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Toxicity of the lampricide TFM (3-trifluoromethyl-4-nitrophenol) to the black sandshell mussel (*Ligumia recta*)

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INTRODUCTION

The Vermont Department of Environmental Conservation (VTDEC) draft Aquatic Nuisance Control Permit (Application Number C96-06) issued August 23, 1996, required the applicant to determine the toxicity of lampricide TFM (3-trifluoromethyl-4-nitrophenol) to two freshwater mussel species and one fish species prior to lamprey control on the Poultney River, Lake Champlain watershed. Juvenile and adult eastern floater mussels (*Pyganodon cataracta*) were tested by the U.S. Fish and Wildlife Service, and the results have been submitted to VTDEC separately. The New York State Department of Environmental Conservation (NYSDEC) tested juvenile and adult pocketbook mussels (*Lampsilis ventricosa; ovata*) as a surrogate for the black sandshell mussel (*Ligumia recta*) and the channel darter (*Percina copelandi*) as specified in the VTDEC draft Aquatic Nuisance Control Permit (Neuderfer 1996). The pocketbook mussel surrogate was tested because adequate numbers of black sandshell mussels could not be collected in the Lake Champlain watershed.

The Vermont Endangered Species Committee required testing of black sandshell mussels for the Endangered Species Permit required for the proposed Poultney River TFM treatment. This report contains the results of that testing on organisms obtained from Kentucky.

The NYSDEC Aquatic Nuisance Control Permit allows the use of TFM at a concentration equal to 1.0 times MLC (minimum lethal concentration resulting in 99.9% mortality of sea lamprey ammocoetes - *Petromyzon marinus*) as determined by the pretreatment bioassay to control sea lamprey in the Poultney River. The draft VTDEC Permit allows a potentially similar TFM concentration, based on results of mussel and channel darter bioassay results. Sea lamprey ammocoetes were tested along with these non-target species, allowing comparison of the toxicity data as a proportion of sea lamprey MLC as required in the permits.

MATERIALS AND METHODS

This toxicity test was performed using NYSDEC's mobile aquatic toxicology laboratory on-site at the Poultney River from September 26 - October 2, 1996. The mobile lab was located adjacent to the Poultney River at Central Vermont Public Service's (CVPS) Carvers Falls hydroelectric station. Water was pumped continuously from the upstream side of Carvers Falls dam through coolers used for test organism acclimation to Poultney River water and the serial diluter in the mobile lab. The test method was identical to that used in the standard pretreatment bioassay procedure outlined below to determine the MLC prior to all TFM treatments in the Lake Champlain watershed, except sediment was used in the test containers. The methodology used in these pretreatment tests is consistent with USEPA and ASTM toxicity test methods.

The mobile lab diluter delivers 0.75 l/minute of stream water continuously to ten 40-l polyethylene test containers equipped with standpipe overflows. Test organisms are acclimated to test stream water for a minimum of 48-hours prior to initiation of the toxicity test. Approximately 24-hours before exposure to TFM begins, test animals are placed in the test system. Ten lamprey ammocoetes are placed in a stainless steel wire basket that is suspended in the test container. Non-target test

organisms are placed in the polyethylene tank.

Nine of the test containers receive serially diluted concentrations of TFM (0.805 between concentrations), and one receives stream water without TFM (control). The TFM concentrations used are based on water chemistry comparison to pH/alkalinity charts and past stream experience. The predicted sea lamprey MLC concentration is used as the middle TFM concentration. Just prior to initiation of the TFM exposure period, the test container standpipes are removed to lower the water level to approximately 7 - 10 cm depth. A metering pump is then turned on, and a predetermined concentration of TFM stock solution is injected into the diluter. The standpipes are then replaced. This results in a TFM concentration pattern in the test chambers similar to an in-stream sea lamprey treatment. Water chemistry (pH and alkalinity) and mortality/morbidity data are recorded in each test container hourly during the TFM exposure. Water temperature is recorded continuously in one test container during the acclimation and test period. TFM exposure duration is dependant upon the anticipated stream treatment strategy, which usually involves a 12-hour TFM feed to establish a 9-hour lethal block. Therefore, the bioassay test organisms are usually exposed to TFM for a 12-hour period.

Because the the test organisms might harbor immature stages of Zebra mussels (*Dressina polymorpha*), bioassay test water was filtered through a sand filter and/or the sand and gravel stream bank at Carvers Falls before discharge via groundwater to the Poultney River.

The no observed effect concentration (NOEC) were determined empirically. Standard operating procedures for acute toxicity testing (ASTM and USEPA) allow 10% mortality (morbidity) in control exposures. At TFM concentrations that exceed 10% mortality (morbidity), it was assumed to be significant mortality. The highest TFM concentration that exhibited $\leq$10% mortality (morbidity) was the NOEC.

A total of 80 adult and juvenile black sandshell mussels were collected by a commercial fisherman dragging braille in the lower Kentucky River and Ohio River in Kentucky on September 25 - 27, 1996. It had been the plan to collect 75 each of adults and juveniles and perform bioassays on each life stage, but that number of specimens could not be collected in the allotted time frame. Individuals that were $\geq$10.2 cm total length were considered to be adults. Acclimation to Poultney River water began at 1900-hours on September 28, 1996. Sea lamprey ammocoetes were collected from the Salmon River by electrofishing on September 26, 1996, and held in Poultney River water for 16-hours prior to test initiation.

Seven to ten centimeters of gravel substrate from the Missisquoi River, collected at a site where black sandshells were recently observed, was added to the test exposure chambers the afternoon of September 29, 1996, and diluter water flow from the Poultney River was initiated. Ten black sandshell mussels and ten sea lamprey ammocoetes were placed in the test containers the afternoon of September 29, 1996. The 80 mussel specimens were sorted into four size categories; <10.2 cm (11 specimens), $\geq$10.2 - <12.7 cm (24 specimens), $\geq$12.7 - 15.2 cm (40 specimens), and $\geq$15.2 cm (6 specimens). The available specimens were evenly distributed by size category into the 7 highest

TFM concentration test containers, plus the control container. Before placing the mussels into two randomly selected test chambers, the twenty specimens were measured for total length, total height, and weight prior to placing them in the test containers.

