120.5	7.6
Cheboygan River Cheboygan County	4.0	2.0	18.0	9.0	250.6	1.4	184.0	8.1
Trout River Presque Isle County	4.0	2.0	17.0	8.5	268.6	3.3	210.0	7.8
Carp Creek Presque Isle County	4.0	2.5	22.0	9.0	202.0	1.0	181.5	8.4
Crystal River Leelanau County	5.0	3.0	14.0	10.0	227.9	1.0	130.0	8.4

(continued)

Table 3. - Continued

Source of test water	Minimum lethal concentration (ppm)		Maximum allowable concentration (ppm)		Properties of test water			
	TFM	TFM-2B	TFM	TFM-2B	Conductivity (μ mhos/18° C.)	CO_2 (ppm)	Alkalinity (ppm $CaCO_3$)	pH
Acme Creek Grand Traverse County	5.0	3.0	22.0	11.0	247.5	2.9	154.0	7.7
Good Harbor Creek Leelanau County	5.0	3.0	10.0	7.0	250.7	1.0	132.0	8.4
Betsie River Benzie County	5.0	3.0	14.0	10.0	260.9	1.0	148.5	8.2
Davenport Creek Mackinac County	5.0	3.5	18.0	11.0	214.8	1.0	172.0	8.3
Carp Lake River Emmet County	5.0	3.5	22.0	12.0	268.5	1.0	196.5	8.4
Manistee River Manistee County	6.0	3.0	20.0	9.0	260.5	1.0	150.0	8.4
Platte River Benzie County	6.0	3.0	20.0	10.0	266.0	1.2	155.0	8.1
Pere Marquette River Mason County	6.0	3.0	22.0	11.0	297.3	1.4	145.0	8.0
Jordan River Charlevoix County	6.0	3.0	20.0	8.0	303.8	1.4	221.0	8.2

(continued)

Table 3. - Continued

Source of test water	Minimum lethal concentration (ppm)		Maximum allowable concentration (ppm)		Properties of test water			
	TFM	TFM-2B	TFM	TFM-2B	Conductivity (μ mhos/18° c.)	CO_2 (Ppm)	Alkalinity (ppm $CaCO_3$)	PH
Au Gres River Arenac County	6.0	3.0	18.0	9.0	544.0	1.5	240.0	8.2
Little Manistee River Manistee County	6.0	3.5	18.0	10.0	223.1	1.0	140.0	8.3
Mitchell Creek Grand Traverse County	6.0	4.0	26.0	13.0	311.6	1.0	250.0	8.5
Pentwater River Mason County	7.0	2.5	24.0	12.0	320.6	1.0	163.0	a.2
Boardman River Grand Traverse County	7.0	3.5	22.0	10.0	250.5	1.0	136.0	8.1
Lincoln River Mason County	7.0	3.5	20.0	11.0	297.6	1.2	156.0	8.1
Crow River Mackinac County	9.0	4.5	22.0	12.0	338.7	1.2	203.0	8.2
Hospital Creek Grand Traverse County	10.0	4.0	30.0	15.0	438.8	2.1	217.0	8.0
Hound Creek Benzie County	10.0	5.0	28.0	14.0	385.9	2.2	230.0	8.0

Table 4. - Comparison of toxicity, working range, and permissible additional flow of TFM and TFM-2B in dilution waters from 26 sources

[Based on same bioassays as Table 3.]

Source of test water	Minimum lethal concentration for larval lamprey (ppm)		Maximum allowable concentration for rainbow trout (ppm)		Working range (ppm)		Permissible additional flow[1] (cfs)	
	TFM	TFM-2:	TFM	TFM-2:	TFM	TFM-2B	TFM	TFM-2B
Milligan Creek Presque Isle County	1.0	1.0	5.0	4.0	4.0	3.0	4.00	3.00
Lake Huron Presque Isle County	1.5	1.25	9.0	4.5	7.5	3.25	5.00	2.60
Devils River Alpena County	3.0	1.5	13.0	8.5	10.0	7.0	3.33	4.67
Little Fishdam River Delta County	4.0	2.0	18.0	9.0	14.0	7.0	3.50	3.50
Cheboygan River Cheboygan County	4.0	2.0	18.0	9.0	14.0	7.0	3.50	3.50
Trout River Presque Isle County	4.0	2.0	17.0	8.5	13.0	6.5	3.25	3.25
Carp Creek Presque Isle County	4.0	2.5	22.0	9.0	18.0	6.5	4.50	2.60
Crystal River Leelanau County	5.0	3.0	14.0	10.0	9.0	7.0	1.80	2.33

