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Great Lakes Fishery Commission

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ABSTRACT

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been used in the United States and Canada for more than 30 years to control populations of sea lamprey *(Petromyzon marinus)* in the Great Lakes. There is concern that sea lamprey might become resistant to TFM. Lampricide toxicity tests have been conducted at the Hammond Bay Biological Station, Millersburg, Michigan, since the 1950s and examination of TFM toxicity data for larval lamprey from 1963 to 1987 indicated that sea lamprey have not developed increased resistance to TFM. Maintenance of current control practices are unlikely to cause the development of TFM-resistant sea lamprey strains in the foreseeable future.

INTRODUCTION

Studies were initiated at the Hammond Bay Biological Station (HBBS) in 1953 to identify a chemical that would be acutely toxic to larval sea lamprey *(Petromyzon marinus)* and nontoxic to other aquatic organisms. Tests on more than 6,600 chemical compounds (listed in part by Applegate et al. 1957) indicated that halogenated nitrophenols were selectively toxic to larval sea lamprey (Applegate et al. 1958). From this chemical group, 3-trifluoromethyl-4-nitrophenol (TFM) was later developed for field use (Applegate et al. 1961). From 1958 to 1983, about 1,265,000 kg of TFM were applied *to* tributaries of the Great Lakes to control larval sea lamprey (National Research Council of Canada 1985). The use of TFM is expected to continue as the principal lampricide licensed for controlling sea lamprey in the United States and Canada.

Because of the long use of TFM (>30 yrs) and the fact that some sea lamprey survive TFM treatments (Purvis 1979), concern has been expressed that some of the animals may be developing increased resistance to TFM (Lamsa et al. 1980). Development of resistance to pesticides is common among insect pests: nearly 450 species of insects and mites have been classified as resistant to certain pesticides (Carrow 1987). The exposure of insect populations to sublethal doses of pesticides sometimes results in an increase in the frequency of some or all of the genes that transmit resistance (Miller 1987) and eventually results in populations that are resistant to a pesticide. The objective of the present study was to determine if sea lamprey have developed increased resistance to TFM by comparing results of toxicity tests conducted in the 1960s with those of tests conducted in the 1980s.

METHODS

Raw data from static TFM toxicity tests conducted at the HBBS were sorted into two comparable data sets covering the periods from 1963 to 1970 (herein termed the 1960s) and 1985 to 1987 (the 1980s). Test data were selected which met the following criteria: 1) the toxicity test must have been conducted in aerated aquaria containing Lake Huron water, 2) temperature at about 12°C (± 1°C), 3) free-swimming larval lamprey, and 4) the sodium salt of TFM obtained from Hoechst Chemical Company, Frankfurt, West Germany. The larval lamprey used in the tests were collected with electroshockers from tributaries of the Great Lakes, held in flowing Lake Huron water, and fed by the method described by Hanson et al. (1974). The pH of the Lake Huron water ranged from 7.9 to 8.3 and the alkalinity from 80 to 90 mg/L as $CaCO_3$.

The toxicity data were analyzed to estimate the LC (lethal concentration) values, according to the method described by Litchfield and Wilcoxon (1949). Even though the precision of the LC50 values is substantially better than that of the LC99.9 values, the LC99.9 values are good estimates of the "minimum lethal concentration" used by the sea lamprey control personnel (Kanayama 1963). Because the imprecision of the LC99.9 values might mask a change in the toxicity of TFM to larval sea lamprey, we determined both the LC50 and LC99.9 values and their 95% confidence limits. The nonparametric Mann-Whitney U-test was used to compare the LC values for the 1960s with those for the 1980s. We calculated linear regression lines for time (years 1963-1987) versus LC50 and LC99.9 values. A t-test was used to compare the slope of the lines to a slope of zero-a zero slope indicating no changes in toxicity.

RESULTS AND DISCUSSION

We examined the data from more than 450 TFM toxicity tests conducted at the HBBS, but only those from 19 tests-l 3 tests from the 1960s and 6 from the 1980s - met the criteria for this study. The toxicity data from the 1960s tests were based on a nominal 24 h exposure because the tests were terminated at 21-24 hours. For larval lamprey exposed to TFM in the 1960s the LC50 values (mg/L) ranged from 1.0 to 2.8 and averaged 1.7; and the LC99.9 values ranged from 1.6 to 4.0 and averaged 2.6 (Table 1). In the 1980s the comparable LC50 values ranged from 1.0 to 1.7 and averaged 1.4 mg/L; the LC99.9 values ranged from 1.6 to 2.7 and averaged 2.2 (Table 2). A comparison of the toxicity data from the 1960s and 1980s indicated no significant differences between either the LC50 ($P=0.33$) or the LC99.9 values ($P=0.20$).