Mortality/morbidity checks on sea lamprey were performed hourly during the 12-hour TFM exposure period, and on visible black sandshell mussels after 3-, 6-, 9-, and 12-hours of exposure. The majority of the mussels were not buried in the sediment. After the 12-hour exposure check, the mussels in each exposure chamber were dug-out of the substrate, and data was recorded regarding mortality/morbidity on all the mussels. The mussels were then returned to the test chambers, and the diluter was left running with Poultney River water until the 12-hour post-exposure check. Mortality/morbidity checks were then performed at 12-, and 36-hours post-exposure, and then on Mondays, Wednesdays, and Fridays for two weeks post-exposure.

Sea lamprey mortality was determined by standard pretreatment protocol, where a test animal is assumed to be dead if there is no tail movement. Data on black sandshell mortality/morbidity during the exposure period followed the three stages of intoxication described by Bills et al. (1992). Stage 1 mussels were buried in the substrate; Stage 2 mussels were lying on the substrate with foot extended and responsive to physical stimuli; and Stage 3 mussels were lying on the substrate with foot extended, valves gaping, and non-responsive to physical stimuli. For the post-exposure period, mortality/morbidity checks followed a modified Bills et al. (1992) procedure. Stage "1" mussels had valves nearly closed or closed and foot not extended; Stage "2" mussels had foot extended and respond to external stimuli; Stage "3"mussels had foot extended, valves gaping, and were non-responsive to external stimuli.

The black sandshell mussels were left in the test chambers prior to the first (12-hour) post-exposure mortality/morbidity check. After this check, the mussels from each test container were placed in a labeled nylon-mesh bags with draw-strings and placed in flowing Poultney River water in a large tank. The bags were opened for the 36-hour and subsequent post-exposure mortality/morbidity checks.

RESULTS

The black sandshell mussel bioassay was performed on September 30, 1996. Length and weight data are contained in Table 1.

The nine target diluter TFM concentrations ranged from 1.5 - 8.3 mg/l, plus the Poultney River control, for the black sandshell mussel bioassay. The sea lamprey MLC for the black sandshell bioassay was 3.2 mg/l (Table 2).

Table 3 presents the mean pH, alkalinity, and TFM concentration data for the black sandshell mussel bioassay. The sea lamprey 9-hour MLC for this bioassay was 3.2 mg/l TFM or 1.3 times the predicted pH/alkalinity prediction chart value of 2.4 mg/l TFM. This result is slightly lower but consistent with past experience on the Poultney River (Neuderfer 1996). The TFM concentration data is also presented as a portion of the sea lamprey bioassay MLC.

The mussel mortality/morbidity data is summarized in Tables 3 and 4. Table 3 contains the data from the 3-, 6-, 9-, and 12-hour exposure checks, and 12- and 36-hours post-exposure checks. (NOTE- Data for post-exposure checks beyond 36-hours was not yet available when this draft report was written. It will be included in a later draft.) Table 4 contains the data from the dug-out check after 12-hours of TFM exposure (or 0-hour post-exposure check).

The undisturbed mussels in the four exposure period checks had few mussels at a stage 2 level of TFM intoxication, but they were above the 1.0 x MLC TFM concentration proposed for Poultney River treatment. The Table 4 dug-out data after 12-hours of TFM exposure showed a similar incidence of mussel intoxication at TFM concentrations exceeding 1.3 x MLC. Unlike adult and juvenile pocketbook mussels (Neuderfer 1996), the majority of the black sandshell mussels did not bury themselves in the gravel substrate.

The 36-hour post-exposure data (Table 3) showed that most of the intoxicated mussels quickly recovered. Additional post-exposure mortality/morbidity data will be reported when it is available.

Based on the 36-hour post-exposure recovery data, the NOEC TFM concentration is greater than 2.6 x MLC for black sandshell mussels. On a qualitative observation basis, there appeared to be no significant difference in sensitivity between juvenile (<10.1 cm) and adult (≥10.1 cm) black sandshell mussels. If anything, the largest adults (≥15.2 cm) were the most sensitive size category; the two individuals at the stage 3 level after 36 hours post-exposure were in this category.

DISCUSSION

Bills et al. (1992) observed that many of the pink heelsplitter mussels (*Potamilus alatus*) exposed to ≥MLC concentrations of TFM exhibited Stages 2 and 3 of intoxication, and the mussels unburied themselves from the substrate. Neuderfer (1996) observed that after 12-hours of exposure, most of the juvenile and adult pocketbook mussels exposed to 1.9 and 2.3 x MLC concentrations of TFM, respectively, remained buried, but their foot was extended when they were dug out of the gravel substrate. Very few of the black sandshell mussel specimens used in this study buried themselves in the gravel substrate, unlike the pocketbook mussels tested (Neuderfer 1996). The braille collection method used to collect these test organisms was most likely more stressful than the hand-picking method used to collect the pocketbook mussels. This, along with a longer transport to the test site and a shorter acclimation to Poultney River water, most likely put these black sandshell mussels under considerably more pre-test stress than the pocketbook mussels. This may have been reflected in a slower burying rate for the black sandshells versus the pocketbook mussels.

The NOEC TFM concentration for black sandshell mussels at the 36-hour post exposure time was greater than 2.6 x MLC. Based on these results, the proposed Poultney River treatment at 1.0 will have no significant adverse impact on the black sandshell mussel population.

