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Relatively Rapid Loss of Lampricide Residues from Fillet Tissue of Fish after Routine Treatment

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The selective sea lamprey (*Petromyzon marinus*) larvicide 3-trifluoromethyl-4-nitrophenol (TFM) is currently used to control parasitic sea lampreys in tributaries to the Great Lakes basin. The concentration and persistence of TFM and its major metabolite, TFM glucuronide (TFM-glu), was determined in fillet tissue of fish after a typical stream application. Rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) were exposed to a nominal concentration of 12.6 nmol/mL TFM for about 12 h during a sea lamprey control treatment of the Ford River in Michigan. Concentrations of TFM and TFM-glu were greatest in the fillet tissues during the exposure period, with greater residues in channel catfish (wet wt; mean, 6.95 nmol/g TFM; mean, 2.40 nmol/g TFM-glu) than in rainbow trout (wet wt; mean, 1.45 nmol/g TFM; mean, 0.93 nmol/g TFM-glu). After the exposure period, residues in both species decreased by 90−99% within 6−12 h and were less than the quantitation limit (<0.03 nmol/g) within 36 h.

KEYWORDS: TFM; TFM glucuronide; sea lamprey; lampricide; rainbow trout; channel catfish

INTRODUCTION

Registered in 1964, the selective sea lamprey (*Petromyzon marinus*) larvicide 3-trifluoromethyl-4-nitrophenol (TFM; **Figure 1A**) is currently the principal chemical applied to tributaries of the Great Lakes basin to control populations of the parasitic sea lamprey. Although TFM effectively reduces the abundance of sea lampreys, its repetitive and continuing use raised concern over its effects on nontarget organisms in the aquatic environment (*1*). This concern resulted in the testing of over 20 species of fish for sensitivity to TFM (*2*, *3*, *4*, *5*, *6*).

Other studies addressed questions about the fate of TFM in fish. Sills and Allen (*7*) determined that pH of the exposure water had the greatest influence on TFM concentrations in the fillet tissues of eight freshwater fish species. Increasing the pH of the exposure water resulted in decreased TFM concentrations in the fillet tissue. They also reported that 24 h after exposure, parent TFM concentrations in the fillet tissue were near or less than the detection limit of 0.01 μg/g. A subsequent field study reported that 99% of the parent TFM was eliminated from (whole body) rainbow trout (*Oncorhynchus mykiss*), sculpin (*Cottus* sp.), and cyprinids (*Rhinichthys cataractae* and *Notropis* sp.) within 96 h after lampricide treatment (*8*). Researchers have demonstrated that TFM is metabolized to TFM glucuronide (TFM-glu; **Figure 1B**) (*9*, *10*, *11*) and that the liver and kidney of fish are sites of TFM biotransformation (*9*, *12*). The selective toxicity of TFM to lampreys appears to be the result of lower glucuronyltransferase activity in lampreys, which results in greater parent TFM concentrations in lampreys when compared to rainbow trout (*11*). Kane et al. (*13*) determined that in vitro hepatic efficiency of TFM biotransformation is inversely related to TFM toxicity in four species of fish, with biotransformation efficiency in the following order: bluegill (*Lepomis macrochirus*) > rainbow trout > channel catfish (*Ictalurus punctatus*) > sea lamprey.

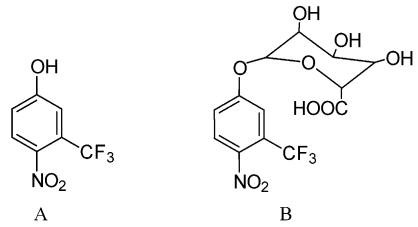

Figure 1. Structure of (A) technical grade TFM, MW = 207.11 and (B) TFM-glu, MW = 383.23.