Regression analysis of LC50 and LC99.9 values over time (years 1963-1987) yielded lines with slopes of -0.017 for the LC50s and -0.027 for the LC99.9s; neither slope differed significantly from zero ($P=0.14$ and 0.085, respectively). This comparison indicated that the toxicity of TFM to sea lamprey has not changed and that sea lamprey have not developed increased resistance to TFM during the interim.

TABLE I. LC-50 and LC-99.9 values (95% confidence limits in parentheses) for static TFM toxicity tests on larval sea lamprey in Lake Huron water at 12°C during the 1960s (1963-1970).

Time[a] (years)	Date	Test duration (hrs)	LC 50 (mg/L TFM)	LC 99.9 (mg/L TFM)
0.40	27 May 63	21	1.0 (0.8-1.2)	2.0 (1.1-3.8)
0.58	1 Aug 63	21	2.8 (2.6-3.2)	4.0 (3.3-4.8)
0.78	9 Oct 63	21	2.7 (2.6-2.8)	3.7 (3.4-4.0)
2.15	23 Feb 65	21	1.6 (1.4-1.9)	2.5 (1.9-3.2)
2.43	7 Jun 65	24	1.7 (1.4-2.2)	3.0 (2.0-4.6)
2.82	27 Oct 65	21	1.7 (1.5-1.9)	3.4 (2.1-5.5)
2.84	1 Nov 65	21	1.7 (1.7-1.8)	(2.2-2.6)
2.88	18 Nov 65	21	1.7 (1.6-1.8)	2.2 (2.0-2.6)
3.07	24 Jan 66	21	1.5 (1.4-1.6)	2.0 (1.8-2.3)
5.73	24 Sep 68	23	1.1 (0.9-1.3)	(1.2-2.1)
6.06	21 Jan 69	22	1.3 (1.2-1.4)	2.2 (1.8-2.9)
7.05	20 Jan 70	21	1.1 (0.9-1.3)	2.0 (1.6-2.4)
7.07	27 Jan 70	21	1.6 (1.4-1.8)	2.5 (2.0-3.2)
Average			1.7	2.6

[a] From the first year of data (1963)

Insects commonly develop resistance to a single pesticide because of an increase in the frequency of certain genes that confer resistance (Schreiber 1987). Changes in genes frequently result when pesticides are applied in sublethal doses over many generations, thereby allowing animals with resistant genes to survive and reproduce. Because some insects may have more than one generation per year, frequent exposure to sublethal doses of a pesticide sometimes leads to increased resistance within a few years. For example, mosquitoes have several generations per year. As a consequence, the malarial mosquito *Anopheles arabiensis* in Sudan developed resistance to malathion after just two years of house spraying (Lines et al. 1984).

Holloway (1986) predicted that it would require 43 to 600 generations for an animal to increase a resistant Mendelian trait to the level that resistance could be detected in 1% of the population. Because a generation of sea lamprey requires about five to seven years, probably only six generations of sea lamprey have been exposed to TFM during the entire 30-year control program. In addi-

TABLE 2. LC-50 and LC-99.9 values (95% confidence limits in parentheses) for static TFM toxicity tests on larval sea lamprey in Lake Huron water at 12°C during the 1980s (1985-1987).

Time" (years)	Date	Test duration (hrs)	LC 50 (mg/L TFM)	LC 99.9 (mg/L TFM)
22.50	2 Jul 85	24	1.6 (1.4-1.8)	2.5 (2.0-3.1)
23.14	21 Feb 86	24	1.7 (1.6-1.9)	2.7 (2.3-3.2)
23.84	3 Nov 86	24	1.4 (1.3-1.6)	2.0 (1.7-2.4)
24.04	14 Jan 87	24	1.4 (1.3-1.5)	2.0 (1.8-2.3)
24.29	15 Apr 87	24	1.3 (1.1-1.5)	2.1 (1.6-2.8)
24.31	23 Apr 87	24	1.0 (0.9-1.1)	1.6 (1.2-2.2)
Average			1.4	2.2