Literature Cited

Bills, T.D., J.J. Rach, L.L. Marking, and G.E. Howe. 1992. Effects of the lampricide 3-trifluoromethyl-4-nitrophenol on the pink heelsplitter. U.S. Fish and Wildlife Service, Resource Publication 183. 7pp.

Neuderfer, G.N. 1996. Toxicity of the lampricide TFM (3-trifluoromethyl-4-nitrophenol) to juvenile and adult pocketbook mussels *(Lampsilis ventricosa: ovata)* and the channel darter *(Percina copelandi)* (draft). New York Department of Environmental Conservation, Administrative Report, Avon, NY.

Table 1. Length and weight data for the black sandshell mussel bioassay performed on the Poultney River on September 30, 1996.

Parameter Measured	Mean	Range
Total Length (mm)	125	90-149
Total Height (mm)	50	36-60
Wet Weight (g)	174	67-307

Table 2. Summary of sea lamprey mortality data from September 30, 1996, Poultney River black sandshell mussel TFM bioassay.

Exposure Chamber Number	Mean pH	Mean Alk. (mg/l)	Mean 12-hr. TFM Concentration (mg/l)	Percent Sea Lamprey Mortality at 9 hr.
10	8.10	98	8.3	100
9	8.10		6.6	100
8	8.10		5.4	100
7	8.10		4.3	100
6	8.09		3.5	100
5	8.08		2.8	60
4	8.09		2.3	0
3	8.15		1.9	0
2	8.15		1.5	0
1	8.13		0.0	0

Note: 100% sea lamprey mortality was observed after 3.5 mg/l at 6-hours of exposure, and 60% mortality after 9-hours exposure at 2.8 mg/l. Therefore, the sea lamprey MLC was 3.2 mg/l TFM.

Table 3. Exposure and post-exposure mortality/morbidity data summary for black sandshell mussels from a 12-hour TFM lampricide exposure on September 30, 1996, with comparison to 9-hr. sea lamprey MLC.

Exposure Chamber Number	Mean TFM (mg/l)	Mean pH	Mean Alk. (mg/l)	Mean D.O. (mg/l	TFM Conc. as Portion of 9-hr. MLC	Number of Mussels at Modified Bills et al. (1992) Intoxication Stage Number:															
						Hours of Exposure to TFM												Hours of Post-Exposure			
						3-hours			6-hours			9-hours			12-hours			12-hours			36-hours	
						1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
10	8.3	8.10	98	9.5	2.6	10			8	2		5	5		4	6		9		1	9		1
9	6.6	8.10			2.1	9	1		8	2		8	2		8	2		9		1	9		1
8	5.4	8.10			1.7	10			9	1		8	2		7	3		9		1	10		
7	4.3	8.10			1.3	10			10			8	1		9	1		10			10		
6	3.5	8.09		9.4	1.1	10			10			10			10			10			10		
5	2.8	8.08			0.9	10			10			10			10			10			10		
4	2.3	8.09			0.7	10			10			10			10			10			10		
3	1.9	8.15			0.6																		
2	1.5	8.15			0.5																		
1	0.0	8.13		9.5	0.0	10			10			10			10			9		1	9		1

Key to the modified Bills et al. (1992) stages of intoxication used in this table:

Stage "1" Mussels buried or unburied, valves nearly closed or closed, and foot not extended.
Stage "2" Mussels unburied with foot extended, and respond to external stimuli.
Stage "3" Mussels unburied with foot extended, valves gaping, and non-responsive to external stimuli.

NOTE: Sea lamprey MLC was 3.2 mg/l. No mussels in containers 2 and 3.

Table 4. Summary of black sandshell mussel mortality/morbidity data collected after 12-hours of exposure to TFM (when the mussels were dug-out of the substrate), with comparison to 9-hr. sea lamprey MLC.

Exposure Chamber Number	Mean TFM (mg/l)	TFM Conc. as Portion of 9-hr. MLC	Number of Mussels at modified Bills et al. (1992) Intoxication Stage Number:					
			Stage 1		Stage 2		Stage 3	
			Unburied	Buried	Unburied	Buried	Unburied	Buried
10	8.3	2.6	4		6			
9	6.6	2.1	6	2	2			
8	5.4	1.7	6	1	2	1		
7	4.3	1.3	7	1	1	1		
6	3.5	1.1	9			1		
5	2.8	0.9	6	4				
4	2.3	0.7	9	1				
3	1.9	0.6	No mussels					
2	1.5	0.5	No mussels					
1	0.0	0.0	10					

Key to the modified Bills et al. (1992) stages of intoxication used in this table:

Stage "1" Mussels with valves nearly closed or closed, and foot not extended.
Stage "2" Mussels with foot extended, and respond to external stimuli.
Stage "3" Mussels with foot extended, valves gaping, and non-responsive to external stimuli.

DRAFT

Toxicity of the Lampricide TFM (3-trifluoromethyl-4-nitrophenol) to Juveniie and Adult Pocketbook Mussels *(Lampsilis ventricosa; ovata)* and the channel darter (*Percina copelandi*)

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INTRODUCTION

The Vermont Department of Environmental Conservation (VTDEC) draft Aquatic Nuisance Control Permit (Application Number C96-06) issued August 23, 1996, required the applicant to determine the toxicity of lampricide TFM (3-trifluoromethyl-4-nitrophenol) to two freshwater mussel species and one fish species prior to lamprey control on the Poultney River, Lake Champlain watershed. Juvenile and adult giant floater mussels (*Pyganodon grandis*) were tested by the U.S. Fish and Wildlife Service, and the results have been submitted to VTDEC separately. The New York State Department of Environmental Conservation (NYSDEC) tested juvenile and adult pocketbook mussels (*Lampsilis ventricosa; ovata*) as a surrogate for the black sandshell mussel (*Ligumia recta*) and the channel darter (*Percina copelandi*) as specified in the VTDEC draft Aquatic Nuisance Control Permit. The pocketbook mussel surrogate was tested because adequate numbers of black sandshell mussels could not be collected in the Lake Champlain watershed. This report contains test results for these species.