TFM has undergone re-registration as a regulatory requirement to ensure the safety of pesticides used in the United States. Data were needed to elucidate the magnitude and persistence of TFM and major metabolites (metabolite > 10% total residue) in the fillet tissue of representative predator and bottom-feeding fish species during an actual field treatment (*14*). Although previous work did not detect TFM-glu in rainbow trout muscle (*11*), unpublished radiolabeled studies at the Upper Midwest Environmental Sciences Center (UMESC) revealed that TFM-glu is a major metabolite in muscle tissue after TFM exposures. The objective of this study was to quantify, over time, both TFM and TFM-glu in the fillet tissue of fish under typical sea

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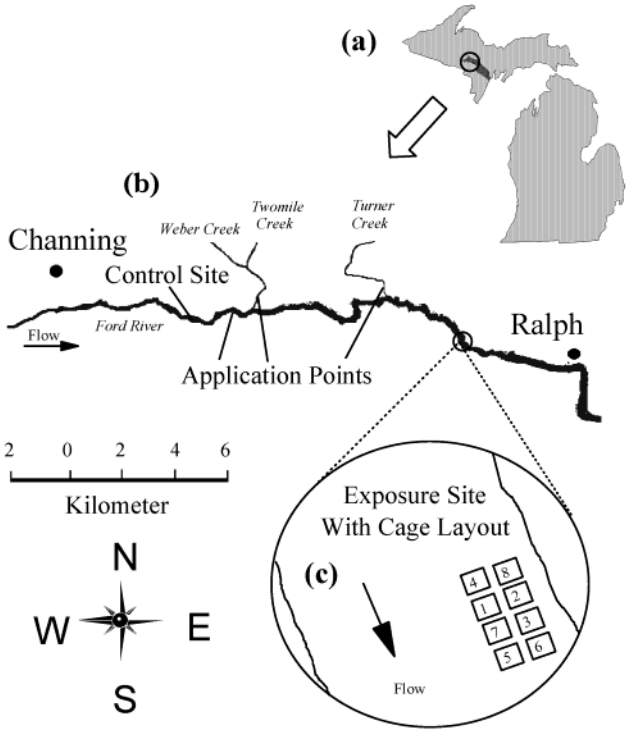

Figure 2. Map of the Ford River with (a) the location of the Ford River basin in the Upper Peninsula of Michigan, (b) the locations of the control site, exposure site, and lampricide application points, and (c) an expanded view of the exposure site that shows the position and sampling order of the cages.

lamprey control treatment conditions. Rainbow trout were used as a representative predator species, and channel catfish were used as a representative bottom-feeding species.

MATERIALS AND METHODS

Test and Reference Substances. The field grade TFM (Lamprecid, Hoechst AktienGesellschaft, Frankfurt, Germany) used in the sea lamprey control treatment of the Ford River contained the active ingredient formulated with 38% 2-propanol and 25% water by weight. The TFM analytical standard obtained from Aldrich Chemical Co. (Milwaukee, WI) was 99% pure.

Test Organisms. Rainbow trout were obtained as eyed-eggs from Ennis National Fish Hatchery (Ennis, MT) and reared at UMESC (La Crosse, WI) until one year old (mean, 310 ± 72 g; range, 154−460 g). Three-year-old channel catfish (mean, 270 ± 76 g; range, 144−472 g) were obtained from Osage Catfisheries (Osage, MO). Fish were transported to the testing site in aerated, temperature-controlled tanks.

Test Conditions. This study was conducted during a regularly scheduled U. S. Fish and Wildlife Service (FWS) lampricide treatment of the Ford River (Dickinson County, Michigan; **Figure 2**a) on June 16, 1996. The Ford River is about 250 km long with riffles, runs, and pools. Average flow during the treatment was about 13.2 m^3/s near the exposure site. The exact locations of the control site (87° 58′ 58.79″ long., 46° 07′ 30.89″ lat; **Figure 2b**) and the exposure site (87° 50′ 48.23″ long., 46° 06′ 36.59″ lat; **Figure 2b**) were determined by a Global Positioning System. The substrate at the control and exposure sites was sand and gravel. A two-chambered mesh cage was placed in the river at the control site, located upstream from the lampricide application points (**Figure 2b**). Twenty-four rainbow trout were placed into one chamber, and twenty-four channel catfish were placed into the other. Eight identical mesh cages were placed in the river at the exposure site (**Figure 2c**), located a sufficient distance downstream from the lampricide application points to ensure uniform distribution of the TFM across the stream. Five rainbow trout and five channel catfish were placed into each cage (40 fish of each species total). All fish were allowed to acclimate in the river for 3 days before initiation of the lampricide treatment.