"From the first year of data (1963)

tion, because of the protocol of use, it is unlikely that many sea lamprey are exposed to sublethal doses of TFM. Generally, sea lamprey control managers schedule infested streams to be treated on a three- to five-year cycle (Smith et al. 1974). Often, just before a stream treatment, toxicity tests are conducted to determine the minimum concentration of TFM needed to kill larval sea lamprey and the maximum concentration that can be used without causing significant mortalities to nontarget organisms. During a stream treatment, TFM is applied only by licensed federal or state personnel. Concentrations of TFM are monitored every one or two hours and adjusted to maintain lethal concentrations through the treatment. After treatment, chronic exposure of larval lamprey to TFM residuals does not occur because of the relatively rapid degradation of TFM by microbial and photolytic processes (National Research Council of Canada 1985). Thus, few larval sea lamprey are exposed to sublethal doses of TFM. If the current treatment practices are maintained, no TFM-resistant strains of sea lamprey would be expected to develop.

REFERENCES

APPLEGATE, V. C., J. H. HOWELL, A. E. HALL, JR., AND M. A. SMITH. 1957. Toxicity of 4346 chemicals to larval lamprey and fishes. U.S. Fish Wildl. Serv., Spec. Rep., Fish. 207.

APPLEGATE, V. C., J. H. HOWELL, J. W. MOFFETT, B. G. H. JOHNSON, AND M. A. SMITH. 1961. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 1.35 pp.

APPLEGATE, V. C., J. H. HOWELL, AND M. A. SMITH. 1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science *127:336-338.*

CARROW, J. R. 1987. Review of pesticide resistance: strategies and tactics for management. J. Environ. Qual. 16:287.

HANSON, L. H., E. L. KING, JR., J. H. HOWELL, AND A. J. SMITH. 1974. A culture method for sea lamprey larvae. Prog. Fish-Cult. 36:122-127.

HOLLOWAY, G. J. 1986. A theoretical examination of the classical theory of inheritance of insecticide resistance and the genetics of time to knockdown and dry body weight in Sitophilus oxyzal (L.) (Coleoptera: Curculionidae). Bull. Entomol. Res. 76:661-670.

KANAYAMA, R. K. 1963. The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluoromethyl-4-nitrophenol required for treating lamprey streams. Great Lakes Fish. Comm. Tech. Rep. 7:1-10.

LAMSA, A. K., C. M. ROVAINEN, D. P. KOLENOSKY, AND L. H. HANSON. 1980. Sea-lamprey-Where to from here? Report of the SLIS Control Theory Task Force. Can. J. Fish. Aquat. Sci. 37:2175-2192.

LINES, J. D., M. A. E. AHMED, and D. F. CURTIS. 1984. Genetic studies of malathion resistance in *Anopheles arabiensis* Patton (Diptera: Culicidae). Bull. Entomol. Res. 74:317-325.

LITCHFIELD, J. T., JR., and F. WILCOXON. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99-113.

MILLER, J. 1987. Sublethal doses of insecticides. Letter to Chemical Engineering News. 65(25):3.

NATIONAL RESEARCH COUNCIL OF CANADA. 1985. TFM and Bayer 73: lampricides in the aquatic environment. Environ. Sec. Publ. NRCC 22488. 184 pp.

PURVIS, H. A. 1979. Variations in growth, age at transformation, and sex ratio of sea lampreys reestablished in chemically treated tributaries of the upper Great Lakes. Great Lake Fish. Comm. Tech. Rep. 35.36 pp.

SCHREIBER, F. 1987. Pesticide resistance. Letter to Chemical and Engineering *News. 65(25):3.*

SMITH, B. R., J. J. TIBBLES, and B. G. H. JOHNSON. 1974. Control of the sea lamprey *(Petromyzon marinus)* in Lake Superior, 1953-70. Great Lakes Fish. Comm. Tech. Rep. 26.60 pp.