The NYSDEC Aquatic Nuisance Control Permit allows the use of TFM at a concentration equal to 1.0 times MLC (minimum lethal concentration resulting in 99.9% mortality of sea lamprey ammocoetes - *Petromyzon marinus*) as determined by the pretreatment bioassay to control sea lamprey in the Poultney River. The draft VTDEC Permit allows a potentially similar TFM concentration (based on alkalinity-pH chart or bioassay, whichever is lowest), and conditions the TFM concentration based on results of mussel and channel darter bioassay results. Sea lamprey ammocoetes were tested along with these non-target species, allowing comparison of the toxicity data as a proportion of sea lamprey MLC as required in the permits.

MATERIALS AND METHODS

These toxicity tests were performed using NYSDEC's mobile aquatic toxicology laboratory on-site at the Poultney River between September 12 - 18, 1996. The mobile lab was located adjacent to the Poultney River at Central Vermont Public Service's (CVPS) Carvers Falls hydroelectric station. Water was pumped continuously from the upstream side of Carvers Falls dam through coolers used for test organism acclimation to Poultney River water and the serial diluter in the mobile lab. The test methods were identical to those used in the standard pretreatment bioassay procedure outlined below to determine the MLC prior to all TFM treatments in the Lake Champlain watershed, except sediment was used in the test containers for the mussel bioassays. The methodology used in these pretreatment tests is consistent with USEPA and ASTM toxicity test methods.

The mobile lab diluter delivers 0.75 l/minute of stream water continuously to ten 40-l polyethylene test containers equipped with standpipe overflows. Test organisms are acclimated to test stream water for a minimum of 48-hours prior to initiation of the toxicity test. Approximately 24-hours before exposure to TFM begins, test animals are placed in the test system. Ten lamprey ammocoetes are placed in a stainless steel wire basket that is suspended in the test container. Non-target test organisms are placed in the polyethylene tank.

Nine of the test containers receive serially diluted concentrations of TFM (0.805 between concentrations), and one receives stream water without TFM (control). The TFM concentrations used are based on water chemistry comparison to pH/alkalinity charts and past stream experience. The predicted sea lamprey MLC concentration is used as the middle TFM concentration. Just prior to initiation of the TFM exposure period, the test container standpipes are removed to lower the water level to approximately 7 - 10 cm depth. A metering pump is then turned on, and a predetermined concentration of TFM stock solution is injected into the diluter. The standpipes are then replaced. This results in a TFM concentration pattern in the test chambers similar to an in-stream sea lamprey treatment. Water chemistry (pH and alkalinity) and mortality/morbidity data are recorded in each test container hourly during the TFM exposure. Water temperature is recorded continuously in one test container during the acclimation and test period. TFM exposure duration is dependant upon the anticipated stream treatment strategy, which usually involves a 12-hour TFM feed to establish a 9-hour lethal block. Therefore, the bioassay test organisms are usually exposed to TFM for a 12-hour period.

Because there was remote possibility that the test organisms might harbor immature stages of Zebra mussels (*Dressina polymorpha*), bioassay test water was filtered through a sand filter and/or the sand and gravel stream bank at Carvers Falls before discharge via groundwater to the Poultney River.

The lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) were determined empirically. Standard operating procedures for acute toxicity testing (ASTM and USEPA) allow 10% mortality (morbidity) in control exposures. At TFM concentrations that exceed 10% mortality (morbidity), it was assumed to be significant mortality. The highest TFM concentration that exhibited ≤10% mortality (morbidity) was the NOEC. The next higher TFM concentration that exhibited >10% mortality (morbidity) was the LOEC.

Adult Pocketbook Mussel Bioassay- September 14, 1996

Adult pocketbook mussels were collected by hand-picking in the Missisquoi River upstream from the Swanton dam, Swanton, Vermont, between September 9 - 12, 1996. Individuals that were ≥10cm total length were considered to be adults. Acclimation to Poultney River water began at 1400-hours on September 12, 1996. Sea lamprey ammocoetes were collected from the Poultney River by electrofishing on September 12, 1996, and held in Poultney River water until tested.

Seven to ten centimeters of gravel substrate from the Missisquoi River collection site was added to the test exposure chambers the afternoon of September 12, 1996, and diluter water flow from the Poultney River was initiated. Ten adult pocketbook mussels and ten sea lamprey ammocoetes were randomly placed in the test containers the morning of September 13, 1996. Twenty randomly selected mussels were measured for total length, total height, and weight prior to placing them in the test containers.

Mortality/morbidity checks on sea lamprey were performed hourly during the 12-hour TFM exposure period, and on visible pocketbook mussels after 3-, 6-, 9-, and 12-hours of exposure. The majority

of the mussels were buried in the sediment. After the 12-hour exposure check, the mussels in each exposure chamber were dug-out of the substrate, and data was recorded regarding mortality/morbidity on all the mussels. The mussels were then returned to the test chambers, and the diluter was left running with Poultney River water until the 12-hour post-exposure check. Mortality/morbidity checks were then performed at 12-, 36-, and 60-hours post-exposure, and then on Mondays, Wednesdays, and Fridays for two weeks post-exposure. Sea lamprey mortality was determined by standard pretreatment protocol, where a test animal is assumed to be dead if there is no tail movement. Data on pocketbook mortality/morbidity during the exposure period followed the three stages of intoxication described by Bills et al. (1992). Stage 1 mussels were buried in the substrate; Stage 2 mussels were lying on the substrate with foot extended and responsive to physical stimuli; and Stage 3 mussels were lying on the substrate with foot extended, valves gaping, and non-responsive to physical stimuli. For the post-exposure period, mortality/morbidity checks followed a modified Bills et al. (1992) procedure. Stage "1" mussels had valves nearly closed or closed and foot not extended; Stage "2" mussels had foot extended and respond to external stimuli; Stage "3"mussels had foot extended, valves gaping, and were non-responsive to external stimuli.

