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United States Department of the Interior
Fish and Wildlife Service

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INVESTIGATIONS IN FISH CONTROL

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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

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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 reaeration 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 μCi 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 (±)
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 μg/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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