EFFECTS OF CHANGES IN DISSOLVED OXYGEN ON THE TOXICITY OF 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) TO SEA LAMPREY AND RAINBOW TROUT

by James G. Seelye and Ronald J. Scholefield

ABSTRACT

The toxicity of TFM to larval sea lamprey *(Petromyzon marinus)* and other aquatic organisms is influenced by chemical factors such as pH, alkalinity, conductivity, and hardness. Oxygen levels as low as 30% saturation did not affect the toxicity of TFM to larval sea lamprey, but its toxicity to rainbow trout fingerlings *(Oncorhynchus mykiss)* increased as the oxygen concentration decreased at 13°C but not at 20°C. To help insure safe, effective chemical control of sea lamprey, treatment teams should monitor dissolved oxygen as well as other pertinent water chemistry variables in streams just prior to treatment.

INTRODUCTION

The toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to larval sea lamprey *(Petromyzon marinus)* and other aquatic organisms is influenced by chemical and physical characteristics of streams they inhabit (Applegate et al. 1961). Treatment teams in the United States and Canada have routinely used measurements of alkalinity, pretreatment toxicity tests, and past treatment histories to set concentrations of TFM.

Streams that are highly productive or that receive inputs of contaminants sometimes undergo short-term changes in pH, oxygen, and other variables that might influence the toxicity of chemicals to aquatic organisms (Lloyd 1961). The influence of pH on the ionization of TFM in water was described by Hunn and Allen (1974); and the effects of changes in pH on the toxicity of TFM to larval sea lamprey was reported by Dawson et al. (1975) and Bills et al. (1988). In the present study, we investigated the influence of decreased dissolved oxygen concentrations (DO) on the toxicity of TFM to larval sea lamprey and rainbow trout fingerlings *(Oncorhynchus mykiss).*

METHODS AND MATERIALS

Flow-through toxicity tests were conducted in seven 15-L aquaria supplied with a constant flow of Lake Huron water (average pH 8.0; alkalinity 89 mg/L; hardness 105 mg/L). Identical toxicity tests were conducted at 13 and 20°C.

These temperatures are frequently encountered during treatments with TFM, especially in productive streams where DO fluctuations would be likely to occur.

Larval sea lamprey (80-120 mm long) were collected by electroshocking from the Chippewa River, Michigan, and rainbow trout (44-80 mm long) were obtained from Cedarbrook Hatchery, Harrisville, Michigan. Both species were held in Lake Huron water for at least 30 days before testing. Ten trout fingerlings or ten larval lamprey were put into each aquarium for the toxicity tests.

We measured pH, alkalinity, and hardness at the beginning and end of each test. Procedures specified by ASTM (1985) were used throughout the study. A nitrogen degassing system similar to one described by Whitmore et al. (1960) was used to reduce concentrations of DO in a flow of about 4.0 L/min of lake water. The water passed downward through a vertical PVC column 11 cm in diameter, 170 cm long, and two-thirds filled with polyethylene spheres 2.0 cm in diameter; nitrogen gas flowed upward in the column. Concentrations of DO were monitored in the dilution water throughout the study with a YSI[1] polarographic meter and were measured at 0, 6, 12, and 24 h in the control and two randomly selected test aquaria by the modified Winkler method (APHA 1975).

The formulation of TFM used in this study contained 35.7% active ingredient (free nitrophenol, dimethylformamide carrier; American Hoechst Corporation). Solutions of TFM and water were delivered at a rate of 0.4 L/min into each of the six test aquaria by an Ecodyne Mec-O-Matic Company pump. The control aquarium received the same flow rate of water only. We used the spectrophotometric method of Smith et al. (1961) to measure concentrations of TFM hourly in each tank for the first 9 h and at 12 and 24 h. Six concentrations of TFM were used in each test over a range of 0.7 to 4.4 mg/L for larval sea lamprey and 3.9 to 20.3 mg/L for rainbow trout.

Mortalities were recorded hourly for the first 9 h of each test and at 12 and 24 h. The method of Litchfield and Wilcoxon (1949) was used to calculate toxicity values (LC25, LC50, and LC99.9). The LC50 values were correlated with DO concentrations and slopes were compared to zero by analysis of variance ($P<0.5$).