The adult mussels were left in the test chambers prior to the first (12-hour) post-exposure mortality/morbidity check. After this check, the mussels from each test container were placed in a labeled nylon-mesh bags with draw-strings and placed in flowing Poultney River water with aeration in a large tank. The bags were opened for the 24-hour and subsequent post-exposure mortality/morbidity checks.

Juvenile Pocketbook Mussels - September 16, 1996

Juvenile pocketbook mussels were collected by hand-picking in the Missisquoi River upstream from the Swanton dam, Swanton, Vermont, between September 9 - 13, 1996. Individuals that were <10cm total length were considered to be juveniles. Acclimation to Poultney River water began at 1400-hours on September 13, 1996. Sea lamprey ammocoetes were collected from the Great Chazy River by electrofishing on September 13, 1996. Acclimation to Poultney River water began at 1100-hours on September 14, 1996.

Seven to ten centimeters of gravel substrate from the Missisquoi River collection site was added to the test exposure chambers the afternoon of September 12, 1996, and diluter water flow from the Poultney River was initiated. This sediment had been used for the adult pocketbook mussel bioassay, so the TFM concentration in overlying water was tested before the juveniles were placed in the test chambers. All traces of TFM had been flushed from the test chambers during the 12-hour post-exposure period. Ten juvenile pocketbook mussels and ten sea lamprey ammocoetes were randomly placed in the test containers the morning of September 15, 1996. Twenty randomly selected mussels were measured for total length, total height, and weight prior to placing them in the test containers.

The mortality/morbidity checks methods were the same for sea lamprey and juvenile pocketbook mussels as they were for the adult pocketbook mussel bioassay.

Channel Darter Bioassay - September 18, 1996

Channel darters were seined by Kentucky and Vermont fisheries staff from the Kentucky River watershed in eastern Kentucky. They were collected on September 12 and 13, 1996, and transported via truck to the Poultney River on September 14, 1996. They were transported in four large plastic bags under oxygen. The bags were placed in coolers and lightly iced. They arrived at Carvers Falls at 1900-hours on September 14, 1996. The water temperature upon arrival was 17°C, and Poultney River water was 20°C. The bags with the channel darters were floated in the Poultney River water until the temperatures were equal, and then the darters were placed in a flow-through cooler with Poultney River water and aeration at 2000-hours. Out of 125 channel darters collected, 124 were alive upon arrival at Carvers Falls.

On September 17, 1996, ten channel darters were placed in each of the ten exposure chambers without sediment, and ten sea lamprey ammocoetes were placed in stainless steel baskets in each chamber. This is the standard pretreatment bioassay protocol. They were exposed to TFM for 12-hours, with mortality/morbidity, pH, alkalinity, and TFM concentration data collected hourly during the test.

RESULTS

The nine target diluter TFM concentrations ranged from 1.5 - 8.5 mg/l, plus the Poultney River control, for the mussel bioassays; and 1.7 - 9.5, plus Poultney River control, for the channel darter bioassay. The sea lamprey MLC in these three bioassays ranged from 3.5 - 5.0 mg/l for all three of these bioassays. The changes in MLC were due to changes in Poultney River water pH.

Adult Pocketbook Mussels

The adult pocketbook mussel bioassay was performed on September 14, 1996. Length and weight data are contained in Table 1.

Table 2 presents the mean pH, alkalinity, and TFM concentration data for the adult pocketbook mussel bioassay. The sea lamprey 9-hour MLC for this bioassay was 3.5 mg/l TFM or 1.6 times the predicted pH/alkalinity prediction chart value of 2.2 mg/l TFM. This result is consistent with past experience on the Poultney River. The TFM concentration data is also presented as a portion of the sea lamprey bioassay MLC.

The mussel mortality/morbidity data is summarized in Tables 2 and 3. Table 2 contains the data from the 3-, 6-, 9-, and 12-hour exposure checks, and 12-, 36-, and 60-hours post-exposure checks. (NOTE- Data for post-exposure checks beyond 60-hours was not yet available when this draft report was written. It will be included in a later draft.) Table 3 contains the data from the dug-out check after 12-hours of TFM exposure (or 0-hour post-exposure check).

The undisturbed mussels in the four exposure period checks showed a few mussels displayed stage

2 and 3 levels of TFM intoxication, but they were above the 1.0 x MLC TFM concentration proposed for Poultney River treatment. The Table 3 dug-out data after 12-hours of TFM exposure showed a higher incidence of mussel intoxication at TFM concentrations exceeding 1.0 x MLC. Except for four individual mussels at 1.2 x MLC, all adult pocketbook mussels exposed to greater than 1.0 x MLC concentrations of TFM exhibited either stage 2 or 3 levels of intoxication, but the majority of the mussels remained buried in the substrate.

The 60-hour post-exposure data (Table 2) showed that most of the intoxicated mussels quickly recovered. Additional post-exposure mortality/morbidity data will be reported when it is available.

Based on the 60-hour post-exposure recovery data, the NOEC TFM concentration is 1.5 x MLC for adult pocketbook mussels.

Juvenile Pocketbook Mussels

The juvenile pocketbook mussel bioassay was performed on September 16, 1996. Length and weight data are contained in Table 1. Table 4 presents the mean pH, alkalinity, and TFM concentration data for the juvenile pocketbook mussel bioassay. The sea lamprey 9-hour MLC for this bioassay was 4.5 mg/l TFM or 1.6 times the predicted pH/alkalinity prediction chart value of 2.8 mg/l TFM. This result is consistent with adult pocketbook bioassay and past experience on the Poultney River. The TFM concentration data is also presented as a portion of the sea lamprey bioassay MLC.