(continued)

Table 4. - Continued

Source of test water	Minimum lethal concentration for larval lamprey (ppm)		Maximum allowable concentration for rainbow trout (ppm)		Working range (ppm)		Permissible additional flow[1] (cfs)	
	TFM	TFM-2B	TFM	TFM-2B	TFM	TFM-2B	TFM	TFM-2B
Acme Creek Grand Traverse County	5.0	3.0	22.0	11.0	17.0	8.0	3.40	2.67
Good Harbor Creek Leelanau County	5.0	3.0	10.0	7.0	5.0	4.0	1.00	1.33
Betsie River Benzie County	5.0	3.0	14.0	10.0	9.0	7.0	1.80	2.33
Davenport Creek Mackinac County	5.0	3.5	18.0	11.0	13.0	7.5	2.60	2.14
Carp Lake Emmet County	5.0	3.5	22.0	12.0	17.0	8.5	3.40	2.43
Manistee River Manistee County	6.0	3.0	20.0	9.0	14.0	6.0	2.33	2.00
Platte River Benzie County	6.0	3.0	20.0	10.0	14.0	7.0	2.33	2.33
Pere Marquette River Mason County	6.0	3.0	22.0	11.0	16.0	8.0	2.67	2.67
Jordan River Charlevoix County	6.0	3.0	20.0	8.0	14.0	5.0	2.33	1.67

(continued)

Table 4. - Continued

Source of test water	Minimum lethal concentration for larval lamprey (ppm)		Maximum allowable concentration for rainbow trout (ppm)		Working range (ppm)		Permissible additional flow[1] (cfs)	
	TFM	TFM-2:	TFM	TFM-2B	TFM	TFM-2B	TFM	TFM-2B
Au Gres River Arenac County	6.0	3.0	18.0	9.0	12.0	6.0	2.00	2.00
Little Manistee River Manistee County	6.0	3.5	18.0	10.0	12.0	6.5	2.00	1.86
Mitchell Creek Grand Traverse County	6.0	4.0	26.0	13.0	20.0	9.0	3.33	2.25
Pentwater River Mason County	7.0	2.5	24.0	12.0	17.0	7.5	2.43	3.80
Boardman River Grand Traverse County	7.0	3.5	22.0	10.0	15.0	6.5	2.14	1.86
Lincoln River Mason County	7.0	3.5	20.0	11.0	13.0	7.5	1.86	2.14
Crow River Mackinac County	9.0	4.5	22.0	12.0	13.0	7.5	1.44	1.67
Hospital Creek Grand Traverse County	10.0	4.0	30.0	15.0	20.0	11.0	2.00	2.75
Hound Creek Benzie County	10.0	5.0	28.0	14.0	18.0	9.0	1.80	1.80

[1] Per 1 cfs of flow at point of treatment at the maximum allowable concentration.

Raceway Tests

Methods

Experiments with TFM and TFM-2B in running-water raceways measured their biological activity under conditions approaching a natural stream environment. Facilities and techniques were essentially the same as those used by Applegate, et al., (1961) for a series of "simulated stream" tests with TFM. The raceways are a pair of concrete troughs, each 65 feet long, 6 feet wide, and 30 inches deep. A "head" flume, common to both races, delivers a stabilized flow of Lake Huron water to them from a surge tank. The volume of Water entering each raceway was measured through a "V" weir. Water depth was maintained at 18 inches by stopboards in the discharge flumes.

Rainbow trout, brook trout, *Salvelinus fontinalis,* and brown trout, *Salmo trutta,* were used in the raceway tests. The trout, which ranged from 10 to 14 inches in total length, were allowed to move unrestricted within the 65-foot-long test area. Larval lampreys were confined in 4 cylindrical screen cages. The solid-walled lower 6 inches of each cage contained washed beach sand. Larvae were placed in the cages and allowed to burrow before each test was started. Two cages were placed at the mid-point of the race and 2 at the lower end. Confinement of larval lampreys to cages permitted rapid and accurate determinations of mortality. Hourly observations were made on all animals during the 22-hour test period.