RESULTS AND DISCUSSION

Inasmuch as this study was intended primarily for use in sea lamprey control, we reported the LC99.9 values after 9 h for larval sea lamprey and the LC25 values after 24 h for rainbow trout. These values were defined as the "minimum lethal concentration" and "maximum allowable concentration" of TFM by Kanayama (1963). Data collected after 9 h of exposure was used to calculate the LC99.9 for sea lamprey because 9 h generally represents the average time TFM concentrations are kept at or above the minimum lethal concentration during treatment of a stream to kill sea lamprey. Data collected after 24 h of exposure

[1]References to trade names or manufacturers do not constitute U.S. Government endorsement of commercial products.

was used to calculate the LC25 for rainbow trout because 24 h generally represents the maximum amount of time that TFM would be present at a point in a stream during a treatment to kill sea lamprey. The LC25 after 24 h represents the "worst case" situation and is intended to provide a conservative estimate of the maximum allowable concentration. Because LC50 values can be estimated with more precision than either the LC99.9 or LC25 values (Tables 1-4), we also examined the relation between DO and LC50 values for both species at each temperature. This process helped insure that no subtle relations were missed between DO and the toxicity of TFM.

Decreased DO did not measurably affect the toxicity of TFM to larval sea lamprey at either 13 or 20". For larval sea lamprey at 13°C the LC50 values ranged from 2.0 to 2.2 mg/L and the LC99.9 values from 2.6 to 3.0 mg/L within a DO saturation range of 105% to 31% (Table 1); at 20°C the LC50's ranged from 1.8 to 3.2 mg/L and LC99.9 values from 2.3 to 4.1 mg/L within a DO saturation range of 112% to 31% (Table 2). There were, thus, no significant correlations between DO and the toxicity of TFM to larval sea lamprey at either temperature (Fig. 1).

The consumption of oxygen is lower in lamprey than in many other fishes (Randall 1970). Low DO increases the branchial pulse rate in lamprey (Potter et

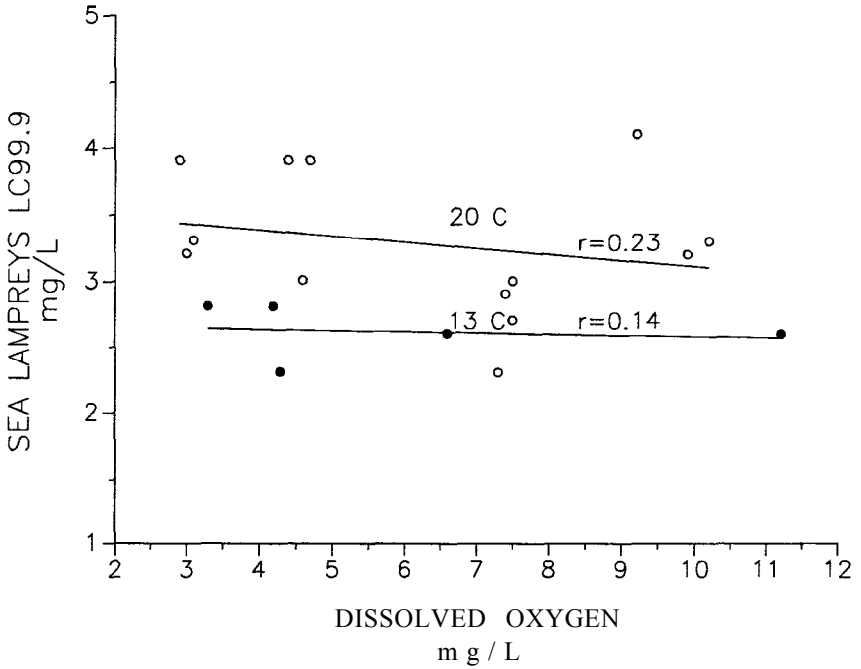

Fig. 1. Relation between dissolved oxygen and LC99.9 values for TFM and sea lamprey at 13" (dots) and 20°C (open circles)

TABLE I. Lethal concentrations (95% confidence intervals in parentheses) of TFM for larval sea lamprey at 13°C after 9 h.

Concentration (mg/L)	Average oxygen content Saturation (%)	LC50 (mg/L)	LC99.9 (mg/L)
11.2 (± 0.09)	105	2.0 (1.82-2.13)	2.6 (2.30-2.95)
6.4 (± 0.08)	61	2.0 (1.82-2.14)	2.6 (2.22-3.05)
4.3 (± 0.14)	40	2.0 (1.77-2.24)	(2.20-4.09)
4.2 (± 0.19)	40	3.0 (2.07-2.33)	(2.64-3.29)
3.3 (± 0.11)	31	2.1 (1.90-2.28)	2.8 (2.31-3.49)

TABLE 2. Lethal concentrations (95% confidence intervals in parentheses) of TFM for larval sea lamprey at 20°C after 9 h.