The mussel mortality/morbidity data is summarized in Tables 4 and 5. Table 4 contains the data from the 3-, 6-, 9-, and 12-hour exposure checks, and 12-, 36-, and 60-hours post-exposure checks. (NOTE- Data for post-exposure checks beyond 60-hours was not yet available when this draft report was written. It will be included in a later draft.) Table 5 contains the data from the dug-out check after 12-hours of TFM exposure (or 0-hour post-exposure check).

The undisturbed mussels in the four exposure period checks had only one mussel at a stage 2 level of TFM intoxication and no stage three mussels, but it was above (1.9 x MLC) the 1.0 x MLC TFM concentration proposed for Poultney River treatment. The Table 5 dug-out data after 12-hours of TFM exposure showed a higher incidence of mussel intoxication at TFM concentrations exceeding 0.8 x MLC. However, all of the mussels remained buried, and except for those at 1.9 x MLC and 1.5 x MLC, all recovered by 60-hours post exposure.

The 60-hour post-exposure data (Table 2) showed that most of the intoxicated mussels quickly recovered. Additional post-exposure mortality/morbidity data will be reported when it is available.

Based on the 60-hour post-exposure recovery data, the NOEC TFM concentration is 1.5 x MLC for juvenile pocketbook mussels.

Channel Darters

The channel darter bioassay was performed on September 18, 1996. Length and weight data are contained in Table 1. Table 6 presents the mean pH, alkalinity, TFM concentration data as mg/l and portion of pH/alkalinity chart MLC, and the 12-h exposure channel darter mortality data at each TFM concentration. The sea lamprey 9-hour MLC for this bioassay was 5.0 mg/l TFM or 1.5 times the predicted pH/alkalinity prediction chart value of 3.3 mg/l TFM. This result is consistent with the two pocketbook bioassays and past experience on the Poultney River.

Based on strictly empirical evaluation, the channel darter NOEC was equal to the sea lamprey MLC, or 5.0 mg/l. When the TFM concentration/morality was analyzed by linear regression without data transformation, the NOEC (i.e. - 20% channel darter mortality concentration) was 5.3 mg/l. When these data are plotted on semi-logarithmic graph paper, which is more appropriate for toxicity data, the NOEC was also 5.3 mg/l.

DISCUSSION

Bills et al. (1992) observed that many of the pink heelsplitter mussels (*Potamilus alatus*) exposed to ≥MLC concentrations of TFM exhibited Stages 2 and 3 of intoxication, and the mussels unburied themselves from the substrate. After 12-hours of exposure, most of the juvenile and adult pocketbook mussels exposed to 1.9 and 2.3 x MLC concentrations of TFM, respectively, remained buried, but their foot was extended when they were dug out of the gravel substrate. One concern with intoxicated mussels that lie on the surface of the substrate with their feet extended, is that they would be exceptionally vulnerable to predation by crayfish, birds, racoons, and etc. In this study, the foot on many of the intoxicated mussels was extended, but the majority of the mussels remained buried. These mussels looked just like the buried control organisms, and would be much less susceptible to predation.

The NOEC TFM concentrations for juvenile and adult pocketbook mussels at the 60-hour post exposure time were both 1.5 x MLC. The proposed Poultney River treatment at 1.0 will have no observable adverse impact on the pocketbook mussel population.

The channel darter margin of safety is somewhat narrower. The sea lamprey MLC and empirical NOEC were both 5.0 mg/l. Linear regression and semi-logarithmic plotting of the TFM concentration and mortality data both result in a refined NOEC (i.e. - 20 % mortality) of 5.3 mg/l TFM, or 1.1 x MLC.

This is a worst-case analysis, and the margin of safety for channel darters during the proposed 1.0 x MLC treatment of the Poultney River is expected to be much greater than is reflected in this bioassay. There several reasons for this assumption:

- The channel darters started to get "tail rot" (tail fin rotted-off and caudal peduncle tissue necrotic) the day before the test. Two dead darters were found with tail rot on

September 17, 1996. On September 18, 1996, an average of about two darters/test chamber were removed and replaced with seemingly healthy ones just before the test was initiated. This condition is not unusual, especially with darters, when fish are seined from their natural habitat, transported, and then held until testing. This is the same problem that the author experienced with Eastern sand darters (Neuderfer, 1987). These darters had an especially stressful transit from Kentucky over a two-day period of time.

The individual dead channel darters that died in exposure chambers 7 and 4 at 5- and 9-hours of exposure, respectively, each has tail rot. The darter that died in chamber number 8 at 8-hours of exposure, had a external parasite embedded in it's tail. Although other dead individuals were not closely examined, it is likely that some had tail rot. One or two individuals in each exposure chamber, including the control, showed signs of developing tail rot by the latter half of the 12-hour exposure period.

- The channel darters in the bioassay were exposed to target bioassay TFM concentrations within one-hour of test initiation until test termination 12-hours later. This does not give non-target organisms much time to begin to conjugate and excrete TFM. This will not happen in the Poultney River during an actual 1.0 x MLC treatment. Because the 1.0 x MLC TFM concentration cannot be appreciably exceed, the initial TFM feed rate will start at less than 1.0 x MLC, then be slowly increase up to 1.0 x MLC over several hours. This will ensure that 1.0 x MLC is not exceeded, and allow non-target organisms to begin to conjugate and excrete TFM. The leading and trailing TFM plume edges attenuate as well. That is the reason that TFM is proposed to be feed for 12-hours at Carvers Falls, so that a 9-hour lethal TFM block will occur at downstream station.

Literature Cited

Bills, T.D., J.J. Rach, L.L. Marking, and G.E. Howe. 1992. Effects of the lampricide 3-trifluoromethyl-4-nitrophenol on the pink heelsplitter. U.S. Fish and Wildlife Service, Resource Publication 183. 7pp.

