ASSESSMENT OF POSSIBLE PESTICIDE ACCUMULATION IN THE SEDIMENT OF THE BATTEN KILL RIVER, 2008.

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INTRODUCTION:

The Batten Kill is one of Vermont's premier trout fisheries, but has undergone a decline in trout in recent years. Various efforts by the U.S. Forest Service (USFS), Vermont Department of Environmental Conservation (VTDEC), Vermont Agency of Agriculture, Food and Markets (VTAAFM), and others have investigated this problem over the past several years. Previous efforts by the VTAAFM in the Batten Kill watershed have concentrated on investigating possible pesticide runoff from the use of herbicides to control vegetation on railroad beds, and on the use of herbicides and fungicides on golf courses. These earlier pesticide investigations concentrated on pesticides dissolved in the water column which have washed into surface waters during rain storms. A summary of the results of these studies can be seen in Table 1. The majority of detections (46 of 68 or 68%) were of the fungicides Chlorothalonil and Pentachloronitrobenzene (PCNB) used on golf courses to control winter snow mold. Five of these Chlorothalonil detections were above the VTDEC Maximum Allowable Concentration (MAC) indicating levels of possible environmental concern. The timing and purpose of these fungicide applications leads to the likelihood that they will runoff after application. These fungicides are applied in late fall in an attempt to control 'snow mold' growth during the winter. The fungicides are applied as late in the fall as possible, with the aim of having them on the ground when snow comes to stay in the winter. Sampling was aimed at worst case conditions; a hard rain in late November or December before snow covered the ground or in early spring during snowmelt.

The current project has been aimed at a different group of pesticides; those which are only slightly water soluble and therefore bind to sediments rather than stay in the water column. These pesticides will not wash off the terrain dissolved in water, but will stay adsorbed to solid particles so they will only end up in the stream if particulate matter washes off site during large rainstorms. The pesticides under investigation were selected based on their use in the Bennington County area, and their chemical characteristics.

The VTAAFM maintains a database of the annual use of pesticides by commercial pesticide applicators by county in Vermont. Table 2 lists some of the major pesticides used in Bennington County in 2006 by use pattern, as well as chemical characteristics of each. In order to prioritize possible pesticides to concentrate on, it was decided to choose those used in excess of 100 pounds in 2006 in Bennington County, and those with a Koc of greater than 100. The Koc is the the soil adsorption coefficient which is a measure of how likely the compound will tend to bind to soil. The larger the number the more likely the compound will attach to soil/sediment organic matter. The next filter was to choose those which were most toxic, the toxicity to rainbow trout was used as an indicator of aquatic toxicity. A rainbow trout LC50 toxicity value of less than 1000 ppb was chosen as the cutoff, which means that it takes less than 1000 ppb in the water to kill 50% of the trout in a controlled study. Based on these criteria, the final analyte list was selected. In summary, these compounds were selected because they were used in significant amounts in Bennington County in 2006, are likely to be associated with sediment rather than be dissolved in the water column, and are relatively toxic to aquatic organisms.

Table 3 contains the final list of compounds selected for analysis for this project. Initially, chlorothalonil was to be included in this list, but it was not possible to develop a method for the extraction of chlorothalonil from sediments which was compatible with extraction of the other pesticides, and timing/funding was not sufficient to develop a method for chlorothalonil by itself. So chlorothalonil was not part of the final analyte list.

The state of California and the U.S. Geologic Survey have devoted significant resources to developing methods for the analysis of pyrethroid insecticides, such as permethrin and bifenthrin, in sediment, because they are highly toxic to aquatic insects (Hladik 2007). Hladik stressed the importance of developing methods for the analysis of pyethroid insecticides in sediment to the low parts per billion (ppb or ng/g) level because these are the environmentally relevant concentrations for aquatic toxicity. Table 4 (from Hladik 2007) shows that several pyrethroids are very toxic to Hyalella azteca, an aquatic insect often used in standardized sediment toxicity tests. Because some of these pesticides are so potentially toxic to aquatic organisms, it has been important to have a very sensitive analytical method, it is not useful to have a method which can't detect the compounds at levels where they may cause problems. The goal for this study was to use the methods in Hladik (2007) as a starting point for developing methods suitable to the compounds of interest and the technology available to the VTAAFM, and hopefully being able to detect the pesticides at a level of 1.0 ppb (ng/g)

MATERIALS AND METHODS:

Sampling site selection:

A preliminary assessment of potential sampling sites was undertaken using topographic maps, aerial photographs and the locations of golf courses, condominium and other concentrated residential development. This information was used during a site visit to select final sampling sites. Within these sampling sites, one or more specific sampling locations were chosen based on visual observation of areas where fine sediment deposition was occurring.