Concentrated, aqueous solutions of TFM and TFM-2B were metered into the raceways by fluid-proportioning devices. TFM was fed directly to the incoming water as it passed through the "V" weir by means of a fuel-pump feeder (Anderson, 1962). The TFM-2B concentrate was pumped by a motor-driven, dual-piston proportioning device and delivered, diluted with flush water, to a perforated pipe located at the head of the raceway.

The proportioning devices were checked hourly to assure a constant feed rate. Water flow through the raceways was controlled at 0.336 cfs. The concentration of TFM or TFM-2B was regulated by varying the strength of the stock solution. Concentrations in each test were checked by periodic colorimetric analysis of samples from the head, middle, and tail of each raceway.

Ten pairs of simulated stream tests were made with TFM and TFM-2B. In each pair, the concentrations of the two materials in the two raceways were identical. Concentrations tested

Table 5. - Comparative toxicity of TFM and TFM-PB in raceway tests

[The figures in parentheses indicate the number of specimens of each species exposed; test water from Hammond Bay, Lake Huron.]

Concentration (ppm)	Mean water temperature (° F.)	TFM Percentage mortality Larval lamprey				TFM-2B Percentage mortality Larval lamprey			
		Larval lamprey	Rainbow trout	Brown trout	Brook trout	Larval lamprey	Rainbow trout	Brown trout	Brook trout
1.0	64.5	(100)	0 (5)	0 (5)	0 (5)	0 (100)	0 (5)	0 (5)	0 (5)
1.5	62.5	(1000)	0 (5)	0 (5)	0 (5)	97 (96)	0 (5)	0 (5)	0 (5)
2.0	66.0	(98)	0 (5)	0 (5)	0 (5)	98 (102)	0 (5)	0 (5)	0 (5)
2.5	62.0	(100)	0 (5)	0 (5)	0 (5)	100 (100)	0 (5)	0 (5)	0 (5)
3.0	66.0	(93)	0 (5)	0 (5)	0 (5)	100 (100)	0 (5)	0 (5)	0 (5)
4.0	63.0	100 (98)	0 (5)	0 (5)	0 (5)	100 (98)	0 (5)	40 (5)	0 (5)
5.0	64.5	100 (100)	0 (5)	0 (5)	0 (5)	100 (100)	0 (5)	20 (5)	0 (5)
8.0	63.0	. . .	0 (10)	0 (10)	0 (10)	. . .	10 (10)	20 (10)	0 (10)
10.0	61.5	. . .	0 (10)	0 (10)	0 (10)	. . .	0 (10)	0 (10)	20 (10)
12.0	63.5	. . .	0 (10)	0 (10)	20 (10)	. . .	70 (10)	90 (10)	60 (10)

ranged from 1.0 to 12.0 ppm. Larval lampreys were used in tests at each concentration up to 5.0 ppm. Trout were used in all tests.

Chemical and physical characteristics of the test water were checked daily during the 2-week test period. Values were: oxygen, 8.3 to 8.5 ppm; carbon dioxide, less than 1.0 ppm; methyl-orange alkalinity, 83.0 to 97.0 ppm; phenolphthalein alkalinity, trace to 4.5 ppm; pH, 8.0 to 8.3; conductivity, 176.4 to 192.6 μ mhos/18° C.

The results of the raceway tests (Table 5) are similar to those from the laboratory bioassays. The MLC_{100} for TFM was 4.0 ppm, whereas only 2.5 ppm of TFM-2B were required to kill all larvae. No trout died at any of the TFM concentrations from 1.0 to 10.0 ppm, but 2 of 10 brook trout died at 12.0 ppm. TFM-2B was more toxic to trout than was TFM. Two of 5 brown trout died at 4.0 ppm and trout of at least one species died at each higher concentration. Mortality at 12.0 ppm was 60 to 90 percent.

Comparison of the records of time to death of larval lampreys at various concentrations of TFM and TFM-2B (Table 6) again indicate the much greater toxicity of the mixture. At 2.5 ppm, for example, TFM-2B killed all the larvae in 9 hours, whereas 5.0 ppm of TFM were required for 100-percent mortality in the same time interval. Records for other concentrations bring out similar contrasts.

Stream Treatments

The final phase in the development of TFM (Applegate, *et al.*, 1961) was a series of 3 experimental stream treatments. These treatments were necessary to develop and test various field methods. It was not considered necessary to follow the same procedure with TFM-2B since no basic changes were required in application procedures. The early treatments with TFM-2B are best described as "semi-experimental."