Neuderfer, G.N. 1987. Relative sensitivity of several fish species to TFM, with special emphasis on eastern sand darter, muskellunge, logperch, and landlocked Atlantic salmon. New York Department of Environmental Conservation, Administrative Report, Avon.

Table 1. Length and weight data for the juvenile and adult pocketbook mussel, and channel darter bioassays performed on the Poultney River in September, 1996.

Parameter Measured	Adult Pocketbook Mussels		Juvenile Pocketbook Mussels		Channel Darters	
	Mean	Range	Mean	Range	Mean	Range
Total Length (mm)	122	103-145	75	40-66	39	31-49
Total Height (mm)	81	72-97	52	31-66		
Wet Weight (g)	331	188-505	76	9-191	0.61	0.28-1.11

Table 2. Exposure and post-exposure mortality/morbidity data summary for adult pocketbook mussels from a 12-hour TFM lampricide exposure on September 14, 1996, with comparison to 9-hr. sea lamprey MLC.

Exposure Chamber Number	Mean TFM (mg/l)	Mean pH	Mean Alk (mg/l)	Mean D.O. (mg/l	TFM Conc. as Portion of 9-hr. MLC	Number of Mussels at Modified Bills et al. (1992) Intoxication Stage Number:																				
						Hours of Exposure to TFM												Hours of Post-Exposure								
						3-hours			6-hours			9-hours			12-hours			12-hours			36-hours			60-hours		
						1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
10	8.1	8.05	106	8.4	2.3	9	1		7	3		7		3	7		3	8	2		9		1	9		1
9	6.5	8.01			1.9	10			9	1		9		1	9		1	7	2	1	8		2	8		2
8	5.2	7.97			1.5	10			9	1		9	1		9	1		9		1	9	1		9		1
7	4.2	7.96			1.2	10			9	1		9	1		9	1		9	1		10			10		
6	3.4	7.93		7.9	1.0	10			10			10			10			10			10			10		
5	2.7	7.99			0.8	10			10			10			10			10			10			10		
4	2.2	8.01			0.6	10			10			10			10			10			10			10		
3	1.8	8.06			0.5	9	1		10			10			10			10			10			10		
2	1.4	8.06			0.4	10			10			10			10			10			10			10		
1	0.0	8.08		8.4	NA	10			10			10			10			10			10			10		

Key to the modified Bills et al. (1992) stages of intoxication used in this table:

Stage "1" Mussels buried or unburied, valves nearly closed or closed, and foot not extended.
Stage "2" Mussels unburied with foot extended, and respond to external stimuli.
Stage "3" Mussels unburied with foot extended, valves gaping, and non-responsive to external stimuli.

Table 3. Summary of adult pocketbook mussel mortality/morbidity data collected after 12-hours of exposure to TFM (when the mussels were dug-out of the substrate), with comparison to 9-hr. sea lamprey MLC.

Exposure Chamber Number	Mean TFM (mg/l)	TFM Conc. as Portion of 9-hr. MLC	Number of Mussels at modified Bills et al. (1992) Intoxication Stage Number:					
			Stage 1		Stage 2		Stage 3	
			Unburied	Buried	Unburied	Buried	Unburied	Buried
10	8.1	2.3					3	7
9	6.5	1.9				3	1	6
8	5.2	1.5			1	9		
7	4.2	1.2		4	1	5		
6	3.4	1.0		10				
5	2.7	0.8		10				
4	2.2	0.6		10				
3	1.8	0.5		10				
2	1.4	0.4		10				
1	0.0	NA		10				

Key to the modified Bills et al. (1992) stages of intoxication used in this table:

Stage "1" Mussels with valves nearly closed or closed, and foot not extended.
Stage "2" Mussels with foot extended, and respond to external stimuli.
Stage "3" Mussels with foot extended, valves gaping, and non-responsive to external stimuli.

Table 4. Exposure and post-exposure mortality/morbidity data summary for juvenile pocketbook mussels from a 12-hour TFM lampricide exposure on September 16, 1996, with comparison to 9-hr. sea lamprey MLC.

Exposure Chamber Number	Mean TFM (mg/l)	Mean pH	Mean Alk. (mg/l)	Mean D.O. (mg/l)	TFM Conc. as Portion of 9-hr. MLC	Number of Mussels at Modified Bills et al. (1992) Intoxication Stage Number:																				
						Hours of Exposure to TFM												Hours of Post-Exposure								
						3-hours			6-hours			9-hours			12-hours			12-hours			36-hours			60-hours		
						1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
10	8.4	8.21	105	9.0	1.9	10			9	1		9		1	9		1	4	1	5	5		5	7		3
9	6.7	8.18			1.5	10			10			10			10			8	2		9		1	9	1	
8	5.3	8.18			1.2	10			10			10			10			9	1		10			10		
7	4.3	8.14			1.0	10			10			10			10			10			10			10		
6	3.5	8.15		8.5	0.8	10			10			10			10			10			10			10		
5	2.8	8.16			0.6	10			10			10			10			10			10			10		
4	2.3	8.18			0.5	10			10			10			10			10			10			10		
3	1.8	8.20			0.4	10			10			10			10			10			10			10		
2	1.5	8.23			0.3	10			10			10			10			10			10			10		
1	0.0	8.23		8.9	NA	10			10			10			10			10			10			10		

Key to the modified Bills et al. (1992) stages of intoxication used in this table:

Stage "1" Mussels buried or unburied, valves nearly closed or closed, and foot not extended.
Stage "2" Mussels unburied with foot extended, and respond to external stimuli.
Stage "3" Mussels unburied with foot extended, valves gaping, and non-responsive to external stimuli.

Table 5. Summary of juvenile pocketbook mussel mortality/morbidity data collected after 12-hours of exposure to TFM (when the mussels were dug-out of the substrate), with comparison to 9-hr. sea lamprey MLC.