Exposure of Test Organisms. The lampricide treatment was conducted in accordance with policies and procedures set forth in the U. S. FWS sea lamprey control standard operating procedures (*15*). Lamprecid was metered into the stream at the application points. Measurements of TFM in the river water were taken spectrophotometrically (*15*) upstream from the exposure site to verify uniform distribution of the lampricide across the water column and at the exposure site to determine the exposure period. Water samples were collected at the control and exposure sites and then injected directly onto a high-performance liquid chromatograph (HPLC) to determine the TFM exposure concentration. The caged fish at the exposure site were exposed to a mean TFM concentration of 12.6 nmol/mL (2.6 mg/L) for about 12 h. Temperature (range, 17.1−18.3 °C), pH (range, 7.94−8.10), and dissolved oxygen (range, 7.3−8.0 mg/L) were monitored at the control and exposure sites during the exposure period.

Sample Collection and Analysis. Fish were collected from the control and exposure sites 1 day before treatment and at 6, 12, 18, 24, 48, 96, and 192 h after the arrival of the lampricide at the exposure site. During each collection period, three fish of each species were removed from the cage at the control site and all fish in one randomly picked cage at the exposure site (five of each species) were removed. The fish were euthanized by a blow to the head, weighed, measured, and filleted. Skin-on fillets were dissected from rainbow trout, and skinless fillets were collected from channel catfish. The fillets were rinsed thoroughly with fresh well water, dried with a paper towel, and then weighed. Individual fillets were wrapped in aluminum foil, bagged in polyethylene bags, and stored frozen at ≤−20 °C. TFM and TFM-glu were extracted from fillet tissue (wet wt) with water-methanol (80:20, v/v), purified using reversed-phase solid-phase extraction, and then quantified utilizing reversed-phase HPLC (*16*).

RESULTS

Concentrations of TFM and TFM-glu in fillet tissues of rainbow trout and channel catfish increased rapidly during the lampricide treatment and reached maximum concentrations during the exposure period (**Table 1**). The maximum total residue concentration in the fillet tissue reached about 19% of the nominal TFM concentration in the water for rainbow trout and 68% for channel catfish. Parent TFM concentrations were greater than TFM-glu in the fillet tissues of both species and reached a mean 1.45 nmol/g in rainbow trout and 6.95 nmol/g in channel catfish. After the 12-h exposure period, residues of TFM and TFM-glu in both fish species decreased by 90−99% to less than 0.2 nmol/g (mean) within 6−12 h and were less than the quantitation limit (<0.03 nmol/g) within 36-h postexposure. Residues of TFM and TFM-glu persisted at greater than detectable levels in channel catfish at least 36 h after exposure, while the residues in rainbow trout were greater than the detection limit more than 84 h after the exposure.

DISCUSSION

During the 12-h exposure period, the concentration of TFM was up to 5 times greater and the concentration of TFM-glu was up to 2.8 times greater in the fillet tissue of channel catfish than in the rainbow trout. The ratio of TFM-glu to parent TFM (**Table 1**), however, was about 1.4−1.8 times greater in rainbow trout suggesting greater biotransformation efficiency in trout. This is in agreement with previous observations about TFM toxicity and glucuronidation efficiency; higher steady-state concentrations and lower glucuronyltransferase activity result in a higher toxicity to TFM (*11*, *13*). Our data also support the general conclusion drawn by others that elimination of TFM from fish is relatively rapid (*1*, *7*, *8*).

Table 1. Concentrations of TFM and TFM-glu in Fillet Tissues of Rainbow Trout and Channel Catfish before, during, and after Treatment of the Ford River in Michigan with the Lampricide TFM[a]