Six streams tributary to the eastern shore of Lake Michigan were treated with TFM-2B in the fall of 1963 as part of a continuing program to control sea lampreys in Lake Michigan. Most relevant to the present report is a comparison of the actual chemical cost with TFM-2B and the estimated cost with TFM in each of these treatments (Table 7).[4] The cost of chemical for treating these 6 streams was reduced by $27,094. This saving exceeds 50 percent of the estimated cost of $53,091 for treating the same waters with TFM.

[4] Interim Report on Sea Lamprey Control, June to November 1963, Bureau of Commercial Fisheries, Ann Arbor, Michigan.

Table 6. - Percentage mortality of sea lamprey larvae at each observation period in raceway tests
[The data for 1.0 ppm have been omitted since no larvae were killed with either compound.]

Time in hours	Concentration (ppm)											
	1.5		2.0		2.5		3.0		4.0		5.0	
	TFM	TFM-2B	TFM	TFM-2B	TFM	TFM-2B	TFM	TFM-2B	TFM	TFM-2B	TFM	TFM-2B
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	5
3	0	0	0	0	0	0	0	13	0	34	1	66
4	0	0	0	0	0	8	0	51	2	78	35	95
5	0	0	0	0	0	42	0	85	12	96	66	100
6	0	0	0	2	0	80	0	95	31	100	84	...
7	0	0	0	12	0	94	6	99	62	...	92	...
8	0	2	0	39	3	99	14	99	74	...	95	...
9	0	6	0	66	7	100	24	99	74	...	100	...
10	0	9	0	77	9	...	35	99	83	...	...	...
11	0	23	0	88	11	...	52	99	92	...	...	...
12	0	33	0	90	17	...	60	100[1]	96	...	...	...
13	0	51	0	94	20	...	68	...	96	...	...	...
14	0	61	1	95	26	...	72	...	98	...	...	...

(Continued)

Table 6. - Continued

Time in hours	1.5 TFM	1.5 TFM-2B	2.0 TFM	2.0 TFM-PB	2.5 TFM	2.5 TFM-2B	3.0 TFM	3.0 TFM-2B	4.0 TFM	4.0 TFM-2B	5.0 TFM	5.0 TFM-2B
15	0	76	5	95	29	...	76	...	98	...	...	...
16	0	79	6	96	32	...	78	...	100	...	...	...
17	0	87	6	96	35	...	78	...	...	...	...	...
18	0	92	6	97	38	...	78	...	...	...	...	...
19	0	93	6	98	42	...	82	...	...	...	...	...
20	0	96	8	...	46	....	82	...	...	...	...	...
21	0	96	9	...	49	...	83	...	...	...	...	...
22	0	97	9	...	52	...	85	...	...	...	...	...

[1] From the 7th to 12th hour only 1 larvae continued to survive. This larvae probably had received a lethal dose of TFM-2B prior to its death at the 12th hour.

Table 7. - Comparison of actual chemical costs with TFM-2B (98 percent by weight 3-trifluormethyl-4-nitrophenol and 2 percent of the synergist, 5,2'-dichloro-4'-hitro-salicylanilide) and estimated cost with TFM

Stream	Discharge at mouth (cfs)	TFM				TFM-2B				
		Concentration (ppm)		Pounds of active ingredient	Estimated cost	Concentration (ppm)		Pounds of active ingredient		Actual cost
		Minimum effective	Maximum allowable			Minimum effective	Maximum allowable	TFM	Synergist	
Mitchell Creek	35	11	20	353	$ 1,045	5.0	12.0	162	4.5	$ 502
Boardman River	236	10	24	7,900	23,384	4.5	8.0	3,585	100.0	10,385
Good Harbor Creek[1]	12	8	16	413	1,223	3.5	7.0	180	5.0	558
Crystal River[1]	16	6	14	431	1,276	3.5	6.0	216	6.0	669
Upper Platte River	129	6	16	3,721	11,014	3.5	8.0	1,782	49.5	5,700
Betsie River[1]	170	6	14	5,118	15,149	3.5	7.0	2,559	68.5	8,183
Total	598	. . .	. . .	17,936	$53,091	. . .	. . .	8,484	233.5	$25,997

[1]Bioassay range estimated for TFM.

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