Concentration (mg/L)	Average oxygen content Saturation (%)	LC50 (mg/L)	LC99.9 (mg/L)
10.3 (± 0.04)	112	2.3 (2.10-2.44)	3.3 (2.90-3.83)
9.9 (± 0.00)	108	2.3 (2.09-2.42)	3.2 (2.80-3.66)
9.2 (± 0.08)	100	4.1 (2.76-3.31)	(3.53-4.76)
7.5 (± 0.11)	82	2.4 (2.26-2.60)	3.0 (2.72-3.49)
7.4 (± 0.04)	82	1.9 (1.80-2.05)	2.7 (2.32-3.14)
7.4 (± 0.13)	81	2.9 (2.24-2.51)	(2.65-3.18)
7.3 (± 0.15)	80	1.8 (1.72-1.97)	2.3 (2.08-2.59)
4.7 (± 0.27)	51	2.4 (2.16-2.62)	3.9 (3.05-4.91)
4.6 (± 0.09)	50	2.4 (2.22-2.52)	3.0 (2.67-3.32)
4.4 (± 0.07)	48	3.2 (3.06-3.41)	3.9 (3.63-4.26)
3.1 (± 0.07)	34	2.3 (2.17-2.49)	3.3 (2.82-3.80)
3.0 (± 0.20)	33	2.4 (2.34-2.54)	3.2 (2.97-3.55)
2.9 (± 0.10)	31	2.7 (2.45-2.89)	3.9 (3.20-4.88)

TABLE 3. Lethal concentrations (95% confidence intervals in parentheses) of TFM for rainbow trout at 13°C after 24 h.

Concentration (mg/L)	Average oxygen content Saturation (%)	LC50 (mg/L)	LC25 (mg/L)
10.9 (± 0.13)	103	11.7 (10.49-13.05)	10.5 (9.35-l 1.80)
9.6 (± 0.22)	92	12.0 (10.75-13.39)	10.7 (9.52-12.03)
8.6 (± 0.12)	80	11.3 (10.42-12.26)	10.3 (9.44-l 1.23)
6.4 (± 0.03)	60	12. I (11.03-13.27)	11.1 (10.09-12.22)
6.3 (± 0.09)	59	11.7 (9.99-13.70)	10.4 (8.76-12.34)
5.2 (± 0.14)	49	11.4 (10.34-12.56)	10.0 (9.00-11.11)
4.1 (± 0.09)	39	10.8 (9.63-12.11)	9.6 (8.56-10.77)
3.3 (± 0.04)	31	9.8 (8.57-l 1.10)	8.4 (7.35-9.66)
3.3 (± 0.03)	31	10.7 (9.59-l 1.93)	9.5 (8.41-10.68)

al. 1970) and this higher rate could have increased the exposure of the gill tissue to TFM in our studies. If the exposure of the gills to TFM was the only factor mediating the toxicity of TFM, lowering the DO should have increased the toxicity; however, this effect was not observed. Lloyd (196 1) noted that changes in DO do not strongly affect toxicants that are influenced by changes in pH. He suggested that a reduction in DO would decrease the amount of carbon dioxide excreted by the gill. This decrease would, in turn, increase the pH of water at the gill surface resulting in decreased toxicity of TFM to sea lamprey (Dawson et al. 1975). This situation might compensate for the effect of lowered DO and result in no net change in the toxicity of TFM to sea lamprey.

Decreased levels of DO increased the toxicity of TFM to rainbow trout at 13°C but not at 20°C. For rainbow trout fingerlings at 13°C, the LC50s ranged from 9.8 to 12.1 mg/L and LC25s from 8.4 to 11.1 mg/L within a DO saturation range of 103% to 31% (Table 3); at 20°C, the LC50s ranged from 9.1 to 15.0 mg/L and LC25s from 7.4 to 12.6 mg/L within a DO saturation range of 109% to 31% (Table 4). Considerable imprecision was observed in the toxicity data presented for rainbow trout. These tests were conducted over a period of about one month, and the sensitivity of the trout to TFM might have changed over that period. We used a method described by Litchfield and Wilcoxon (1949) to analyze the data from each toxicity test and this procedure does not necessarily eliminate results that are imprecise. Because the toxicity of TFM was not influenced strongly by changes in DO, we did not attempt to maximise the precision

TABLE 4. Lethal concentrations (95% confidence intervals in parentheses) of TFM for rainbow trout at 20°C after 24 h.

