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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)

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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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