A single set of samples was collected on September 10, 2008, when a significant rain occurred after several weeks of dry weather. At each sampling location a clean metal trowel was used to collect the fine, superficial sediment from the top approximately 2-5 cm of sediment in an area where fine sediment was accumulating. The sediment was collected into pre-cleaned 2000 ml wide mouth glass jars, labeled and kept in a cooler for transport to the lab. Sampling equipment was cleaned between sampling locations using water to wash off visible dirt and methanol to rinse off potential organic contamination.

Sediment sample prep:

Bulk sediment samples were kept at 4C until preliminary processing could take place the next day. Each sample jar was swirled to mix the sediment/water mixture and then allowed to settle. Excess water was poured off, then the top, fine layer of sediment was carefully removed with a stainless steel scoop into a separate clean jar and allowed to air dry in a fume hood overnight then frozen until analysis. The remainder of the sediment was retained and frozen for a wet analysis. So each sample was analyzed once as a dry/fine sediment analysis and once as a wet/bulk analysis.

Dry/Fine Sediment Extraction:

Pyrethroids and Pendimethalin: Dry sediment extraction for bifenthrin, permethrin and pendimethalin was performed using a Dionex Accelerated Solvent Extractor (ASE). ASE conditions were as follows: solvent 50% hexane/50% acetone plus 1% acetic acid, extraction temperature 100C, with two 15 minute extractions. 10g of air dried sediment was weighed into a 250ml glass jar and mixed with 10g of clean Ottawa sand and transferred to a 33cc stainless steel cell containing two cellulose filters plus about 1cm of clean Ottowa sand. The cell was topped off with sand plus a cellulose filter, before sealing. The resulting extract was evaporated just to dryness using an N-EVAP brand nitrogen evaporator at 40C. Twenty milliliters of toluene was added to the dried extract, sonicated for 1 min. and centrifuged for 5 minutes at 3000 rpm. The clear supernatant was pipetted into a clean 30ml vial and

evaporated just to dryness using the N-EVAP and re-dissolved in 10ml of petroleum ether. The petroleum ether solution was filtered thru a florisil cartridge conditioned with petroleum ether and the filtrate was discarded. The florisil cartridge was eluted with 25ml of 15% ethyl ether in petroleum ether, which was then evaporated just to dryness using the N-EVAP and re-dissolved in 2ml of toluene for analysis.

PCNB: PCNB was not adequately recovered using the above extraction, so a second extraction of dried sediment was performed for PCNB. 4.0g of dried sediment was weighed into a 40ml vial, 25 ml of 50% hexane/50% acetone plus 1% acetic acid was added and the sample was shaken for 20 minutes using a wrist action shaker. Samples were then sonicated for 20 minutes and shaken again for 20 minutes. The samples were then centrifuged at 3000rpm for 10 minutes and the clear supernatant was pipetted into a clean 30 ml vial before evaporating just to dryness using the N-EVAP and re-dissolving the sample in 2ml toluene for analysis.

Wet/Bulk Sediment Extraction: The wet sediment samples were thoroughly mixed prior to subsampling using a stainless steel scoop. At the same time a portion of the sample was weighed into an aluminum weigh dish and dried overnight at 100C to determine percent moisture on each sample. Approximately 80g of wet sediment was weighed into a 200ml Teflon centrifuge bottle. The resulting slurry was extracted using 150ml of 50% hexane/ 50% acetone plus 1% acetic acid by shaking for 20 minutes, sonicating 20 minutes and shaking twenty minutes. The sample was then centrifuged at approx. 4000 rpm for 10 minutes, and the top, hexane layer was pipetted into a 100ml graduated cylinder to measure the volume of extract.