Concentration (mg/L)	Average oxygen content Saturation (%)	LC50 (mg/L)	LC25 (mg/L)
9.9 (± 0.10)	109	9.6 (8.46-10.90)	8.4 (7.27-9.59)
9.3 (± 0.07)	102	15.0 (12.73-17.67)	12.6 (10.33-15.37)
1.3 (± 0.05)	80	9.6 (8.66-10.64)	8.6 (7.67-9.64)
5.5 (± 0.16)	62	9.1 (7.83-10.57)	7.4 (6.21-8.82)
5.5 (± 0.12)	62	10.9 (9.59-12.39)	9.1 (7.87-10.52)
5.4 (± 0.16)	59	10.0 (8.84-11.20)	7.9 (6.96-8.96)
5.4 (± 0.06)	59	10.0 (8.90-1 1.24)	8.8 (7.79-9.94)
4.6 (± 0.10)	50	13.1 (10.89-15.75)	11.3 (8.66-14.74)
3.6 (± 0.07)	40	11.0 (9.48-12.76)	9.7 (8.21-11.45)
3.5 (± 0.06)	38	12.1 (10.95-13.37)	10.9 (9.60-12.38)
2.8 (± 0.06)	31	11.9 (9.73-14.55)	8.7 (6.47-11.68)

of our measurements by repeating the toxicity tests. In tests conducted at 13°C, the correlation coefficient for the relation between DO and LC25s was 0.71 (Fig. 2); between DO and LC50s it was 0.72. Consumption of oxygen is generally higher in teleost fishes than in lamprey (Randall 1970) and might explain why rainbow trout were more sensitive to TFM as the DO decreased at 13°C (Fig. 2). In toxicity tests conducted at 20°C with rainbow trout, no significant correlations were observed between DO and LC25 (Fig. 2), nor were significant correlations observed between DO and LC50 values. The temperature of 20°C is above the optimum of 16.8-18.6°C (Wismer and Christie 1987) for juvenile rainbow trout. Although the fish were acclimated to 20°C according to ASTM (1985) procedures, high temperature stresses might have masked any effect of the lowered DO on the toxicity of TFM. The lowest LC25 measured for rainbow trout at 13°C and 3 1% DO saturation was 8.4 mg/L. The concentration of TFM required to kill all the larval sea lamprey was still sufficiently below the LC25 for rainbow trout to allow safe treatment, even at extremely low DO (Table 1 and 2). Fish such as the yellow bullhead *(Ictalurus natalis),* white sucker *(Catostomus commersoni),* and walleye *(Stizostedion vitreum vitreum)* are more sensitive than rainbow trout to TFM (Applegate and King 1962) and could suffer some mortality from normally safe TFM treatments at lower DO levels.

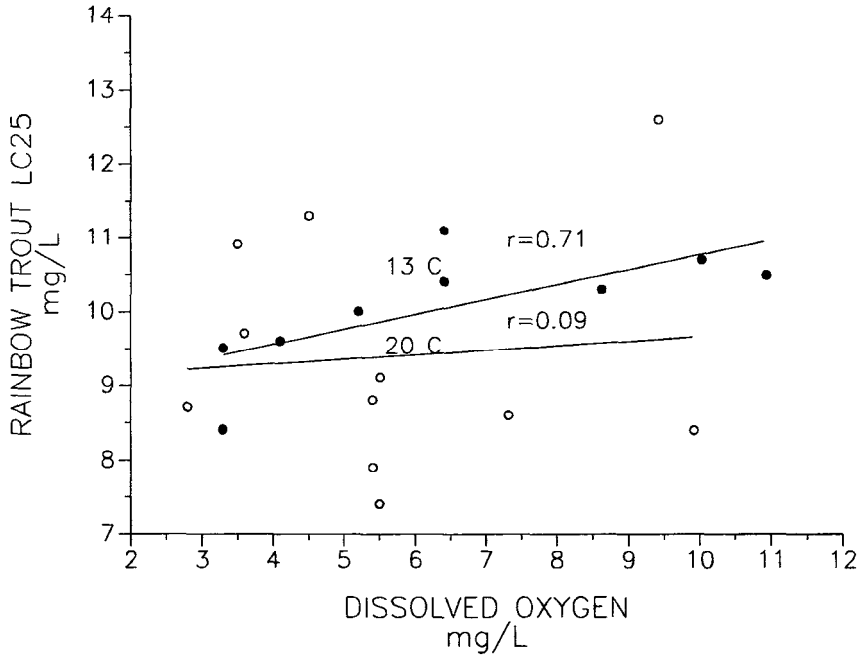

Fig. 2. Relation between dissolved oxygen and LC25 values for TFM and rainbow trout at 13" (dots) and 20°C (open circles).