		rainbow trout fillet			channel catfish fillet		
sampling time (hours)	TFM in water (nmol/ mL)	TFM (nmol/g)	TFM-glu (nmol/g)	TFM-glu/ TFM ratio	TFM (nmol/g)	TFM-glu (nmol/g)	TFM-glu/ TFM ratio
			Preexposure				
<0	<MDL[c]	<MDL	<MDL		<MDL	<MDL	
			Exposure				
3	17.4	NS[d]	NS		NS	NS	
6	9.8	1.38 ± 0.44	0.57 ± 0.21	0.41	6.95 ± 1.18	1.60 ± 0.36	0.23
9	11.4	NS	NS		NS	NS	
12	11.9	1.45 ± 0.64	0.93 ± 0.46	0.64	5.07 ± 0.83	2.40 ± 0.93	0.47
			Postexposure				
6	0.36	0.168 ± 0.066	0.28 ± 0.15	1.67	0.385 ± 0.045	0.49 ± 0.16	1.27
12	0.14	0.052 ± 0.034	0.080 ± 0.070	1.54	0.095 ± 0.089	0.18 ± 0.20	1.89
36	0.04	<LOQ[b]	<LOQ		<LOQ	<LOQ	
84	<MDL	<LOQ	<LOQ		<MDL	<MDL	
180	<MDL	<MDL	<MDL		<MDL	<MDL	

[a] Values represent the mean ± standard deviation of five fish at each time interval. [b] LOQ = limit of quantitation (rainbow trout: TFM = 0.048 nmol/g, TFM-glu = 0.060 nmol/g; channel catfish: TFM = 0.031 nmol/g, TFM-glu = 0.031 nmol/g; water = 0.02 nmol/mL). [c] MDL = method detection limit (rainbow trout: TFM = 0.014 nmol/g, TFM-glu = 0.018 nmol/g; channel catfish: TFM = 0.012 nmol/g, TFM-glu = 0.009 nmol/g; water = 0.004 nmol/mL). [d] NS = no sample collected.

The relatively high concentration of TFM-glu in nonexcretory tissues such as muscle was unexpected. Our data revealed TFM-glu concentrations comprised up to 39% of the total measured fillet tissue residues (TFM and TFM-glu) in rainbow trout and 32% in channel catfish during the exposure period. Other researchers have also reported that glucuronide conjugates comprise notable proportions of total xenobiotic residues in fish fillet tissues (*17*, *18*, *19*, *20*). Three possible explanations for these high ratios of TFM-glu to TFM are (1) a relatively high TFM-glu concentration in the blood that is in the muscle vasculature, (2) conjugation of TFM within the fillet tissue, and (3) diffusion of TFM-glu from the blood into the fillet tissue.

Estimated TFM-glu in the vascular space would account for less than 30% of the TFM-glu measured in the trout fillet tissue in this study. This is based on an estimated blood concentration of 9.19 nmol/g TFM-glu (lethal lamprey dose exposure of rainbow trout) (*11*) and an estimated fillet tissue blood volume of 26−28 μL/g (*21*). Although extrahepatic glucuronidation occurs in fish (*22*, *23*, *24*), glucuronyltransferase activity in muscle has rarely been examined in any animal and muscle tissue has not been reported as a major conversion site (*25*, *26*). The TFM-glu concentration in rainbow trout blood during TFM exposure reported by Lech and Statham (*11*) is up to 10 times greater than the concentration in fillet tissue measured in this study. Therefore, TFM-glu in the fillet tissue may predominately result from diffusion of TFM-glu from the bloodstream.

Although several studies report TFM residues in fish tissue (*7*, *8*, *11*, *27*), they do not adequately address regulatory requirements for the magnitude and persistence of the pesticide in both predator and bottom-feeding fish during actual field treatments. Gilderhus et al. (*8*) was the only field study to report TFM concentrations in fish. Previous field and laboratory studies did not provide information on TFM-glu residues in fish fillet tissue. The current study was the first to document both the concentration and persistence of TFM and its major metabolite TFM-glu in the fillet tissue of fish during a typical lampricide treatment. The rapid depuration of TFM residues from tissues of fish in this study supports the conclusion of the U. S. Environmental Protection Agency that TFM, under current use patterns, does not pose a risk to human health (*28*).

ABBREVIATIONS USED

FWS, Fish and Wildlife Service; TFM, 3-trifluoromethyl-4-nitrophenol; TFM-glu, 3-trifluoromethyl-4-nitrophenol glucuronide; UMESC, Upper Midwest Environmental Sciences Center, USGS, BRD, La Crosse, WI; HPLC, high-performance liquid chromatograph.

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