Pyrethroids and Pendimethalin: One half of the hexane extract was evaporated just to dryness and re-dissolved in 10 ml petroleum ether before being cleaned using the florisil cartridge as described above with a final volume of 2ml toluene for analysis.

PCNB: One half of the remaining extract, one quarter of the original solution, was evaporated just to dryness using the N-EVAP and re-dissolved in 2 ml of toluene for analysis.

Extract Analysis:

Pyrethroids and Pendimethalin: These pesticides were analyzed by Gas Chromatography with Mass Spectrometry (GC/MS), in order to get adequate sensitivity it was necessary to utilize the MS/MS capabilities of the Thermo Trace GC with Polaris Q MS/MS instrument. The analytical column used was a 30m x 0.25mm x 0.25µm DB-5ms column. The GC conditions were a temperature gradient from 70C to 270C in 23 minutes, with a 1.0 µl splitless injection. The MS/MS transitions are listed in Table 5.

PCNB: PCNB was able to be detected at lower levels using a GC with Electron Capture Detector (ECD) because this detector is highly sensitive to chlorinated compounds. So a GC/ECD was utilized using the same column and GC conditions.

RESULTS and DISCUSSION:

SAMPLE LOCATIONS: Sampling took place on 10 September 2008. This date was selected because it was just after the first significant rainstorm after Labor Day, when homeowners might be expected to be doing lawn work and utilizing pesticides. There had also been a several week dry period prior to this rain event (see Figure 1.) The combination of a rainstorm after a dry period and a holiday weekend make this a likely time to detect pesticide runoff if any is occurring.

Sample locations were preliminarily chosen using aerial photographs and topographic maps (GOOGLE EARTH). Criteria were main stem or tributaries downstream of major urban/residential areas, golf courses, or condominiums. Final site selections were made by site visits to find accessible areas where fine sediment was collecting in the stream bottom in two feet of water or less. Figure 2 depicts where the sample sites were located and Table 6 describes them.

EXTRACTION: When this project was initiated, it was assumed that several sampling trips would be undertaken, and that each sample would be analyzed once for the suite of pesticides of interest. It turned out that the extraction process was more complicated than expected, and that not all pesticides could be extracted from sediment at the same time. Pendimethalin, bifenthrin, and permethrin were able to be extracted together, but the fungicides chlorothalonil and PCNB were not compatable with that extraction. A separate extraction was developed for the fungicides, but chlorothalonil extraction efficiency was still not satisfactory so it had to be excluded from this study.

Each sample as received at the lab was a slurry of water and sediments. In order to maximize the likelihood of detecting any pesticides in the sediment collected, each sample was well mixed and then allowed to settle and the water poured off. Then the top, fine sediment was carefully scooped off the top into a separate container. This fine sediment was allowed to air dry to ensure complete extraction of the non-water soluble pesticides from the sediment. Previous research (Gan et al. 2005) has shown that hydrophobic pesticides, like the pyrethroid insecticides, are primarily associated with fine sediments of high organic content so selecting the finest sediments to analyze separately should maximize the likelihood of detecting trace amounts of these pesticides. The remaining sediment/water slurry was well mixed and a portion removed for moisture analysis. Then a portion was weighed to be extracted wet, to ensure that nothing was missed or lost during the dry extraction.

ANALYSIS: The pyrethroid insecticides bifenthrin and permethrin, as well as the herbicide pendimethalin, were analyzed by Gas Chromatography/Mass Spectrometry/Mass Spectrometry. This technique proved most sensitive for these compounds. Gas Chromatography with an Electron Capture Detector was found to be more sensitive for PCNB due to the five chlorine atoms present in this pesticide.

As can be seen in Table 5, it was possible to optimize the methodology to get a detection limit (MDL, which is the lowest level one can statistically differentiate from random noise) of near 1.0 ng/g for all compounds of interest. Obtaining this detection limit took a concerted effort, and the decision was made to work on pushing this as low as possible rather than collecting more samples and analyzing them with less sensitivity. Since bifenthrin has a reported aquatic insect LC50 of 5ng/g for sediment (Table 4), it was deemed most important to be able to reliably detect and quantify this compound at levels below 5 ng/g. This goal was reached, but at the expense of analyzing more samples, or more pesticides per sample.