Sea lamprey treatment crews do not treat streams that have DO levels below 60% saturation. No differences in the toxicity of TFM were measured between 100-60% DO saturation (Tables 1-4), making it unlikely that DO levels would ever cause nontarget mortality during treatment of a stream to control sea lamprey. A nonlinear model might have provided higher correlations between DO and the toxicity of TFM, but this would not have affected the conclusions drawn in this paper. Therefore, a linear model was used for the analysis of the data.

Although our results indicate that low DO by itself would not affect TFM treatments, observation of diurnal changes in DO should alert sea lamprey control personnel to the likelihood of fluctuations in other important chemical factors, such as pH. For example, if nighttime respiration in a eutrophic stream decreases DO and increases CO, production, there could be a coincident drop in pH. This decrease in pH will result in an increase in the toxicity of TFM (Dawson et al. 1975) and could cause mortality of organisms other than sea lamprey. Such an increase in the toxicity would not be expected if the treatment rate was based on water chemistry taken only during the day. Monitoring the DO, pH, and other variables for at least one daily cycle would allow adjustment in either the TFM concentration or the timing of the treatment to minimize potential damage to the stream biota.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). 1975. Oxygen (Dissolved). Pages 440-454 *in* Standard methods for the examination of water and wastewater. 14th edition. American Public Health Associations, Washington, DC.

AMERICAN SOCIETY FOR TESTING AND MATERIALS (ASTM). 1985. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians, pp. 282-306. *In* Annual Book of ASTM Standards Volume 11.04. Philadelphia, PA.

APPLEGATE, V. C., J. H. HOWELL, J. W. MOFFETT, B. G. H. JOHNSON, AND M. A. SMITH. 1961. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 1.35 pp.

APPLEGATE, V. C., AND E. L. KING, JR. 1962. Comparative toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to larval lamprey and eleven species of fishes. Trans. Am. Fish. Soc. 91:342-345.

BILLS, T. D., L. L. MARKING, G. E. HOWE, AND J. J. RACH. 1988. Relation of pH to toxicity of lampricide TFM in the laboratory. Great Lakes Fish. Comm. Tech. Rep. 53:9-17.

DAWSON, V. K., K. B. CUMMING, AND P. A. GILDERHUS. 1975. Laboratory efficacy of 3-trifluoromethyl-4-nitrophenol (TFM) as a lampricide. U.S. Fish Wildl. Serv., Invest. Fish Control No. 63. 13 pp.

HUNN, J. B., AND J. L. ALLEN. 1974. Movement of drugs across the gills of fishes. Ann. Rev. Pharmacol. 14:47-55.

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

LITCHFIELD, J. T., JR., AND F. WILCOXON. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99-113.

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

POTTER, I. C., B. J. GENTLEMAN, AND S. GENTLEMAN. 1970. Survival and behavior of ammocoetes at low oxygen tensions. J. Exp. Biol. 53:59-73.

RANDALL, D. J. 1970. Gas exchange in fish, pp. 253-291. *In* W. S. Hoar and D. J. Randall [ed.]. Fish Physiology Volume 4. Academic Press, London.

SMITH, M.A., V. C. APPLEGATE, AND B. G. H. JOHNSON. 1961. Physical properties of some halo-nitrophenols. J. Chem. Eng. Data 6:607-608.

WHITMORE, C. M., C. E. WARREN, AND P. DOUDOROFF. 1960. Avoidance reactions of salmonid and centrarchid fishes to low oxygen concentrations. Trans. Am. Fish. Soc. 89:17-26.

WISMER, D.A. AND A. E. CHRISTIE. 1987. Temperature relationships of Great Lakes fishes: A data compilation. Great Lakes Fish. Comm., Spec. Pub. No. 87-3, 195 pp.

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