Exposure Chamber Number	Mean TFM (mg/l)	TFM Conc. as Portion of 9-hr. MLC	Number of Mussels at modified Bills et al. (1992) Intoxication Stage Number:					
			Stage 1		Stage 2		Stage 3	
			Unburied	Buried	Unburied	Buried	Unburied	Buried
10	8.4	1.9				2	1	7
9	6.7	1.5		2		4		4
8	5.3	1.2		9		1		
7	4.3	1.0		8		2		
6	3.5	0.8		8		2		
5	2.8	0.6		10				
4	2.3	0.5		10				
3	1.8	0.4		10				
2	1.5	0.3		10				
1	0.0	NA		10				

Key to the modified Bills et al. (1992) stages of intoxication used in this table:

Stage "1" Mussels with valves nearly closed or closed, and foot not extended.
Stage "2" Mussels with foot extended, and respond to external stimuli.
Stage "3" Mussels with foot extended, valves gaping, and non-responsive to external stimuli.

Table 6. Summary of channel darter mortality data from September 18, 1996, Poultney River TFM bioassay, with comparison to 9-hr. sea lamprey MLC.

Exposure Chamber Number	Mean pH	Mean Alk. (mg/l)	Mean 12-hr. TFM Concentration (mg/l)	TFM Concentration as Portion of 9-hr. MLC	Percent Channel Darter Mortality
10	8.21	108	9.7	1.9	100
9	8.23		7.7	1.5	100
8	8.25		6.3	1.3	50
7	8.25		5.0	1.0	10
6	8.24		4.2	0.8	0
5	8.25		3.4	0.7	0
4	8.26		2.7	0.5	10
3	8.26		2.3	0.5	0
2	8.26		1.8	0.4	0
1	8.26		0.0	NA	0

Agency of Natural Resources
Department of Environmental Conservation

Water Quality Division
Biomonitoring and Aquatic Studies U.
R.A. LaRosa Laboratory
802-244-4520

MEMORANDUM

To: John Anderson

From: Steve Fiske

Date: 9/29/96

Subject: Lampsilis ovata ageing TFM Bioassay

Attached is the raw data for both juvenile and adult mussels used in the TFM bioassay on Lampsilis ovata. All animals aged were L.ovata except one in the adult bag #8 which was an eight year old L.radiata. I have summarized the juvenile data to the right of the raw data by number of 3+ animals per treatment dose. 3+ animals are in their fourth year of growth. Please give me a call if you have any questions on the ageing. I can be at the Poultney next Friday 10/4/96 to age the L.recta. If the L.recta do not need to be returned to Kentucky the native mussel management group would like to allocate some of the adults to be used for host fish work on this species next spring. I will discuss possible options with Mark Fergusen, Madeleine Lyttle, and Chris Fichtel.

7/25/96

ER = Eroded

Pictures #1 = 1 adlt #4

#2 = 1 juv #5

#3 = 2 adlt #6 = 0

Lampsilis ovata

Adults

Range 5–13

Bag #														
	1	9	6	5	10	10	7	7	ER 7+	9	9		5	10
	2	9	10	6	9	10	9	10	12	11	11		6	10
	3	8	6	6	8	7	11	8	9	8	9		5	11
	4	6	8	8	11	ER 13	10	8	12	7	–		6	13
	5	9	5	10	8	11	12	9	10	10	8		5	12
QA →	6	6	6	10	9	6	10	6	7	ER 9+	7		6	10
	7	ER 10	9	8	8	10	6	5	8	8	9		5	10
LR →	8	6	LR 9	8	9	10	11	8	9	9	–		6	11
	9	8	8	6	6	10	8	6	8	–	–		6	10
	10	8	6	9	7	8	7	9	7	8	–		6	9
Bottom of Box		13	10	7	10	10							7	13

Total 1+ 1–8

% Mortality of 3+ & 4+

3+ #	%	4+ #	%
5	0	9	0
9	0	10	0
6	0	6	0
6	0	8	0
5	0	8	0
5	0	6	0
4	0	7	0
5	0	7	0
6	0	7	0
7	44%	10	30%

Juveniles

1	2	2	3	3	3	4	5	4	4	4		2–8	
2	4	3	3	3	2+	2+	2+	2+	3	2+		2–4	
3	3	2	2	2	2	3	5	5	6	5		2–6	
4	1+	1+	2	2	5	4	3	3	4	6		1–6	
5	1+	1+	2	3	5	5	4	4	4	2		1–5	
→6	1+	2+	3+	3+	5	4	6	5	8	3		1–8	
7	2+	2+	3	3	4	6	5	4	4	5		2–6	
8	1+	1+	1+	5	5	4	6	4	3	2+		1–6	
9	3	5	5	4	1	1	2	2	2	(5) → almost dead		1–5	
10	(2)	2	3	4	4	3	(4)	–	–	–		2–4	(2 not closers complete)

splayed foot, not pulling it in.

?

Dead (shells) 3 2 2 7

Dead

#6 adult bag

152nd mussel = L. radiata ♀

Agency of Natural Resources
Department of Environmental Conservation

Water Quality Division
Biomonitoring and Aquatic Studies U.
R.A. LaRosa Laboratory
802-244-4520

MEMORANDUM

To: Dick Neves, Virginia F&W Unit

From: Steve Fiske, Vt DEC

Date: 10/02/96

Subject: TFM Toxicity Draft reports on two native mussels

Attached are two draft reports submitted to the Vt DEC as required in a draft TFM Lampricide treatment pesticide permit. Thanks again for your assistance in locating the sandshell populations for our F&W Dept. It appears that Barry Whicklow has offered to do some host fish work on the sandshell for the Poultney River, Vt. He will only need about 24 animals for his work. The rest we will gladly send to you after the full two week post exposure observations are made. This should be about October 14 we will be in touch again before we send them.