RESULTS: All samples were analyzed four times: wet and dry extractions for PCNB, and wet and dry extractions for bifenthrin, permethrin, and pendimethalin. Results are listed in Table 7. No pendimethalin, permethrin, or PCNB was detected in any samples. Bifenthrin was detected in both the wet and dry extracts from PMP 8863, the site on the West Branch by Riverside Heights. Also, a trace of bifenthrin was detected at the next site downstream from there (PMP 8864, dry extraction), the Union St. site by the water supply station.

Bifenthrin is a widely used insecticide, for lawn treatments by homeowners and professionals, and for insect control such as residential ant control and landscaper tent caterpillar control. Any combination of these uses could have contributed to the bifenthrin seen in these samples. Although the amount of bifenthrin detected was quite low, and near the limit of detection of the current method, it is still of concern. Hladik (2007) list an LC50 for *Hyalella azteca* in sediment of 5 ng/g (see Table 3). This means that a concentration of 5 ng/g bifenthrin in sediment was sufficient to kill 50% of the insects during the course of the 10 day experiment. While none of the sediments in this

study exceeded this value, the bifenthrin concentration of 2-3 ng/g in PMP 8863 is high enough that it may be causing some toxicity to benthic organisms. Other researchers have found that bifenthrin binds quite tightly to fine organic sediments, so it is not likely to be found in the water column. It was initially thought that this meant bifenthrin and other pyrethroids were not likely to wash off site during rain events. It is now recognized that significant amounts of hydrophobic pesticides like these can wash off with fine sediment to enter the aquatic environment, and accumulate in areas where fine sediments settle out. Being bound to sediment they may not be highly bioavailable, and can be buried under fresh sediment and effectively removed from the system, until re-suspended during scouring runoff events. Being hydrophobic, it is also possible that pyrethroid insecticides may bioaccumulate within the food chain.

Since bifenthrin is not likely to be in the water column, it is probably not going to effect the fish population of the Batten Kill directly. It is possible though that if there is chronic exposure of the aquatic insects to bifenthrin, that toxicity may be causing a change of food availability for fish, or that bifenthrin may biomagnify through the food chain to have an effect on predatory fish. According to Fecko (1999), partial mortality of gizzard shad, a detritus feeder, has been observed as low as 0.185 ng/g bifenthrin, with complete mortality at 7.75 ng/g.

Bifenthrin was only detected at quantifiable levels at one site on one day during this study, so the environmental significance is unknown. But, because bifenthrin is so highly toxic it would probably be prudent for the VTAAFM and/or USFS to follow up on this detection by undertaking further sampling and testing in the area in 2010. If further detections are observed, then sediment toxicity testing with the aquatic insect Hyalella azteca may be warranted.

REFERENCES:

Fecko,A. 1999. Environmental fate of bifenthrin. California Department of Pesticide Regulation.

Gan, J., J.S. Lee, W.P. Liu, D.L. Haver, and J.N. Kabashima. 2005. Distribution and Persistence of pyrethroids in Runoff Sediments. J. of Environmental Quality. 34: 836-841.

Hladik, M. 2007. Methods Development for Pyrethroid Pesticides in Environmental Samples. Final report for CALFED.

TABLE 1. Summary of Battenkill area Water Sample results, 2001-2007.

Pesticide	# of samples analyzed	# (%) detected	MAC (ppb)*	#(%) above MAC
Railroad				
Diuron	69	2	160	0
Imazapyr	69	1	1500	0
Sulfometuron methyl	69	1	5000	0
Metsulfuron methyl	69	2	365	0
Golf				
Chlorothalonil	60	15	0.47	5
Chlorpyrifos	60	1	0.083	1
Iprodione	60	5	6.5	0
PCNB	60	31	5.0	0
Triadimefon	60	6	24	0
2,4-D	33	3	120	0
Dacthal	33	0	??	0
Dicamba	33	1	420	0
MCPA	33	0	3	0
MCPP	33	0	1860	0
Triclopyr	33	0	1860	0

*** MAC = Maximum Allowable Concentration (VTDEC).**

Table 2. Characteristics of major pesticides used in Bennington County, 2006.
(* = use > 100#, and Koc > 100)**

Pesticide	pounds used (in Bennington County)	toxicity (Rainbow Trout LC50, ppb)	KOC (soil absorption coefficient)
Herbicides			
Glyphosate	956	72,000	
Atrazine	799	14,000	
2,4-D	451	410,000	60
Dimethenamid	402	3,300	
Diuron ***	348	10,000	400
Pendimethalin ***	194	350	5000
Triclopyr	186	5300	59
Fosamine ammonium	216	238,000	
Fungicides			
Chlorothalonil ***	2807	35	1380
PCNB ***	1860	656	5000
Propiconazole ***	1641	5700	650
Methyl isothiocyanate	518	94	
Iprodione***	404	4200	700
Thiophanate methyl	300	11,400	
Copper naphthenate	252	161	
Mancozeb	190	1,500	
Thiabendazole	174	2,400	
Chloroneb	103	3,700	
Insecticides			
Bifenthrin***	1046	0.2	237,000
Permethrin***	686	24	86,000

TABLE 3. CHEMICAL CHARACTERISTICS OF FINAL ANALYTE LIST.

PESTICIDE	2006 BENNINGTON COUNTY USE (pounds)	TOXICITY Rainbow Trout (LC50) (parts per billion)	SOIL ABSORPTION COEEFICIENT (Koc)	SOIL HALF LIFE (days)	OCTANOL/WATER PARTITION COEFFICIENT (Kow)
Bifenthrin	1046	0.2	237,000	122+	1,000,000
Permethrin	686	24	86,000	42	1,260,000
Pendimethalin	194	350	5,000	165	151,000
PCNB	1860	656	5,000	65	28,800

TABLE 4. TOXICITY VALUES OF PYRTHOIDS FOR ORGANISMS IN WATER AND SEDIMENT.

Pyrethroid	**Fresh Water**		**Salt Water**		**Sediment**	
	48 hr LC_{50} (ng/L)	**Organism**	**96 hr LC_{50} (ng/L)**	**Organism**	**10day LC_{50} (ng/g)**	**Organism**
Bifenthrin	70	*Ceriodaphnia dubia*	4	*Americamysis bahia*	5	*Hyalella azteca*
Cyfluthrin	140	*Ceriodaphnia dubia*	2	*Americamysis bahia*	10	*Hyalella azteca*
?-Cyhalothrin	300	*Ceriodaphnia dubia*	4	*Americamysis bahia*	5	*Hyalella azteca*
Cypermethrin	130	*Ceriodaphnia dubia*	5	*Americamysis bahia*	3-6	*Hyalella azteca*
Deltamethrin	37	*Daphnia magna*	17	*Americamysis bahia*		
Esfenvalerate	240	*Daphnia magna*	38	*Americamysis bahia*	15	*Hyalella azteca*
Permethrin	75	*Daphnia magna*	20	*Americamysis bahia*	110	*Hyalella azteca*

Water data from: EPA Ecotox, CDPR Ecotox. Sediment data from: Amwag et al., 2005, Environ. Toxicol. Chem., 24, 966-972; Maund et al., 2002, Environ. Toxicol. Chem., 21, 9-15

(table from Hladik 2007)

TABLE 5. ANALYTICAL PARAMETERS.

COMPOUND	METHOD DETECTION LIMIT (MDL) (ng/g = PPB)	AVERAGE PERCENT RECOVERY	LOWER LIMIT OF QUANTITATION (ng/g = PPB)	RETENTION TIME (minutes)	GC/MS/MS transition
Bifenthrin	1.16	82%	2.0	15.65	181->166
Permethrin	1.38	92%	4.0	19.50/19.81	183-> 165
Pendimethalin	1.11	84%	2.0	9.25	252->162
PCNB	1.72	53%	5.0	9.72	NA

TABLE 6. SAMPLE LOCATIONS:

SAMPLE NUMBER	GPS SITE NUMBER	SITE DESCRIPTION
8861	13	Railroad bridge behind“GWC MOTORS” west side
8862	14	Railroad bridge behind“GWC MOTORS” east side
8863	15	West Branch at Riverside Heights
8864	16	Union Street by water supply station
8865	17	Richville Rd. at River Rd.
8866	18	Walking path at Riverbend Rd.
8867	19	Bridge abutment on River Rd. near Sunderland
8868	20	Upstream of Manchester at “A Safe Place”

TABLE 7. RESULTS:

SAMPLE NUMBER	WET/DRY	PENDIMETHALIN	BIFENTHRIN	PERMETHRIN*	PCNB
8861	Wet	ND**	ND	ND	ND
8861	Dry	ND	ND	ND	ND
8862	Wet	ND	ND	ND	ND
8862	Dry	ND	ND	ND	ND
8863	Wet	ND	3.17 ng/g	ND	ND
8863	Dry	ND	2.20 ng/g	ND	ND
8864	Wet	ND	ND	ND	ND
8864	Dry	ND	Trace***	ND	ND
8865	Wet	ND	ND	ND	ND
8865	Dry	ND	ND	ND	ND
8866	Wet	ND	ND	ND	ND
8866	Dry	ND	ND	ND	ND
8867	Wet	ND	ND	ND	ND
8867	Dry	ND	ND	ND	ND
8868	Wet	ND	ND	ND	ND
8868	Dry	ND	ND	ND	ND

* Permethrin results are based on the sum of the two isomers of permethrin.

** ND = none detected

*** trace = detectable, but below the MDL and below a quantifiable level.

FIGURE 1. WALLOOMSAC RIVER FLOW DURING SUMMER 2008.

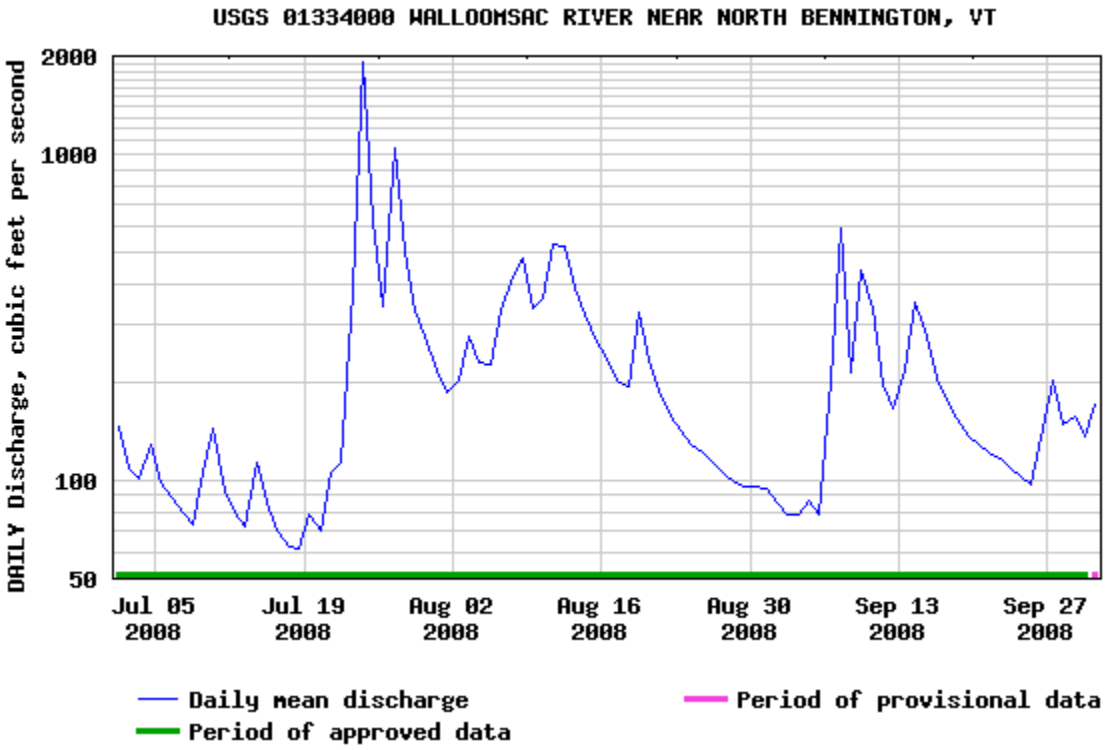

FIGURE 2. SAMPLING LOCATIONS, MANCHESTER, VT 9/10/08